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Congenital Toxoplasmosis: A Neglected Disease? –Current Brazilian public health policy

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Abstract. Toxoplasmosis is a cosmopolite disease caused by the protozoan parasite, *Toxoplasma gondii*. The infection may be contracted through the ingestion of raw or undercooked meat or unpasteurised milk, organ transplants, blood transfusion, through the placenta in vertical transmission or by direct contact with the faeces of infected felids or even through the ingestion of sporulated oocysts in water or food. In immunocompetent individuals, it is generally asymptomatic. However, in patients with various degrees of immunodeficiency and in pregnant women, it may cause severe sequelae and can be fatal. In pregnant women, the prenatal diagnosis should be made as early as possible, so that therapy may be applied to reduce parasitemia and avoid transplacental infection. Despite the high prevalence of infected individuals around the world (20-90%), in some European Union countries, such as France and Austria, the average incidence of foetal toxoplasmosis was reduced from 40% to 7% by means of specific programs. **In Brazil,** seroprevalence may vary between 40% and > 80% (South/Southeast-North/Centre-West/Northeast), reflecting a significant disparity between the public health policies and resources applied in the different regions of the country and the human development index (HDI) of each of them. The lack of consistent and periodical data on seroprevalence per region makes it difficult to understand the significance of this infection and to plan specific public health policies and strategies. Also important is the fact that Hemotherapy Units are not required to screen blood components for toxoplasmosis, not even for immunocompromised patients and pregnant women, or organ transplants. Regarding basic and clinical research, toxoplasmosis is not a priority if compared to the allocation of government funds and incentives to other tropical illnesses, such as: Chagas disease, Leishmaniasis, Dengue fever. There are also few study groups in Brazil that focus in this basic area, for genomics and proteomics studies of *T. gondii* strains–for the design of new methods of diagnosis, antiparasitic drugs and a vaccine model for humans and animals. This article reviews the historical, taxonomic and epidemiologic aspects, diagnosis methods, treatment and technical-scientific aspects of *Toxoplasma gondii* and toxoplasmosis in the Brazilian context.

Keywords. Toxoplasmosis, *toxoplasma gondii*, epidemiology, public health policies.

1 Historical aspects of toxoplasmosis

Toxoplasma gondii was described by Splendore, in 1908 in **Brazil**, affecting lab rabbits, and by Nicole Manceaux, in the same year, in a rodent of the *Ctenodactylus gondii* species, in the Tunisian Pasteur Institute (Neves, 1994; Cimerman, 1999). It was initially named "*Leishmania gondii*" due to its similarity to the *Leishmania* sp protozoa. The correction of the nomenclature took place in 1909 (Nicolle Manceaux, 1909). The first description of human infection by this parasite was made by Jankü, in 1923, with the report of a child deceased in Prague (Cimerman, 1999). Torres *et al*., in 1927, described in **Rio de Janeiro** the presence of microorganisms they identified as *Toxoplasma* in histological sections of the brain, myocardium and skeletal muscle of a newborn deceased 29 days after birth. Wolf Cohen and colleagues, in 1937, were the first authors to describe the congenital infection in man, reporting the occurrence of toxoplasmosis in a newborn with encephalitis, meningitis and myelitis.

In the United States, Pinkerton and Weinman, in 1940, and Pinkerton and Henderson, in 1941, recorded the occurrence of toxoplasmosis in adults, with the isolation of the parasite. However, it was only after a serological test was developed, Sabin and Feldman's classic dye test, in 1948, that it became possible to demonstrate the high prevalence of the disease around the world, contributing immensely to the laboratory diagnosis of toxoplasmosis and allowing the execution of

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epidemiologic studies (Neves, 2000). Finally, Frenkel *et al*., 1970, pointed out that oocysts represent the sexual stage of the agent. Miller and colleagues, 1972, proved that the only mammals capable of supporting the intestinal sexual cycle of *T. gondii* and excrete the oocysts are the felines, both domestic and wild cats. The studies on this disease are abundant and the importance of this protozoosis is now clearly characterized (Villeneuve, 2003). After a careful review of the existing medical literature on toxoplasmosis, we found it could be divided in four stages according to the evolution of the knowledge on the matter: the first characterized by the discovery of the etiologic agent; the second by the description of the infection in man; the third by the introduction of serological tests for diagnosis purposes and, lastly, by the identification of the definitive host.

