RUNNING TITLE: CONGENITAL TOXOPLASMOSIS

TITLE: CONGENITAL TOXOPLASMOSIS IN A PRENATAL CARE STATE PROGRAM

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ABSTRACT

Background: Control programs have been executed in attempt to reduce vertical transmission and the severity of the congenital infection in regions with a high incidence of toxoplasmosis in pregnant women. Objective: To evaluate whether the treatment of pregnant women with spiramycin associated with a lack of monitoring for toxoplasmosis seroconversion affected the prognosis of the patients.

Methods: Prospective cohort study with 246 newborns (NB) at risk for congenital toxoplasmosis, in Goiânia-GO (Brazil), between October 2003 and October 2011, in addition to statistical analysis carried out to test the efficacy of maternal treatment with spiramycin.

Results and discussion: 40.7% (66/162) of the children were born seriously affected. Vertical transmission associated with reactivation during pregnancy occurred in 5.5% (9/162), with one showing severe infection (systemic). The presence of specific immunoglobulins (fetal IgM and NB IgA) suggested the worst prognosis. The treatment of pregnant women resulted in reduced vertical transmission. When infected pregnant women did not undergo proper treatment, the risk of severe infection (neural-optical) in the NB increased significantly. The fetal IgM was associated to an ocular impairment in 48.0%(12/25) of the fetuses and the neonatal IgA-specific was related to the neuro-ophthalmologic and systemic forms of the disease. When the acute toxoplasmosis was identified in the postpartum period, a lack on monitoring seronegative pregnant women resulted in higher risk of severe congenital infection.
**Conclusion:** The treatment of pregnant women with spiramycin decreased the possibility of transmission of infection to the fetus, whereas the lack of proper treatment favored significantly the onset of neural-optical form of congenital infection. It is necessary to amplify the primary preventive measures for all pregnant women during prenatal period and introduce secondary prophylaxis through seroconversion surveillance in seronegative pregnant woman, in order to reduce the severity of congenital infection in our environment.

**KEYWORDS** - Congenital toxoplasmosis; Toxoplasmosis during pregnancy; Treatment of pregnant women with acute toxoplasmosis.
BACKGROUND

Congenital Toxoplasmosis is an important cause of eye, hearing and mental deficiencies [1-16]. In Brazil, this fact was unknown until 2010, when a mandatory reporting was implemented requiring the assessment of a program to control congenital toxoplasmosis throughout the country [17].

Goiânia, capital of Goiás, is located in the central-western region of Brazil. It has a high prevalence of toxoplasmosis in women of reproductive age (65.8%). Moreover, the pregnant women in this city have one of the highest serological conversion rates in the world (8.6%) [18], which represents a predisposition to congenital toxoplasmosis. This happens because seronegative pregnant women undergoing immunological changes - typical of pregnancy [19] - and living in a place with a high prevalence of the disease are more likely to acquire the infection [18, 20]. This epidemiological risk stimulated the establishment of a state program to control the congenital toxoplasmosis in October 2003. This program was linked to another pregnant women care program created to help prevention of vertical transmission through primary and secondary prophylactic measures. These programs were created as an attempt to reduce vertical transmission and the severity of the congenital infection.

Analyzing the screening programs’ effectiveness is essential for the decision-making in public health politics. Even in an environment with a low incidence of the infection, toxoplasmosis has proved to be important [4-14, 21-38]. Countries that do not perform prenatal control program for congenital toxoplasmosis exhibit higher frequencies of severe forms of congenital infection [39, 40]. Recently, large European multicenter studies have questioned the effectiveness of preventive treatment of maternal infections in pregnancy [41-
Furthermore, prophylactic strategies against toxoplasmosis adopted by different public health systems are not always homogeneous [11, 12, 14, 21-30, 32-38]; they differ even within the same country. There is a high prevalence of the infection in France [14, 27, 30] and Austria [26], where seroconversion surveillance is performed monthly. Also they implemented a program of prenatal screening in Slovenia [28] and some other countries with a low incidence of the disease, such as Denmark [35] and Poland [36]. In the United States [39] and United Kingdom [40], congenital toxoplasmosis is a rare condition, so they do not conduct any program of serological screening. There had been much discussion concerning the significance of these government programs to control toxoplasmosis in pregnancy [11-14, 16, 21-30, 32-38]. Several facts hinder the enforcement of these protocols: (a) it is difficult to identify the acute phase of the infection in pregnancy when seroconversion is not performed; (b) a proper interpretation of the results of the IgG avidity test is difficult to achieve if it is carried out after 16 weeks of pregnancy [45-47]; (c) treatment-compliance problems; (d) inappropriate NB assistance according to the reference service; (e) the treatment provided for the pregnant patients does not prevent the vertical transmission of the protozoan infection [21, 22, 38, 41-43]; and (f) the type of drug used in order to treat infected women causes international controversy as well [11, 14, 21-30, 32, 33, 38].

