Congenital Toxoplasmosis in Southeastern Brazil: Results of Early Ophthalmologic Examination of a Large Cohort of Neonates

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Objective: To report results of early ophthalmologic examinations in a large cohort of newborns with congenital toxoplasmosis (CT) after neonatal screening.

Design: Cross-sectional analysis of a cohort.

Participants: A total of 178 newborns with confirmed CT from 146,307 screened babies (95% of live births) from Minas Gerais state, southeastern Brazil.

Methods: From November 2006 to May 2007, newborns underwent neonatal screening by immunoglobulin (Ig)M capture of dried blood samples. On all positive or suspected cases, confirmative serology was performed on babies and their mothers. Congenital toxoplasmosis was confirmed in newborns who had IgM and/or IgA and IgG, or IgG associated with suggestive ocular lesions (with IgM and IgG in the mother). Ophthalmologic evaluation consisted of indirect ophthalmoscopy with a lid speculum. Pediatric examination and radiologic studies of the central nervous system were also performed. In selected cases, biomicroscopy of the anterior segment, fundus photographs, or ultrasonography (B-scan) was performed.

Main Outcome Measures: Prevalence of retinochoroidal lesions, either cicatricial or active, and their location and associated findings, such as vascular sheathing, hemorrhage, vitreous opacities, and retinal detachment, were evaluated. The occurrence of cataract, microphthalmia, microcephaly, intracranial calcification, and hydrocephalus was also recorded.

Results: Of 146,307 neonates screened, 190 had CT, yielding a prevalence of 1 in 770 live births, of whom 178 (93.7%) underwent standardized ophthalmologic examination at an average age of 55.6 ± 16.6 days. Of these 178 infants, 142 (79.8%) had retinochoroidal lesions consistent with CT in at least 1 eye. Bilateral involvement was noted in 113 patients (63.5%). Macular involvement was seen in 165 eyes (46.3%) of 111 patients (62.4%). Active lesions were observed in 142 eyes (39.9%) of 85 patients (47.8%). These lesions were located in the macula of 75 eyes (21.1%) and were associated with retinal vascular sheathing in 44 eyes (12.4%).

Conclusions: A high prevalence of CT was encountered (1/770) with high rates of early retinochoroidal involvement (~80%) and many active lesions (in ~50%), indicating a possibly more severe ocular involvement by CT in Brazil than in other parts of the world. The hypotheses of higher parasite virulence and increased individual susceptibility are being currently investigated.

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Toxoplasma gondii is an extremely ubiquitous parasite, infecting up to one third of the human population.1,2 Although disease caused by T. gondii is usually subclinical, it may be severe, especially in immunosuppressed subjects and in fetuses after vertical transmission.3–4

Congenital toxoplasmosis (CT) presents asymptptomatically in most newborns. However, retinochoroidal involvement is of concern, being seen in up to 85% of infected subjects before adulthood.4–6 Bilateral and macular disease may be responsible for significant morbidity in these patients,7–10 with CT being a leading cause of childhood low vision and blindness in many countries, including Brazil.11,12

Despite some evidence of higher prevalence and severity of CT in Brazil,12 large population-based studies have not been done. Reported prevalences vary from 3 to 20 cases per 10,000 live births, with highly variable rates of retinochoroidal involvement; as a rule, however, these figures
have been based on a relatively small number of infected subjects.\textsuperscript{13-17} The aim of the study reported was to investigate ocular involvement of newborns with CT who were identified by a large comprehensive neonatal screening program in the state of Minas Gerais (MG), in southeastern Brazil.

Materials and Methods

This study is part of a prospective investigation on neonatal screening for CT in the Brazilian state of MG. It is being carried out by a multidisciplinary research group (UFMG-Congenital Toxoplasmosis Brazilian Group) under the coordination of one of the authors (GMQ-A). The study protocol followed the precepts of the Declaration of Helsinki and was approved by the Ethics Committee at our institution (Ethics Committee at Universidade Federal de Minas Gerais).

