Congenital toxoplasmosis in South American children

Toxoplasmose congênita em crianças sul-americanas

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ABSTRACT

Aims: To review the present knowledge about congenital toxoplasmosis in South America and to advance some hypothesis for future research. **Source of data:** Medline and Scielo database search for papers reporting clinical characteristics of cohorts of children in South America and comparative studies between South America and other continents. **Summary of findings:** Systematic analysis of primary data obtained during screening programs showed that the risk of ocular lesions in congenital toxoplasmosis was much higher in the South American cohorts (47%; 18/38) than in Europe (14%, 79/550). The crude risk of intracranial lesions was much higher in the cohorts from South America (53%, 20/38) than those from Europe (9%, 49/550). In a Colombian cohort it was found 11% of mortality. Additionally, a comparative prospective cohort of congenitally infected children from Brazil and Europe found that in Brazilian children eye lesions were larger, more numerous and more likely to affect the area of the retina responsible for central vision that their counterpart in Europe. The presence of *Toxoplasma* strains genetically different to those found in North America and Europe could explain the higher severity of congenital toxoplasmosis in South America. **Conclusions:** Congenital toxoplasmosis in South America seems to be more frequent and infected children are more symptomatic than in Europe and in North America. Research for new drugs and candidate vaccines are a priority to improve indicators of health in children of South America.

Keywords: *Toxoplasma gondii*/genetics; *Toxoplasma gondii*/pathogenicity; *Toxoplasma gondii*/classification; POLYMORPHISM, GENETIC; GENETIC PREDISPOSITION TO DISEASE; TOXOPLASMOSIS, CONGENITAL/epidemiology; TOXOPLASMOSIS, CONGENITAL/complications; TOXOPLASMOSIS, CONGENITAL/virulence; TOXOPLASMOSIS, CONGENITAL/mortality; TOXOPLASMOSIS, OCULAR; SIGNS AND SYMPTOMS; SOUTH AMERICA; COLOMBIA; BRAZIL

RESUMO

Objetivos: revisar o conhecimento atual sobre toxoplasmose congênita na América do Sul e traçar algumas hipóteses para futura pesquisa. **Fonte de dados:** busca nas bases de dados Pubmed e Scielo por artigos sobre características clínicas de coortes de crianças com toxoplasmose congênita na América do Sul e estudos com parativos entre América do Sul e outros continentes. **Síntese dos dados:** uma análise sistemática de dados primários obtidos durante programas de triagem mostrou que o risco de lesões oculares foi muito maior na coorte de crianças da América do Sul (47%, 18/38) do que nas européias (14%, 79/550). O risco bruto de lesões intracranianas foi muito maior na coortes da América do Sul (53%, 20/38) do que nas da Europa (9%, 49/550). Em uma coorte colombiana constatou-se 11% de mortalidade. Adicionalmente, uma coorte prospectiva, que comparou crianças com toxoplasmose congênita do Brasil e da Europa, mostrou que nas crianças brasileiras as lesões oculares foram maiores, mais numerosas e com maior probabilidade de atingir o polo posterior da retina do que nas européias. A presença de cepas de *Toxoplasma gondii* diferentes das da Europa e dos Estados Unidos pode explicar a maior gravidade da toxoplasmose congênita na América do Sul. **Conclusões:** a toxoplasmosis congênita na América do Sul parece ser mais frequente e as crianças infectadas são mais sintomáticas do que na Europa e na América do Norte. A pesquisa sobre novas drogas e vacinas deve ser prioritária, para melhorar os indicadores de saúde nas crianças da América do Sul.

Descritores: *Toxoplasma gondii*/genética; *Toxoplasma gondii*/patogenicidade; *Toxoplasma gondii*/classificação; POLIMORFISMO GENÉTICO; PREDISPOSIÇÃO GENÉTICA PARA DOENÇA; TOXOPLASMOSE CONGÊNITA/epidemiologia; TOXOPLASMOSE CONGÊNITA/complicações; TOXOPLASMOSE CONGÊNITA/virulência; TOXOPLASMOSE CONGÊNITA/mortalidade; TOXOPLASMOSE OCULAR; SINAIS E SINTOMAS; AMÉRICA DO SUL; COLÔMBIA; BRASIL

