Positivity rate of antibodies anti-Toxoplasma gondii in pregnant women from Curitiba-PR

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ABSTRACT

The estimative of women population in Curitiba city, state of Paraná (Brazil) in the year of 2014 was 916,792 women, being 593,203 of them in childbearing age. When the women of this group are pregnant, the infection by *Toxoplasma gondii* is dangerous, since toxoplasmosis can cause health problems to the fetus. Serology to toxoplasmosis seeks to prevent and diagnose this disease, and as it follows, the congenital toxoplasmosis. In this manner, this work aimed to verify the rate of IgG and IgM antibodies anti-*Toxoplasma gondii* in pregnant women assisted in the basic health units from Curitiba between the years of 2010 and 2014. A total of 117,668 pregnant women with ages from 12 to 61 years old were evaluated and the results showed that 28.9% of them were positive for IgG and negative for IgM anti-*Toxoplasma gondii*, whereas 0.8% were positive for both IgG and IgM anti-*Toxoplasma gondii*. Seronegative women accounted 70.3% of the evaluated pregnants. This profile indicates a high susceptibility to infection, a reduced previous immunization, and a low rate of acute infection in these women. Once there is a high rate of people liable to infections, the implementation of awareness campaigns is of great importance, seeking inform people and avoid a possible contamination by *Toxoplasma gondii*, not only for pregnant or women in childbearing age, but also for all population.

Keywords: Toxoplasmosis. Pregnant women. IgG. IgM. *Toxoplasma gondii.*

INTRODUCTION

In 2004, approximately 500 million people in the world had positive serological reaction to *Toxoplasma gondii*, which is an "opportunistic" universal protozoan, resistant to several weather conditions, which leads to pathology in immunocompromised people (COSTA *et al.,* 2010; LEITE *et al.,* 2008; MAROBIN *et al.,* 2004).

The transmission of this protozoan may occur by fecal-oral route, by intake of uncooked or undercooked meat contaminated with cysts of *T. gondii* or by intake of oocysts present in contaminated water and food, or manipulation of contamined soil. The transplacentary route is also a form of this protozoan transmission (CÂMARA *et al.,* 2015; COSTA *et al.,* 2010).

Congenital toxoplasmosis is the transmission of this protozoan to the fetus. Transmission rates to the fetus during pregnancy vary between 15%, 25% and 65% in the first, second and third trimester of pregnancy, respectively. However, the severity of fetal lesions is inversely proportional to the gestational period (FERREIRA *et al.,* 2007; MOREIRA, 2012).

Even though the toxoplasmosis is asymptomatic in adults, the disease may cause miscarriage, and also serious neurological and ocular alterations to the fetus, as eye lesions, psychomotor changes, microcephaly and hydrocephaly. Usually this disease may generate chorioretinitis and an inflammatory response of obstructive and destructive character in brain tissue, cerebral calcifications, and mental retardation, characterizing the most severe form of the disease - Tetrad of Sabin (LOPES-MORI *et al.,* 2011; LUCAS *et al.,* 1998; SOUZA *et al.,* 2010).

Severe inflammations, such as meningoencephalitis, and necrosis, calcification and formation of cysts damnify placenta and several organs, such as heart, lung, kidney, adrenal gland, pancreas, testicles, ovary, striated muscle, eye and central nervous system. T*oxoplasma gondii* causes necrosis as main injury (FORTUNOV, 2008; MCLEOD *et al.,* 2006; MOREIRA, 1988; REMINGTON, 2011).

Detanico and Basso (2006) confirm the importance of conducting routine serological tests of IgM and IgG anti-*Toxoplasma gondii* in pregnant women and in women in childbearing age to prevent congenital toxoplasmosis, because these serological

markers can differentiate latent infection from recent infection in the mother and in the newborn.

IgM antibodies appear in the first week after infection, with maximum concentration in 15 days, remaining in residual levels for 12 to 18 weeks. In contrast, IgG antibodies appear between 2 to 4 weeks after infection, reaching maximum level in 2 to 3 months, being kept in low concentrations throughout life. Transformation from reactive IgG to non-reactive IgG is rare (GOMES, 2004; PRADO *et al.*, 2011).

Neonatal screening searching for IgM and IgG anti-*Toxoplasma gondii* antibodies, with the interpretation in accordance with Table 1, serves to diagnose congenital toxoplasmosis. If the result of IgG antibodies is positive, serological tests of toxoplasmosis (IgG and IgM) are performed in the mother and in the newborn, since that it is possible to verify the presence of maternal IgG antibodies immunizing in the newborn even in the absence of infection. However, the presence of IgM in serum of neonates indicates primary infection because this class of antibody does not cross the placenta (GOMES, 2004).

