

SEROPREVALENCE OF TOXOPLASMOSIS IN PREGNANT WOMEN IN A CITY IN RIO GRANDE DO NORTE STATE, BRAZIL

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ABSTRACT

Toxoplasmosis is caused by *Toxoplasma gondii*, which is particularly relevant in pregnant women, due to the risk of congenital infection. The aim of this study was to study the serological profile of pregnant women and analyze some factors related to infection in this group. A descriptive and retrospective study was performed analyzing the results of 356 pregnant women in the Mixed Health Unit of Jaçanã-RN from 2009 to 2014. Results of serological tests for IgG and IgM anti-*T. gondii* antibodies were provided by LACEN-RN. The chi-square test (χ^2) was calculated being considered statistically significant for $p < 0.05$. The results showed that: 59% of the pregnant women tested presented serology IgG anti-*T. gondii* reagent (IgG+) and IgM anti-*T. gondii* non-reactive (IgM-), 0.6% reagent for both (IgG+ and IgM+) and 40.4% susceptible to infection (IgG- and IgM-). 71.3% of the women were tested in the first trimester of pregnancy; the 20-29 years of age range was the most prevalent (62.5%) and the 11-19 years of age range was the most susceptible (47%). The rural women had the highest incidence of positive serology (60.2%) and those from the urban area were the most susceptible (40.7%). There was no statistical association between serology and the age group ($p = 0.282$) and origin ($p = 0.881$) variables. There is need for investment in prenatal care and health education for the population about toxoplasmosis.

KEY WORDS: Toxoplasma; toxoplasmosis; seroepidemiologic studies.

INTRODUCTION

Toxoplasmosis is a zoonosis caused by the protozoan *Toxoplasma gondii* and it is particularly relevant in pregnancy, considering the high risk of congenital infection. *T. gondii* may cause fetal infection through transplacental

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passage when the mother is infected during gestation or, less commonly, when women chronically infected are immune-compromised (McAuley, 2014). There are also records of fetal infection caused by the reactivation of the chronic infection in immune-competent pregnant women (Andrade et al., 2010).

Among the anatomic and functional data survey regarding congenital toxoplasmosis, the following examples were described: restriction of intrauterine development, fetal death, prematurity and/or clinical manifestations and sequelae as microphthalmia, eye damage, microcephaly or macrocephaly, hydrocephaly, brain calcifications, pneumonitis, hepatosplenomegaly, rash and mental retardation (Pomares & Montoya, 2016, Brasil, 2005).

The globally estimated incidence of congenital toxoplasmosis is approximately 1.5 cases per 1.000 live births. The incidence of congenital toxoplasmosis in the Americas varies from 0.6 to 3.4 and the prevalence of seropositivity in pregnant women varies according to geographic regions, since it is associated with climatic characteristics, cultural factors and eating habits (Torgerson & Mastroiacovo, 2013).

The Brazilian Ministry of Health recommends serological testing for anti-*Toxoplasma* IgM (immunoglobulin M/marker of acute infection by the parasite) and IgG (immunoglobulin G/marker of immunity to the parasite). This test should be done on the first prenatal appointment or in the first trimester, with repetition if the IgG is not reagent (Brasil, 2005).

The presence of specific IgG antibodies indicates the presence of infection but does not distinguish a recent infection from an infection acquired previously or from a latent infection (Villard et al., 2016). The results of the reagent IgG and non-reagent IgM indicate an infection that may have occurred at least six months before. The detection of specific IgM antibodies for *T. gondii* was used to determine acute infection; however, due to higher sensitivity in the currently available diagnosis methods, the interpretation of the serology results with reagent IgM has become dubious, considering that these antibodies can be detected later than 18 months after infection, known as residual IgM antibodies. Thus, other methods of diagnosis should be used such as the avidity IgG antibodies (Souza-Júnior et al., 2010). The specific IgA class antibodies, detectable in about 80% of the cases, become positive after 14 days of infection and remain so for an average three to six months, varying from one to eighteen months. Specific IgE antibodies can remain positive for about four months, or even as much as eight months (Mitsuka-Breganó et al., 2010).