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INTRODUCTION

Congenital toxoplasmosis occurs as a consequence of transplacental transmission of the protozoan parasite *Toxoplasma gondii* after a primary infection during pregnancy.[1] Placental transmission is less frequent when infection is acquired before the tenth week of pregnancy and is very rare when infection is acquired before conception. In immunocompetent mothers who have been infected by *Toxoplasma gondii* before conception, the immune mechanisms prevent transmission of the infection to their fetuses, although there are exceptional reports of transmission during chronic infection (six in total).[2] Public health programs for diagnosis and treatment of toxoplasmosis during pregnancy were established in France and in other European countries to detect and to treat primary infection. Another strategy, also first established in some European countries, is the newborn screening program.[3] In other continents the neonatal strategy was first evaluated in the region of New England in the United States,[4] then in Brazil,[5-8] and more recently in Colombia.[9,10] As a consequence of the implementation of these programs in different continents, it was possible to obtain comparable clinical data of congenital toxoplasmosis, which resulted in an unexpected difference in severity between South American and European cohorts. The aim of this review is to summarize the new knowledge afforded by theses studies and to advance some hypothesis for future research work.

REVIEW OF PUBLICATIONS

A review of the literature was conducted using the databases Medline and Scielo, covering the years 1970-2009. MESH terms used were '*Toxoplasma*', 'congenital toxoplasmosis', 'epidemiology' and 'South America'. Articles in English and Spanish were included. Other articles were identified from the reference lists of articles found by the PubMed search. Articles were analyzed depending of the method of recruitment. Hence, analysis is made differently for children referred to the tertiary level hospital and for those detected during neonatal and prenatal screening.

Congenital toxoplasmosis in cohorts of referred South American children

All South American reports have trends for a high prevalence of symptomatic children in referred patients.[11-14] A clinical consultation for congenital toxoplasmosis was open in September 2000 at the University of Quindio, located in the Center-Western region of Colombia (534,552 inhabitants, 7,734 liveborn infants in 2006).[11] As prenatal program is recent in the region of Quindio and there are multiple administrative barriers for prenatal treatment, a natural history of untreated infection was obtained for a significant number of cases. During this time, 25 congenitally infected children were referred to the consultation from Quindio and other regions in Colombia, for confirmation of diagnosis and treatment. At the end of the first year of life, 57% of cases in this cohort had ocular involvement and 64% of cases had neurological involvement.[11] Other reports in South America described clinical symptoms in referred cases to tertiary level hospitals in similar percentage of ocular and neurological involvement. In Chile, in 11 of 15 (73.3%) symptomatic children, ocular manifestation was present.[12] In Brazil, in 43 children, 77% had neuroradiological alterations, and retinochoroiditis was found in 95% of cases.[13]

Frequency and clinical severity of congenital toxoplasmosis in cohorts detected during neonatal and prenatal screening in South America compared to other geographical regions

The frequency of congenital toxoplasmosis as determined by newborn screening programs in South America varies in rates of 0.6% reported in Colombia to 0.01% in some regions of Brazil (Table 1).[5-11] This frequency is at least 10 times higher in most of the studies performed in South America than in those reported in Europe and United States.[15,16]

The frequency of primary infection during pregnancy in Colombia has been established by mathematical models in a representative sample in a national study in 1980, then in the Quindio region in 1993, and by a new analysis in this same region in 2002.[17] These analyses showed that age related change in prevalence was the same at the national and regional levels and that it were unchanged between 1993 and 2002. This revealed that women in the group of 10-15 years of age had an annual risk of 1.5% whereas the risk in women of 35-40 years was 0.4%. This information is now more relevant because teenage pregnancy has importantly risen during the last years in Colombia and represent near of 20% of new pregnant women (13% in 1990, 17% in 1995 and 19% in 2000).[18] As a result of that study, prenatal programs were initiated in the public and private health services.[11] In addition, two pilot programs of newborn screening were performed in 2003[9] and in 2004.[10] In an analysis of 17 children congenitally infected and detected in prenatal and neonatal screening programmes, 41%

Table 1. Prevalence of congenital toxoplasmosis in South American newborn screening programs.

