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Toxoplasma gondii Infection in Pregnant Women: Seroprevalence and Associated Risk Factors in Ouagadougou, Burkina Faso

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Abstract *Toxoplasma gondii* (*T. gondii*) infection can lead to abortion in pregnant women. Unfortunately, very limited information is available concerning the seroprevalence and associated risk factors in pregnant women in Burkina Faso. Therefore, the present study aimed to estimate the seroprevalence of *T. gondii* among pregnant women attending for antenatal care. A cross-sectional study was conducted containing 579 pregnant in Ouagadougou, Burkina Faso. Serological patterns were assessed by enzyme-linked fluorescent immunoassay and the study utilized univariate analysis to identify the potential risk factors for *T. gondii* infection. Of the 579 pregnant women investigated, 29.71% were tested as *T. gondii*-seropositive, with 25.91% seropositive for *T. gondii* IgG antibodies, 1.9% positive for IgM and 1.9% positive for both IgM and IgG. We found firstly that pregnant women consuming unpasteurized cow's milk had a significantly higher seroprevalence than individuals who did not consuming (30.15% *vs* 06.67%; p=0.0039). Then, age was associated with an increased risk of being seropositive for *T. gondii*, seroprevalence increases significantly with age, ranging from 18% for 15-25 year olds to 45% for those over 35 years old, p<0.001. Finally, we found that pregnancy number and living children number were associated with an increased risk of being seropositive for *T. gondii* (p=0.0065 and 0.023, respectively). The other risk factors like contact with soil, drinking water source, contact with cats and eating some under-cooked meat were not significantly associated with *T. gondii* infection. Altogether, our study showed that seroprevalence of *T. gondii* was mainly related to consumption of unpasteurized cow's milk, to patient age, to the number of pregnancies and to living children. The findings will provide key and baseline data for prevention and control of toxoplasmosis among pregnant women and other people.

Keywords: *Toxoplasma gondii, seroprevalence, risk factors, pregnant women, ouagadougou, Burkina Faso*

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1. Introduction

Toxoplasmosis is a public health problem worldwide caused by *Toxoplasma gondii (T. gondii)*, an intracellular, parasitic protozoan. This parasite is capable of infecting nearly all warm-blooded animals including humans, but cats are the only known definitive host [1]. Human infections occur following the ingestion of raw meat infected with tissue cysts, food or drink contaminated with oocysts, or by direct assimilation from the environment [2,3]. Vertical transmission from the infected mother to the fetus may also occur [2]. In most adults it does not cause serious illness, blindness and mental retardation can result in congenitally infected children and severe disease in those with depressed immunity [4,5].

In pregnant women, the time of onset of infection is crucial, especially because post-conceptional acquisition represents a risk for the fetus and the degree of severity of clinical signs vary depending on the stage of gestation at the time of infection. The risk of transmission is lower in the first trimester and higher during the last trimester [6]. Primary infection during pregnancy can result in severe damage to the fetus manifested as mental retardation, seizures, blindness, or even death [5]. Thus, early diagnosis

of *T. gondii* infection during pregnancy is very important for prevention of congenital toxoplasmosis.

In Burkina Faso, a study of Tialla et al., 2019 revealed a prevalence of 92.5% and 75%, respectively for porcine and bovine toxoplasmosis [7]. The prevalence of *T. gondii* infection was also evaluated at the slaughterhouse of Bobo-Dioulasso and the prevalence of carcasses positive for anti-Toxoplasma IgG was 29% [8]. These studies imply the endemicity of toxoplasmosis in Burkina Faso and highlight the need for routine testing and increased public awareness especially for immunocompromised people and pregnant women. Thus, pregnant women are commonly tested for anti-*T. gondii* immunoglobulin M (IgM) and IgG in the first trimester of gestation, and again in the second and third trimesters for those who are seronegative. Nevertheless, the predisposing factors of *T. gondii* infection are not completely elucidated in our context and relatively little is known about its epidemiology in Burkina Faso. The aim of the present study was to assess the seroprevalence and evaluate associated risk factors of *T. gondii* infection in pregnant women from Ouagadougou, in Burkina Faso.