Table 1. Summary of interpretation of the results to serology anti-*Toxoplasma gondii*

IgM non-reactive IgG reactive	Immune pregnant
IgM non-reactive IgG non-reactive	Susceptible pregnant * Repeat serology quarterly
IgM reactive IgG non-reactive or undetermined	Infection
IgM undetermined or non-reactive IgG undetermined	Possible infection
IgM reactive or undetermined IgG reactive	IgG avidity test

Modified from BRASIL, 2006.

In Curitiba city, state of Paraná (Brazil), a program entitled "Mãe Curitibana" was implemented in 1999 by Municipal Secretariat of the city aiming to assist women during the pregnancy. Serological tests for toxoplasmosis figure between the several free offered exams. If the pregnant does not have IgG anti-*Toxoplasma gondii* or if she is at high risk of infection, these tests can be performed during the three trimesters of pregnancy. If the

pregnant presents immunization or has low risk of infection, these tests are performed after the first obstetric consultation and between the twenty-sixth and twenty-eighth weeks of pregnancy. However, if the mother is IgG and IgM reactant, the presence of infection must be confirmed by the IgG avidity test (CURITIBA, 2012; LOPES-MORI *et al.*, 2011).

The IgG avidity test differentiates a recent infection from an old infection comparing the affinity of antibodies to specific antigens. Recent infection - or infection with less than four months - has IgG antibodies with low avidity. In contrast, IgG antibodies with high avidity indicate their presence in the circulation for longer time, or, in other words, an older infection (CAMARGO *et al.,* 1991).

The monitoring of toxoplasmosis during pregnancy is carried out continuously, which follows a protocol established by Mãe Curitibana Program, with specific treatment for each case. For example, if a pregnant woman presents acute infection for toxoplasmosis during pregnancy, she will be subjected to a treatment to prevent congenital toxoplasmosis (CURITIBA, 2012).

Due to severity of the disease in its congenital form, important measures to prevent toxoplasmosis may be implemented, mainly for non-immune pregnant women (SOUZA *et al*., 2010). According to Logar et al. (2002), countries using advertisements and programs to prevent *T. gondii* and toxoplasmosis have low prevalence of this pathology. In order to define strategies of prevention, association between antibody titration and cultural habits must be made. In prevention campaigns, these tactics should be expressed in oral and written forms. During antenatal care, they must be reinforced by the doctor (AMENDOEIRA and CAMILLO-COURA, 2010).

The population of Curitiba is estimated at 1,864,416 habitants, of which 916,792 are women and 593,203 of them are in reproductive age, being capable to get pregnant. The number of pregnant women is estimated by the number of live births. In 2013, the Municipal Secretariat Curitiba estimated that there were approximately 24,906 births in the city (BRASIL, 2004; DATASUS, 2012; IBGE, 2014; PARANÁ, 2011).

Considering the scarce published data encompassing the occurrence of toxoplasmosis in pregnant women from Curitiba, Paraná (Brazil), the present study aims to estimate the rate of positivity of IgG and IgM anti-*Toxoplasma gondii* antibodies in

pregnant women in this city from 2010 to 2014, in order to know a current overview of the vulnerability to congenital toxoplasmosis.

MATERIAL AND METHODS

The present research was approved by the Research Ethics Committee (CAAE No 44879315.2.0000.0093) from Universidade Positivo (Curitiba-PR), meeting the requirements required by the National Health Council.

A cross-sectional prevalence study was performed through the analysis of toxoplasmosis tests from pregnant women attended in the basic health units (UPAs) from Curitiba city (state of Parana, Brazil) during 2010-2014.

Tests for toxoplasmosis screening in the pregnants employ the technique of Chemiluminescence Microparticles Immunoassay (CMIA) in kit and Abbott apparatus for quantitative and qualitative detection of IgG and IgM anti-*Toxoplasma gondii* respectively.

Serum samples from pregnant women were collected in laboratories of the basic health units of Curitiba and evaluated in Laboratório Municipal de Curitiba. Samples are considered IgG reagent for values above 2.9 IU/mL. Indexes above 0.599 are considered for IgM reactivity.

