Seroprevalence and classification of cases by probability of acute toxoplasmosis among pregnant women from Northeast Brazil.

Soroprevalência e classificação de casos por probabilidade de toxoplasmose aguda em gestantes do Nordeste do Brasil.

DOI:10.34117/bjdv6n6-034

Recebimento dos originais: 08/05/2020
Aceitação para publicação: 02/06/2020

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ABSTRACT
Introduction: Toxoplasmosis rates vary significantly between countries. Acute toxoplasmosis during pregnancy can cause congenital infections. Objective: to estimate the prevalence of toxoplasmosis among pregnant women from Northeast Brazil and to classify the participants by the probability of acute infection and vertical transmission based on the criteria of the Ministry of Health of Brazil. Methods: Toxoplasmosis prevalence and incidence was studied through 1716 serologic examinations in pregnant women in a tertiary hospital in Fortaleza, state of Ceará, northeast Brazil, between October 2015 and October 2017. The risk of vertical transmission was classified as not infected, possible, probable and definite cases based on IgG and IgM serologies and IgG avidity test. Results: There were 1248 (67.75%) susceptible cases and 468 (27.27%) suggestive of chronic infection. We found 105 cases (6.12%) with positive IgM suggestive of acute toxoplasmosis; however, 12 were excluded as false-positive results. Another 10 cases were categorized as chronic infections by high IgG avidity before 16 weeks. The other cases were divided in the probable (13), possible (69) and definite (1) groups. Conclusions: Toxoplasmosis is a prevalent disease among pregnant women (27.27%) in Northeast Brazil. The higher IgM values, at least 5 times above cutoff, suggest greater evidence of acute infection in pregnancy. Most of the cases with positive IgM belonged to the possible group and the evidence of acute infection is incomplete in this group. Therefore, the number of patients of this group could have been reduced if the IgG avidity test had been performed in the first trimester.

Keywords: Toxoplasmosis, Vertical Transmission of Infectious Disease, Maternal-Fetal Infection Transmission, Serology, Prenatal Care

RESUMO
Introdução: As taxas de toxoplasmose variam significativamente entre os países. A toxoplasmose aguda durante a gravidez pode causar infecções congênitas. Objetivo: estimar a prevalência de toxoplasmose em gestantes do Nordeste do Brasil e classificar as participantes pela probabilidade de infecção aguda e transmissão vertical com base nos critérios do Ministério da Saúde do Brasil. Métodos: A prevalência e incidência de toxoplasmose foram estudadas através de 1716 exames sorológicos em gestantes de um hospital terciário de Fortaleza, estado do Ceará, nordeste do Brasil, entre outubro de 2015 e outubro de 2017. O risco de transmissão vertical foi classificado como não infectado, possível e provável e casos definitivos baseados em sorologias IgG e IgM e teste de avidade de IgG. Resultados: Houve 1248 (67,75%) casos suscetíveis e 468 (27,27%) sugestivos de infecção crónica. Foram encontrados 105 casos (6,12%) com IgM positiva sugestiva de toxoplasmose aguda; no entanto, 12 foram excluídos como resultados falso-positivos. Outros 10 casos foram classificados como infecções crônicas por alta avidade de IgG antes de 16 semanas. Os demais casos foram divididos nos grupos provável (13), possível (69) e definido (1). Conclusões: A toxoplasmose é uma doença prevalente entre gestantes (27,27%) no Nordeste do Brasil. Os valores mais altos de IgM, pelo menos 5 vezes acima do ponto de corte, sugerem maior
INTRODUCTION

Toxoplasma gondii is one of the most common parasites worldwide. This parasite infection can be acquired through contaminated food ingestion, blood transfusion, organ transplantation, and by vertical transmission. Toxoplasmosis during pregnancy can cause congenital infection, and newborns affected may present a broad spectrum of clinical manifestations, such as the classic triad (hydrocephaly, chorioretinitis, and intracranial calcifications), abortion, fetal death, low birth weight, blindness, hearing loss, severe cognitive deficiencies, and hydrops. Congenital transmission can only occur in previously seronegative women. The determination and correct interpretation of the immune status are essential for the implementation of treatment or appropriate prophylactic measures. Since pregnant women are generally asymptomatic during T. gondii infection, the diagnosis is based on serological methods. Toxoplasmosis is diagnosed by the detection of T. gondii immunoglobulin M (IgM) and immunoglobulin G (IgG) in patient’s serum samples through enzyme linked immunosorbent assay (ELISA).