2. Materials and Methods

2.1. Study Design and Population

A cross-sectional study was conducted in Ouagadougou at Burkina Faso. From June to December 2018 and June to December 2020, a total of 579 blood samples were collected among pregnant women in Hôpital Protestant Shiphra and Laboratoire national de Santé Publique. Questionnaires have been administrated to identify risk factors and possible routes of toxoplasmosis transmission.

2.2. Sample Collection and Serological Tests

Pregnant women attending public and private health centers at Ouagadougou for antenatal care were recruited for this study. About 5mL of venous blood was collected aseptically from each of the study participants into non-anticoagulation tubes and kept at room temperature for 2 hours. Then serum was separated from the whole blood by centrifugation at 3000 rpm for 15 minutes, which was labeled and frozen at -20 °C or -80°C until use.

All sera were tested for the detection of anti-*T. gondii* IgG and IgM antibodies using commercial enzyme immunoassay kits, on the instruments of the VIDAS family (VITEK® ImmunoDiagnostic Assay System). The ELISA kits (Toxo IgG and Toxo IgM) were provided by BIOMÉRIEUX, Marcy l'Étoile, France. Toxoplasma IgM and IgG detection were performed according to the manufacturer's instructions. An automated enzyme-linked fluorescent immunoassay (ELFA) was used for the presumptive qualitative detection of anti-*T. gondii* IgM and IgG antibodies in human serum in the diagnosis of acute, recent, or reactivated *T. gondii* infection. It is intended for use as an aid in determination of immune statut. Positive, negative and blank controls were included in every plate. Optical densities were measured by photometer at a wavelength of 450 nm. Values higher than the cut-off (10 IU/mL) were considered positive

2.3. Questionnaire Survey

A structured questionnaire was used to assess risk factors, which included age, marital status, residential area, education level, stage of pregnancy, total number of pregnancies, children remained alive, previous dead babies, baby malformed history, history of aborted pregnancies, presence of cats and dogs in home, contact with cats and dogs, consumption of raw/undercooked meat, consumption of raw vegetables and fruits, consumption of unpasteurized cow's milk, source of drinking water and exposure to soil. These variables had been selected based on the literature.

2.4. Diagnosis

The challenge in diagnosing toxoplasmosis is to establish the acute (primary) infection and distinguish it from past or chronic infection. Results of serologic tests measuring immunoglobulin (IgM and IgG) are often difficult to interpret when differentiating between acute and chronic infections. The absence of IgG and IgM antibodies before or early in pregnancy indicates no previous infection and identifies women at risk of acquiring the infection during pregnancy [9]. IgM antibody titers rise starting on day 5 and reach the maximum level at 1 to 2 months. At this point, IgM antibodies decline more rapidly than IgG antibodies [10]. However, *T. gondii*-specific IgM can sometimes persist for years, giving rise to false-positive diagnoses of acute infection when no additional tests for *T. gondii*-specific IgG were conducted, prior to conception or in the first trimester, to exclude women already infected before pregnancy [11]. So, in many cases the IgM antibodies persist for years following acute infection.

In contrast, IgG antibodies are usually detectable within 1 to 2 weeks after acute infection, peak within 12 weeks to 6 months, and usually remain detectable throughout life [12]. The detection of IgG antibodies and absence of IgM antibodies indicates an old infection. However, if test results are positive for both IgG and IgM, interpretation is difficult, as the positive results might be owing to either a recent infection or low levels of IgM antibodies from a previous infection [13].

2.5. Data Analysis

The data of questionnaire covered socio-demographic information such as age, resident area, source of drinking water and behavioral factors including cat at home, consumption of raw vegetables and fruits, and consumption of raw/undercooked meat and drinking of cow milk. The information of questionnaire and experimental results was entered on to an excel spreadsheet and the data were analyzed using the R software (R version 3.2.5 (2016-04-14). Univariate analysis was used to analyze the association between variables and *T. gondii* infection. Probability (p) value < 0.05 was considered as statistically significant in the analysis and the odds ratios (OR) and its 95% confidence interval were calculated.