1.1 Classification

According to Levine (1977, 1980), *Toxoplasma gondii* is a protozoan parasite of the:

Phylum Protozoa

Subphylum Apicomplexa

Class Sporozoa

Family Sarcocystidae

Subfamily Toxoplasmatinae

Genus Toxoplasma (Nicolle and Manceaux, 1909)

Species *T. gondii* (Nicolle and Manceaux, 1909)

Since the classification proposed by the Society of Protozoologists (Levine *et al*., 1980), there were no significant changes regarding the understanding of the phylogenetic lineages of eukaryotes. However, with the advent of molecular biology, many studies have focused on supra-group and infra-group genetic variability. Adl and colleagues, 2005, proposed a new system for eukaryote organisms. These authors proposed the organization in six main phylogenetic clusters: (1) Opisthokont (animals, fungi, choanoflagellata and mesomycetozoea); (2) Amoebozoa (amoebae, amoebae-flagellates); (3) Excavate (Euglenozoa, Heterotrophic Flagellates, Diplomonads); (4) Rhizaria (foraminifera); (5) Archaeplastida (plants and algae) and (6) Chromalveolate (Ciliates, Dinoflagellates and Apicomplexans). *Toxoplasma gondii* falls in the Chromalveolate group, Alveolate: Apicomplexan: Coccidia subgroups.

2 Introduction

Toxoplasmosis is a very common infection in humans and its prevalence ranges from 20 to 90% of the adult world population. Seroprevalence varies according to regions, especially when related to sanitation conditions and socioeconomic indexes (Hill and Dubey, 2002; Spalding *et al*. 2003). The severity of the infection caused by *T. gondii* may vary depending of the cellular and humoral immune condition of the individual, spanning from very mild symptoms (similar to flu) or no symptoms at all, to exuberant clinical symptoms. The severe clinical forms generally occur in immunocompromised individuals and pregnant women. Immunocompromised individuals (AIDS patients, cancer patients, transplant subjects or patients with genetic immunodeficiencies and diabetes), may present high levels of morbidity and mortality (Ho-Yen, 1992; Israelski and Remington, 1993; Lewden *et al*., 2005; Khurana *et al*., 2005). When these individuals become infected, the parasite displays tropism with the nervous system. The disease is manifested more frequently through neurological symtpoms and may be fatal when treatment is not administered quickly. Transplacental transmission may occur by primary infection during the entire pregnancy (Pelloux *et al*., 2002; Remington *et al*., 2001). When the infection is contracted during gestation, the parasite may attach itself to the placenta and develop there throughout the rest of the pregnancy. In a large number of cases it may affect the foetus. The severity of the lesions is related to the stage of the gestation and the consequences may be more severe the younger the foetus, and may result in miscarriage, stillbirth or hydrocephalus. If the infection occurs at a later stage it may result in eyesight or hearing disorders or mental retardation. The immunoenzymatic methods allow the analysis of the humoral immune profile of the pregnant woman and determine the risk of infection according to the gestational age, allowing the inclusion of pregnant women in therapeutic protocols designed to inhibit parasitemia (Pinon, 2001; Remington, 2004). The combination of antiparasitic toxic drugs, such as pyrimethamine and spiramycin, does not always prevent contagion nor does it guarantee the total elimination of tachyzoites (Peyron *et al*., 2001; Binquet, 2004). Molecular methods, such as qualitative PCR for the *B1* gene (repeated 35 times in the *T. gondii* genome), are used to assess the efficacy of treatment in clinical samples such as: amniotic fluid, umbilical cord blood, placenta, acqueous humour and various tissues infected by *T.gondii* (Burg *et al*., 1989; Grover *et al*., 1990; Hohfeld *et al*., 1994; Pelloux *et al*., 1996; Jones *et al*., 2000; Spalding *et al*., 2002; Remington *et al*., 2004). The persistence of parasites in biological samples from pregnant women undergoing treatment may indicate the resistance of the parasite to the drugs, possibly a result of genetic differences between the *T. gondii* strains isolated (Ajzenberg *et al*., 2002; Dardé *et al*., 2004; Vaz, 2006; Vaz, 2010). The molecular characterization of *T. gondii* strains by techniques such as isoenzymatic tests, restriction fragment length polymorphism (RFLP-PCR), automated sequencing and random amplification of polymorphic DNA (RAPD) shows a correlation between the genotype and virulence of the strain isolated, in addition to the correlation of the strains and resistance to the drugs used in treatment of the infection (Sibley *et al*., 1992; Dardé *et al*., 1992; Cristina *et al*., 1995; Guo *et al*., 1995; Howe *et al*., 1997; Dardé *et al*., 2004). The clonal lineages identified by these techniques are of the types I, II and III; they are related to the SAG2 gene of *T. gondii* and are found in strains isolated in humans and animals (Sibley *et al*., 1992; Fuentes, 2001; Vaz, 2010). Phylogenetically, the type II and III genotypes belong to the same group; however, the type II genotype prevails most often in mice and strains maintained in cellular culture. The type I genotype is most associated with congenital

