Real-Time PCR as a Prognostic Tool for Human Congenital Toxoplasmosis

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Real-time PCR (qPCR) was positive in 72/150 (48%) blood samples of newborns with congenital toxoplasmosis. Among infants with active retinochoroiditis, 68% had positive qPCR results, while positivity was 29% (P = 0.009) in the absence of ocular involvement. Positive qPCR was associated with the presence of retinochoroidal lesions, with an odds ratio of 2.8.

Toxoplasma gondii is responsible for significant clinical morbidity following congenital infection in humans. Consequences of fetal infection may range from subclinical infection at birth to severe neurological abnormalities and retinochoroiditis. Retinochoroidal involvement is particularly of concern, being seen in up to 85% of infected subjects before adulthood (1, 2). New lesions or recurrences may occur unpredictably and at any time long after birth (3). T. gondii causes more severe ocular disease in congenitally infected children in Brazil than in Europe, with marked differences in frequency, size, and multiplicity of retinochoroidal lesions (4). A recent population-based study involving the entire state of Minas Gerais (Brazil) revealed one case of congenital toxoplasmosis in every 770 live births (1.3/1,000), with 79.8% of infected newborns displaying retinochoroidal lesions in at least one eye (5). Several factors can be related to the severity of congenital toxoplasmosis, including parasite strain and load, host genetic variability, and immune response.

The aim of this study was to identify and quantify T. gondii DNA by real-time PCR (qPCR) in peripheral blood of newborns with congenital toxoplasmosis, also analyzing the results in the light of ocular manifestations of the disease.

This study is part of a prospective investigation on neonatal screening for congenital toxoplasmosis conducted by a multidisciplinary research group (UFMG Congenital Toxoplasmosis Brazilian Group) in the Minas Gerais state, southeastern Brazil. A total of 146,307 children were tested for anti-T. gondii IgM antibodies, according to previous studies on neonatal screening for toxoplasmosis (6, 7), in dried blood samples on filter paper (Toxo IgM kit, Q-Preven; Symbiosis, Leme, Brazil) (5, 8). Subsequent confirmative serologic tests (IgG, IgA, and IgM Elfa-Vidas; bio-Mérieux SA, Lyon, France) were performed in 220 infants with positive or undetermined screening results in a reference center in Belo Horizonte, the capital of Minas Gerais. Out of these 220 infants, 190 tested positive by confirmative tests and for persistence of anti-T. gondii IgM antibodies in serum at the age of 12 months. Ophthalmologic examinations were performed in these children according to the method described previously (5). The protocols used in this study were approved by the local Human Research Ethics Committee (COEP-UFMG, protocol 298/06).

Peripheral blood samples from 150 children diagnosed with congenital toxoplasmosis were collected during confirmatory tests, when children had an average age of 55.8 ± 15.8 days old. These samples were frozen (−20°C), and DNA was extracted from 300 μl of blood using the Wizard genomic DNA purification kit (A7280; Promega, Madison, WI, USA) according to the manufacturer’s instructions. For DNA quantification, a homogeneous solution was prepared with 1 × 10⁶ tachyzoites (RH strain) in 1 ml of donor blood (with negative serology and PCR for T. gondii). This suspension was frozen (−20°C), and DNA was extracted as a clinical sample. Serial 10-fold dilutions of this DNA were made ranging from 6 × 10² to 6 × 10⁻¹ parasites/μl to establish a standard curve of parasites. Parasite quantification for each blood sample was performed in duplicate from independent experiments, and values were expressed as the number of T. gondii organisms per milliliter. PCR was performed on an ABI Prism 7500 DNA sequence detection system using SYBR Green PCR Master Mix (PE Applied Biosystems, Foster City, CA, USA), targeting the T. gondii 529-bp repetitive genomic sequence (rep529) (9). The reaction mixture (10 μl) included 2 μM (each) primer (10) and 100 ng of DNA sample. β-Globin qPCR was performed in parallel for each sample as described previously (11) in order to confirm DNA integrity and to verify qPCR inhibitors. Samples were incubated at 95°C for 10 min and then submitted to 40 cycles of 95°C for 15 s and 60°C for 1 min, when fluorescence data were collected. Reproducibility was considered good (83.2%) when 20% of samples were tested again in an independent experiment.