The importance of establishing the serologic profile of pregnant women is due to the possibility of adopting prophylactic and therapeutic measures to reduce vertical transmission and damage to fetal development (Castilho-Pellosso et al., 2005; Cortesa et al., 2017).

Furthermore, the awareness of seropositivity in pregnant women within a certain geographic area may encourage primary prevention measures, reducing parasite contact with the community, and secondary measures to screen and identify reagent cases of toxoplasmosis and provide adequate treatment (Amendoeira & Camillo-Coura, 2010).

Considering that awareness regarding the prevalence of a disease is important to the collective health and the well-being of a community, prevention procedures, diagnosis and clinical protocols in accordance to the data obtained (Maia et al., 2012) are imperative. Therefore, the aim of this study was to investigate the serologic profile of the pregnant women in Jaçanã/RN from 2009 to 2014 to verify the prevalence of *T. gondii* in the target population. Associations between the methods and the serology results and certain risk factors were analyzed, as well as evaluations of the serological monitoring of seronegative pregnant women in the second and third trimesters of gestation.

MATERIAL AND METHODS

A descriptive and retrospective cross-sectional study was performed to analyze the results of serologic tests to detect antibodies IgG and IgM anti-*T. gondii* in 356 pregnant women seen in the Mixed Health Unit in Jaçanã in the State of Rio Grande do Norte (06° 25' 33" S, 36° 12' 18" W) Northeast of Brazil, from 2009 to 2014. Data stored in the database of the Central Laboratory of the State of Rio Grande do Norte (LACEN-RN) were analyzed. The variables studied were: serology for toxoplasmosis: IgG and IgM; diagnostic methods used by LACEN-RN: Electrochemiluminescence (Roche Elecsys® Toxo IgG and IgM,) used from June 2013 to June 2014, the Chemiluminescence method, used from October 2009 to February 2013, and the Enzyme-Linked Immunosorbent Assay, used in July 2009 and March to May 2011; age; origin of the pregnant woman: rural or urban residence, as well as distribution of the frequency of seropositivity per year. The pregnant women were considered seropositive for toxoplasmosis when presenting reagent result for IgG anti-*Toxoplasma* followed or not by reagent result for IgM anti-*Toxoplasma*. Patients were considered susceptible to the infection when they presented non-reagent results for antibodies IgG and IgM anti-*Toxoplasma*. The project was approved by the Ethics Research Committee of the Alcides Carneiro University Hospital /PB (number 1.012.990).

The data utilized in this study were obtained from the Laboratory Environment Manager (an online platform in which the patients' requisitions are registered, known in Brazil by the acronym GAL) of the State of Rio Grande do Norte, linked to DATASUS (Computing Department of SUS). The serological results contained in GAL were included by LACEN-RN. The research data were computed in an accessory database, Microsoft Office Excel® 2007, and then transferred to the program SPSS Statistic® v.13.0, in which the statistical

analyses were performed. Simple percentages were calculated to obtain the frequency of the variables. Contingency tables were made and the chi-square test (χ^2) was utilized to verify the association between the variables, in which $p < 0.05$ was accepted as a statistically significant criteria for rejection of a null hypothesis.

RESULTS

A total of 356 pregnant women in Jaçanã-RN underwent prenatal screening tests for toxoplasmosis in the Central Laboratory of the State of Rio Grande do Norte (LACEN-RN) from 2009 to 2014.

Table 1 shows the number of pregnant women tested positive and susceptible to *T. gondii*, where a higher prevalence of positive pregnant women (59%) was noted. None of the pregnant women presented a serological profile of non-reagent IgG and reagent IgM.