toxoplasmosis (Fuentes *et al*., 2001; Ajzenberg *et al*., 2002; Vaz, 2010). **In Brazil** studies demonstrate the variations in the prevalence of toxoplasmosis in the adult immunocompetent population and in pregnant women, as well as the relationship and comparison of various diagnostic methods (immunological and parasitological), including *T. gondii* DNA detection methods, in various clinical samples. However, these studies are isolated and do not reveal the consistency and periodicity necessary to the planning of public health policies with a view to reverting seroprevalence indexes strongly associated to the HDI per region, sanitary infrastructure. Few Brazilian research groups also publish articles addressing the relationship between isolated genotypes, protocol drugs used and the persistence of parasites and severity associated with infections during pregnancy (Jaquier, 1995; Camargo *et al*., 1996; Cantos, 2000; Lopez *et al*., 2000; Coppens *et al*., 2001; Spalding and Amendoeira, 2003). The probable cause of this situation may be the failure to give a higher priority to toxoplasmosis compared to other diseases, such as Chagas' disease, leishmaniasis and Dengue fever, all of which receive substantial public funding.

3 Epidemiology of congenital toxoplasmosis in Brazil

The prevalence of *T. gondii* infection may vary greatly from country to country, in different regions in the same country, and among different population groups of the same region (Remington *et al*. 2001). These differences are related to the characteristics of the parasite, such as the level of infection potency and peaceful coexistence with the host, the capacity to infect thousands of animal species, including aquatic, land and winged animals, and the capacity to inhabit various regions of the globe (in lower proportion in very cold areas, arid regions and at high altitudes). Other factors that interfere with the epidemiology of *T. gondii* are feeding habits and cultural characteristics.

The prevalence of toxoplasmosis in pregnant women in Brazil varies a great deal. This fact is related to weather, cultural and, especially, socioeconomic differences found in the population (Couto *et al*., 2003; Vaz e Thomaz-Soccol, 2010). Recently, Neto and colleagues (2010) conducted an estimate of the regional distribution of congenital toxoplasmosis in Brazil based on the results of neonatal triage. The results pointed to a general prevalence of congenital toxoplasmosis of 1/1.613, varying from 1/1547 to 1/495 in different States. According to Table 1 of the above mentioned work, the States afflicted at highest rates are Pará, Rondônia, Mato Grosso and Maranhão. São Paulo, Paraná, Mato Grosso do Sul and some northeastern States presented a low prevalence. These studies reveal the need for health and education policies that target the prevention and control of congenital toxoplasmosis in Brazil while respecting the different characteristics of each State.

