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Full Length Research Paper

Toxoplasmosis Seroprevalence in pregnant women and in sheep and goats intended for human consumption in Dakar, using Direct Agglutination High sensitivity techniques

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Many studies have identified meat as an important vehicle for *Toxoplasma gondii* human contamination. In Senegal, meat consumption is important, consequently, humans are exposed. However, few data are available on toxoplasmosis infection in women and animals, especially, sheep and goats. This survey assessed toxoplasmosis prevalence in pregnant women in the suburb and in sheep and goats, using the Direct Agglutination High Sensitivity (DAHS) in order to better estimate the potential risk carried out by women meat consumption. The serology using DAHS test detected anti toxoplasma IgG antibodies in 123 out of 313 pregnant women [39.30%, 95% CI: 33.8-44.7%]. The infection tends to increase with age. In animals, a sample of 198 sheep and 65 goats was picked from the official slaughter site of Dakar. Anti-t*oxoplasma* IgG were found in 55% of sheep and 55.4% of goats. In both species, females were more affected than males without any statistically significant difference. These results indicate that toxoplasmosis is present in Dakar with a high prevalence in pregnant women but also in sheep and goats, which are an important parasite reservoir. The consumption of meat, without proper cooking, might be pregnant women's contamination factor.

Keywords: Toxoplasmosis, pregnant women, sheep, goats, Direct-Agglutination-High-Sensitivity.

INTRODUCTION

Toxoplasmosis is caused by *Toxoplasma gondii*, an obligate intracellular protozoan parasite (Rippert, 1996). For more than 50 years, the medical and veterinary significance of toxoplasmosis has motivated a large number of sero-epidemiological surveys aimed at identifying the reservoirs and modes of the parasite transmission **(**Dubey and Jones, 2008; Shaapan *et al*., 2008; Dubey, 2009). The consumption of raw or undercooked meat containing cysts of the parasite and the ingestion of oocysts with water or unclean food are the two main modes of contamination. Seroprevalence of toxoplasmosis varies considerably from country to country, between different geographical areas within a country and between different ethnic groups living in the same area (Tenter *et al*., 2000). In general, it's estimated that about one third of the global population is infected with T. gondii (Montoya et al., 2008; Zemene et al., 2012).

Humans and animals are generally infected by ingestion of contaminated undercooked meat, unwashed vegetables

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or water containing oocysts of *T. gondii*. Infections are usually asymptomatic or uncomplicated in immune competent adults and children. However, the infection can be severe or fatal in immune-compromised people such as AIDS patients or pregnant women. Indeed, a wide range of manifestations can occur in congenitally acquired infection including spontaneous abortion, newborn with neurologic and ocular complications (AFSSA, 2005; Khan *et al*., 2006). Therefore, it's become important to correctly manage toxoplasmosis in pregnant women to avoid these severe consequences in the foetus. In addition, the parasite therefore, survives in raw meat after slaughter, until it is killed either by thorough cooking or prolonged freezing. For sheep and goats prevalence may vary from 3.5 to 62% (Tonouhewa et al. 2017, Al-Kappany et al., 2018). To assess the risk of transmission of toxoplasmosis, it's needed to know the seroprevalence of toxoplasmosis in herbivores, especially small ruminants (sheep and goats) that are an important part of the food chain in Senegal.

Additionally, a high prevalence of the infection have been reported among pregnant women and women of childbearing age from different foci in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa (Pappas *et al*., 2009).

In Senegal, the seroprevalence of toxoplasmosis among pregnant women varied from 33.3% to 37.2%, between 1993 to 2006. Most of the studies showing these figures during this period were conducted in the central city. However, few studies have been carried out in peripheral health facilities e.g. in the suburb of the capital city characterized by a high level of poverty.

This study aimed to evaluate the seroprevalence of toxoplasmosis in sheep, goats and pregnant women attending antenatal clinics in the suburb of Dakar.

MATERIAL AND METHODS

Study site

For Women

The present study was conducted among pregnant women attending consultation at the maternity of Roi Baudouin hospital. This level 1 health centre, located in the suburb of the capital city Dakar, records over 7,000 deliveries per year (Diouf *et al*., 2004). This was an observational, descriptive and cross-sectional survey carried out from February to June 2012. Sociodemographic data of patients were collected, including the order number, name, address, and phone number. The age of the women, as well as pregnancy age, the IPT/SP (chemoprophylaxis) intakes during pregnancy were recorded.