From November 1, 2006, to May 31, 2007, 146,307 newborns underwent screening for CT using a capture enzyme-linked immunosorbent assay for anti-\textit{T. gondii} immunoglobulin (IgM) detection (TOXO IgM-Q-Preven, Symbiosis, Leme, Brazil). Blood samples were collected from 1560 public health care centers located in 853 cities within the state; these were then sent for analysis to the laboratory of Núcleo de Ações e Pesquisa em Apoio Diagnóstico in the capital of the state, Belo Horizonte.

Confirmative plasma/serum tests were run on cases with positive or undetermined screening results. These mothers and babies were tested for IgA (enzyme-linked immunosorbent assay) and IgG and IgM anti-\textit{T. gondii} (Enzyme-Linked Fluorometric Assay, ELFA-VIDAS, BioMérieux SA, Lyon, France). Babies also underwent a thorough pediatric examination, neuroimaging studies (cranial radiographs or transfontanel ultrasound; computer-assisted tomography in selected cases), hearing assessment, and ophthalmologic evaluation.

Cases of confirmed CT were defined as those infants with positive testing of the serum/plasma for IgM and/or IgA and IgG, and those with IgG associated with suggestive retinochoroidal lesions and positive IgM and IgG test results on their mothers. Each ophthalmologic evaluation of the babies was performed without sedation by 2 retina/uveitis specialists (DVV-S and DOMA) with the help of a trained nursing professional, according to a standardized protocol. After external inspection, pupils were dilated with 2 drops of tropicamide 0.5% and 1 drop of phenylephrine 2.5%. All newborns, after being placed on an appropriate bed, underwent indirect ophthalmoscopy with a 20-diopter lens, with careful immobilization of the body with a cloth sheet, of the head with the hands of the nurse, and after topical anesthesia and placement of a tailored lid speculum. Most examinations were recorded with a video charged-coupled device camera coupled to the ophthalmoscope. When possible, fundus photographs were also recorded; lesions were carefully drawn on an appropriate form (Fig 1A; available at http://aaojournal.org). In cases in which the fundus examination was limited because of media opacity, echographic evaluation of the posterior segment of the eye was performed. Biomicroscopy of the anterior segment with a slit-lamp was also performed in these cases, as well as in those with active retinochoroiditis, looking for signs of anterior segment inflammation.

All cases with confirmed or strongly suspected \textit{T. gondii} infection were started on classic treatment with sulfadiazine (100 mg/kg/d [in 2 divided doses]), pyrimethamine (1 mg/kg/d for the first 6 months followed by the same dose 3 times per week thereafter), and folic acid (7.5 mg 3 times per week) from the time of diagnosis until completing 1 year of treatment, with careful bi-monthly monitoring of the complete blood count. Cases of active retinochoroiditis in the macular area and of neurologic involvement with increased protein content in the cerebrospinal fluid also received oral prednisolone (1 mg/kg/d) in a tapering fashion.

After the first examination, follow-up visits were scheduled at 6 and 12 months of life and yearly thereafter, unless active retinochoroiditis lesions were detected. In this case, more frequent examinations were performed, with the intervals between examinations individualized for each case.

Information from pregnancy cards on which the assistant obstetrician had registered data from the mother and the fetus, along with results of serologic tests during pregnancy, were also collected.

Data were stored and analyzed by a statistical package (SPSS for Windows, Version 12, Chicago, IL), with frequency and descriptive statistics. Age at the first ophthalmologic examination was compared between infants with active and those with cicatricial retinochoroidal lesions using the Mann–Whitney \textit{U} test. A \textit{P} value \leq 0.05 was regarded as statistically significant.

Results

A total of 146,307 newborns were screened during the 6-month period (November 1, 2006 to May 31, 2007), corresponding to approximately 95% of the live births in the state of MG during those months. Of these screened babies, 235 had suspected CT and, along with their mothers, underwent confirmative serology. Infection was confirmed in 190 cases, corresponding to a prevalence of 1 in 770 liveborn infants.