Some States have public health programs that aim to reduce the mortality of women and children during pregnancy and birth. One example is the *Programa Mãe Curitiba* [Curitiba Mother Program] developed in the city of Curitiba, capital of the State of Paraná. Created in 1999, on International Women's Day, this Program strives to improve the access and quality of prenatal, childbirth, postnatal and newborn assistance in maternities and health units. In terms of prevention and treatment of maternal and congenital infections the *Mãe Curitibana* program presents an intervention protocol for toxoplasmosis. Any cases of suspected Toxoplasmosis are submitted to serologic investigation and, depending on the results, are considered: 1- Discarded cases (RN with negative IgG and IgM); 2- Confirmed cases (child with or without clinical manifestation and and/or positive IgM after one week of life, persistently high or rising IgG, presence of *T. gondii* in placental tissue); 3- Cases under investigation (children with decreasing IgG and negative IgM at 30 days of life are followed up until the serology is negative).

Brazil has a prenatal program, implemented by the Ministry of Health, that includes the clinical-laboratorial diagnosis of pregnant women and children exposed to *T. gondii* risk, available for all users who seek medical assistance. However, there are many difficulties related to the technical and operational parts, as well as laboratory diagnosis interpretation problems. Thus, by knowing the prevalence of pregnant women susceptible to toxoplasmosis in various Brazilian states and by considering the risk factors in each region, it is possible to implement effective control strategies.

4 Toxoplasmosis–treatment

Despite the importance of toxoplasmosis, there are still few effective treatments for this disease, their main goal being the reduction of the parasite replication rate so as to avoid more extensive damage to the organs involved. It is therefore clear that continuous drug therapy is essential to guarantee the prevention of severe complications. The ideal drugs for the treatment of toxoplasmosis should be effective, easily obtained at affordable cost and have no toxicity or hypersensitivity reactions. In addition, they cannot pose risks of teratology or malformation, allowing their use in pregnant women, and should be available in parenteral presentations for patients unable to ingest the medication. In addition, they need to be effective against all strains of *T. gondii*, be capable of killing tachyzoites and have a high ocular and cerebral penetration (Mui, *et al*., 2008). However, many of these ideal characteristics are not existent, which compromises the treatment of the disease. Since the beginning of the nineties, the standard treatment remains unaltered, focusing only on the metabolism of the parasite's nucleotides.

The therapy consists of a synergic combination of pyrimethamine and sulfadiazine, drugs that cause the blockage of the synthesis and reduction of the folic acid levels in tachyzoites through the inhibition of dihydrofolate reductase and dihydropteroate synthetase, respectively (Martins-Duarte, *et al*., 2009; Boothroyd, 2009). However, the effectiveness of this therapy is limited, with approximately 50% of all patients not responding to treatment (Safarjalani, *et al*., 2010). Moreover, the drugs used only act on the tachyzoites, not affecting the tissue cysts and thus allowing resurgence of the disease after treatment is over (Martins-Duarte, *et al*., 2011). We also verify that prolonged exposure to the medication regime currently in use is often associated with various adverse

Table 1. Prevalence of congenital toxoplasmosis in each State of Brazil based on a screening of newborns that went through triage in the period from September 1995 to July 2009.