For Animals

The samples were taken between February and March 2012 at the new official slaughter site of the capital city of Senegal, which is also entrusted with the health, and commercial management of meat consumption in the Dakar area.

Sample collection

For Women

The number of patients needed to be included in this study was calculated with an expected prevalence of 34.5% from previous study (Ndiaye *et al.*, 2006) a confidence level of 95% of the desired accuracy of 80% and a cluster effect 2. The sample size calculation estimated the needed number at 246 patients. Taking into account the expected non-responses rate, we decided to include 300 women. For each patient,100 microliters of blood sample at the finger was collected on a filter paper Whatmann 3A®.

For Animals

The sample size was determined for an expected prevalence of 40%, a 5% alpha risk and 80% of power. Thus, 250 animals should be selected. As sheep are more consumed in Senegal than goats, we adopted a 3 sheep for 1 goat ratio.

The blood was collected upon the slaughtering in sterile EDTA tubes. The tubes were opened only at collection time and closed immediately after.

Study description

Design

It was a cross-sectional observational study conducted from February to June 2012. Socio-demographic data of the pregnant women attending antenatal clinics (ANC) were collected, including identification, address, age, as well as the age of the pregnancy, number of ANC visit the Intermittent Preventive treatment against malaria (IPTp) with SP

Laboratory method

DAHS (Direct Agglutination High Sensitivity) Testing

The DAHS test was used in this study. It's one of the method used widely. It has high sensitivity and specificity (Desmonts and Remington, 1980; Valtaud *et al*., 1991, Bamba *et al. b* 2012), also relatively rapid and does not require complex laboratory equipment. Therefore, this is a reference method in the study of Toxoplasmosis in animals (Pappas *et al*., 2009). It's been considered as a method of choice in resource-limited setting, particularly in developing countries.

Methodology for Women

The filter papers were suspended in a dilute solution of PBS (Phosphate Buffer Saline). After serum preparation,

three dilutions were made: serum with PBS, the antigen with BABS (Buffer Albumin Bovine Serum) and DDT (dithiotréitol) with PBS. The final solutions were mixed and deposited on the plate in cascade dilutions. Interpretations were made 24 hours after according to the manufacturer's instructions. The last positive dilution was the dilution that has a veil covering at least 50% of the well of the plate. The title was calculated using the conversion: Title =Dilution divided by 8.

Methodology for Animals

The DAHS was performed as described by Bamba *et al.* (Bamba *et al. b*, 2012) to detect antitoxoplasma *IgG* in the serum of the animals. In our survey, positive control was the serum of pregnant women who were positive to toxoplasmosis with IgG. During our manipulations, we performed 4 dilutions at $1/6^{th}$, $1/25^{th}$, $1/100^{th}$ and $1/400^{th}$ and the calculated title was inversely proportional to the dilution. (Title calculation: T = dilution x 1/8).

Statistical methods

All data were entered into Excel and analysis was performed with STATA software 11. Quantitative variables were described as mean and standard deviation. Intergroup comparisons were performed using Mann-Whitney test for the conditions of application. Categorical variables were described in terms of numbers and percentage. Contingency tables were analyzed using Pearson Chi 2. The significance threshold of the statistical tests was 5%.

Ethical aspects

Field briefing was organized with gynecologist and midwives from the hospital to explain all the aspects considered in the context of this study. The agreement of the director of the hospital and the head of midwives was also obtained. The informed consent of each woman participating in the study was obtained before the start of any investigation. The protocol was explained and women were free not to participate in this study. We provided free treatment for any prescription given to pregnant women during ANC. The institutional independent review board of Cheikh Anta Diop University gave its ethical clearance.

RESULTS

Serology

For Women

A total of 313 pregnant women were included in this study. The age varied from 16 to 44 years with a mean age at 27.20 years. The coverage of malaria chemoprevention with SP during their antenatal visit was 39.22%. Of these, 34.05% took one dose (first visit) while only 5.17% have taken the two doses of SP.

Out of the 313 women tested, DAHS detected anti toxoplasma IgG antibodies in 123 of them (39.30%; 95% CI: 33.8% -44.7%).

We found that the infection tends to increase with age in the first three age groups, without any statistically significant difference (table 1). However, we observed a slight decrease in older patients whose average age is over 30years old (NS).