Place of the study	Number of screened newborns	Assay	Prevalence (%)	CI 95%	Author (Year)
Brazil (all states)	364,130	IgM FEIA	0.05	0.05-0.05	Neto et al (2004)[5]
Uberlândia, Minas Gerais, Brazil	805	IgM-IgA EIA	0.5	0.1-0.9	Segundo et al. (2004)[6]
Riberão Preto, São Paulo, Brazil	15,162	IgM FEIA	0.03	0.01-0.07	Carvalheiro et al. (2004)[7]
Porto Alegre, Rio Grande do Sul, Brazil	10,000	IgM FEIA	0.06	0.02-0.13	Lago et al. (2007)[8]
Quindio, Reference Hospital (Colombia)	200	IgM-IgA Western blot	0.5	0.2-0.8	Gallego-Marin et al (2006)[9]
Quindio, Community Hospital, Colombia	322	IgM EIA	0.62	0.19-2.2	Gomez-Marin et al. (2007)[10]
Cordoba, Argentina	673	Persistence of IgG one year after birth	0.45	0.18-0.72	Del Vado et al (1997)[14]

were symptomatic, of whom seven received prenatal *in utero* treatment.[11] Four of 13 children (30%) had retinochoroidal lesions, and four of 11 (36%) had neurological involvement (in ultrasound examination). Two children (11.7%) died before six months of age.[11]

Besides the high prevalence, congenital cases in South America are more symptomatic. The higher severity of South American cases was an unexpected result of the Systematic Review on Congenital Toxoplasmosis (SYROCOT) international collaborative study.[19] For this analysis, 25 cohorts of infected mothers identified during prenatal screening were selected. The risk of ocular lesions was much higher in the South American cohorts (47%; 18/38) than in Europe (14%, 79/550). The crude risk of intracranial lesions detected by cerebral tomography scan was much higher in the cohorts from North (19%, 19 of 103) and South America (53%, 20 of 38) than those from Europe (9%, 49 of 550), where cranial ultrasound was used. Another finding was that the risk of transmission decreased significantly with higher latitude (odds ratio [OR]=0.71 for 5° higher, 95% confidence interval [CI] 0.53-0.96). Theses results were then confirmed by a comparative prospective cohort of children with congenital toxoplasmosis identified by universal neonatal screening in Brazil and neonatal or prenatal screening in Europe between 1992 and 2003, using the same protocol in both continents.[20] The median follow up was 4.1 years in Europe and 3.7 years in Brazil. Relatively more children had retinochoroiditis during the first year in Brazil than in Europe: 15/30 (50%) versus 29/281 (10%). The risk of lesions by four years of age was much higher: the hazard ratio for Brazil versus Europe was 5.36 (95% CI 3.17-9.08). Children in Brazil had larger lesions, which were more likely to be multiple and to affect the posterior pole. In Brazil, visual impairment was predicted for most affected eyes (87%, 27/31), but not in Europe (29%, 20/69); p=0.0001. Therefore, the results of that study showed that Brazilian children had a five times higher risk than European children of developing eye lesions, and their lesions were larger, more numerous and more likely to affect the area of the retina responsible for central vision.[20] Also, in a maternal screening in 2,513 mothers during delivery, in Rio Grande do Sul, Brazil, all the three infected newborns were symptomatic with active ocular lesions.[21] Altogether, these reports of children detected during prenatal and newborn screening confirm a higher percent of symptomatic children in South America.

New world and old world strain types of *toxoplasma*: relevant for congenital infection?

Recent findings about the genetic diversity of *Toxoplasma* have revealed the extraordinary structure of this successful parasite, and at the same time generated a debate about the origin of the parasite and about what markers would be useful to its genetic typing. Many studies revealed that strains from South America are genetically distinct from strains from North America and Europe.[22] This conclusion was first obtained by using a "SAG2 clonal genotype classification" of type I, type II and III[23] and then confirmed by multilocus analysis[24] and by serogenotyping, which identifies specific antibodies for clonal lineages I, II and III.[25]

The three clonal lineages classified by using the SAG2 genotyping can be found indistinctly in congenital or in immunosupressed patients.[23] Thus, SAG2 genotyping is not useful for clinical correlates, but it was the first technique that demonstrated different genetic structure between New World and Old World *Toxoplasma*. Also, methods that classify strains by serotypes of clonal lineages failed to demonstrate a clinical correlate with ocular toxoplasmosis, but remains

Table 2. Practical application of genotype markers of *Toxoplasma gondii*.