Studies have estimated that 30% of the global population has been exposed and may be chronically infected with T. gondii, although infection rates vary significantly among countries. A high seroprevalence of T. gondii IgG in Brazilian pregnant women has been reported. In Brazil, 5-23 newborns for every 10,000 live births are infected with T. gondii. The risk of vertical transmission increases with gestational age, with the highest rates (60%–81%) in the third trimester compared to 6% in the first trimester. Disease severity, however, decreases with gestational age, with infection in the first trimester resulting in abortion and fetal loss or major sequelae. The overall risk of mother-to-child transmission in pregnant women with acute T. gondii infection has been reported as 20%–50% without treatment. However, after maternal treatment, the vertical transmission rate has been reported to reduce to 9.9%, regardless of the gestational age.

The European Research Network on Congenital Toxoplasmosis has classified the immunocompetent pregnant women as definite, probable, possible, and not infected by the
probability of acute infection with toxoplasmosis. The Ministry of Health of Brazil has adopted this classification system as well. The recommendation of Health Ministry of Brazil is to carry out the screening with IgM and IgG serologies at the first trimester. In seronegative pregnant women, the test must be repeated in each trimester. For those with positive IgM and IgG titers, the IgG avidity test must be performed, preferentially before 16 gestational weeks.

Definite cases must present seroconversion of T. gondii IgG and IgM antibodies during the gestational period; or detection of T. gondii DNA in amniotic fluid sample or in placental, fetal or organ tissue by anatomopathological examination, tissue culture or bioassay; or child with confirmed congenital toxoplasmosis diagnosis. Probable cases are characterized by positive IgM and IgG with low or intermediate IgG avidity preferentially before 16 gestational weeks or positive IgM and up-titers of IgG in serial samples with a minimum interval of two weeks, or first serology performed after 16 gestational weeks presenting positive IgM and high levels (>300 IU/dL) of IgG. Any pregnant woman who presents a positive or indeterminate T. gondii IgM result, or a clinical history compatible with toxoplasmosis, or an obstetrical ultrasound (USG) or imaging tests suggestive of congenital toxoplasmosis, or is identified in clinical and epidemic situations of this disease belongs to the possible group. The subjects with positive IgG for more than three months before conception, or high IgG avidity in the preceding 16 weeks are considered as chronically infected and included in the not-infected group. Despite positive IgM, subjects with two samples of negative T. gondii IgG performed two to three weeks apart must be considered as susceptible pregnant women (false positive IgM) and also included in the not-infected group.

The definite and not infected categories are considered absolute and leave no room for doubt. The other categories are subjective, however in the probable group, strong evidence of infection is available.

The objective of the current study was to estimate the prevalence of toxoplasmosis in a selected population of pregnant women from northeast of Brazil and to classify the subjects in categories of the probability of acute maternal infection.
2 METHODS

An observational, transverse study was carried out from October 5, 2015, to October 4, 2017, based on the analysis of medical records, among pregnant women visiting the Assis Chateaubriand Maternity School (MEAC), tertiary public maternity of Federal University of Ceara, at Fortaleza, Ceara, located in the northeast of Brazil. Those women were referenced from basic units of the Brazilian unified health system to the MEAC’s specialized high-risk prenatal service. Some epidemiological characteristics of the subjects such as age, years of schooling and residential area were also collected from the medical records.

This research was approved by the Ethics Committee in Human and Animal Medical Research of the Clinical Hospital of Federal University of Ceara (No. 2.627.886). The study respected and followed the precepts established in Resolution 466/12 of the National Health Council dated October 10, 1996.