In our study, none of the sera tested positive is beyond the dilution of 1:6400th. 41.46% of positive sera are in the 1:10 dilution, 3.25% at 1:800 dilutions and 0.6% at 1:6400 dilutions. This corresponds respectively to one arbitrary unit (1 AU), 100 AU, and 800 AU (titters) anti-Toxoplasma antibodies. The positivity threshold was set up at 10 AU (dilution), meaning the second well (table 2). The mean positivity titter was 15.22.

Seroprevalence and the gestational age

The rate of Toxoplasma infection increased with the duration of the pregnancy from 35.03% to 52.77% from the first to the last trimester of pregnancy respectively (table 3).

Toxoplasma infection according to malaria chemoproprevention

Women with negative serology and who did not take chemoprevention were relatively a high proportion (63.12%) compared to negative women who did take with SP (59.49%) but the difference was not statistically significant. By comparing the proportion of women with positive serology, we found those who had taken the SP during their pregnancy were slightly higher than those who did by taking, but the difference was not significant (figure 1).

Women with high titters

Among positive patients, 5 had relatively high titters corresponding to100uA (4 women) and 800AU (1woman). Their mean age was 25.2±4.99. The mean gestational age of these patients was 4.9 ± 2.35 months. Three-fifths of the women heavily infected (100uA), did not take the SP during pregnancy.

Analysis of animal's data

Prevalence

A total of 198 sheep and 65 goats had been sampled, the DAHS analysis was positive in 109 sheep (55%) IC 95% [47.1-69.2] and in 36 goats (55.4%) IC 95% [38.7-76.6]. Regarding gender distribution, 89 females out of 160

Table 1. Age range of pregnant women and Toxoplasma IgG antibodies depending.

Age (year)	Pregnant women	Number of Positives	Percentage (%)	p value
≤20	61	22	36,06	
21-25	72	27	37,50	NS
26-30	98	43	43,87	
>30	82	31	37,80	NS
Total	313	123	39,29	

Table 2. Positivity tests based on pregnant women antibody titters.

Dilution	1:10	1:25	1:50	1:100	1:200	1:400	1:800	1:6400	Total
Titers(AU)	1	3	6	10	25	50	100	800	
Positive(number)	51	15	31	14	4	3	4	1	123
%	41,46	12,19	25,20	11,38	3,25	2,43	3,25	0,60	

Table 3. Toxoplasma infection distribution depending on the gestational age.

Age (months)	women examined	Women positives	percentage	P value
3	137	48	35,03%	
4-5	89	34	38,20%	
6-7	51	22	43,13%	
8-9	36	19	52,77%	
Total	313	123	39,29%	

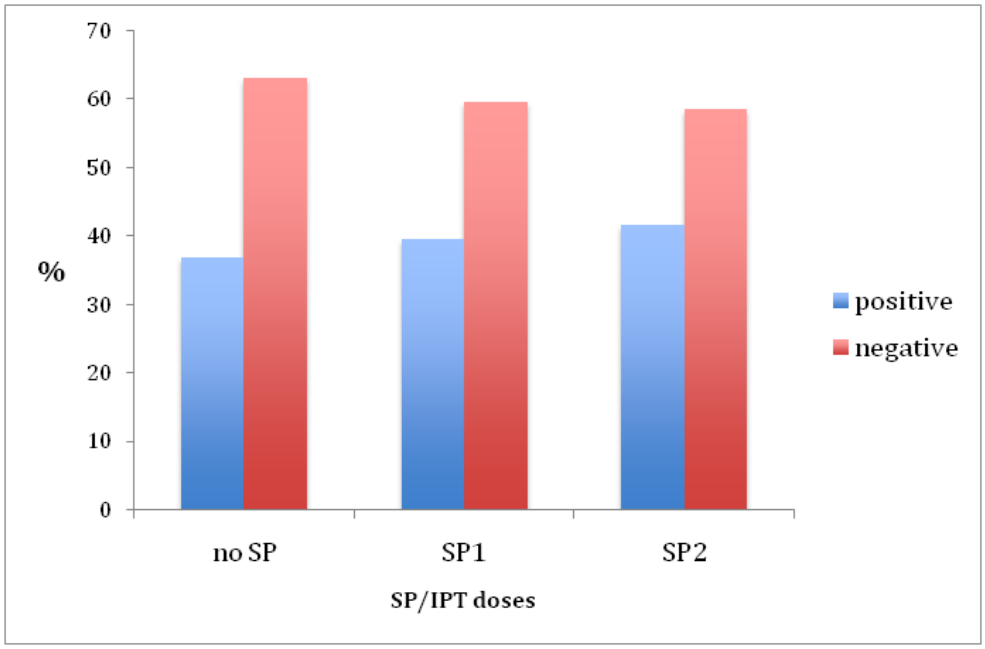

Figure 1. Toxoplasma infectionand SP/IPTchemoprevention intakes.

sheep were positives representing 55.6% IC 95% [44.6-68.4] and 20 males out of 38 were positives representing 52.7% IC 95% [32.1-81.2]. Among the goats, 54.5% (12/22) IC 95% [28.2-95.2] males were tested positive and 55,8% (24/43) IC95% [35.7-83.0] of females were found to be positive (table 4).