Therefore, this study aimed to assess whether the treatment of the mother and the lack of serological conversion surveillance in the seronegative pregnant women affected the prognosis.
METHODS

1. The population

This research is a prospective cohort study in 246 NB at risk of congenital toxoplasmosis, conducted between October 2003 and October 2011 at the Clinical Hospital (CH), Federal University of Goiás (UFG). This institution is a reference to the Control of congenital toxoplasmosis in Goiás. The CH is located in Goiânia, capital of the state of Goiás, in the central-west region of Brazil. NB from pregnant women with the specific IgM against *T. gondii* identified in the first serological examination of the prenatal and NB from seronegative pregnant women who did not undergo surveillance seroconversion during pregnancy were considered at risk for toxoplasmosis.

Mothers were selected by the Pregnancy Protection Program in the state of Goiás (PPGGO) and indicated to the prenatal service at CH/UFG in order to continue the treatment initiated, once the presence of IgM in the peripheral blood of pregnant women and the fetal infection was confirmed. Those patients were followed up until they gave birth at the CH/UFG Maternity. After birth, their children were subjected to additional tests to diagnose the presence of congenital toxoplasmosis, according to the protocol 039/2002 approved by the Ethics Committee of the CH/UFG for Human and Animal Research.

NB were selected through: (1) routine postnatal screening in cord blood for detection specific IgG and IgM antibodies to *T. gondii*; (2) postnatal screening (specific IgM detected by Guthrie test); and (3) through serological screening of peripheral blood of infants who were born at other public Maternity Hospitals that participate in the PPGGO. In (1), the screening was performed in infants who were born at CH/UFG Maternity, and their mothers had specific IgM
anti-\textit{T. gondii} identified during the prenatal (by screening test or serological monitoring). In (2), selection was performed in blood collected on filter paper from the digital pulp of the NB between the 5th and 7th days of life. The sample through paper-based ELISA was analysed with a sensitivity close to 100.0%. The test was carried out at the Association of Parents and Friends of Exceptional Children (APAE) in Goiânia and Anápolis. In (3), mothers were diagnosed with acute infection although they had shown serological and clinical signs of congenital toxoplasmosis.

The detection of anti-\textit{T. gondii} IgG and IgM in cord blood of NB suspected of congenital toxoplasmosis (mothers with specific IgM or seronegative who did not undergo seroconversion surveillance during prenatal) is routinely performed at CH/UFG Maternity. The specific antibodies were confirmed in peripheral blood of NB and of their mothers and then compared to one another. This procedure identified, by seroconversion, women who have had acute toxoplasmosis in pregnancy but were not diagnosed in the prenatal.

2. The Medical and Animal Research Ethics Committee Approval

The study was approved by the Human and Animal Experimentation Ethics Committee of the CH at UFG (protocol n. 092/2001) and the mothers of the NB who agreed to participate signed the free informed term of consent after they have been made aware of the importance of the research. The protocol for the congenital infection diagnosis and treatment was approved by the Medical and Animal Research Ethics Committee from CH/UFG (039/2002).
3. Inclusion criteria

Vertical transmission diagnosis was confirmed in NB at risk of congenital toxoplasmosis whose mothers accepted to participate in the research. Exclusion criteria were: (a) mothers who refused to take part in the research; (b) inconclusive diagnosis of congenital infection and NB whose mothers did not exhibit seroconversion during pregnancy and who underwent neonatal screening (46 NB).

4- Protection of Pregnant Women Program (PPG/GO) of the state of Goiás

Women with acute infection were selected by serologic test for toxoplasmosis, performed on filter paper collected from digit pulp blood at the first time in the prenatal control. The pregnant patients were labeled as having an acute infection when specific IgM was confirmed and the avidity of IgG (<30%) was low. The tests were performed before 16 weeks of pregnancy. These criteria are also used by Camargo et al. [46], Jenum et al. [47] and Figueiró-Filho et al. [48]. Acutely infected patients were treated with spiramycin 3g/day (from diagnosis to delivery). When the patients started the prenatal after 16 weeks of pregnancy and the presence of specific IgM anti-\textit{T. gondii} antibodies was confirmed, they underwent treatment since identifying the acute phase of protozoal infection was not possible. The diagnosis of fetal infection was carried out by identification of \textit{T. gondii} through: (a) polymerase chain reaction (PCR) or isolation of \textit{T. gondii} from mice; (b) presence of specific IgM antibodies in amniotic fluid and/or fetal blood from biological material collected after the 20\textsuperscript{th} week of gestation by amniocentesis and cordocentesis, respectively. The procedure was performed by a specialist in fetal medicine.
Moreover, a serologic survey of other infections that can be transmitted to the fetus was included in the PPGGO: syphilis, rubella, cytomegalovirus, human immunodeficiency virus (HIV), hepatitis B and C, human cell lymphotropic virus (HTLV), and Chagas' disease.

5- Congenital Toxoplasmosis Control Program (CTCP)

Children who met the criteria were included in the CTCP, taking place in CH/UFG where NB suspected of congenital infection were subjected to laboratory tests and routine procedures for the diagnosis of congenital infection, according to the service approved by the Ethics Committee of the CH/UFG (039/2002).