Immunocompetent pregnant women who underwent serologic investigation for toxoplasmosis immunoglobulins during pre-natal care visits were included. Consequently, subjects who did not confirm pregnancy, immunocompromised women, and those who had no serologic evaluation for toxoplasmosis were excluded.

The detection of *T. gondii* IgG and IgM antibodies and the avidity test were performed using the chemiluminescent technique (CMIA, chemiluminescent microparticle immunoassay), using Abbott Architect I4000 equipment (Abbott, Chicago, IL, USA). The cut-off values considered for this study were equal to or higher than 0.300 IU/mL and 0.600 IU/mL for IgG and IgM, respectively. The IgG avidity test values <30.00% were considered low avidity; 30.00%–59.90%, moderate avidity; and ≥60.00%, high avidity.

Subjects with suspect of acute toxoplasmosis underwent bi-monthly ultrasound screening and could choose to perform a polymerase chain reaction (PCR) of the amniotic fluid in order to investigate fetal infection. Amniocentesis were performed in subjects between 17 and 30 gestational weeks and were guided by ultrasound. The amniotic fluid samples (10-20 ml) were sent to laboratory for PCR diagnosis. As the Brazilian Ministry of Health protocol does not include amniocentesis for the investigation of fetal infection, the subjects had to pay for the test and sign an informed consent form. Some patients were unable to afford the PCR. The probability of acute infection of toxoplasmosis was classified as not infected, possible, probable, and definite cases based on the criteria of the Ministry of Health of Brazil. The data obtained were digitized in Microsoft Excel (2012). Statistical analysis was
conducted with Stata statistical program version 12.0. Descriptive statistics, chi squares, and t-tests were conducted to determine differences between groups. The differences were considered as statistically significant at p < 0.05.

3 RESULTS

The subjects had a mean age of 26.0 ± 6.4 years (range: 13-45 years), the vast majority (88.5%) were from the capital and metropolitan area, and had low schooling (<8 years of study).

Among 1842 pregnant subjects analyzed, 1716 (93.16%) showed serologic tests to toxoplasmosis. Most of the serologies were performed during the first trimester, at a mean gestational age of 13 ± 6 weeks (range: 5-35 weeks) (Figure 1).

The mean initial IgM and IgG values of the groups were, respectively: 1.79 and 261.50 IU/ dl in the not infected group, 3.40 and 379.06 IU/ dl in the possible group and 5.35 and 271, 22 IU/ dl in the probable group. There were no statistically significant differences between the mean IgG values. However, the mean IgM value of the probable group was significantly higher than the mean IgM value of the not infected group (p=0.03).

Figure 1: Distribution of the subjects of the present study who underwent serologic tests and avidity test to toxoplasmosis according to the classification of the Ministry of Health of Brazil.

IgG: Immunoglobulin G; IgM: Immunoglobulin M; AT: Avidity Test; W: gestational weeks, 2x: Those cases presented two samples of negative T. gondii IgG performed two to three weeks apart. *These subjects are susceptible to T. gondii infection.
The subjects were categorized into not infected, possible, probable, and definite groups according to the probability of acute toxoplasmosis during pregnancy based on the criteria of the Ministry of Health of Brazil (Figure 1).

Negative IgG was found in 1248 subjects (72.72%), indicating that they were susceptible to *T. gondii* infection. Most of them (1235 subjects) also presented negative IgM, implying that they have never been infected by *T. gondii* and were included in the not-infected group.

Positive IgG was found in 468 subjects (27.27%), of which 376 had negative IgM, which means that they were chronically infected by *T. gondii* and had no risk of vertical transmission of toxoplasmosis. Therefore, these cases were also included in the not-infected group.

Positive *T. gondii* IgM was found in 105 subjects (6.12%), which presented an increased risk of vertical transmission of toxoplasmosis. These subjects were young, with mean age of 24.1 ± 5.9 years (range, 13-41 years). They did not relate recent clinical signs of toxoplasmosis. Furthermore, no cerebral abnormalities were detected in their obstetric ultrasound scans.