Table 4. Seroprevalence of anti Toxoplasma IgG in sheep and goats.

Sheeps	Total n/N (%)	Males n/N(%)	Female n/N (%)	p
Toxoplasmosis Positive	109/198(55%) [47.1-69.2]	20/38 (52.7%) [32.1-81.2]	89/160 (55.6%) [44.6-68.4]	0.73
Goats				
Toxoplasmosis Positive	36/65(55.4%) [38.7-76.6]	12/22 (54.5%) [28.2 – 95.3]	24/43 (55.8%) [35.7- 83.1]	0.92

In terms of age, the number of positives was higher than 50% among the young animals (below 2 years) and adults (above 2 years), and this applied to both sheep and goats (table 5). Regarding IgG titters, 77 sheep scored 0.75 U/ml (70.6%), 27 scored 3.25 U/ml (24.7%) and 5 showed a score that equalled 12.5 U/ml (4.6%). No sample was positive with a 50U/ml title. Among the goats, 31 had a titter equal to 0.75U/ml, 4 had 3.12U/ml and one came out with a titter equal to 12.5U/ml. No result with a 50U/ml title was found.

DISCUSSION

For women

Faced with the consequences of *Toxoplasma gondii* infection in pregnant women and the risk for the fetus, the prevalence of this disease has been estimated in Dakar using the DAHS technique. This study is the first evaluation using this technique in Senegal in pregnant women.

As a preliminary study, patients were not monitored throughout their pregnancy until delivery, and did not observe either seroconversion or congenital infections. Second samples which can look for IgM and IgA and estimate the prevalence toxoplasmosis according to the season, have not been made either. Another limitation was the absence of avidity test, which did not allow us to date the infection.

In this study, it was reported that among 313 pregnant women, 123 or 39.30% are carrying antitoxoplasmic antibodies. These negligible results can be explained in part by the economic status of pregnant women who are mostly occupied and have a high income compared to farmers. They also tend to live in cities and can afford to eat poultry and red meat that happen to be a major source of *Toxoplasma gondii* transmission. These results are similar to those reported by Ndiaye *et al.* (34.5%) in 2006 among pregnant women who come for screening during their pregnancy in Le Dantec hospital (Ndiaye *et al*., 2006). They are also consistent with results obtained in 1993 using immunofluorescence, 40.3% of antitoxoplasmic antibodies carriers in 729 women of childbearing age (Pappas *et al*., 2009). In Senegal, the first investigation of toxoplasmosis in pregnant women was conducted by Garin *et al.* (Garin *et al.*, 1971). By the Dye test method, they observed among 144 samples collected in women, 18% had antitoxoplasmic antibodies. Subsequently, in 1987, Dumas *et al.* obtained a prevalence of 33.3% among pregnant women in Dakar using the agglutination method with latex particles (Dumas *et al*., 1990).

The seroprevalence of *T. gondii* IgG, therefore obtained in this study was higher than those reported from some Sahelian areas like Bamako (Maiga *et al*., 1984), Mauritania (Monjour *et al*., 1983), Niger (Develoux *et al*., 1988), Nigeria (Olusi *et al*., 1996). We noticed the same in studies conducted in Italy (De Paschale *et al*., 2010), Thailand (Nissapatorn *et al*., 2011). The United States of America and South East of Asia (Welch *et al*., 1980), Tanzania (Berno *et al*., 2013). In contrast, it was lower when we compare to studies conducted in Ethiopia (Endalew *et al*., 2012), Lome (Lapierre *et al*., 1984; Tourte-Schaeffer *et al*.,1987) Cameroon (Deniau *et al*., 1987), Burkina Faso (Bamba *et al*. a, 2012) Congo (Candolfi *et al*., 1993; Makuwa *et al*., 1992), Gabon (Nabias *et al*., 1998).