The data were obtained through reports extracted from the Program Informatics Laboratory Municipal Curitiba (Labsystem system - Automation System Laboratories). By this program, the results of all anti-toxoplasma antibodies tests performed by pregnant women from basic health units in the city of Curitiba, Paraná, between the years 2010-2014 were selected.

The collected data were tabulated, processed and statistically analyzed by GraphPad Prism 6 program. Results of pregnant women who underwent tests more than once in the same trimestrer and duplicated data were excluded.

RESULTS

Municipal Laboratory of Curitiba is responsible for the laboratorial tests of patients attended in the basic health units (UPAs) of the city. As shown in Table 2, from the total

tests effectuated there between 2010 and 2014, 71.0% were performed in women, 14.4% of which being pregnants. Serology to toxoplasmosis accounted 48.6% of the tests in pregnants. So, the present work evaluated the results of these 117,668 pregnant women who frequented basic health units between 2010 and 2014 in Curitiba, Paraná (Brazil).

Table 2. Total laboratorial tests of patients from the UPAs performed in Laboratório Municipal of Curitiba between 2010 and 2014

	2010	**2011**	**2012**	**2013**	**2014**	**TOTAL**
Performed tests	497,375	511,244	396,357	496,364	466,616	2,367,956
Women patients	353,468	361,884	283,290	351,056	331,645	1,681,343 (71.0%)
Pregnant women	52,909	51,032	40,107	48,821	49,220	242,089 (14.4%)
Toxoplasmosis tests	25,372	24,515	18,984	24,416	24,381	117,668 (48.6%)

Between the performed serologic tests for toxoplasmosis in pregnant women from 2010 to 2014, 29.7% presented positivity. Figure 1 shows the distribution of ages of pregnant women in relation to the positive serology to toxoplasmosis. The study comprises women between 12 and 61 years old. Women between 22 and 31 years old represented the majority of the positivity, with 48.7% of the total, followed by the group between 12 and 21 years old (29.4%); the group between 32 and 41 years old (20.6%); and the group between 42 and 61 years old (1.3%).

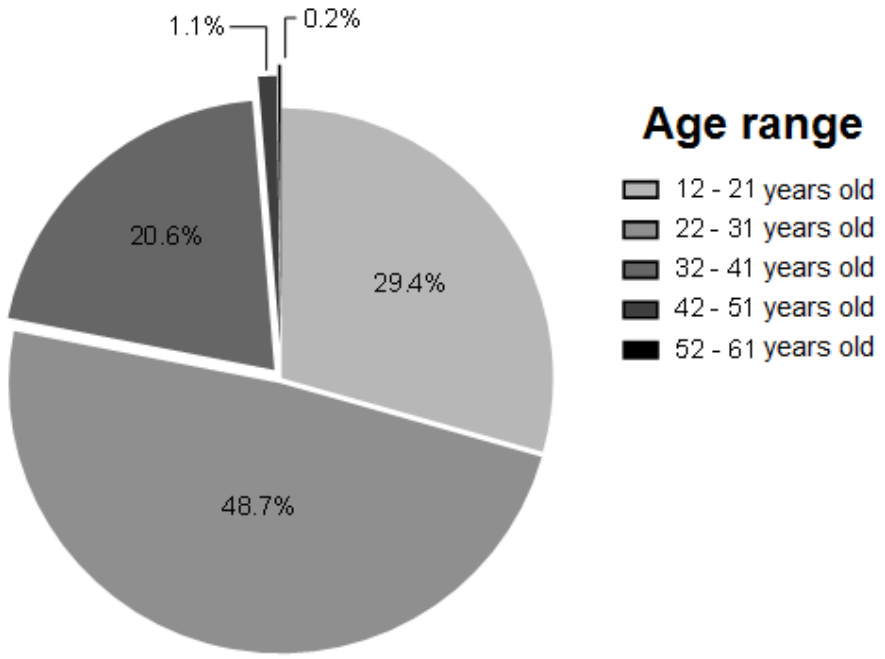

Figure 2. Age distribution of the total amount of seropositivity for toxoplasmosis pregnant women attended in UPAs in Curitiba, Paraná (Brazil) between the years of 2010 and 2014.

The relationship of analysed pregnant women and the reactivity of IgG and IgM anti-*T. gondii* per year is demonstrated in Table 3. The prevalence of IgM in pregnant women who conducted the serology to toxoplasmosis during the studied period was about 0.8%, while prevalence of IgG was 28.9%. Approximately 70.3% of them presented negative serology for *T. gondii.*

Table 3. Relationship between the amount of pregnant women and the reactivity of IgG and IgM anti-*T. gondii* per studied year.