STATE	Total population per State	Number of samples	Number of positives	Number of newborns tested for each positive case	Number of positives per each 10,000 newborns tested
Pará (PA)	7,065,573	14,835	30	495	20
Rondônia (RO)	1,453,756	2,021	4	505	20
Mato Grosso (MT)	2,854,652	9,875	19	520	19
Maranhão (MA)	6,118,995	4,033	6	672	15
Espírito Santo (ES)	3,351,669	4,158	6	693	14
Sergipe (SE)	1,939,426	829	1	829	12
Santa Catarina (SC)	5,866,252	20,517	21	977	10
Bahia (BA)	14,080,654	22,659	22	1,030	10
Roraima (RR)	395,725	2,124	2	1,062	9
Piauí (PI)	3,032,421	6,409	6	1,068	9
Amapá (AP)	587,311	1,086	1	1,086	9
Tocantins (TO)	1,243,627	1,109	1	1,109	9
Rio Grande do Sul (RS)	10,582,840	280,962	200	1,405	7
Goiás (GO)	5,647,035	14,804	10	1,480	7
Minas Gerais (MS)	19,273,506	61,661	38	1,623	6
Ceará (CE)	8,185,286	20,788	11	1,890	5
Distrito Federal (DF)	2,455,903	11,420	6	1,903	5
Pernambuco (PE)	8,485,386	64,915	28	2,318	4
Paraíba (PB)	3,641,395	26,264	11	2,388	4
Paraná (PR)	10,284,503	32,318	12	2,693	4
Rio de Janeiro (RJ)	15,420,375	65,585	23	2,852	4
Alagoas (AL)	3,037,103	18,105	6	3,018	3
São Paulo (SP)	39,827,570	94,712	29	3,266	3
Rio Grande do Norte (RN)	3,013,740	5,442	1	5,442	2
Mato Grosso do Sul (MG)	2,265,274	10,894	2	5,447	2
Amazonas (AM)	3,221,939	2,402	0	–	–
Acre (AC)	655,387	237	0	–	–
Total	183,987,303	800,164	496	1,613	6

Source: Neto *et al*., 2010.

reactions, especially in AIDS patients, which include suppression of bone marrow and cytopenia caused by pyrimethamine (Martins-Duarte, *et al*., 2010) and hypersensitivity reactions to sulfadiazine in the form of skin allergies (Maubon, *et al*., 2010), leucopoenia, thrombocytopenia and fever (Jiang, *et al*., 2008) and also kidney stones, hepatotoxicities and nephrotoxicities (Mui, *et al*., 2005). In general, folinic acid is added to the treatments to reduce the risk of bone marrow suppression (Montoya and Liesenfeld, 2004). Other limitations to this therapy include low tolerance, the large number of tablets involved in treatment, the unavailability of the drugs in some countries, the high cost of the drugs, and the absence of an intravenous presentation for these compounds (Béraud, *et al*., 2009). Taking into account all these difficulties, some alternative therapies were developed and some drug combinations are available to replace the classical therapeutic model. In this context, an alternative therapy is the combination of clindamycin and pyrimethamine, which has an efficacy similar to that of the combination with sulfadiazine, and which is also associated with various side effects (Martins-Duarte, *et al*., 2010).

Antibiotics such as co-trimoxazole and clindamycin have been used as a second treatment option (Fung and Kirschenbaum, 1996) in addition to other drugs such as esperamicin and atovaquone, used with limited success, particularly in the long-term treatment of toxoplasmosis patients (Safarjalani, *et al*., 2008). Other drugs, such as dapsone, trimethoprim, pentamidine and azithromycin, have also displayed anti-*T. gondii* activity and have thus been used despite their side effects (Jiang, *et al*., 2008). In light of this scenario, we see that the search for new drugs for the treatment of toxoplasmosis is extremely important, since the therapeutic arsenal available continues to be defective. In the last few years, a new therapeutic proposal using co-trimoxazole has shown potential and proven more effective in the treatment and prophylaxis of encephalitis, lymphadenitis and eye infections caused by *T. gondii* (Alavi and Alavi, 2010). Co-trimoxazole (trimethoprim/sulfamethoxazole) is an affordable medicine, widely available in developing countries, exists in the parenteral form and has a high diffusion rate throughout the central nervous system, in addition to being better tolerated than the classic therapy with pyrimethamine/sulfadiazine (Béraud, *et al*., 2009). **In Brazil** we find that the classical drug treatment still remains unaltered, using the sulfadiazine, pyrimethamine and folinic acid combination, alternating with esperamicin, both in paediatric patients and in pregnant women (Higa, *et al*., 2010; Sáfadi, *et al*., 2003). The therapeutic models used vary according to the initial levels of IgM; in general, seropositive women (IgM+/IgG-) are treated more often (Castilho-Pelloso, *et al*., 2007). However, recent studies using genetic characterization have shown that the *T. gondii* strains found in certain areas of Brazil are atypical, mostly corresponding to subtype I and not to the subtypes II and III seen in Europe and in many English-speaking countries in the Northern Hemisphere (Vaz, 2006; Vaz, 2010; Vaz e Thomaz-Soccol, 2010). Furthermore, they were found to be genetically polymorphic and associated with more severe manifestations of disease in humans (Mui, *et al*., 2008). Therefore, additional studies involving the parasite's proteomics and functional genomics are necessary for the development of new drugs, a viable and safe vaccine, and more specific diagnosis methods.