Epidemiological studies from 5 continents reflected a universal distribution of the parasite in humans (Welch *et al*., 1980, Alsammani et al., 2016). Large incidence and prevalence among other changes attributed to geographic, cultural and temporal factors were observed between regions and within a region (Welch *et al.,* 1980). In addition, being a cosmopolitan zoonosis, *Toxoplasma gondii* can be a permanent danger to humans consuming meat, but also using certain animals as pets.

Different Toxoplasma serological prevalence rates were observed among pregnant women who took SP chemoprevention and the oneswho did not take. But the differences are not significant. However, age is a factor known to play a role in the infection occurrence.

For animals

The species surveyed (sheep and goats) were selected due to the fact that they are well known to be highly sensitive to *Toxoplasma gondii* as; intermediary hosts to the parasite on the one hand and because they are indispensable for the development of animal farming which is an important sector of the economy in Senegal

Table 5. Age ranges and toxoplasmosis seroprevalence in sheep and goats.

	< 2 ans	> 2 ans	p
Sheep			
Positive	45/83	64/115	
% IC 95	54.2% [39.5 – 72.5]	55.2% [42.5 – 70.7]	0.97
Goats			
Positive	15/28	20/27	
%IC95	53.5% [29.9- 88.4]	74.1% [46.9-100]	0.11

on the other hand. Indeed, the consumption of meat from these animals, especially the sheep, is significant. These two species are considered the most toxoplasmosis contaminated animal's meat (Euzeby J. 1998).The DAHS used, as a method for analysis is a good technique for studying toxoplasmosis in animals, with good sensitivity and specificity. Moreover, the neutralization of natural IgG helps avoid false positives. We noted in our survey that half of the sheep slaughtered at the official site and consumed in the Dakar area have been in contact with the parasite. Indeed the percentage of positives is higher than 50% in female and male, young and aged animals. The only survey previously performed on this matter in 1992 found a 38.5% rate in sheep (Dia M. 1992)**,** which is an indication that disease transmission has been raising over the past 20 years among sheep. In West Africa, prevalence rates as high as 58.8% have been observed in Burkina Faso in 2010 (Bamba *et al*. b, 2012)but are lower than those noted in North Africa: 43.7% in Egypt and 27.6% in Morocco **(**Shaapan *et al.,* 2008; Sawadogo *et al*., 2005).In Europe, significant rates have been recorded: 40.6% in Spain (Mainar-Jaime *et al*., 2007) and 32% in France (Rozette *et al*., 2005) despite stricter farming conditions compared to Africa where livestock circulate more freely. Also, goats seem to be very prone to infection given the percentage of positive individuals, which is as significant as in sheep. The same thing was observed with the 1992 data regarding sheep with a prevalence of 33.7% against 55.4% in our survey. Contrary to the 1992 survey, which showed prevalence rate much higher in females (42.1% against 30% males), our survey showed that gender has little bearing on infection with 52.7% against 55.6% in sheep (p=0.73). We therefore, believe that *Toxoplasma gondii* infection is not influenced by gender because all animals can be in contact with the parasite and develop the diseases. Bamba *et al.* b (2012) in Burkina Faso also demonstrated this low variation in positivity based on gender with seroprevalence rates at 63.2% in males and 58.1% in females (p=0.47). Euzeby (1998) also showed in France the absence of variability between male and female in terms of *Toxoplasma* sensitivity. The infection does not seem either to discriminate by age as both young and adults animals are affected at a significant rate. However, the survey carried out in Senegal in 1992 showed a higher prevalence in adults (40.4% against 30.2%). This was confirmed in Burkina Faso where it was found that the number of positives rises with age (Bamba *et al*., 2012).However, the significant rates observed in young animals are an indication that contamination is possible at any age as shown previously (Dubey and Jones, 2008). As half of the sheep and goat slaughtered at the official site and marketed have been in contact with *Toxoplasma* and they probably host cysts of the parasite in their muscles, especially the heart muscle that seems to be the most affected (Villena *et al.,* 2012).

CONCLUSION

Our study confirmed the presence of toxoplasmosis in Senegal where pregnant women may be contaminated during pregnancy because of their lack of antitoxoplasmic immunity. Thus, the risk of infection in the foetus is also important. The establishmentof systematic serological screening of women of childbearing age or during the premarital examination or upon the declaration of pregnancy to save the consequences is needed. This study showed also, toxoplasmosis is endemic in the Senegalese livestock. A broader survey in animals, taking into account their geographical origin is however needed to better assess the risks for human health.

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