Year	Pregnant women	IgG reactant (%)	IgG and IgM reactant (%)	Susceptibility (%)
2010	25,372	7,856 (31.0%)	160 (0.6%)	17,36 (68.4%)
2011	24,515	7,232 (29.5%)	239 (1.0%)	17,04 (69.5%)
2012	18,984	5,515 (29.1%)	168 (0.9%)	13,30 (70.1%)
2013	24,416	6,675 (27.3%)	220 (0.9%)	17,52 (71.8%)
2014	24,381	6,680 (27.4%)	175 (0.7%)	17,53 (71.9%)
Total (Average)	117,668	33,958 (28.9%)	962 (0.8%)	82,75 (70.3%)

Figure 2 shows the positivity rate for antibodies anti-*T gondii* in the evaluated pregnant women from the present study. In Figure 2A, a significant increased level of IgG is observed in 2010 in relation to the other years, where the levels were statistically similar between each other (p<0.0001). In contrast, Figure 2B denotes comparable levels of IgM positivity along the studied period.

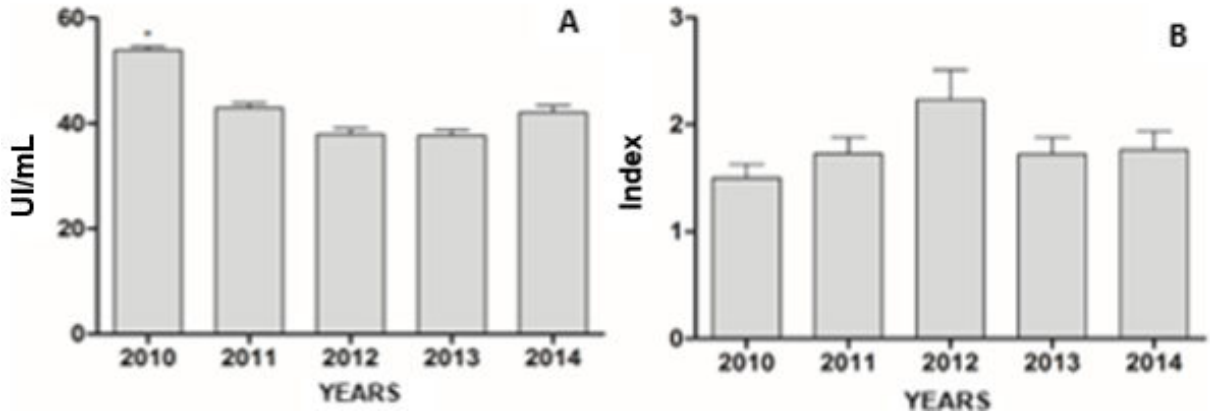

Figure 2. Positivity rate for antibodies anti-*T. gondii* in pregnant women attended in the basic health units (UPAs) in Curitiba, Paraná (Brazil) between the years of 2010 and 2014. (A): IgG, (B): IgM. Note: *represent significant levels at $p < 0.0001$.

The division of positivity of antibodies anti-*T. gondii* per age of pregnant groups in each studied year is shown in Table 4. Similar annual trends of positivity are observed, with the highest prevalence in young pregnants, from group between 22 and 31 years old, followed by that between 12 and 21 years old. The rate of positivity did not show statistically differences during the studied years when analyzing the same age group.

Table 4. Division per age group of seropositive for toxoplasmosis pregnant women attended in UPAs in Curitiba, Paraná (Brazil) between the years of 2010 and 2014.

	12 – 21 years old	22 – 31 years old	32 – 41 years old	42 – 51 years old	52 – 61 years old	Total
2010	2,521 (31.45%)	3,864 (48.20%)	1,526 (19.04%)	104 (1.30%)	1 (0.01%)	8,016
2011	2,224 (29.77%)	3,648 (48.83%)	1,502 (20.10%)	97 (1.30%)	0 (0.00%)	7,471
2012	1,719 (30.25%)	2,801 (49.29%)	1,100 (19.36%)	62 (1.09%)	1 (0.02%)	5,683
2013	1,974 (28.63%)	3,351 (48.60%)	1,784 (21.67%)	75 (1.09%)	1 (0.01%)	6,895
2014	1,829 (26.68%)	3,352 (48.90%)	1,600 (23.34%)	74 (1.08%)	0 (0.00%)	6,855

DISCUSSION

The prevalence of toxoplasmosis in pregnant women varies according to the study area and it is directly related to food habits of the population, intake of untreated water, age, multiparity, schooling and family income (AVELINO et *al.*, 2004; DIAS et *al.*, 2011).