Seventy-two of 150 samples (48%) tested positive on rep529-qPCR. Of infants with any retinochoroidal involvement, 54% (61/113) were qPCR positive, while positivity was only 29% (11/37) in those who had no retinochoroidal lesions (P = 0.013). In addition, among newborns with active lesions, 68% (13/19) were qPCR positive, in contrast to 29% of those without any retinochoroidal lesions (P = 0.009) (Fig. 1). This high qPCR positivity suggests that parasitemia may be associated with lesion activity, as...
### FIG 1 Positivity rates of rep529-qPCR for *Toxoplasma gondii* in congenitally infected infants without ocular involvement (no lesion [NL]), with active retinochoroidal lesions (ARL), with concomitant active and cicatricial retinochoroidal lesions (ACRL), and with cicatricial retinochoroidal lesions (CRL). *, $P < 0.05$; **, $P < 0.01$ (Fisher’s exact test).

previously reported (12). Differences in qPCR positivity were also observed between children with retinochoroidal lesions and those with only retinochoroidal scars (11/37, 29%, versus 21/37, 57%, respectively; $P = 0.034$). Positive qPCR results in patients with toxoplasmonic retinochoroidal scars have already been observed (12, 13), suggesting subclinical parasitemia. Ongoing parasitemia in such patients might help to explain ocular recurrences. In this study, odds ratios further corroborated the association between retinochoroidal involvement and qPCR positivity, so that infants with ocular involvement had 2.8-fold greater odds of presenting positive qPCR results. Considering only infants with active retinochoriditis, the odds ratio was 5.1 (Table 1).

A very low parasite load was observed in peripheral blood of the infants with congenital toxoplasmosis (mean, 0.133 parasites/ml, ranging from 0.005 to 6.140 parasites/ml). The detection limit of *T. gondii* qPCR targeting rep529 was approximately 1/30 to 1/50 of 1 parasite genome (14). Forty-nine samples (68%) presented less than 0.5 parasite/ml. This was an expected result, considering the small amount of blood analyzed (300 μl), the transience of parasitemia, and the low parasite number in clinical samples (15, 16). Parasite load was also not associated ($P = 0.677$) with age in our study (Spearman’s correlation).

Parasite load on blood was not correlated with ocular involvement in these infants, so that there was no difference between the parasitemia medians in those with and those without retinochoroidal lesions ($P = 0.413$). It is important to consider that such lesions could be associated with exacerbated immune responses that control parasitic burden (17). Although rep529 has been regarded as a highly conserved nucleotide sequence and copy number (18), a large variation in its copy number, as well as in that of the B1 gene, was recently described in different strains of *T. gondii* (19). This implies technical limitations in quantifying parasites. Attempts to genotype *T. gondii* in DNA extracted from blood of infants with significant parasitemia are in progress.

We conclude that *T. gondii* qPCR positivity is higher in infants with ocular involvement, particularly in those with active retinochoriditis. The parasite load in peripheral blood of congenitally infected infants within the first 2 months of life is low, with no association between degree of parasitemia and the presence of retinochoroidal lesions.

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### REFERENCES


### TABLE 1 Association between qPCR-rep529 and clinical signals presented by the infants with confirmed congenital toxoplasmosis

<table>
<thead>
<tr>
<th>Ocular involvement (no. positive/total no. of infants submitted to qPCR)</th>
<th>qPCR-rep529*</th>
<th>OR</th>
<th>CI (95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ocular injury (113/150)</td>
<td>2.773</td>
<td>1.250–6.148</td>
<td>0.013</td>
<td></td>
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<tr>
<td>Active retinochoroiditis lesion (19/150)</td>
<td>5.121</td>
<td>1.547–16.96</td>
<td>0.009</td>
<td></td>
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<tr>
<td>Active and cicatricial retinochoriditis lesion (57/150)</td>
<td>2.127</td>
<td>0.885–5.109</td>
<td>0.131</td>
<td></td>
</tr>
</tbody>
</table>

* CI, confidence interval; OR, odds ratio.