Among the 45 cases that were not confirmed, 35 (77.8%) had the infection ruled out because anti-\textit{T. gondii} IgG antibodies decreased during the follow-up, ultimately reaching undetectable levels (passively transferred antibodies from their mothers). Of the remaining 10 cases, 9 could not be evaluated because of neonatal death (4 newborns), loss to follow-up (3 newborns), or refusal to participate (2 newborns whose parents opted for private care). The other had decreasing specific IgG antibodies, but these had not reached undetectable levels.

Of the 189 women who delivered infected newborns (one delivered dizygotic twins, both of whom were infected), only 11 (5.8%) had been treated during pregnancy, 10 of whom received spiramycin and 1 of whom was treated with sulfadiazine, pyrimethamine, and folic acid.

Of the 190 patients with confirmed CT, 103 were male (54.2%) and 87 were female (45.8%). Pediatric examination results were normal (absence of hepatomegaly, splenomegaly, microphthalmia, microcephaly, and convulsions) in 89 patients (46.8%) at a mean age of 57.3 days. In 9 patients (4.7%), there had been clinical suspicion of a congenital infection soon after birth. Neuroimaging revealed intracranial calcifications in 39 patients (20.5%). Microcephaly was observed in 53.0% (10 patients) and hydrocephalus in 6.3% (12 patients).

Standardized ophthalmologic examination was performed at a mean age of 55.6±16.6 days (median: 56 days) in 178 of the 190 patients (93.7%). The remaining 12 infected newborns (6.3%) were examined by local ophthalmologists in their home towns and are not included in this report. Retinochoroidal lesions consistent with CT were found in 142 infants (79.8%), being bilateral in 63.5% (113 cases). The macula was affected in 165 eyes (46.3%) of 111 patients (62.4%) (Fig 1A–D; available at http://aaojournal.org). Involvement of the foveal center (which posed an ominous visual prognosis) was observed in 120 eyes (33.7%) of 88 infants (49.4%). Isolated peripheral lesions were seen in 51 eyes (14.3%) (Fig 1E; available at http://aaojournal.org) and were bilateral in 9 patients (5.1%). Fundus examination was not possible in 7 eyes.
(2.0%) of 5 infants (2.8%) because of a cataract (2 eyes of 2 patients), pupillary seclusion (4 eyes of 3 patients), a dense vitreous opacity, either inflammatory (7 eyes, 4 patients) or hemorrhagic (3 eyes, 2 patients), or a combination of these factors. Microphthalmia was seen in 14 eyes (3.8%) of 8 patients (4.5%). In 11 eyes (3.1%) of 7 patients (3.9%), retinal detachment was observed by indirect ophthalmoscopy (Fig 1F; available at http://aaojournal.org) or echographic examination (Fig 1G; available at http://aaojournal.org; Table 1).

Active retinochoroidal lesions were seen in 142 eyes (39.9%) of 85 patients (47.8%) and were associated with retinal scars in 66 eyes (18.5%) of 53 patients (29.8%). These exudative retinochoroidal lesions involved the macula in 75 eyes (21.1%) (Fig 2A, B), with associated features being retinal vascular sheathing in 44 eyes (12.4%) (Fig 2C) and vitreous or retinal hemorrhage in 11 eyes (3.1%) (Fig 2D). Vitreous opacities were relatively rare (26 eyes, 7.3%) and mild (1–2+, ++) because babies with active lesions were examined at an age comparable to that of babies with only retinochoroidal scars (median of 58 and 57 days, respectively; P = 0.393).

Ophthalmoscopic examination was decisive in 28 infants (15.7%) who had suspected CT at the neonatal screening (positive or undetermined IgM) but who did not have a confirmative serology at that time (positive IgG but negative IgA and IgM, along with maternal seroconversion). These infants, indirect ophthalmoscopy disclosed retinochoroidal lesion(s) suggestive of toxoplasmosis, allowing confirmation that they had, in fact, been congenitally infected.