The CTCP considers a patient infected when: (a) *T. gondii* is isolated from peripheral blood or cerebrospinal fluid (CSF) by experimental inoculation in mice, or DNA analysis for PCR; (b) specific anti-*T. gondii* IgM and/or IgA in fetal or NB blood is identified; (c) specific antibodies (IgG and/or IgM) are found in the CSF of NB; (d) the IgG-specific is larger (4x) than maternal IgG; (e) NB IgG-specific levels increase or remain positive after 12 months of life; and (f) a clinical alteration is compatible to the congenital infection in the absence of other diagnosis (Chagas' disease, syphilis, rubella, cytomegalovirus, HIV, HTLV, hepatitis B and C). The specific IgM and IgA were confirmed with a new blood sample collected between the fifth and tenth day of life. The eye fundus examination was performed at the Reference Center of Ophthalmology (CEROF), at the Department of Ophthalmology, Faculty of Medicine of UFG.

NB infected or suspected of being infected were treated according to the service approved by the Ethics Committee of the CH/UFG (039/2002): sulfadiazine (100-150mg/kg/day) 4 times a day (every 6 hours); pyrimethamine
(1-2mg/kg/day) twice a day (every 12 hours); and folic acid orally given in a daily dose of 3.5 mg. When the protein levels in the CSF were ≥150 mg/dl or when there was acute ophthalmologic impairment, a daily dose of prednisone (2-3mg/kg/day) was added to the treatment. These conditions were maintained until the certainty of no vertical transmission could be reached. For the infected patients, however, the duration of the treatment depended on the presence of clinical abnormalities at birth (2 years) or on the absence of clinical signs (1 year).

6- Laboratory Techniques

6.1. Screening in pregnant women - performed at the Institute of Diagnostic and Prevention (IDP) in APAE/Goiânia.

For the identification of specific anti- *T. gondii* in the blood of pregnant women blood samples were collected by finger prick on filter paper S&S 903, according to the standard procedures of the IDP in APAE/Goiânia. Serological screening for toxoplasmosis in eluate of filter paper was performed through ELISA kits, registered at the Ministry of Health by the National Agency for Sanitary Surveillance (ANVISA). The sensitivity of the filter paper technique for the identification of IgM anti-*T. gondii* was 99.8% [48], while the serum sensitivity was 97.9% (determined by manufacturer). A similar study conducted in pregnant women from Mato Grosso do Sul state, in the midwest of Brazil, found IgM sensitivity in the filter paper of 99.4% [48]. The test was repeated in the peripheral blood of pregnant women and confirmed by ELISA kits registered at ANVISA. The IgG avidity test (before 16 weeks of gestation) was performed to determine if acute infection occurred before pregnancy (with high
avidity). Low avidity test was considered as an indicative of acute infection, which was also used in other scientific researches [46-48].

The analysis of blood on filter paper technique was described by Minuzzi [49] 3 microliters of serum were used for the extraction of the IgM anti-\textit{T. gondii} antibody, placed in 200 microliters of elution. The plate was homogenized at 1000 rpm at room temperature for 1 hour and the tests were accomplished using automated ELISA analyzer. The antibody capture method is based on ELISA. The presence of specific anti-IgM \textit{T. gondii} allows the conjugated connect to the solid phase through the presence of \textit{Toxoplasma} antigen. The enzyme activity is proportional to the concentration of specific IgM present in the samples or controls. The enzymatic activity is analyzed by adding a colorless solution of chromogen/substrate, measured with a photometer. The technique is summarized below: place 100 microliters of calibrator and controls to the corresponding wells; elution of filter paper blood spots (the strips-sensitized) with 200 microliters of the eluent itself; incubate the plate for 60 minutes at 37\(^\circ\)C and wash with washing buffer 5 times; dispersing 100 microliters of conjugate in each well, incubate the plate for 60 minutes at 37\(^\circ\)C, washing with washing buffer 5 times; place 100 microliters of conjugate in each well, incubate the plate for 30 minutes at room temperature protected from light; add 100 microliters of the blocking solution to each well, mix gently for 30 seconds; use immediately a 450 nanometers microplate reader filter for the results.

6.2. Techniques used for the neonatal diagnosis of congenital toxoplasmosis - performed at the Laboratory of Immunology CH/UFG
The following tests were defined as markers of congenital infection with *T. gondii*: (a) serological tests, such as the presence of specific anti-*T. gondii* IgM and IgA antibodies; and (b) parasitological identification tests, such as the experimental inoculation in mice and/or PCR to analyze the biological samples suspected of being contaminated (fetal blood, amniotic fluid, blood and CSF of the NB).