Table 1. Seroprevalence for toxoplasmosis in pregnant women examined in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Seroprevalence	n	%
Seropositive		
IgG (+) and IgM (-)	210	59.0
IgG (+) and IgM (+)	2	0.6
Susceptible		
IgG (-) e IgM (-)	144	40.4
Total	356	100.0

Table 2 shows gestational period serological screening, evidencing that the highest number of pregnant women underwent the first screening in the first gestational trimester (71.3%), followed by 13.8% in the second trimester, and only 1.7% in the third trimester. There was no information about the gestational trimester of 47 pregnant women (13.2%). Of 144 pregnant women who presented negative serology in the first test, only 12 (8.3%) repeated the serology, with no record of serology alterations in comparison with the first test.

Table 2. Frequency of pregnant women who underwent their first serological test for toxoplasmosis according to the gestational trimester. These pregnant women were seen in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Trimester (1° exam)	n	%
1° trimester	254	71.3
2° trimester	49	13.8
3° trimester	6	1.7
Not informed	47	13.2
Total	356	100.0

Table 3 shows age groups, according to seroprevalence of pregnant women. The largest number of pregnant women seen was in the 20-29 age group, with a percentage of 58.9% seropositive results in relation to the total number of pregnant women that presented positive serology in their first gestational test. This age group also presented the highest percentage of seropositive pregnant women for toxoplasmosis with 62.5%. The 11-19 age group presented the highest percentage (47%) of pregnant women susceptible to infection by toxoplasmosis. The relationship between the seroprevalence of pregnant women and their age group was also analyzed during screening, with no statistically significant association noted between these variables ($p=0.282$).

Table 3. Seroprevalence of pregnant women who underwent their first toxoplasmosis test and statistical association to age groups. These pregnant women were seen in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Age Group		Seropositive	Susceptible	Total	p
	n	53	47	100	
11-19	%	53.0	47.0	100	
	n	125	75	200	0.282
20-29	%	62.5	37.5	100	
	n	34	22	56	
30-50	%	60.7	39.6	100	
Total	n	212	144	356	
	%	59.6	40.4	100	

The pregnant women were classified according to their urban or rural origin. Of 356 pregnant women, 75.3% lived in urban zones of which 59.3% were seropositive and 40.7% were susceptible. Of the 88 pregnant women (24.7%) that lived in rural zones, 60.2% were seropositive and 39.8% were susceptible. Analyzing the association between origin and seroprevalence, no statistically significant association between these variables was noted ($p=0.881$) (Table 4).

Table 4. Association between origin, seropositivity and susceptibility in pregnant women seen in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Origin		Seropositive	Susceptible	Total	p
Rural Zone	n	53	35	88	0.881
	%	60.2	39.8	100	
Urban Area	n	159	109	268	0.881
	%	59.3	40.7	100	
Total	n	212	144	356	0.881
	%	59.6	40.4	100	

The serological methods applied for diagnosis were the Electrochemiluminescence (Roche Elecsys® Toxo IgG e IgM), the Chemiluminescence method and the Enzyme-Linked Immunosorbent Assay (specifications not informed by LACEN-RN). The method most utilized during the period 2009-2014 was the Immunoassay by Chemiluminescence, which diagnosed 72.6% seropositive pregnant women in their first test. No statistically significant association was noted ($p=0.892$) between the serological methods utilized and seroprevalence, (Table 5).

Of the 39 pregnant women who underwent their first serological test for toxoplasmosis in 2009, 59% were reagent and 41% were susceptible. In 2010, of the 76 pregnant women who underwent the test, 65.8% were reagent and 34.2% were susceptible. In 2011, of the 107 pregnant women tested, 55.1% were reagent and 44.9% were susceptible. In 2012, of the 62 pregnant women tested, 61.3% were reagent and 38.7% were susceptible. In 2013, of the 40 pregnant women tested, 52.5% were reagent and 47.5% were susceptible; and in 2014, of the 32 pregnant women tested, 65.6% were reagent and 34.4% were susceptible. Therefore, 2010 presented the highest percentage of pregnant women reagent for toxoplasmosis (Table 6).