Discussion

The prevalence of CT (1/770 live births) is higher in the present study than in most other reported series in the rest of the world19–23 and in Brazil.13–17 These few published reports on the prevalence of CT in Brazil13–17 have involved limited geographic areas and had selection and referral bias that may limit interpretation and extrapolation of the resultant data. In contrast, our study had coverage of approximately 95% of liveborn infants in a large geographic area: a Brazilian state with approximately 20 million people distributed over 853 cities in an area of 586,528 square km. Furthermore, the use of a well-established public network for neonatal screening allowed for prompt screening, referral, treatment, and follow-up of both children and their mothers, with relatively few losses (3.8%, representing 9/235 suspected of being infected).

In agreement with other reports on neonatal14,19,23 and prenatal24–26 screening, but not on referred patients (who are theoretically more severely affected), only 4.7% of newborns in our study had a suspected congenital infection, and a large proportion (46.8%) was completely asymptomatic, even after careful pediatric examination.

Retinochoroidal lesions consistent with toxoplasmosis were present in approximately 80% of our cases; these infants underwent ophthalmologic examination at an average of 55.6±16.6 days (median: 56 days) after birth. The prevalence rates of retinochoroidal lesions at early evaluation in the literature are significantly lower, ranging between 4% and 24%.5,19,20,23,26 As the children grow up, months or even years later, the reported prevalence rates increase to 30% to 90%5,6,8,9,27–29 because of either occurrence of new lesions or better visualization of the retina. Ophthalmoscopic examination was decisive for the early diagnosis of CT in 15.7% of newborns in our study, who had negative confirmatory serology for IgM and IgA but who were positive for IgG anti-T. gondii. These newborns had retinochoroidal lesions that were consistent with CT, allowing confirmation of the diagnosis by the end of the first year of life, before completion of the IgG kinetics study. This suggests that a careful ophthalmologic examination may in fact help, not only in evaluating the extent of ocular

### Ophthalmologic Findings

<table>
<thead>
<tr>
<th>Ophthalmologic Findings</th>
<th>No. Eyes (%)</th>
<th>95% CI</th>
<th>No. Patients (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinochoriditis</td>
<td>255 (71.6%)</td>
<td>66.9%–76.3%</td>
<td>142 (79.8%)</td>
<td>73.9%–85.7%</td>
</tr>
<tr>
<td>Unilateral lesions</td>
<td>29 (16.3%)</td>
<td>10.9%–21.7%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral lesions</td>
<td>113 (63.5%)</td>
<td>56.4%–70.6%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active lesions</td>
<td>142 (39.9%)</td>
<td>34.8%–45.0%</td>
<td>85 (47.8%)</td>
<td>40.4%–55.1%</td>
</tr>
<tr>
<td>Inactive lesions</td>
<td>173 (48.6%)</td>
<td>43.4%–53.8%</td>
<td>108 (60.7%)</td>
<td>53.5%–67.9%</td>
</tr>
<tr>
<td>Macular lesions</td>
<td>165 (46.4%)</td>
<td>41.2%–51.5%</td>
<td>111 (62.4%)</td>
<td>55.2%–69.5%</td>
</tr>
<tr>
<td>Foveal lesions†</td>
<td>120 (33.7%)</td>
<td>28.8%–38.6%</td>
<td>88 (49.4%)</td>
<td>42.1%–56.8%</td>
</tr>
<tr>
<td>Isolated peripheral lesions†</td>
<td>51 (14.3%)</td>
<td>10.7%–18.0%</td>
<td>42 (23.6%)</td>
<td>17.4%–29.8%</td>
</tr>
<tr>
<td>Cataract</td>
<td>2 (0.6%)</td>
<td>0.0%–1.3%</td>
<td>2 (1.1%)</td>
<td>0.0%–2.7%</td>
</tr>
<tr>
<td>Microphthalmia</td>
<td>14 (3.9%)</td>
<td>1.9%–6.0%</td>
<td>8 (4.5%)</td>
<td>1.5%–7.5%</td>
</tr>
<tr>
<td>Vascular sheathing</td>
<td>44 (12.4%)</td>
<td>8.9%–15.8%</td>
<td>32 (18.0%)</td>
<td>12.3%–23.6%</td>
</tr>
<tr>
<td>Retinochoroidal lesions</td>
<td>111 (60.7%)</td>
<td>53.5%–67.9%</td>
<td>108 (60.7%)</td>
<td>53.5%–67.9%</td>
</tr>
<tr>
<td>Vitreous opacities mild</td>
<td>26 (7.3%)</td>
<td>4.6%–10.0%</td>
<td>16 (9.0%)</td>
<td>4.8%–13.2%</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>11 (3.1%)</td>
<td>1.3%–4.9%</td>
<td>7 (3.9%)</td>
<td>1.1%–6.8%</td>
</tr>
</tbody>
</table>

CI = confidence interval.