Among the 105 cases of positive IgM, 12 were found to be false positives, because they presented two samples of negative *T. gondii* IgG performed two to three weeks apart. The remaining 93 cases presented both positive IgG and IgM. Among 93 subjects, the IgG avidity test was performed in 23, however, only twenty subjects were less than 16 weeks pregnant the appropriate period to determine whether the infection occurred during pregnancy. Low and moderate avidity was detected in thirteen subjects, out of which ten were less than 16 weeks pregnant and three were more than 16 weeks pregnant. The mean value of low avidity test was 36% ± 15% (range from 18% to 59%). These thirteen cases composed the probable group. High avidity in less than 16 gestational weeks was found in ten cases, which were included in the not-infected group and returned to low-risk prenatal care. The remaining 69 patients who did not undergo the IgG avidity test were included in the possible group (Figure 2).

Thus, the not-infected group included 1,633 patients, of which 1,611 had negative IgM, 12 had false-positive IgM, and 10 had positive IgM but presented high avidity before 16 gestational weeks. Spiramycin was being unnecessarily prescribed for many of these 22 positive IgM patients.
There was only one definite case in our study, which presented seroconversion of *T. gondii* IgG and IgM antibodies during the gestational period. The serology to toxoplasmosis was negative at 10th gestational week and anti-*T. gondii* IgG became positive at 29th gestational week.

The Brazilian Ministry of Health protocol does not include PCR of the amniotic fluid in the investigation of fetal infection. The reasons for this decision may be the low availability of laboratories with PCR technology, the high operational costs and the possible maternal-fetal risks. Nevertheless, PCR was performed for sixteen subjects, of which eight belonged to the probable group and the rest belonged to the possible group. Positive PCR for toxoplasmosis was not found among the patients of this study.

Among the 105 IgM positive cases, spiramycin had been prescribed in 96 cases (91.42%). Eleven of them belonged to the not-infected group; thus, their treatment was suspended. The triple scheme, composed of sulfadiazine, pyrimethamine, and folinic acid, was used in three cases, one in the probable group and two in the possible group.

### 4 DISCUSSION

One-third of the world's population is infected with *T. gondii*\(^{23}\). The prevalence of positive IgG in our study was 27.27%, and this value is similar to the worldwide rate.
Researchers have found a seroprevalence of anti-\textit{T. gondii} antibodies ranging from 10% to 30% in other regions such as North America, South Asia, and Northern Europe\textsuperscript{24,25}.

In industrially developed, temperate-climate countries, a decline in seroprevalence has been observed in recent decades\textsuperscript{12,26}. In France, it declined from 83% in 1965 to 37% in 2010\textsuperscript{27}. In the United States, the seroprevalence among women of childbearing age was 9% from 2009 to 2010 compared with 11% from 1999 to 2004\textsuperscript{28}.

In Brazil, the prevalence of IgG antibodies among pregnant women is variable. The toxoplasmosis seroprevalence in this study was lower than that estimated by other studies performed in Brazil. Several studies have reported toxoplasmosis seroprevalence in several cities of Brazil, such as 64% in Goiania (Goiás), 77% in Caxias (Maranhão), 68% in Gurupi, (Tocantins), and 57% in Parana\textsuperscript{28-31}. However, lower prevalence (16%) has been reported in Lages (Santa Catarina)\textsuperscript{31}. This difference in prevalence can be explained by the vast area of Brazil, with several regional differences in health, food, and labor habits.

It is important to highlight that 72.72% (1248/1716) of the participants were susceptible to toxoplasma infection, which confirms the importance of preventive measures, such as avoiding exposure to cats during pregnancy; consuming only cooked foods, treated water and pasteurized dairy products; washing hands, foods and kitchen utensils; and wearing gloves when handling soil.