2.6. Ethical Considerations

The ethical committee for health research in Burkina Faso approved this study. The purpose of and procedures

involved in the study were explained and written informed consent was obtained from all participants. Sera were collected with the consent of the volunteers.

3. Results

A total of 579 pregnant women aged 18 - 47 years (mean age: 31.93 years; std. dev: 6.009 years) were enrolled in this study after informed consent. We first analyzed the level of pregnant women knowledge about toxoplasmosis. Only 16 (2.76%) women had some knowledge about Toxoplasmosis.

3.1. Prevalence of *T. gondii* infection in Pregnant Women

Of the 579 investigated pregnant women, 172 (29.71%, 95% CI = 25.91-33.51) were tested as *T. gondii*-seropositive. Among the pregnant women seropositive for *T gondii*, 150 (25.91%, 95% CI = 22.27 - 29.55) were positive for *T. gondii* IgG antibodies only. A total of 11 (1.9%, 95% CI = 0.76 - 3.03) IgM positive were found in this study while 11 (1.9%, 95% CI = 0.76 – 3.03) pregnant women were also positive for both IgG and IgM antibodies. Detailed information is summarized in Table 1. Most of the seropositive women in our study had only IgG antibodies (87.20%) and only 6.40% had IgM only.

Table 1. Combined IgG and IgM anti- *T. gondii* antibodies seroprevalence in pregnant women

Seroreaction	Positive	% (95% CI)
Positive for IgG only	150	25.91 (22.27 - 29.55)
Positive for IgM only	11	1.9 (0.76 - 3.03)
Positive for IgG and IgM	11	1.9 (0.76 – 3.03)
Negative for IgG and IgM	407	70.29 (66.49 – 74.09)
Positive for either IgG or IgM	172	29.71 (25.91-33.51)

3.2. Risk Factors Associated with *T. gondii* Infection in Pregnant Women

Several potential risk factors associated with Toxoplasma infection have been investigated in pregnant women at Ouagadougou. According to a study, from Changchun in 2008, in the north of China, major risk factors identified by multivariate analysis include eating raw or undercooked meat, unwashed raw vegetables or fruits, contact with cats, living in rural areas and low educational level [14]. Cow milk usually consumed in Burkina Faso is also suspected to be a risk of factor.

3.2.1. Sociodemographic Characteristics

We first analyzed sociodemographic characteristics association with the seroprevalence of *T. gondii*. Statistical analyses revealed no significant differences in seropositivity associated with education level and marital status. However, age was significantly associated to *T. gondii* seropositivity. Age was associated with an increased risk of being seropositive for *T. gondii* in pregnant women with odds ratios of 1.73 (1.30-2.16) for patients over 36 years of age. *T. gondii* seroprevalence increases significantly with age, ranging from 18% for 15-25 year olds to 25% for 26-35 year olds and 45% for those over 35 years old, p<0.001 (Table 2).

3.2.2. Pregnancy Characteristics

Number of pregnancies, living children, history of stillborn babies, malformed baby history, history of aborted pregnancies and time of pregnancy (trimesters) were analyzed in order to identify their association with *T. gondii* prevalence. While history of stillborn babies, malformed baby history, history of aborted pregnancies and time of pregnancy (trimesters) were not significantly associated with seropositivity to *T. gondii*, the number of pregnancy and consequently the number of living children were all found to be significantly associated with seropositivity to *T. gondii*. Women who had at least one pregnancy and women with at least one living child had higher seroprevalence. The number of pregnancy and the number of living children were associated with an increased risk of being seropositive for *T. gondii* in pregnant women with odds ratios of 3.40 (95% CI = 1.24-9.31, p=0.0065) and 2.36 (95% CI = 1.00-5.52, p=0.023) respectively (Table 3).