*Examination performed at a mean of 55.6±16.6 days after birth (median: 56 days).
†Involving the foveal center.
‡In zone 2 or 3 of Holland’s scheme,18 with no lesion in zone 1.
involvement in CT but also in making an early diagnosis in uncertain cases.

Active lesions also were frequently observed in 39.9% of eyes (47.8% of patients). This has been described only rarely in other series, in approximately 4% of newborns who were examined early.\textsuperscript{8,23,30} Notably, the vitreous body was usually clear, even in the presence of relatively large exudative retinochoroidal lesions (Fig 2A, B). Involvement of the macula in our series (46.3% of eyes, and 62.4% of patients) is similar to that of other reports,\textsuperscript{8,23,31} and this macular tropism (considering the relatively small area of the macula) is thought to be due to anatomic, developmental, and immunologic factors.\textsuperscript{7,32} We also reported involvement of the foveal center, which is more apt to be associated with significant visual impairment, especially if the involvement is bilateral.\textsuperscript{33} Unilateral and bilateral foveal involvement were seen in 33.7% (120) and 18.0% (32) of newborns, respectively, in our study, but further follow-up should provide more information as to its true functional impact, because relatively large central lesions in CT may be associated with reasonably good visual acuity.\textsuperscript{9}

The high prevalence of retinochoroidal lesions that we found, many of which were active, may have been influenced by the neonatal screening strategy, as well as by the lack of prenatal treatment. Only 10 of the women (5.7%) had received spiramycin during their pregnancy, which did

**Figure 2.** Active retinochoroiditis in newborns with congenital toxoplasmosis (CT). A, Fundus photograph of the right eye of a 42-day-old female with an active macular lesion and adjacent retinal edema and pigment abnormalities. B, Fundus photograph of the right eye of a 30-day-old male showing an active macular lesion with circumjacent retinal edema. C, Fundus photograph of the left eye of a 30-day-old male showing vascular sheathing (phlebitis) superiorly in an area with multiple punctate exudative lesions. D, Fundus photograph of the left eye of a 48-day-old female with vitreous hemorrhage and an exudative lesion inferonasally.
T. gondii demonstrated to be “seeded” with lesions, in either normal-appearing retina (which has been the congenital form, and detection of new retinochoroidal and their mothers is currently under way, and it is hoped that study of the immune response in the children in our cohort DQ3 has been reported. 37 An association between ocular results. Correlation between disease outcome in CT and HLA- genotypes have been observed infecting intermediate hosts, as well as humans with ocular disease.34–36 This is an issue that is currently being addressed by the UFMG Congenital Toxoplasmosis Brazilian Group, because all babies had blood collected at the initial evaluation, which was inoculated into mice for isolation, phenotyping, and genotyping of T. gondii. Preliminary results show that 27 infants had a positive mouse inoculation (Carneiro ACAV, Andrade GMQ, Januário JN, et al. Isolation and characterization of T. gondii from newborns with CT identified by the neonatal screening program in MG, Brazil. Poster presented at: Toxoplasma Centennial Congress, September 23, 2008; Búzios), but further results from our parasitology team should better elucidate these findings.

Individual susceptibility also likely influenced our results. Correlation between disease outcome in CT and HLA-DQ3 has been reported. 37 An association between ocular toxoplasmosis and a polymorphism of interleukin-10 gene at position −1082 was also recently demonstrated in adults attending a university uveitis referral center in Brazil. 38 A study of the immune response in the children in our cohort and their mothers is currently under way, and it is hoped that this study will answer some questions.

