

Contributions to the history of ocular toxoplasmosis in Southern Brazil

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*The evolution of knowledge regarding ocular toxoplasmosis over the last 30 years is described based on studies and observations performed in Southern Brazil. The isolation of *Toxoplasma gondii* established the definitive diagnosis of the disease. It was proven that in most cases, the disease was acquired after birth, a concept supported by the description of numerous familial cases and observation of the disease many years after primary infection. Epidemiological studies showed important regional variations in the prevalence of the disease due to different factors, including the types of strains involved, of which type I predominates. The large number of patients also enabled detailed study of the different forms of clinical presentation of the disease and its complications. New parameters have been established for the use of steroids and the management of pregnant women with active lesions. Studies on the epidemiology of toxoplasmic infection in pregnant women and newborns showed a high prevalence of infection. The different factors of exposure to infection have also been studied. Gradually, preventive actions have been developed in the sphere of public health, although they have not been sufficiently effective. Trends for future research over the next few years are also outlined.*

Key words: toxoplasmosis - ocular toxoplasmosis - acquired toxoplasmosis - congenital toxoplasmosis - epidemiology - public health

Apparently, *Toxoplasma gondii* has existed in Brazil for a long time. Certainly, it was not by chance that its discovery occurred in São Paulo (SP) a century ago. The generous land, a climate without harsh cold or snow, the constant humidity and the presence of definitive hosts - wild and domestic - certainly aided in its very successful evolution.

The flourishing national research since the early days when toxoplasmosis was discovered is evidence of the frequent contact between the parasite and man and animals in Brazil. Up to 1975, over 300 articles were published on the topic (Hyakutake & Mearim 1975, 1976). Since then, about the same numbers of new papers and academic theses have been added.

Soon after my arrival in Porto Alegre, in 1978, while working in the Department of Ophthalmology and Otorhinolaryngology at the Medical School of the Federal University of Rio Grande do Sul, I had my first contact with *Toxoplasma* and the disease it causes. Our attention was immediately called to the large number of cases of severe uveitis with many retinal and choroid lesions that we attended in Infirmary 25 at Santa Casa de Misericórdia Hospital, headed by the late Prof. Luiz Assumpção Osório. Although the clinical picture was fully compatible with the descriptions published since 1950 for ocular toxoplasmosis in Brazil and abroad, one question remained. Could the disease that we so frequently encountered really be caused by *Toxoplasma*, or could it be due to other infections, such as tuberculosis and syphilis, or even to some other unknown entity?

The available study to perform at that time, specific antitoxoplasmic antibody detection in peripheral blood, confirmed both in the patients (Melamed 1982) and in the healthy population (Silva 1979, Melamed et al. 1981, Chaplin 1984 et al. 1984, Nishikawa 1984) a high degree of parasitic infection in the population of Rio Grande do Sul (RS). However, this coincidence did not help distinguish between the two populations; therefore, it did not provide any assistance in identifying the etiological agent (Melamed et al. 1988).

For these reasons, we decided to eliminate the terms “probable” and “compatible” from our patients’ diagnoses and attempted to identify the etiological agent directly. In 1983, in collaboration with Prof. Eunice Chaplin, a veterinary professor who was researching toxoplasmosis at our university, we managed to isolate the agent from the eye of an infected patient. For the first time in Brazil and in Latin America, *T. gondii* was isolated from an affected eye, which confirmed that we were on the right track regarding the diagnosis of our patients (Osório 1986, Melamed 1992).

However, we were not the only ones surprised by the unusual ocular morbidity of the disease. As early as 1969, in the city of Passo Fundo, located in the North of the state, a high prevalence of ocular toxoplasmosis had been reported (Martins 1969). This was then confirmed years later by Dr. Fernando Gomes da Silveira, who practiced in Erechim, another city in that region. Discussing this fact with other colleagues, we verified that the high prevalence found at Santa Casa Hospital in Porto Alegre was also present in the Northeast and Northern region of RS, Western Santa Catarina (SC) and Western Paraná (PR). What could be the cause for this?

At that time, the most accepted hypothesis was that of Perkins, which postulated that most of the cases in adults could be a consequence of the reactivation of con-

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Received 29 October 2008

Accepted 18 November 2008

genital lesions (Perkins 1973). It became very clear to us that transplacental transmission, even at the highest known rates, could not *per se* explain the large number of patients affected by the disease who visited doctors every year, both in Porto Alegre and in other regions of the state. There was only one possible answer: most of the cases of ocular toxoplasmosis in Southern Brazil had been caused by toxoplasmosis acquired after birth. This concept went against the predominant ideas at the time and was received with a certain amount of scepticism when we presented it at the 1st South Brazilian Congress of Uveitis, in 1984 (Melamed 1988, 1989). Later on, the description of familial cases (Silveira 1987, Melamed 1988, Silveira et al. 1988), which included the histological demonstration of the parasite in non-twin brothers, by Silveira et al. (1987) strengthened the hypothesis of acquired toxoplasmosis since only in exceptions does transplacental transmission occur in more than one offspring.