5 Discussion

Until now, Brazil does not have a consistent official program for congenital toxoplasmosis (CT) at a national level to assess and follow-up pregnant women in prenatal triage throughout the pregnancy period, provide childbirth assistance and postpartum follow-up to the mother and newborn; only isolated cases exist, like the *Programa Mãe Curitiba* [Curitiba's Mother Program], in the State of Paraná. Recently, the Government of the State of São Paulo implemented the *Programa Mãe Paulistana* [São Paulo's Mother Program] for pregnant women in the city of São Paulo that follows the model of Mãe Curitibana, which this year celebrates its 12th anniversary and which achieved a reduction of the seroprevalence associated with CT from 53% (2004-2006 data) to < 45% (recent data). The Federal Government and the Ministry of Health intend to replicate these success models in other capitals and, in the medium term, in the public network in all the country's States. Even so, for the new public health policies to be enforced in a more effective manner, it would be necessary to collect seroprevalence data in all regions of the country in a continuous manner, month after month, year after year, to allow the observation of the development of toxoplasmosis in the country as a whole and to define specific strategies for regions with different socioeconomic aspects, sanitary infrastructures and HDI. Generally speaking, we do not know (exactly) the number of pregnant women in the country susceptible of seroconversion potential throughout gestation and, even in regards to the registered miscarriages, what percentage is associated with toxoplasmosis. In terms of scientific research in the basic and clinical area, the country has isolated groups with specific research lines per area. In the area of basic research and technological innovation, the situation is the same. However, in the Conference of the 100th anniversary of *Toxoplasma gondii*–TOXO-100 (Búzios-RJ–September 2008), promoted by the Brazilian, French, European and North American Parasitology Societies, it was found that the Brazilian and South American *T. gondii* strains were very different from the North American and European strains, where in Brazil the most prevailing strain associated with CT is type I, with more aggressive characteristics when compared to the Northern hemisphere strains. Another aspect not well publicized, not only in Brazil but around the world in general, is the matter of screening blood for toxoplasmosis, at least for immunocompromised patients and in transplant patients (Vaz *et al*., 2008). There is no law in Brazil that requires hemotherapy centres to triage blood components for that purpose. This fact may facilitate the infection of patients whose immunity is frail. There are no national studies that demonstrate this fact. For the definition of the prevalence of lineages of *T. gondii* strains in Brazil, it would be necessary to collect clinical samples from the various regions of the country for molecular characterization and, also, to ascertain if there are intragenotypic variations. These data would be very valuable for the development of new diagnosis tools,

since most methods used in the national territory come from European countries and from North America and are created from regional strains, and also to allow the development of new drugs, more effective that those currently used which are not effective in many clinical situations. In addition, there would be the possibility of new vaccine models, both for humans and animals. Generally speaking, the understanding at various levels of this parasite infection and of the specific Brazilian parasite opens several revaluation perspectives for our health system, for appropriate funding of the research groups and for their coverage of the entire national territory. The wider understanding of this infection would allow us to know the impact it has on human and animal health and which specific measures could be implemented in terms of public health so as to revert the current scenario.

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