3.2.3. Consumption Habits and Living Environment

We also analyzed the consumption habits and the living environment of the pregnant women. Source of drinking water, degree of meat cooking, unwashed raw vegetable or fruit consumption, residence area, soil exposition and cat in home or neighborhood were not significantly associated with seropositivity. However, consumption of unpasteurized cow's milk was found to be significantly associated with seropositivity to *T. gondii*. This factors was associated with an increased risk of being seropositive for *T. gondii* in pregnant women with odds ratios of 6.02 (95% CI 4.54 - 7.50) p=0.0039 (Table 4).

Table 2. Analysis of sociodemographic characteristics association with *T. gondii* prevalence in pregnant women

Variables	No. tested	Positive	Prevalence %(95% CI)	OR (95% CI)	*P*-value
Age group (years)					
18 -25	85	16	18.82 (10.51-27.13)	0.59 (0.32-1.13)	
26 -35	172	48	25.13 (18.65-31.61)	Reference	<0.001*
36-46	152	61	45.86 (37.94-53.78)	1.73 (1.30-2.16)	
Educated					
Yes	114	27	23.68 (15.88-31.48)	Reference	0.167
No	52	16	30.76 (18.23-43.31)	1.42 (0.69 - 2.97)	
Married					
Yes	147	41	27.89 (20.64-35.14)	Reference	
No	19	2	10.52 (3.27-24.31)	0.31 (0.07-1.38)	0.052

Table 3. Analysis of pregnancy-related risk factors association with *T. gondii* prevalence

Variables	No. tested	Positive	Prevalence %(95% CI)	OR (95% CI)	*P* -value
Number of pregnancies					
1	43	5	11.63 (8.82-14.44)	Reference	0.0065*
> 1	123	38	30.89 (22.72-39.05)	3.40 (1.24-9.31)	
Living children					
Yes	115	35	30.43 (22.02–38.84)	2.36 (1.00-5.52)	0.023*
No	51	8	15.69 (5.71-25.67)	Reference	
History of stillborn babies					
Yes	7	1	14.28 (11.64–40.20)	0.46 (0.0543–3.973)	0.4734
No	159	42	26.41(19.56-33.26)	Reference	
Malformed baby history					
Yes	1	0	0	NA	NA
No	165	43	26.06 (19.36-32.76)	Reference	
History of aborted pregnancies					
Yes	26	10	38.46 (19.76-57.16)	2.03 (0.84–4.89)	0.056
No	140	33	23.57 (16.54-30.60)	Reference	
Time of pregnancy (trimesters)					
1	40	9	22.5 (9.56-35.44)	0.80 (0.34-1.82)	0.286
2	126	34	26.98 (19.23-34.73)	Reference	

Table 4. Analysis of the association of consumption habits and living environment with the prevalence of *T. gondii* in pregnant women

Variables	No. tested	Positive	Prevalence %(95% CI)	OR (95% CI)	P-value
Source of drinking water					
Tap	117	28	23.93 (16.20-31.66)	Reference	0.185
Well or river	49	15	29.79 (16.71-42.86)	1.402 (0.668-2.943)	
Consumption of unpasteurized cow's milk					
Yes	136	41	30.15 (22.44-37.86)	6.02 (4.54-7.50)	0.0039*
No	30	2	06.67 (2.26-15.60)	Reference	
Undercooked meat					
No	164	43	26.22 (19.49-32.95)	NC	NC
Yes	0	0	0	Reference	
Unwashed raw vegetable or fruit consumption					
Yes	18	5	27.79 (7.09-48.48)	1.113 (0.372-3.33)	0.424
No	148	38	25.67 (12.78-38.56)	Reference	
Residence area					
Peri-urban	4	1	25 (17.43-67.43)	0.95 (0.096-9.41)	0.483
Urban	162	42	25.92 (19.17-32.67)	Reference	
Exposure to soil					
Yes	78	22	28.21(9.99-46.43)	1.253 (0.63-2.51)	0.262
No	88	21	23.86 (14.95-32.76)	Reference	
Cat in home or neighbourhood					
Yes	12	5	41.67 (13.77-69.56)	2.18 (0.65-7.28)	0.098
No	154	38	25.32 (18.45-32.19)	Reference	

4. Discussion

The greatest challenge in diagnosing toxoplasmosis is to establish the acute (primary) infection and distinguish it from past (chronic) infection; and timing the onset of the infection is crucial in pregnant women, especially because post-conceptional acquisition represents a risk for the fetus. This study aimed to investigate *T. gondii* infection among pregnant women in relation to exposure to infection risk factors, age and pregnancy-related risk factors.