The clinical findings in a large sample of patients also supported this hypothesis (Melamed 1991). This new concept was presented over the years in many papers, at many national and international events (Melamed 1991, 1993), and gradually it stopped being considered a regional phenomenon and took on universal status (Nussenblatt & Belfort 1994, Holland 1999, Gilbert & Stanford 2000).

Another concept, which arose more or less at the same time to complement the hypothesis of acquired toxoplasmosis, was that the disease could appear in the eye many years after the primary episode of infection (Melamed 1989). Late onset acquired ocular toxoplasmosis may manifest itself up until at least 13 years after primary infection (Melamed 1994, Silveira et al. 2001) and appears to be the most common form of ocular presentation in our region (Melamed 2002). However, in children up to 10 years old, the congenital forms seem to be predominant (Melamed 1991a, 1993).

Areas were found in the state of RS with one of the highest degrees of toxoplasmic ocular involvement ever described (A Alcântara et al. unpublished observation, Glasner et al. 1992, Melamed et al. 1993), constituting one of the most frequent causes of blindness and visual impairment throughout Southern Brazil (Melamed & Alves 1984, Alves 1985, Lavinsky et al. 1986, Esteves et al. 1996, Santos & Vegini 2006).

Another uncommon characteristic of the disease in RS was the disparity of its geographical distribution. The affected individuals were not distributed homogeneously throughout the state, and thus it was possible to delimit different sub-regions as much as in the degree of infection as in the actual disease (Melamed 1989, Melamed et al. 1991, 1993, 2000a, Raymundi 1991). Even in the Northern region of the state, with a generally high prevalence, small population groups were found where the disease was even more frequent (Melamed et al. 1993).

The reasons explaining these very special characteristics of the disease in our state were not very clear at the time. Geographic, climatic, ethnic, socio-cultural differences and different strains of the parasite were raised as the probable factors involved (Melamed 1989, Melamed et al. 1993, 2000a).

Research conducted over the years found that several factors might be acting together. Many factors of exposure to infection were carefully studied (Spalding 2000, Jones et al. 2006) and the role of water as a vehicle of transmission has taken on great importance during the last few years (Moura et al. 2006). The ethnic differences among the populations studied have not yet been established as a factor of influence and the cultural factors that might contribute to the greater dissemination of the infection have not yet been precisely identified. There is no doubt that the climate as a whole influences the regional findings (Melamed et al. 1993, Sebben et al. 1995), but no seasonality has been found in the presentation of the ocular disease (Sebben et al. 1995). As to the different lineages that might be involved, it has been demonstrated, differently from what happens in other parts of the world, that the type I strain is the most frequent among us (Vallochi et al. 2005), which may also be the reason for the greater severity of the lesions we found as early as the first years of studying the disease. It was also observed in both human beings and animals that an unusual rate of different genotypes of *T. gondii* was involved (Dubey et al. 2003, Neto et al. 2007). Another important fact that must be mentioned is the early age of the infection in the endemic regions when compared to others areas (Melamed et al. 2000a, Giraldo et al. 2002).

Currently, we think that it is likely that the characteristics of the disease in Southern Brazil are due in part to each of the reasons presented, among others that are yet to be discovered.

At the same time as the studies already mentioned, the large number of patients who came to our service (Melamed et al. 2000c) enabled the detailed study of the different forms of ocular presentation of the disease. Thus, it was possible to characterize two main types of lesions: the typical ones, which were found in about 80% of the cases, and the atypical ones, which corresponded to approximately 20% of the cases (Melamed et al. 2000b). While the typical ones behaved classically and evolved favourably in terms of healing and recurrence, the atypical ones escaped the habitual patterns and were generally more severe (Melamed 2002).

The atypical lesions were divided into four main sub-groups: (1) torpid or chronic; (2) extensive-exudative; (3) multiple; and (4) punctate. We finally had parameters to classify the different forms of presentation of the disease, predict its prognosis and adjust the treatment to each particular case (Melamed et al. 2002). Transitory lesions that apparently did not leave any anatomical sequela, but somehow indicated retinal involvement by the parasite, were also described (Holland et al. 1999).

Simultaneously, numerous clinical studies were developed during these years, such as the first description, in Brazil, of ocular toxoplasmosis in AIDS patients (Melamed et al. 1989), pigmentary retinopathy accompanying the disease (Silveira et al. 1989, Degrazia et al. 2007) and toxoplasmosis provoking hypertensive uveitis in a great number of cases (Melamed et al. 2006). Complications of the disease, such as macular hole (Melamed & Borges Filho 1985), secondary detachment of the retina reaching very high percentages and the involvement

of the optic nerve, usually with a good visual outcome (Eckert et al. 2007), were also described. Differences were also found in the immune response between congenital and acquired toxoplasmosis (Yamamoto et al. 2000, Vallochi et al. 2002).