6.2.1. Parasitological Examination - Blood samples were collected before the NB's specific medication, to determine the presence of the parasite by polymerase chain (PCR) or experimental inoculation in mice (second protocol Silva et al. [50]). The PCR was performed according to the following protocol: the DNA was extracted according to specifications from Pure Link Genomic Purification kit – for purification of genomic DNA-Invitrogen. The PCR reactions were performed in the MasterCycler Personal thermocycler. The amplification process consisted of an initial denaturation at 94°C (5 min), 35 cycles of denaturation at 94°C (1 min), annealing at 62°C (1 min) and extension at 72°C (1 min), followed by a final extension at 72°C (10 min). The PCR reactions were performed in duplicate, using a sequence of the B1 gene of *T. gondii*. The following primers were used: Toxo-B5 (5'-TGA AGA GAG GAA ACA GGT GGT CG-3') and Toxo-B6 (5'-CCG CCT CCT TCG TCC GTC GTA-3'). The PCR products were visualized in a 6% polyacrylamide gel electrophoresis and the gel was stained using silver nitrate [51]. Peritoneal fluid from mice infected with *T. gondii* RH strain was used as positive control.
6.2.2. Serological Tests - performed at the Laboratory of Immunology CH/UFG and Laboratory studies of the Host-Parasite Relationship - LAERPH/IPTSP/UFG. These serological tests were used to detect anti-*T. gondii* IgG and IgM in the NB’s blood samples. The neonatal serological screening was performed through the Indirect Fluorescent Antibody Test (IFAT), according to Camargo et al. [52] and the Microparticle Enzyme Immunoassay (MEIA). The IgM detection test for the suspected patients of congenital toxoplasmosis was performed using three techniques - IFAT, MEIA and ELFA (Enzyme-Linked Fluorescent Assay). The methodologies used for detection of anti-*T. gondii* specific antibodies were followed according to the manufacturer's instructions. The specificity for IgM of tests was 100.0% except for IFAT-IgM which was 91.7%. The sensibility of the tests was MEIA-IgM (60.9%), ELFA-IgM (60.9%), IFAT-IgM (59.6%), ELISA-IgA (57.1%), Rodrigues et al. [54].

The technique IFAT was used according to Camargo et al. [52, 53], with Biolab conjugate (Fluoline G and M), at LAERPH/IPTSP/UFG. The presence of IgM was demonstrated by removal of rheumatoid factor, using reagents produced by Biomérieux. The reference values for IFAT were: < 1/10 – nonreactive and ≥ 1/10 – reactive.

The microparticle enzyme immunoassay (MEIA) was used for the quantitative determination of the antibodies IgG and IgM anti-*Toxoplasma gondii* in the CSF (NB) or in the plasma of the pregnant women, NB and puerperal patients, following the instructions manual for the AXsYM ABBOTT immunochemical automated analyzer at the Clinical analysis laboratory, CH/UFG. Reference values for IgG were: > 3 UI/mL – reactive, between 2 and 3 UI/mL – indeterminate, and < 2 UI/mL – nonreactive. Reference values for IgM
were: > 0.600 UI/mL – reactive, between 0.500 and 0.600 UI/mL – indeterminate, and < 0.500 UI/mL – nonreactive.

Enzyme-Linked Fluorescent Assay (ELFA) was used for the quantitative determination of the antibodies IgM anti-Toxoplasma gondii in the plasma of the NB, also following the instructions manual, at the Clinical analysis laboratory, CH/UFG. Reference values for IgM using ELFA were: < 0.55UI/mL – nonreactive, > 0.55 and < 0.65 UI/mL – indeterminate, and > 0.65 UI/mL – reactive (Bio-Mérieux Instruction Manual Toxo-M). ELFA was carried out because it performs the immunocapture of IgM antibodies, which avoids false-positive (due to the presence of rheumatoid factor) and false-negative (due to excess of IgG) reactions and presents sensibility and specificity compared to ISAGA, of 93.5% and 99.3%, respectively.

Enzyme-linked immunosorbent assay (ELISA) was used for the determination of IgA, according to the instructions manual, at the Clinical analysis laboratory, CH/UFG. The specific anti-T. gondii IgA was researched using double-sandwich ELISA (capture). Reference values for specific IgA were: < 4.5 UA/mL – nonreactive, between 4.5 and 5 UA/mL – indeterminate, and > 5 UA/mL – reactive. Blood samples were sent to a private laboratory to detect anti-T. gondii IgM also using ELFA (VIDAS, Bio-Mérieux) and IFAT, Camargo & Leser [52].

7- Statistical analysis

The data was computerized with Excel 2007 and SPSS 15.0 for Windows was used for the statistical analyses. The evaluation was to determine whether
there was association between each of the variables collected or not. The results were compared to the presence and the absence of maternal treatment with Spiramycin. P values < 0.05 were considered as statistically significant and with a 95% confidence interval. The Fisher's Exact Test was used when data were less than 5. Statistical calculations excluded cases where there was no information on the time of occurrence of the diagnosis of toxoplasmosis and the maternal treatment.

**RESULTS**

The PCTC made possible the diagnosis of 162 children infected with *T. gondii* (Table 1). Those children were sent to the Reference Center in the CH/UFG.