In our study, of the 579 pregnant women investigated, 172 (29.71%) were tested as *T. gondii*-seropositive, with 150 (25.91%) seropositive for *T. gondii* IgG antibodies and 11 (1.9%) IgM positive. A total of 11 (1.9%) pregnant women were also positive for both IgG and IgM antibodies. An overall anti-*T. gondi*i antibodies seroprevalence of 29.71% (172/579) recorded among the pregnant women is relatively lower than previously recorded values in other studies in Ouagadougou, Burkina Faso [15]. Ouermi et al., 2009 found more higher prevalence among HIV infected women in Burkina Faso, positive *T. gondii* specfic IgM at 4.7% vs 1.9% and IgG at 27.2% vs 25.91% found in our study and concluded that HIV status seems to be associated with great prevalence of *T. gondii* (31.9% vs 29.71% in our study) [16]. Immune-depressed people are indeed more exposed to toxoplasmosis infection.

However, our results were higher than those found by Wei Cong et al., 2015 in Easter India regarding IgG seroprevalence. Anti-*T. gondii* IgG seroprevalence were at 15.2% vs 25.91% in our study. Although anti-*T. gondii*

IgM antibodies were higher (positive for 2.9% of pregnant women vs 1.9%) than that found in our study [17]. Most of the seropositive women in our study had only IgG antibodies (87.2%), indicating past exposure to the parasite. Only 6.4% had IgM only, which generally denotes a currently active or recent infection [18]. During pregnancy, the likelihood of transmission to the fetus is much lower in chronically infected women than in newly infected women [2]. IgM positive (1.9%) found in this study, could indicates an urgent state, or an infection that has occurred within the last year. In fact, as described by Montoya, 2002, *T. gondii*-specific IgM statute is complex as in many cases the IgM antibodies persist for years following acute infection [11]. That IgM persistence in blood giving rise to false-positive diagnoses of acute infection. Some additional tests for *T. gondii*-specific IgG were appropriated, prior to conception or in the first trimester, to exclude women already infected before pregnancy.

In addition, 1.9% of pregnant women were positive for both IgG and IgM. In this case, results are difficult to interpret as wrote Iqbal J and Khalid N, 2007. If test results are positive for both IgG and IgM, interpretation is difficult, as the positive results might be owing to either a recent infection or low levels of IgM antibodies from a previous infection. If acute infection is suspected, repeat testing is recommended within 2 to 3 weeks [13]. Indeed, IgM can be detected for a long period following the acute infection, and therefore a true-positive result cannot discriminate between acute, recent and past infection. Toxoplasma IgG Avidity ELISA was not done in this present study. This would have indicated if the infection was recent or past [19].

A total of 70.29% (66.49 – 74.09) of pregnant women were seronegative for all *T. gondii* antibodies (IgM and IgG negative), which is higher than that found by Ayi et al., 2009 in Ghana [20]. IgM and IgG negative suggest that they are at high risk for toxoplasmosis infection. Hedman et al., 1989 precised that issue for the fetus. The absence of IgG and IgM antibodies before or early in pregnancy indicates no previous infection and identifies women at risk of acquiring the infection during pregnancy [9]. Moreover, we found that, 97.24% of the women included in this study did not have any knowledge regarding toxoplasmosis. In view of these circumstances, information about toxoplasmosis and advice on its prevention should continue to be disseminated to pregnant women, expectant mothers, and the general population.