Ocular toxoplasmosis is a recurrent disease, especially the congenital form, and detection of new retinochoroidal lesions, in either normal-appearing retina (which has been demonstrated to be “seeded” with T. gondii cysts 39–42) or the margins of preexisting scars (satellite lesions), may be observed in up to 66% of patients. 1,10,43,44 Phan et al 28 recently demonstrated that new lesions occurred in only 31% of infected children who were treated during their first year of life, after a mean follow-up of 11 years. In most of their patients with new lesions (79.4%), lesions were peripherally located, in contrast with 44.1% of their patients with central lesions; 38.2% had new lesions in both eyes. 28 The new retinochoroidal lesions were detected at 2 peaks: one around entry to school (ages 5–7.5 years) and another in adolescence (15–20 years of age); 41% of the patients were 10 years old or older when they developed new lesions. 28 Long-term longitudinal follow-up of our cohort will allow monitoring of recurrences and new lesions.

All children in our cohort received triple therapy with sulfadiazine, pyrimethamine, and folic acid during the first year of life (and those with active macular lesions also received a tapering course of oral corticosteroids). Despite the concerns regarding their negligible activity against T. gondii tissue cysts, and on their erratic pharmacokinetics in newborns, antiparasitic drugs may be beneficial in this setting of an immature or more tolerant immune system. The finding of a significant number of newborns with active retinochoroidal lesions, some of which were in the macula, as well as the preliminary data on parasitemia, reinforces this possibility.

**Study Limitations**

A neonatal screening strategy using detection of IgM may underestimate the prevalence of CT by missing fetal losses and perinatal/early neonatal deaths (which are rare), as well as by false-negative cases, which may be reflected by the smaller number of newborns who were infected in the first gestational trimester 45,46 (and who may ultimately have more severe disease). However, neonatal screening offers the advantages of lower cost and relative simplicity, and thereby allows the study of large samples 3,46 such as reported in this article.

Examination under sedation would have been desirable, because it would have allowed more thorough evaluation of the retina and prevented overlooking of peripheral lesions that may be detected later in life, at which time they may be considered “new.” 23,28,29 We managed to attenuate this potential problem by standardizing the ophthalmologic examination, which was performed by specialists experienced in ocular toxoplasmosis, which accounts for approximately 75% of cases of posterior uveitis in our service. 47 In addition, the use of indirect ophthalmoscopy with a lid speculum allowed careful inspection of the retina. Lesions located in the far periphery of the retina might have been missed, but this would have underestimated the rate of ocular involvement, which was already found to be high. Furthermore, these are early results on the first ophthalmologic examination, and further data will be available because the cohort is to be followed in the long-term.

In conclusion, we found a high prevalence of CT (1/770) in a large population-based cohort of newborns who had undergone neonatal screening in Brazil, with high rates of early retinochoroidal involvement (~80%), often with active lesions (~50%). Whether this may be associated with more virulent parasites or with individual susceptibility is now being investigated.

**References**

Footnotes and Financial Disclosures

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Figure 1. Fundus drawing, photographs, and echography of infants with congenital toxoplasmosis (CT). A, Schematic drawing of the fundus of the left eye of a 72-day-old female with a macular scar and 2 additional pigmented lesions in the periphery. B, Fundus photograph of the same eye showing the macular scar. C, Fundus photograph of the right eye of a 41-day-old male showing a pigmented macular scar, with adjacent (circumferential) retinal pigment epithelium atrophy and a delicate whitish band connecting the scar to the optic disc. D, Fundus photograph of the right eye of a 42-day-old male with an atrophic macular scar and a vitreous opacity surrounding the disc. E, Fundus photograph of the left eye of a 46-day-old male showing an isolated pigmented scar in the superior periphery. F, Fundus photograph of the left eye of a 108-day-old male with extensive retinochoroidal scarring and a tractional retinal detachment. G, B-scan of the right eye of a female infant with a cone-shaped retinal detachment, as well as decreased axial length associated with microphthalmia.