Among the 246 women whose infants were followed up at the Reference Center for Congenital Infections, 30.1% (74/246) were seronegative at the first prenatal visit. Seroconversion was identified during gestation in 16.2% (12/74) of women who underwent pre-natal care at CH/UFG, institution that conducts seroconversion surveillance in pregnant patients at risk of infection or seronegative women. Other women were diagnosed through the identification of specific anti-*T. gondii* IgG and/or IgM in the cord blood of NB, confirmed by the analysis of peripheral blood of both NB and mothers.

The fetal IgM test was performed in 29.0% (47/162) of the cases and 53.2% (25/47) were positive, 48.0% (12/25) within this group having developed ophthalmologic impairment. The neonatal IgM was positive for 38.5% (62/161) of the cases and the neonatal IgA for 20.4% (18/88). Patients with specific anti-
Toxoplasma gondii IgA - 61.1% (11/18) - developed a severe form of infection. 85.7% (6/7) of the children with widespread disease had IgA-specific.

The parasite was identified in 31.7% (38/120), and in 34.2% (13/38) of these cases developed some sort of severe impairment, and CSF was abnormal in 70.9% (78/110) with Toxoplasma gondii-specific IgG associated with nonspecific condition.

The use of spiramycin during pregnancy was conducted either when the diagnosis of acute infection was established before pregnancy (specific anti-\textit{T. gondii} IgM persistence in their bloodstream), or when the serologic screening was conducted in the first pre-natal visit. Alternatively, spiramycin would be also used even when the presence of specific anti-\textit{T. gondii} IgG and IgM was identified in women who were previously seronegative. 120 women were treated - 70 NB born with congenital toxoplasmosis where 18.6% (13/70) of them, were born severely infected. 115 pregnant women were not treated - 84 NB born with congenital infection where 60.7% (51/84) of them, were born severely infected. 11 women did not report on treatment. The neural-optical form of congenital infection occurred in all trimesters of pregnancy, but in 36.9% (31/84) NB of mothers who were not subject to antiparasitic treatment with spiramycin and only 7.1% (5/70) when treated.

45% (9/20) of women who had \textit{T. gondii} infection before pregnancy (months before pregnancy or previous pregnancy) gave birth to infected children; 4 (44.4%) of those children developed an asymptomatic form of the disease; 3 (33.3%) had asymptomatic meningitis; 1 (11.1%) developed the systemic form of toxoplasmosis; 1 (11.1%) an ocular form of toxoplasmosis. 6 cases occurred in previous pregnancies (1 neonatal death) and the other 3 occurred three months before pregnancy. No patient was infected by HIV or any
other infectious disease (Chagas disease, Syphilis, Rubella, CMV, HTLV, Hepatitis B and C).

There were no cases of systemic forms, hearing or lymph node in the third trimester of prenatal diagnosis. Among 62 seronegative pregnant women who were not monitored during pregnancy, and who had acute infection with *T. gondii*, 87.1% (54/62) had children infected and 64.8% (35/54) with severe forms of congenital infection (intracranial calcifications, neural-optical, ocular and systemic).

Furthermore, 4.9% (8/162) of the patients were infected with toxoplasmosis and also with cytomegalovirus showing cerebral impairment characteristic of both infections. Mortality associated with congenital infection was 4.3% (7/162).

**Table 1** - Highlights 162 infected patients, 40.7% (66/162) of them were born seriously sick; among the 7 patients who showed widespread disease, 2 also had neurological and optical impairment. Furthermore, 30.8% (50/162) of the babies were born with ophthalmologic impairment and 76.0% (38/50) of those had also neurological impairment; 20.0% (10/50) developed only the optical form while 4.0% (2/50) only had the systemic form of the disease. Among these children, 52.0% (26/50) were born with poor eyesight, 50.0% (13/26) of those were blind and 50.0% (13/26) had peripheral vision because of bilateral macular impairment; the systemic form was found in 4.3% (7/162); 31.5% (51/162) had intracranial calcifications, and among children with neurological damage, 37.2% (19/51) developed hydrocephalus.

**Table 2** - The transmission of congenital toxoplasmosis was associated significantly with the time of diagnosis of maternal infection.
Table 3- The maternal diagnosis favored significant delay in the appearance of neural-optical forms of congenital infection and systemic. Also favored transmission of congenital infection.

Table 4 - The lack of treatment of pregnant women with spiramycin favored significantly the appearance of vertical transmission and a severe form of congenital infection (neural-optical).

DISCUSSION

Although serological conversion follow-up is recommended in a high prevalence of the infection [4, 5, 7, 14, 22, 24], this study did not performed it. There is a high risk of *T. gondii* contamination for the pregnant or seronegative woman in the current environment. Pregnancy increases the chances for the infection to take place [18], especially in places with high prevalence of infection and major environmental contamination [55], contact with animal reservoirs, poor dietary habits and low level of formal education as it is found in pregnant women served in pre-natal services in public health throughout Brazil [20].

