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Evaluation of serological and molecular tests used to identify *Toxoplasma gondii* infection in pregnant women attended in a public health service in São Paulo state, Brazil

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ABSTRACT

Toxoplasmosis during pregnancy can have severe consequences. The use of sensitive and specific serological and molecular methods is extremely important for the correct diagnosis of the disease. We compared the ELISA and ELFA serological methods, conventional PCR (cPCR), Nested PCR and quantitative PCR (qPCR) in the diagnosis of *Toxoplasma gondii* infection in pregnant women without clinical suspicion of toxoplasmosis (G1 = 94) and with clinical suspicion of toxoplasmosis (G2 = 53). The results were compared using the Kappa index, and the sensitivity, specificity, positive predictive value and negative predictive value were calculated. The results of the serological methods showed concordance between the ELISA and ELFA methods even though ELFA identified more positive cases than ELISA. Molecular methods were discrepant with cPCR using B22/23 primers having greater sensitivity and lower specificity compared to the other molecular methods.

1. Introduction

Toxoplasmosis, an infection caused by the obligate intracellular parasite *Toxoplasma gondii*, affects mammals and birds worldwide (Dubey, 2008; Robert-Gangneux and Dardé, 2012). In humans, the disease can be severe especially during pregnancy, as the parasite can cross the placental barrier and infect the fetus with serious and even fatal consequences (Robert-Gangneux and Dardé, 2012).

The prevalence of gestational toxoplasmosis is high in many regions of Brazil (Câmara et al., 2015; Gontijo et al., 2015; Lopes-Mori et al., 2013; Moura et al., 2013; Porto et al., 2008; Rebouças et al., 2011; Spalding et al., 2005; Sroka et al., 2010) including the northwestern region of São Paulo State (64.4% – Mattos et al., 2011a) (Fig. 1). Early diagnosis and anti-parasite treatment can reduce the severity of the fetal disease, but complications, such as microcephaly, hydrocephalus, cerebral calcifications, retinocoroidites, and mental retardation, can occur if cases remain untreated (Bittencourt et al., 2012; Fochi et al., 2015; McLeod et al., 2012, 2014; Rodrigues et al., 2009; Sroka et al., 2010).

The diagnosis of toxoplasmosis is challenging because the clinical manifestations are often nonspecific. Thus, the use of sensitive serological and molecular tests is extremely important to identify the disease early (Bichara et al., 2012; Lago et al., 2014; McLeod, 2014; Robert-Gangneux and Dardé, 2012). The aim of this study was to compare the serological and molecular methods used to diagnose

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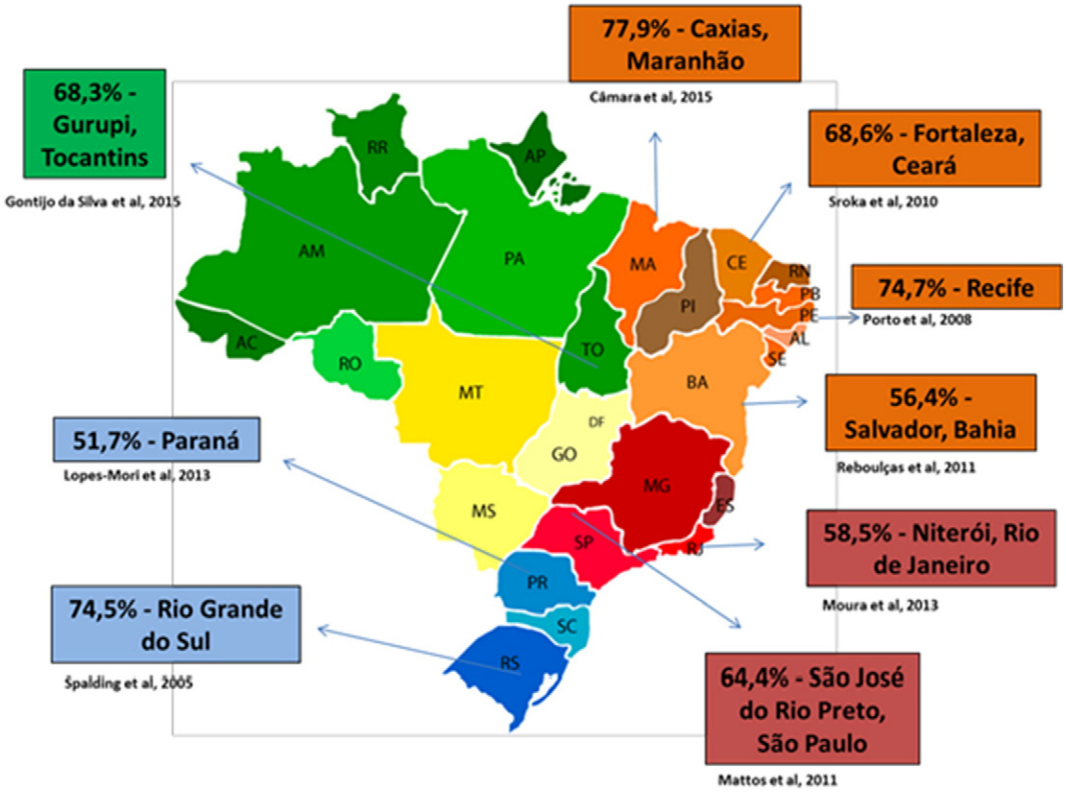