A great number of therapeutic schemes were used to minimize retinal damage and avoid relapses. The treatment of ocular toxoplasmosis was the subject of a wide debate at several meetings of the Brazilian Society of Uveitis in the 80s and 90s, until finally a consensus was reached with regards to the results achieved: no new medication had performed better than the classical drugs: pyrimethamine, sulphadiazine and prednisone (Melamed 1998).

Treating a great number of patients over the years also convinced us that the most effective drug against the parasite is pyrimethamine. This substance, which favours quick healing of lesions in immunocompetent patients, is also highly effective when used alone in AIDS patients with ocular toxoplasmosis, in whom cell immunity is compromised. Faced with special cases with torpid or extensive lesions, we verified that treatment with pyrimethamine could be extended for long periods if it is accompanied daily by folic acid, until the lesions have completely healed (Melamed 1999).

Another modification to the classical treatment scheme that we have used in our clinical practice is the use of steroids distributed throughout the day, in order to maintain a continuous anti-inflammatory effect (Melamed 1999).

We observed, prospectively, that the number of recurrences in our treated patients was very small, mainly when we used sulphadiazine for 30-60 days after the lesion was completely healed. (Melamed 1999, 2003) In this same sense, it has been shown that sulphamethoxazol with trimethoprim is useful in preventing recurrences (Silveira et al. 2002). In special cases, such as patients with outer punctate lesions, the use of sulphonamides seems to be beneficial in preventing relapses.

The large number of patients seen has also made it possible to establish procedures for the management and treatment of pregnant women with active retinochoroiditis lesions (Melamed et al. 1997).

At the same time as the studies previously mentioned, carried out mostly by ophthalmologists, the frequency of congenital toxoplasmosis in the region has been a major concern among pediatricians and other researchers interested in the subject. Over the last 10 years, several studies have been performed focusing mainly on the epidemiology of *Toxoplasma* infection in pregnant women and newborns, as well as the diverse factors of exposure to the infection.

In Porto Alegre, three studies involving large populations of pregnant women showed prevalences of toxoplasmic infection between 54.3% and 61.1%, similar to the highest described internationally (Neves et al. 1994, Varella et al. 2003, Reis 2006). The results indirectly show that pregnant women who have not yet been infected are highly vulnerable to primary infection.

In a study in the region of Erechim, 2,126 pregnant women were screened. A high rate of infection was found - 74.5% - with a significant number of IgM re-

agents - 3.6% - (Spalding et al. 2003). In another study, developed in Passo Fundo, also in the Northern region of RS, a congenital toxoplasmosis prevalence of 8/10,000 live births was found (Mozzatto & Procianny 2003).

In 2002, in Porto Alegre, an IgM study was performed in blood samples collected on filter paper from 10,000 neonates, showing a prevalence of infection of 6/10,000 (Lago et al. 2007). Another study, evaluating the presence of IgM in newborns, showed an approximate prevalence of 2/10,000 in samples from all over RS (Neto 1996).

Specifically regarding congenital ocular toxoplasmosis, it was demonstrated concurrence with cerebral alterations diagnosed by CT scan (Melamed et al. 2001). Recently, in Porto Alegre, during the first medical visit, 44 children with a confirmed diagnosis of the disease and a mean age of 4.25 months were evaluated. Ocular involvement was found in 70.4%, where the retinochoroiditis represents 65.9% of the total. These lesions were active in 17.6% and healed in 82.2% of the cases. In most of these cases - 75.8% - the lesions were bilateral and located in the papilomacular area. Other ocular manifestations, such as cataract, microphthalmia and strabismus, were also present, but at lower percentages. The study showed that ocular involvement of congenital toxoplasmosis is more frequent and occurs earlier than in other places around the world (Melamed et al. 2005, 2008).

The occurrence, in recent years, of at least three epidemic outbreaks, attests to the great exposure to the parasite in Southern Brazil. The first and most important of these epidemic outbreaks occurred in PR, in the town of Santa Isabel do Avaí, in November 2001. A total of 426 people were contaminated by drinking water containing the parasite oocysts, which after being isolated and characterized, proved to belong to the type I-SAG-2 strain. The infected people had ocular lesions in 10% of the cases, and 4.4% had necrotizing retinochoroiditis lesions (Moura et al. 2006).

The second outbreak was familial, and occurred in Santa Vitória do Palmar, RS, from May-June 2005. Ten people acquired the disease. Of the infected, two were infants less than one year old, which may indicate transmission through breast milk. The outbreak was related to eating industrially processed pork, consumed at a family birthday. One patient presented active multifocal retinochoroiditis lesions (Tavares et al. 2006).