The diagnosis of the congenital disease was difficult to establish - the mother suffered acute toxoplasmosis or a recurrent form of the disease - because of the low sensitivity of congenital infection markers. In NB, the diagnostic markers of congenital toxoplasmosis showed low sensitivity - IgM specific (38.5%), IgA specific (20.4%) and identification of *T. gondii* (31.7%) - similar to that found by Gilbert et al. [56] and Bessieres [57] in relation to serological markers, but smaller when compared to the identification of the parasite. Bessières et al. [57] also analyzed the influence of medication provided to pregnant women with acute toxoplasmosis through the results of the
diagnostic markers of the congenital infection. They found significant results when pyrimethamine and sulfadiazine were used together, with little interference when spiramycin was used. It may be related to the strain that congenital toxoplasmosis in this region of Brazil is more sensitive to spiramycin than the strain present in Europe. Therefore the fetal specific IgM proved to be a better marker for congenital infection than the peripheral blood test (53.2% and 38.5%, respectively). However it can be considered a poor marker as it failed to diagnose nearly half of the infected patients. An important aspect is that 48.0% of fetuses who presented IgM in their bloodstream were born with ocular impairment. No reference or description of that fact in the literature was found. The results imply that scientists should research this type of infection more intensely, even though there is an international debate on the subject concerning PCR in amniotic fluid being more important than other tests [5, 7, 58-63]. Bessieres et al. [58], found a higher sensitivity in the identification of the parasite in the amniotic fluid.

The IgA-specific was also associated with the worst prognosis of vertical transmission (neural-optical and systemic forms), just as it was described by Rodrigues et al. [54]. The specificity of tests was 100.0% except for the IFAT-IgM (91.7%), but it was higher than those observed by Pinon et al. [64].

The CSF was abnormal in 70.9% of infected patients according to another research. On the one hand, it is considered an important marker of congenital infection [5, 7, 64, 65]. On the other hand, Wallon et al. [66] found no significance in the CSF examination for the diagnosis of congenital toxoplasmosis in the neonatal period.
A fact has drawn attention: 5.5% (9/162) of congenital infection cases affected immunocompetent children of women who had acute infection prior to pregnancy. According to the literature, the majority of cases of vertical transmission in acute toxoplasmosis during pregnancy took place when maternal infection preceded gestation by a few months [67, 68]. The results show that only 3 out of 9 cases would fit that description, whereas the other 6 cases occurred in previous pregnancies. In addition, 2 children were born with severe infection, one of them with the systemic form (his mother was diagnosed with acute toxoplasmosis earlier in pregnancy), and another child born with ocular form of the disease (mother with acute toxoplasmosis 3 months before pregnancy). This suggests that this situation is likely to have resulted from a reduced cellular response of the host during the gestation period [19]. It can interfere with the parasite load and with the clinical course of maternal infection and consequently increase the risk of vertical transmission. Some authors report that IgG positive pregnant women could not transmit toxoplasmosis except in rare cases of acute toxoplasmosis relapse due to immunodeficiency of the patient [5]. The results show that toxoplasmosis is a complex infection disease during pregnancy. Also, even the presence of prior immunity does not prevent the transmission of infection to the fetus. Furthermore, severe forms of vertical transmission were also found in these women, which supports the establishment of preventive measures of primary nature (in all pregnant women), to avoid contact with T. gondii by avoiding potentially contaminated food (meat, eggs, milk and raw vegetables), contact with animals (cat, dog), and land of gardens [4, 5, 7, 69].
The results - 40.7% (66/162) NB severely affected (table 1) - are better than those found by Olariu et al. [39]. In this study the clinical alterations have been described for children from 0 to 180 days, who were not undergoing treatment. Most children showed evolution of the congenital forms of the disease after birth, which is similar to those results found by Thiebaut et al. [70]. One should note that those severe forms of the congenital infections occurred more frequently when there was no diagnosis of acute infection in pregnant women. This fact could be explained by either the lack of serological conversion surveillance in seronegative pregnant women or the absence of prenatal care.

Neurological manifestations, including asymptomatic meningitis, took place in 51.2% (83/162) (see table 1) and 59% (49/83) of those developed intracranial calcification, where 37.2% (19/51) had hydrocephalus. The results related to hydrocephalus as a complication of congenital infection were similar to that found by Fahnehjelm in Sweden between 1997-1998 and Paul in Poland, between 1996-1998 [71], but much higher than that reported in those studies, the latter with low frequency of this complication [16, 44, 72-74]. Others found no significant result related to hydrocephalus [28, 71, 75]. The intracranial calcifications present in 6.8% of those infected were consistent with those reported in Europe in 2005 [76]. These results are similar to that of Daffos [77], evidence that the infection is more aggressive in our environment.