The host animals are also important in the epidemiology of this disease, since they are involved in toxoplasmosis horizontal transmission to humans. Besides that, vertical transmission of *T. gondii* from the mother to the fetus may occur during pregnancy and represents a serious risk to the fetus to develop congenital toxoplasmosis (AVELINO et *al.*, 2004; CÂMARA et *al.,* 2015; COSTA et *al.,* 2010; DETANICO and BASSO, 2006).

Congenital toxoplasmosis is not uncommon in Brazil. In Minas Gerais state, two researches address the issue (SOAREZ *et al.,* 2011; VASCONCELOS-SANTOS *et al.,* 2009). One of them, with 146,307 newborns and conducted between November 2006 and March 2007, showed 190 newborns infected by the disease, with a prevalence of 1/770 babies born alive in this population (VASCONCELOS-SANTOS *et al.,* 2009). The other study evaluated 58 children who came from "Centro de Referência em Doenças Infecciosas" of Montes Claros, Minas Gerais (Brazil) between the years of 2002 and 2010 (SOAREZ *et al.,* 2011). The study found 21 children (36.2%) diagnosed with congenital toxoplasmosis, and 20 of them (34.5%) showed ocular changes by the end of the first year of life.

Ocular manifestations also are characteristics of systemic postnatal toxoplasmosis, which is acquired after the birth in acute or late phase, having a variable interval of latency and making the diagnosis harder once this pathology could be confused with the tardy emergence of congenital infection (AKSTEIN *et al.,* 1982; COUVREUR e THULLIEZ, 1996; MELAMED, 1991; ORÉFICE *et al*., 2010).

As approximately 90% of the pregnant infected with *Toxoplasma gondii* will have asymptomatic toxoplasmosis, serological tests are of great importance to detect antibodies anti-*Toxoplasma gondii* and to confirm or not the presence of the infection, and a possible risk of congenital toxoplasmosis (BITTENCOURT *et al.,* 2012).

Between these serological tests are the search of IgG and IgM anti-*T.gondii*, enabling also the determination the probable period of infection based on the positivity rate of the tests. When present in high levels, the immunoglobulin IgM is related to acute infection, since it is formed right in the first week after infection, however it may maintain residual levels until 18 weeks after the infection. In contrast, the immunoglobulin IgG appears between the second and fourth weeks, reaching its peak in two or three months, and its positivity can demonstrate previous immunization caused by the contact with the *Toxoplasma gondii* (GOMES, 2004).

Considering the results of the present work (Table 2), a significant rate of the total requested tests in pregnant were found as serology for toxoplasmosis (48%), showing the medical preoccupation with the disease in this group of women.

It was verified that 48.7% of the seropositive pregnant women in this work were between 22 and 31 years old, being the highest prevalence. This relation was similar to other studies, as that of Foschiera's *et al.* (2009), where the women's ages with the highest prevalence of seropositivity for toxoplasmosis were among 16 and 35 years old, or that of Sartori's (2011), where these ages were between 20 and 30 years old.

The rate of positivity for IgG varied among the five studied years, the highest rate was found in the years of 2010, probably due to the higher number of pregnant women in this year. Related to the IgM, the highest index of positivity occurred in 2012, however without statistical difference between the other studied years. The values for IgM vary according to the time of infection, showing the importance of IgG avidity tests in the process of diagnosing the disease (GOMES, 2004).

When Ferreira *et al.* (2007) searched antibodies IgG and IgM anti-*Toxoplasma gondii* in a total of 308 serum samples of pregnants from Bernardino de Campos, São Paulo (Brazil), they found 65.59% of them presenting IgG and IgM non-reactant; 5.84% presenting IgG and IgM reactant and 28.57% presenting IgG and IgM non reactant.

Similar trends were observed by Reis *et al.* (2006) in Porto Alegre, Rio Grande do Sul (Brazil). They evaluated toxoplasmosis's serology in 10,468 pregnant women. IgG positive and IgM negative were found in 58.5% of them, whereas 38.7% of them were IgG and IgM negative; 2.6% were IgG and IgM positive or it was inconclusive; and 0.18% were IgG negative and IgM positive.