Fig. 1. Seroprevalence of *T. gondii* infection in different regions of Brazil.

toxoplasmosis in pregnant women treated at a teaching hospital in the northwestern region of São Paulo State.

2. Material and methods

2.1. Ethics statement

This study was approved by the Ethics Committee of the Medicine School in São José do Rio Preto (FAMERP-CAAE 32259714.8.0000.5415).

2.2. Patients and clinical samples

This is a retrospective study that evaluated pregnant women treated at the High-risk Antenatal Care and Fetal Medicine Outpatient Clinic of the Fundação Faculdade Regional de Medicina, Hospital de Base (FUNFARME), São José do Rio Preto, São Paulo State, Brazil. The pregnant women were characterized in 2 groups: G1 – Pregnant women without clinical suspicion of toxoplasmosis, who had been referred to the clinic due to other complications ($n = 94$) and G2 – high-risk pregnant women with suspicion of toxoplasmosis and/or positive for IgM anti-*T. gondii* antibodies at some time during pregnancy ($n = 53$).

Peripheral blood was collected from all subjects in a dry tube for serological analysis and in a tube with ethylenediaminetetraacetic acid (EDTA) for DNA extraction and molecular tests. Serological and molecular analyses were performed in the Immunogenetics Laboratory, Molecular Biology Department, FAMERP, São José do Rio Preto, São Paulo, Brazil. Of the 53 pregnant women in G2, 50 were also submitted to amniocentesis to investigate *T. gondii* in amniotic fluid. Amniotic fluid was sent to the reference laboratory of the São Paulo State Health Department (Dr. Vera Pereira-Chioccola) for conventional polymerase chain reaction (cPCR). All pregnant women are routinely screened in the High-risk Antenatal Care and Fetal Medicine Outpatient Clinic for TORSCH (Toxoplasmosis, Rubella, Syphilis, Cytomegalovirus, Hepatitis and HIV) (Gonçalves et al., 2010).

2.3. Serological diagnosis

The presence of anti-*T. gondii* was confirmed by enzyme linked immunosorbent assay (ELISA, DiaSorin, Italy) using the ETI-TOXOK-A reverse plus kit for IgA, ETI-TOXOK-M reverse plus kit for IgM and ETI-TOXOK-G plus kit for IgG, and enzyme linked fluorescent assay (ELFA, Biomerieux, France) using the Vidas®Toxo IgM kit for IgM, Vidas®Toxo IgG II kit for IgG and Vidas®Toxo IgG avidity kit for IgG avidity. The detection of IgA and IgM antibodies was performed by capture ELISA. All samples that were positive for IgG and IgM anti-*T. gondii* antibodies by ELFA were also assayed using the ELFA IgG avidity test. Low-avidity antibodies (<25%) are indicative of recent infection. ELFA was performed in automated equipment (Mini Vidas, Biomerieux, France). Both tests were performed according to manufacturer's instructions. Samples were considered positive for IgG antibodies by ELISA when the concentration was >15 IU/mL and negative when the IgG concentration was ≤15 IU/mL. Results were considered positive for IgA antibodies by ELISA when the IgA concentration was >5 AU/mL, and negative when the concentration was ≤5 AU/mL. For the IgM ELISA test, the absorbance values of the samples were compared with the average cut-off point; samples were considered positive when the absorbance values were higher than or equal to the cut-off point with the remaining samples being considered negative. By ELFA, samples were considered positive for IgG antibodies when the value was ≥8 IU/mL, indeterminate from ≥4 to ≤8 IU/mL and negative when <4 IU/mL. For IgM antibodies, ELFA results were positive when the reagent index was ≥0.65 IU/mL, indeterminate from <0.65 to ≥0.55 IU/mL and negative <0.55 IU/mL. Results demonstrated low IgG avidity when the IgG antibody concentration was <0.200, intermediate avidity was between 0.200 and 0.300 and high avidity when the IgG antibody concentration was ≥0.300.

2.4. Molecular diagnosis

2.4.1. Genomic DNA extraction

The genomic DNA was extracted from 5 mL of peripheral blood collected in EDTA using a commercial kit (Qiamp DNA blood mini kit,