Gilbert et al. [15] compared the severity of congenital toxoplasmosis in Brazil and Europe, the former having most severe congenital cases. In this study, 30.8% (50/162) of the infected showed ocular impairment, 52.0% (26/50) of those were blind or they had only peripheral vision. The congenital infection in general was also more severe, since 4.3% (7/162) were born with the systemic form of the disease, and 2 of those also had neuro-optical form of the
disease. 37.2% (19/51) of children with neurological impairment showed hydrocephalus, similar to that found by Daffos et al. [77]. Moreover, 4.3% (7/162) died from the infection and 4.94% (8/162) had concomitant infection with cytomegalovirus, showing intracranial calcifications with characteristics of both infections. These results are similar to those found in the past [1-3, 5, 7] and in locations that do not have control state program for congenital toxoplasmosis [39, 40, 64, 65]. These results pointed out a major flaw in the current toxoplasmosis control program of Goiás. It is extremely necessary to improve these levels of congenital infection severity, through the implementation of primary prophylaxis in all pregnant women (seronegative and seropositive), and conducting seroconversion surveillance in pregnant women through serological tests performed monthly, in order to control the disease and identify maternal infection at very early stages of the infection, thus reducing the transmission and the severe forms of the congenital infection.

Hearing impairment was observed in 2.4% of the infected (table 1); it was transient and disappeared with specific antiparasitic treatment instituted in the NB. 4.3% (7/162) died as a result of the severe congenital infection. This number is higher than that described by Ricci [78].

The lack of seroconversion surveillance in 62 pregnant women at risk during the 8 years of the PCTC in Goiânia resulted in the appearance of 56.4% (35/62) the children with severe forms of the infection (neurological, ocular and/or systemic). This number is much higher than the ones described by Desmonts & Couvrer [2] and Foulon et al. [11] and where no governmental program for controlling infection is available [39, 40, 64, 65]. The finding highlights the need for serological surveillance of the pregnant women, as already recognized by the literature [5, 7, 14, 16, 22-30, 32-36, 38, 79-91].
The transmission of toxoplasmosis was higher when the acute infection was identified postpartum and not during pregnancy; possibly related to the lack of maternal treatment.

In surveys conducted in Europe, when the acute infection occurred in the third trimester, no ocular or intracranial impairment were found in the NB [76]. Different from this study results, they found no systemic, hearing or lymph node impairment when the infection occurred in the third trimester of pregnancy.

The neuro-optical forms found in all trimesters of pregnancy were present in 36.9% (31/84) of children when their mothers were not subjected to antiparasitic treatment with spiramycin during pregnancy and only 7.1% (5/70) of them showed complication. Therefore, maternal treatment with spiramycin protected NB against neuro-optical impairment. The lack of treatment of acutely infected pregnant women also resulted in an increased risk of developing this type complication (table 4). In addition, maternal treatment decreased the prevalence of these severe forms of toxoplasmosis, a reduction from 60.7% (51/84) to 18.6% (13/70). These findings were much higher than those described by Schmidt et al. [35] - 21.8% of untreated pregnant women - in Denmark and they confirm the importance of the treatment of pregnant women with acute toxoplasmosis, even with spiramycin as a single drug [2, 4, 5, 7, 25, 32, 70, 88]. This severe form of congenital infection can manifest very early at birth, confirming the more aggressive strain of *T. gondii* circulating in this study’s region of focus.

Among children with ocular impairment, 52.0% (26/50) were born with poor eyesight one half of them blind and the other half with peripheral vision because of bilateral macular impairment. These results are in accordance with the study
of Gilbert et al. [15], who found more intense aggressiveness of toxoplasmosis in Brazil than in Europe. The maternal treatment with spiramycin prevented neural-optical form of the infection and lack of treatment during pregnancy resulted in a greater risk for this alteration to take place (table 4).

A higher prevalence of the systemic form was found - 4.3% (7/162) - considerably higher than the prevalence for other countries. In studies carried out in Europe, complications such as neonatal death and systemic form were reported but the incidence did not exceed 1.0% of the children in the study [76]. When the patient was treated with spiramycin during pregnancy, there was a protection against this form of congenital infection and a decrease in the frequency of 7.1% (6/84) to 1.4% (1/70).

Moreover, 4.9% (8/162) were infected by toxoplasmosis and cytomegalovirus together. The cerebral impairment is a characteristic of both infections and it made the prognosis of the infected patients even worse.

The neurological and ocular aggressiveness - even in the children of treated pregnant women - larger than those found in places lacking prenatal preventive programs indicate the need for future research, isolation and genetic characterization of the circulating strains in Goiás, and also immune assays of neonates exposed to T. gondii in pregnancy.

Although treatment was made with spiramycin, there was a clinical improvement of the congenital infection consequences. However these results - even in the presence of treatment - are still higher than those found in the absence of treatment in other locations. Schimidt et al. [35], for example, also found higher ocular aggressiveness among untreated women (9.6%) than in those who underwent treatment (2.8%).
There are other studies showing the diagnosis of acute infection during pregnancy and its consequent treatment that can reduce the several forms of congenital infections [4-14, 21-30, 32, 33, 38, 69, 70-95]. These works emphasize the importance of the preventive programs of congenital toxoplasmosis during pregnancy, including seroconversion surveillance in seronegative pregnant. In Brazil, where the congenital infections are more aggressive (which has been described) than elsewhere in the world, a national program for controlling toxoplasmosis infection during pregnancy is highly recommended.