The mean age of the subjects with positive IgM was 24.1 ± 5.9 years, which implies that young women are the most affected group. Another Brazilian study conducted in Goiania, state of Goias, also concluded that the IgG prevalence and the susceptibility to toxoplasmosis were higher among patients younger than 30 years and with fewer than eight years of schooling. Low schooling also was present in our study. This finding supports the hypothesis that low educational levels increase the risk of exposure.\textsuperscript{28}.

In the present study, 6.12% of the patients that underwent serologic tests for toxoplasmosis presented positive anti-\textit{T. gondii} IgM. This rate is higher than that estimated by other studies conducted in other states of Brazil, such as Parana (1.1%) and Rio Grande do Sul (3.92%), and in other countries.\textsuperscript{32-34}

Among 105 positive IgM cases, twelve were excluded due to false-positive results. Another ten cases were diagnosed with chronic infection by high IgG avidity before 16 gestational weeks. The remaining cases were divided into the probable (13), possible (69), and definite (1) groups. Rising IgG titers were not observed among the subjects of this study.
Further, this factor has never been adequately evaluated and is prone to error because of lack of reproducibility\textsuperscript{35}.

The diagnosis of recent toxoplasmosis can be made with greatest confidence when both IgM and IgG seroconversion are documented on serial testing. However, paired screening serologies showing this type of response are uncommon. We found only one case of seroconversion of \textit{T. gondii} IgG and IgM antibodies during the gestational period, and despite being considered in the highest risk criterion for vertical transmission, the child did not present any abnormality during the neonatal period. Probably, there was a false-positive result or the treatment reduced the risk of serious neurological sequel. However, the lack of abnormalities in the neonatal period does not exclude the risk of vertical transmission, because they can appear later.

A systematic review published in 2017 showed that abnormal ultrasonographic cranial findings such as intracranial calcification or ventricular dilatation were found in 6\% (14 of 218) of infected fetuses after 21 gestational weeks\textsuperscript{36-39}. No cerebral abnormalities were detected in the ultrasonographic exams performed during the prenatal period of the subjects of the study.

Half of the negative PCR results was found in subjects that belonged to the possible group, in which there is weak evidence of acute maternal infection, and the other half belonged to the probable group, but was in use of preventive medication to vertical transmission.

Most of the patients were being medicated based on serological diagnosis, without evidence of recent clinical signs of maternal infection. In most cases, the toxoplasmosis transmission occurs in the initial days after maternal infection, during the parasitemic phase, and before the development of a maternal serologic response. Hence, the best result is achieved when the treatment is started within three weeks of seroconversion. Acute clinical cases of toxoplasmosis in pregnant women have not been documented among the cases of this study. Since the treatment was always initiated after maternal serological diagnosis, in seronegative pregnant women, it is necessary to repeat the test in each trimester or sooner.

Toxoplasmosis remains a prevalent disease among pregnant women in northeast of Brazil. The screening of acute toxoplasmosis infection among pregnant women in Brazil could be improved by the training of the professionals who work in the basic units of health to properly analyze the sorological results. They must also provide information to the susceptible pregnant women (72,72\% of the cases) in order to prevent acute infection during pregnancy.
It would be also important for the IgG avidity test to be included in the first trimester pre-natal exams in order to detect the chronic infection cases. Otherwise, many women would be allocated in the possible group. Those women and their offspring would be submitted to more intensive clinical and laboratory surveillance, which generates higher costs for public health services, as well as emotional stress.

One of the strengths of this study is that we were able to demonstrate that the treatment of most of the pregnant women could have been avoided if the tests had been performed in the first trimester of pregnancy, including the IgG avidity test. Another highlight was the finding that higher IgM titles suggest greater evidence of acute infection during pregnancy.

The cross-sectional study design was one of limitations. The follow-up of the cases from pre-natal to the first year of life would be more interesting because it would provide more information about the outcomes.

ACKNOWLEDGMENTS
To the proposing institution and the patients participating in this study.

CONFLICT OF INTEREST STATEMENT
There are no conflicts of interest to disclose.

FINANCIAL SUPPORT
There was no financial support

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