Our study highlights potential risk factors for *T. gondii* infection in pregnant women at Ouagadougou. Consumption of unpasteurized cow's milk, patient age, number of pregnancies and consequently number of living children were identified as possible risk factors associated with *T. gondii* infection. Drinking unpasteurized milk had previously been linked with *T. gondii* infection in America and In Lausanne where 14% of infections were attributed to consumption of unpasteurized milk or milk products [21,22]. We were unable to assess the type of milk consumed by pregnant woman but *T. gondii* have been found in the unpasteurized milk of sheep, goats, and cows and only drinking of unpasteurized goat’s milk seem to pose an elevated risk of infection in human [23].

Analysis of Toxoplasma seroposivity by age showed gradual age associated-increase positivity. Among all pregnant women tested for toxoplasmosis, the prevalence was 18.82% (10.51-27.13) for pregnant women in the age group of 18-25 years, while highest a frequency of 45.86% (37.94-53.78) was found in the age group of 36-46 years Age was significantly associated with *T. gondii* infection. Frequency of *T. gondii* infection was reported to increase in older age groups in other studies [24]. The reasons for *T gondii* increasing risk exposure by age are still unclear. But unlike the present study, previous findings in Burkina Faso reported highest frequency of anti-toxoplasmosis (34.5%) in the age group of 18-25 years [15], while our study recorded 18.82%. Toxoplasmosis prevalence also increased with the number of pregnancies and the number of living children. Pregnant women with more than one child had a higher seroprevalence of toxoplasmosis. These results are similar to those found in France and in Thailand [25,26]. The increasing risk of toxoplasmosis in women with more than one child can be attributed to age, which has been shown to be a risk factor [26].

Unwashed raw vegetable or fruit consumption and especially eating under-cooked meat was not found to be associated to toxoplasmosis in this study, as reported by Ayi et al., 2009 [20]. Although, previous studies had reported infection in most farm animals especially pigs and cattle in Burkina Faso this can lead to human infection [7]; and eating raw or undercooked meat, unwashed raw vegetables or fruit are well defined as a risk factor for toxoplasmosis [14,27]. This risk factor was not found in our study. According to Kijlstra and Jongert, 2008, *T. gondii* infection is associated with the consumption of undercooked meat or meat products, and up to 50% of infections are transmitted by consumption of undercooked meat, making toxoplasmosis one of the clinically most important foodborne diseases in pregnant women [28]. But, the thorough cooking of meat significantly reduces the risk of meat-eating-related Toxoplasma infection and could have contributed to the lack of association of Toxoplasma infection in our study population. We also found that contact with soil, drinking water source and contact with cats were not significantly associated with *T. gondii* infection. A multicentre case-control study conducted in Europe also failed to identify cats as a risk factor for seroconversion in women during pregnancy [29]. Nevertheless, some published studies report contact with cats and their presence at home as a risk for seroconversion for all women, with a greater risk for adolescents and pregnant women [14,20].

5. Conclusion

This study provides key data about the seroprevalence of anti-*T. gondii* antibodies among pregnant women and invested the association between toxoplasmosis and risk factors. Consumption of unpasteurized cow's milk, patient age, pregnancy number and consequently living children number were identified as possible risk factors associated with *T. gondii* infection. In addition, most pregnant women from our study population are susceptible to

primary infection with *T. gondii* and, therefore, the risk of congenital toxoplasmosis remains high. Thus, continuing education about on food and environmental sources of this infection remains essential in the forthcoming years and effective preventive measures must be implemented. It is important to educate pregnant women about the methods of preventing *T. gondii* infection. Results provided by this present work are useful information to the medical and public health authorities when addressing policies for monitoring and controlling infection and disease in Burkina Faso.

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Authors' Contributions

YEH, IPD designed the study; GD carried out interviews and recruited participants and carried out laboratory assessments; YEH, GD analyzed and YEH interpreted the data; YEH and IPD drafted the manuscript. All authors reviewed and approved the final manuscript. YEH and IPD are guarantors of the paper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper

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