This research has also shown the need for improvements in the current program implemented in the state of Goiás. Primary and secondary prophylaxis measures should be intensified (serological surveillance in seronegative pregnant women and change in the treatment of acutely infected patients). It is suggested a monthly serological screening, such as the French program for control of congenital toxoplasmosis [14]. It is worth mention that the money spent on tertiary prophylaxis for severely infected NB and the maintenance of life without quality exceeds what would be spent to carry out the monthly serological tests in seronegative pregnant women (34.2%). According to second Remington et al. [5] the cost is approximately one million dollars per patient with a severe form of the disease.

Since the treatment of pregnant women is a very controversial issue in the literature, this study is in accordance with studies that demonstrated a reduction in severity of the fetal infection when the mother was treated during pregnancy [11, 13, 14, 16, 22, 24, 27, 69, 71-78, 88]. This study also presented a different result from the meta-analysis published by SYROCOT study group [79]. A
statistically significant occurrence has been found in the onset of neural al-optical form in the offspring of women treated during pregnancy.

This study showed that congenital toxoplasmosis is more severe in the study area than elsewhere. The infection occurs at much higher levels than in other locations that do not even have a program of prenatal care. It is recommended the implementation of primary preventive measures (to control infection with \textit{T. gondii}). These measures must be performed regardless of the patient immune status against toxoplasmosis: (a) to implement monthly seroconversion surveillance for seronegative pregnant women or for patients at risk; and (b) replace spiramycin by sulfadiazine after the 20th week of pregnancy (75 mg/kg/day in the first two days, followed by 50 mg/day in two doses) and folinic acid (10 to 20 mg/day) until a week after withdrawal of drugs (up to the moment of birth). This should be done even without a diagnosis of fetal impairment, as the diagnosis of congenital infections is complex and the aggressiveness of the infection in Brazil is intense. It is worth noting that a positive test does not prevent the vertical transmission. The determination of specific anti-\textit{T. gondii} IgM indicated in the new proposal of the Ministry of Health for prenatal programs in public health (Project Stork) is inadequate and ineffective according to the results of this study because it could no longer identify seronegative pregnant women or the risk of acute infection development. In addition to not performing the primary prophylactic measures, which is admittedly effective to decrease acute toxoplasmosis rate among susceptible women [4-14, 16, 21-30, 32, 69-76, 80-95], it still does not perform seroconversion surveillance and fails to identify the majority of the infected children. One can only be sure that the infection has occurred during pregnancy when seroconversion is identified [89, 93, 95]. Moreover, the treatment of the
early infected fetus is delayed (secondary prevention). This study highlighted
the need of preventive measures for all pregnant women, once 5.5% of children
who had congenital infection got the parasite from women immune to *T. gondii*
and 2 of them developed severe forms of the infection (systemic and ocular).

**CONCLUSIONS**

1) The treatment of pregnant women with spiramycin decreased the
possibility of transmission of infection to the fetus, and the lack of treatment
favored significantly the onset of neuro-optical form of congenital infection.
2) The occurrence of severe forms in pregnant women whose chronic
infection is recurrent demonstrated the need to expand the primary prophylactic
program to all pregnant women, regardless of their immune state against *T.
gondii*.
3) The occurrence of a severe form of congenital infection remains important
in Brazil, despite the government programs.
4) The severity of the congenital infection in Brazil reveals the need for
secondary prophylaxis programs with drugs that treat the fetus and pregnant
women acutely infected.

**LIST OF ABBREVIATIONS USED**

- **NB**- newborn
- **T. gondii**- *Toxoplasma gondii*
- **PPGGO**- Pregnancy Protection Program in the state of Goiás
- **CH**- Clinical Hospital
- **UFG**- Federal University of Goiás
All the authors declare that they have no competing interests.

**Author’s contributions**

All authors contributed to the design of the study, prepared and approved the final manuscript. **MMA** – Corresponding author, responsible for the toxoplasmosis project in Goiânia, the collection of results, data analysis and monitoring children suspected of congenital infection. **WNA** – responsible for collecting the biological material from the fetus and the mother with acute toxoplasmosis and for the treatment of the acutely infected pregnant woman; **IMXR** – responsible for conducting laboratory tests of the umbilical cord blood, NB suspected of congenital infection (beyond the 5th day of life) and cerebrospinal fluid, performed in the Laboratory of Immunology CH/UFG and also for laboratory monitoring of patients suspected of congenital infection. **ARR** - responsible for the ophthalmological testing of NB and to draft the manuscript; **MBFG** - partly responsible for of the monitoring of some NB and participated in the design of the study and to draft the manuscript; **TLC** – carried out the immunoassays and responsible for the writing of the manuscript. **AMC** – responsible for identification of *Toxoplasma gondii* by PCR and inoculacion in
mice, participated in the project since its implementation and also responsible for the writing and analysis of the study data.

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Additional files provided with this submission:

Additional file 1: Tables- Congenital toxoplasmosis 05082013.doc, 90K
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