The third outbreak was recorded in Agronômica, SC, where eight people were contaminated, apparently by drinking untreated water. It should be mentioned that in the three outbreaks, the parasites belonged to the type I strain, although with marked differences in the genotypes isolated (Khan et al. 2006).

As a consequence of the clinical and epidemiological studies performed, preventive actions were gradually developed in public health. Since the beginning of the 80s, we repeatedly contacted the appropriate health authorities to warn them about the magnitude of the infection and of toxoplasmic disease in the state. Initially, through meetings at the Division of Zoonosis and Vectors of the RS, State Department of Health, and later at the School of Public Health and in the State Department of Health itself. Thus, the governmental health agencies

slowly became aware of the serious problems and of the great damage that this disease brought to the state.

In 1993, in Porto Alegre, a workshop was held on toxoplasmosis, with the participation of the Ministry of Health, the RS State Department of Health, the State University of Londrina and local researchers. There, the magnitude of the disease and the need for prevention through actions taken by official agencies were emphasized. On this occasion, a strategy was proposed based on four main points: (1) epidemiological surveillance of the disease and the infection; (2) preventive actions in pilot areas of endemic regions; (3) actions to be implemented by the division of Zoonoses and Vectors and the Women's Health Program of the RS State Department of Health; and (4) the establishment of multidisciplinary centres of reference to treat the cases of toxoplasmosis. Unfortunately, none of these recommendations was implemented at the time and only two explanatory booklets were made, which were sent to public health centres to be distributed to the population (FNS a, b 1994).

Finally, in 1998, a state law was passed that made toxoplasmosis a mandatory notification disease and it was specified that the state was responsible for supplying the tests and medications needed to control it (GERGS 1998). Another six years went by before this law was regulated by an administrative ruling of the RS State Department of Health (Secretaria da Saúde Estado do Rio Grande do Sul 2004). In order to apply these measures, it became necessary to write a handbook about toxoplasmosis, which was done by specialized consultants at a workshop in December 2001, in Porto Alegre. The technical standards of the handbook included several protocols: to manage acquired toxoplasmosis in immunocompetent and immunodepressed patients for ocular toxoplasmosis and for gestational and congenital toxoplasmosis, including diagnostic algorithms and therapeutic schemes for each type. Recently, the significance of the *T. gondii*-specific IgG avidity test in pregnant women and the necessity of an effective control program for congenital toxoplasmosis are being stressed (Reis et al. 2006, Lago 2007).

In terms of public health, it should be highlighted that already in the 90s, and for short periods, preventive actions, such as prenatal screening were implemented in isolated municipalities, such as Encantado, Erechim and Porto Alegre. Unfortunately, they were discontinued. Researchers at the State University of Londrina recently implemented a surveillance program for congenital toxoplasmosis in PR. However, it should be recognized that RS was the first in the country to adopt public and legal measures to control the disease.

Knowledge on toxoplasmosis in our region has advanced systematically in the last 30 years. Many important contributions were made by colleagues, researchers from other areas, residents and fellows who, for many years, met on Saturday mornings at the Ophthalmology Service at Hospital de Clínicas, in Porto Alegre, to discuss every aspect of toxoplasmosis.

This review gives special importance to the facts and work done in the Southern most states, but the significant contributions of researchers from other parts of Brazil must also be recognized. Thus, the major contri-

butions of two great schools in uveitis in Brazil should be mentioned: the Federal University of Minas Gerais, in Belo Horizonte, under the leadership of Prof. Fernando Oréfice, and the Federal University of São Paulo, in SP, led by Prof. Rubens Belfort Jr. Likewise, the major role of the Brazilian Society of Uveitis, since it was founded in the 80s, should be highlighted, because it has been the privileged forum where all researchers throughout the country have debated a great number of issues aroused by ocular toxoplasmosis.

In the last 10 years, we have verified a tendency toward clinical benignity of the disease. Currently, there are few patients with aggressive lesions as compared to the past and the same seems to be happening in the endemic areas (Estacia et al. 2007). The prevalence of ocular disease, all over the state of RS, appears to be much lower than 30 years ago. We believe that better and more widespread knowledge of the disease has contributed to this, both among those who deal directly with health care - physicians, health care agents, public agencies - and among the general population as a whole.

The future paths of research will be to study other aspects of the disease, such as the relationship between the different genotypes and the type of lesion; the rate and types of reinfection by different genotypes; molecular methods of diagnosis that can be applied in clinical practice; new forms of treatment and more effective drugs; applicability of the preventive measures by the public health organisms and the molecular mechanisms involved in the pathogenesis of ocular disease.

We must point out that after many years of significant contact with *T. gondii*, the parasite continues to challenge us and even though much has been learned, there are still many questions to be answered.

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