Others researchers also found higher levels of IgG positive: 84.4% in Jataí, Goiás (Brazil) (COSTA *et al.*, 2010) and 77% in Caxias, Maranhão (Brazil) (CÂMARA *et al.*, 2015), demonstrating previous immunization caused by the contact with the *Toxoplasma gondii*. In contrast, the mean prevalence for IgG positive found in the present study was 28.9%, similarly to the results (31.3%) obtained in Caxias do Sul, Rio Grande do Sul (Brazil) (DETANICO and BASSO, 2006).

The divergence between the results of different regions of Brazil could be due to the regional habits, considering factors as age, schooling, culture, uncooked and underdone meat consuming, familiar agriculture and the presence of pets, leading to elevated seropositivity for *T. gondii* (VARELLA, 2003; CÂMARA *et al.*, 2015).

Between 2010 and 2014, prevalence for IgM in the studied pregnant women in Curitiba was very low (about 0.8%), and it is commonly defined as an acute infection. This is in accordance with other studies, as that done in Palotina, Paraná (Brazil) with prevalence of 1.1% of positive IgM (BITTENCOURT et al., 2012), and other done in Uberlândia, Minas Gerais (Brazil), with 0.8% IgM prevalence (SEGUNDO et al., 2004).

A study performed in Goiânia city, state of Goiás (Brazil) with 1,114 women, being 522 pregnant, demonstrated that pregnant have 2.2 times more chances to infect themselves with *T. gondii* (AVELINO et al., 2004). This major vulnerability is due to immunological and hormonals changes occurring during the pregnancy period, usually associated to the protozoan presence.

Approximately 70.3% of the studied pregnant women from Curitiba presented seronegativity for toxoplasmosis, showing a possible susceptibility to the infection. The

majority of conducted studies in distinct regions of Brazil showed lower rates of susceptibility to the infection (Table 5), highlighting a higher number of pregnant immune to the protozoan.

Table 5. Susceptibility rate in pregnant women in many Brazilian regions with sero-surveillance of IgG non reactant.

Researchers	Year	City/State	n	Susceptibility
Câmara *et al.*	2015	Caxias/MA	561	22.1%
Bittencourt *et al.*	2012	Palotina/PR	356	39.0%
Bittencourt *et al.*	2012	Jesuítas/PR	66	39.4%
Costa *et al.*	2010	Jataí/GO	6,077	15.4%
Detanico and Basso	2006	Caxias do Sul/RS	1,080	63.2%
Soccol *et al.*	2003	Curitiba/PR	152	55.6%
Reis *et al.*	2006	Porto Alegre/RS	10,468	38.7%
Varella *et al.*	2003		1,261	40.2%

In Table 5 is possible to note that Soccol et al. (2003) and Detanico and Basso (2006) also found significant susceptibility to toxoplasmosis in pregnant. Soccol *et al.* (2003) evaluated the prevalence of antibodies IgG anti-*Toxoplasma gondii* from pregnants also in Curitiba/PR, but in a specific maternity hospital (Maternidade Nossa Senhora de Fátima) and with a low number of studied individuals (152 pregnants). In their study, 55.6% from the analyzed pregnant women were found as susceptible to infection during the pregnancy.

In contrast, Detanico and Basso (2006) evaluated the serology for toxoplasmosis in both pregnant women and women in fertile age attended in a laboratory in Caxias do Sul, Rio Grande do Sul (Brazil). From 1,080 samples, a susceptibility of 63.2% was found, a value closer to the present work (70.3%).

The results of their studies and of the present work indicate a relevant amount of pregnant women susceptible to the toxoplasmosis infection and confirm the need of serologic tracking during the pregnancy, besides the information about prophylaxis actions capable of minimize the risks of contamination of toxoplasmosis.

The pregnancy is considered a high risk factor to the disease and considering the high rates of susceptibility, the intensification of preventive measures of its diagnosis and control, added to health professional's qualification and population advertisement, could improve the knowledge of the population to prevent the congenital toxoplasmosis.

CONCLUSION

With the data showed in the present work, it was possible to observe the high proportion of susceptible pregnant women to the toxoplasmosis infection (70.3%), once the pregnant period is considered a risk factor to this disease mainly because can cause severe consequences to the fetus. Therefore, it is necessary to implement campaigns of population awareness in relation for safe hygienic and dietary habits to the prevention of acquiring toxoplasmosis, which could benefit not only the pregnants or women in childbearing age, but also all population.

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