Table 5. Association between the methods used for testing for toxoplasmosis and seropositivity of pregnant women according to first test results. These pregnant women were seen in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Method		Seropositive	Susceptible	Total	p
Electrochemiluminescence	n	41	30	71	0.892
	%	57.7	42.3	100	
Immunoassay by Chemiluminescence	n	154	104	258	
	%	59.7	40.3	100	
Enzyme-Linked Immunosorbent Assay	n	17	10	27	
	%	63.0	37.0	100	
Total	n	212	144	356	
	%	59.6	40.4	100	

Table 6. Seroprevalence in pregnant women according to the year of testing. These pregnant women were seen in the Mixed Health Unit from 2009 to 2014, in Jaçanã-RN.

Year		Seropositive	Susceptible	Total
2009	n (%)	23 (59.0)	16 (41.0)	39 (100)
2010	n (%)	50 (65.8)	26 (34.2)	76 (100)
2011	n (%)	59 (55.1)	48 (44.9)	107 (100)
2012	n (%)	38 (61.3)	24 (38.7)	62 (100)
2013	n (%)	21 (52.5)	19 (47.5)	40 (100)
2014	n (%)	21 (65.6)	11 (34.4)	32 (100)
Total	n (%)	212 (59.6)	144 (40.4)	356 (100)

DISCUSSION

In Brazil, research performed on pregnant women has shown a prevalence of high seropositivity for toxoplasmosis, varying from 40% to 80% (Coelho et al., 2003). This was the first epidemiologic study for toxoplasmosis performed in Jaçanã/RN. The high prevalence of seropositivity for IgG-anti-*Toxoplasma* in pregnant women in the studied region (59,6%) was also observed in other parts of the country. In Vitória/ES the prevalence was 72.2% (Areal & Miranda, 2008), in Sergipe/AL 69% (Barreto et al., 2009), in Natal/

RN 66.3% (Barbosa et al., 2009), in Araraquara/SP 58% (Isabel et al., 2007) and in the Northeast of SP, 57.3% (Galisteu et al., 2007).

In the present study, the percentage of pregnant women with reagent IgM antibodies followed by reagent IgG was very low (0.6%). In other areas of Brazil such as in Paraná (Bittencourt et al., 2012) and Ijuí/RS (Pletsch et al., 2010) this positivity was null, signifying none of the pregnant women studied presented seropositivity for IgM and in Sergipe the prevalence was 0.5% (Barreto et al., 2009). The detection of specific IgM antibodies for *T. gondii* was used to determine acute infection. However, due to the increase in sensitivity of the diagnostic methods currently available, the interpretation of the results with reagent IgM became complicated, since these antibodies can be detected for a period longer than 18 months after infection, known as residual IgM antibodies (Souza-Júnior et al., 2010). Therefore, acute phase confirmatory testing was necessary, for which the determination of IgA levels, avidity test for IgG or PCR are currently recommended (Brazil, 2005; Singh, 2016).

This study noted a considerable number of pregnant women with negative serology (40.4%), therefore susceptible to acquiring the infection during gestation, therefore requiring attention regarding hygiene, preventive measures and health care. In Brazil, in Santo Antônio da Patrulha/RS, the susceptibility was 46.7% in 197 pregnant women (Rocha et al., 2014). Recife/PE presented 22.5% of 503 pregnant women (Porto et al., 2008) and in Caxias/MA, 22.1% of 561 pregnant women (Câmara et al., 2015).