Marker	Origin of the species	Clonal groups versus non clonal groups	Clinical correlate	Mouse virulence	Geographical divergence (South America versus North America and Europe)
SAG2	No	No	No	No	Yes
Microsatellites	?	No	No	No	Yes
Introns	?	Yes	No	No	Yes
ROP18 Polymorphism	No	No	?	Yes	No
Mitochondrial or apicoplast sequences	? Probably yes	No	? Probably no	? Probably no	? Probably no

strongly linked to geographical origin.[25] Therefore, it is essential to precisely define the questions to be resolved by each genetic marker (Table 2).

Limits of parasite genotyping on clinical samples and future work

A significant problem to analyze the relation of the *Toxoplasma* genotype with clinical outcome and prognosis in human infection is the difficulty of studying the parasite on clinical samples. The most important series of genotyping of *Toxoplasma* strains on clinical samples of congenital toxoplasmosis were performed in Europe in 86 samples[26] and in South America in 6 Colombian samples.[23] The use of genetic markers in clinical samples could be challenging. Negative amplification was described when the genotyping was tried directly from clinical samples. In Colombia, a SAG2 genotyping was only possible in 33 of 50 (66%) clinical and animal positive samples for B1 specific *Toxoplasma* gene.[23] For SAG2 genotyping, a restriction fragment length polymorphism (RFLP) analysis is made on two products of amplifications, one for the 3' end and another for the 5' end. In the Colombian study, no amplification of 3' end was found in 19 samples (57%) and no amplification of 5' end fragments was found in three samples (9%). This can be explained by presence of polymorphisms in the primers binding site of these isolates. The low sensitivity of these markers could be due to the fact that the target (SAG-2 gene) is present in genome as a single copy, and to the low amount of parasite DNA present in clinical samples.

Theses results on clinical samples showed the urgent need to find a sensitive genetic marker that could be applied for clinical studies. When looking for accurate genetic markers of parasite virulence, a genetic analysis of the whole *Toxoplasma* genome showed a virulence trait that explained the high virulence of the type I lineages in mouse and proved to be largely (90%) due to a single allele at the ROP18 locus, a gene encoding a rhoptry protein kinase.[27] However, the clinical significance for human is unknown.

Additionally to these important barriers for the application of parasite genotyping in human infection, there are some crucial questions about the contribution of host and parasite factors in human *Toxoplasma* infection. This is a critical point to be defined before the implementation of *Toxoplasma* genotyping for clinical application (i.e. to define prognosis of a patient). Not all children who acquire infection *in utero* develop clinical signs of disease. Whilst severity of disease is influenced by trimester in which infection is acquired by the mother, other factors, including genetic predisposition and parasite strain, may also contribute. This has become now more relevant by the discovery of the association of ocular and brain disease in congenital toxoplasmosis with parental polymorphisms in ABCA4 encoding ATP-binding cassette transporter, subfamily A, member 4. Polymorphisms at COL2A1 encoding type II collagen are associated only with ocular disease. Both loci showed unusual inheritance patterns for the disease allele when comparing outcomes in heterozygous affected children with outcomes in affected children of heterozygous mothers.[28] Therefore, the genotyping of the parasite is not enough to define the outcome of a congenital infection.

In this context, genotyping studies applied on clinical samples are now needed, but it would be necessary to determine if the detection of ROP8 insertion/deletion polymorphism is better correlated with clinical outcome than the markers used so far. Future clinical application of genotyping in congenital toxoplasmosis should also define the relative weight of genetic susceptibility (ABCA4 and COL2A1 genotypes in the host) and parasite strain (i.e. ROP18 insertion/deletion), by improving the sensitivity of amplification methods for parasite DNA targets.

CONCLUSIONS AND PROSPECTS FOR RESEARCH IN SOUTH AMERICA

In conclusion, congenital toxoplasmosis in South America is a public health problem with a significant

impact on children morbidity and mortality. Research for new drugs and candidate vaccines are a priority to improve indicators of health in South American children. A practical consequence of the higher frequency of symptomatic congenital toxoplasmosis in South American is that clinical trials to test new vaccine or therapeutic candidates would need smaller samples than in Europe. Altogether, these new data are precious to address the research in this field. Some issues to resolve include whether it is feasible to determine the strain type in clinical samples to predict the prognosis, and whether it is possible to identify virulence proteins for development of new drugs or vaccine approaches.

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