Considering that seronegative pregnant women are susceptible to primo infection by *T. gondii*, primary prevention with periodic serological testing until giving birth is important in order to detect maternal seroconversion (Mitsuka-Breganó et al, 2010; Areal & Miranda, 2008). The results demonstrated that there was no systematic serological segment, as recommended by the Health Ministry of Brazil (2012), during the pre-natal period for 91.7% of the susceptible pregnant women, fact that can enhance the chances of a primo infection not being diagnosed early in the pregnancy. This risk also becomes higher in those women that did not undergo serology in the first trimester (28.7%). Although the reasons for this are unknown since only secondary data is utilized, health education campaigns and incentives regarding early pre-natal testing in pregnant women in Jaçanã/RN is of the utmost importance.

Even though the pregnant women in the 20-29 age group presented a higher percentage of seropositivity (62.5%) and those under 20 years of age were the most susceptible, a statistically significant association was not found between the variables of age and seroprevalence. In Brazil, in Caxias do Sul/RS, pregnant women in the highest age group (37 to 49) presented higher prevalence (51.7%) (Detanico & Basso, 2006). In 32.512 pregnant women in Mato Grosso do Sul - Brazil (Figueiró-Filho et al., 2005) and in 131 pregnant women in Chicago-USA (Boyer et al, 2005), no increase in prevalence was

detected in higher ages. However, in Sergipe – Brazil a statistically significant association between age group and the presence of IgG antibodies against toxoplasmosis ($p=0.007$) was found, revealing an increase in prevalence in higher ages (Barreto et al., 2009).

In the present study, a statistically significant association between the seroprevalence and place of residence (rural or urban) was not detected. The same was found in a study with 226 pregnant women in Palotina/PR and 52 pregnant women in Jesuítas/PR - Brazil, in which there was no association between place of residence and infection (Bittencourt et al., 2012). A different result was seen in Londrina-PR with 5.251 pregnant women, where a statistically significant association was observed ($p<0.001$) between these variable (Mandai et al., 2007). In Rolândia/PR (Dias et al., 2011) and in the region of Alto Uruguai/RS (Spalding et al., 2005) the pregnant women from rural areas presented a higher prevalence of the infection in comparison with those from urban area.

An important factor in serological diagnosis is the methodology used in searching for antibodies. Different methods can be applied when screening pregnant women with previous infection and with acute infection, as well as for estimating the period of infection (Marques et al, 2015). The combination of IgG and IgM Electrochemiluminescence assays, a first line method of serological study, presents high sensitivity and specificity (Prusa et al, 2010). The high sensitivity chemiluminescence detects anti *T. gondii* antibodies through IgG and IgM immunoglobulins marked with a luminescent compound, as well as detecting residual IgM. According to Firouz et al (2014), this method is more sensitive than ELISA. In a systematic review, Marques et al (2015) claims that the most frequent serological methods for screening for prenatal toxoplasmosis in pregnant women were the enzyme immunoassays for IgG and IgM. The ELISA lineage test presents high precision, reproducibility and automation, allowing the performance of high sensitivity tests and specificity on a large scale and in standard form. In the present study no statistical association was noted between the method of diagnosis and seroprevalence, which means there is no difference between the percentage of seropositive pregnant women and susceptible women according to the three serological methods analyzed.

In the present study, the socioeconomic profile of the pregnant women was not evaluated as this is considered secondary data, precluding the evaluation of some risk factors for infection by *T. gondii*, such as educational level and sanitary education of the pregnant women (Dias et al., 2011).

In conclusion, the pregnant women that underwent prenatal testing for toxoplasmosis, in Jaçanã/RN, presented high levels of seropositivity for the antibody IgG anti-*Toxoplasma*, none of which presented only the reagent IgM anti-*Toxoplasma*. Pregnant women aged 20 to 29 presented a higher prevalence of reagents while the most susceptible were those aged 11 to 19, although the data were not significant. The highest percentage of reagent pregnant women was found in the rural zone. A failure was noted in the serological segment

of the pregnant women susceptible to the infection, which probably leads to the decrease in chances of early diagnosis for possible primo infection and increases, on the other hand, the risk of congenital transmission. From these data, the need for investment in information about prevention of toxoplasmosis and the adoption of primary prevention measures by health agencies is emphasized.

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