

EXPERIMENTAL CONGENITAL TOXOPLASMOSIS IN WISTAR AND HOLTZMAN RATS

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Summary :

Congenital toxoplasmosis was evaluated in Wistar and Holtzman rats using two strains of *Toxoplasma gondii* isolated in Brazil. Pregnant rats were inoculated by subcutaneous or intraperitoneal routes with 10^6 or 8×10^6 tachyzoites of N strain (virulent for mice) and by subcutaneous or oral routes with 10^2 or 1.2×10^3 cysts of P strain (avirulent for mice). The tissues of rat pups born from these rats were bioassayed for *T. gondii* infection. *T. gondii* was not observed in the pups born from rats inoculated with N strain. In the animals inoculated with P strain, congenital toxoplasmosis occurred in 22.8 % (Wistar rats inoculated with 10^2 cysts by the subcutaneous route), 11.4 % (Wistar rats inoculated with 10^2 cysts by the oral route), 21.2 % (Wistar rats inoculated with 1.2×10^3 cysts by the oral route) and 2.9 % of fetal infection (Holtzman rats inoculated with 10^2 cysts by the oral route). None of the pups born from chronically infected mother were infected with *T. gondii*.

KEY WORDS : *Toxoplasma gondii*, congenital toxoplasmosis, rats.

Résumé :

La toxoplasmose congénitale expérimentale chez les rats Wistar et Holtzman

La toxoplasmose congénitale a été étudiée chez les rats Wistar et Holtzman en utilisant deux souches de *Toxoplasma gondii* isolées au Brésil. Les femelles gestantes ont été inoculées par les voies sous-cutanée et intra-péritonéale avec 10^6 et 8×10^6 tachyzoïtes de la souche N (virulente pour les souris) et par les voies sous-cutanée et orale avec 10^2 et 1.2×10^3 kystes de la souche P (non virulente pour les souris). Les tissus des nouveaux-nés de ces animaux ont été inoculés chez les souris pour la recherche de *T. gondii*. La transmission congénitale n'a pas été observée chez les animaux inoculés avec la souche N. Par contre, avec la souche P, 22,8 % d'infection fœtale ont été observés chez les rats Wistar gestantes infectées avec 10^2 kystes par voie sous-cutanée, et aussi 11,4 % et 21,2 % d'infection fœtale ont été observés après inoculation par voie orale avec 10^2 et 1.2×10^3 kystes respectivement. Chez les femelles Holtzman infectées avec 10^2 kystes par voie orale, le taux d'infection fœtale de 2,9 % a été obtenu. Les animaux nés lors de la phase chronique ont été sans contamination par *T. gondii*.

MOTS CLÉS : *Toxoplasma gondii*, toxoplasmose congénitale, rats.

Toxoplasmosis is a coccidian infection caused by the obligate intracellular protozoan *Toxoplasma gondii*. For most immunocompetent individuals the infection is asymptomatic but severe toxoplasmosis can occur in immunocompromised patients and in infected fetuses *in utero*. The latter may be aborted or present neurological and ophthalmological disorders (Desmonts & Couvreur, 1974). Congenital toxoplasmosis is also important in animals, because it is considered one of the major causes of abortion and neonatal losses in goats and sheep (Dubey & Beattie, 1988).

The development of a suitable animal model of congenital toxoplasmosis is essential to evaluate the efficacy

of vaccines and new drugs. Dubey & Shen (1991), Zenner *et al.* (1993) and Dubey *et al.* (1997) have used rats as models in the study of congenital toxoplasmosis and have observed that congenital transmission only occurs in rats that are infected during pregnancy and that maternal chronic infection protects the pups against congenital toxoplasmosis. Zenner *et al.* (1993) observed that congenital toxoplasmosis occurred in Fisher rats infected with virulent (RH) or avirulent (Prugniaud and 76K) strains of *T. gondii*. Dubey *et al.* (1997) observed that, unlike mice, repetition of congenital infection by *T. gondii* in successive generations of Sprague Dawley rats occur in less than 1 % of cases. The purpose of this work was to study congenital toxoplasmosis in Wistar and Holtzman rats infected with a virulent (N) and an avirulent (P) strain of *T. gondii* isolated in Brazil, verifying the frequency of congenital transmission in rats infected during pregnancy and the possibility of occurrence of materno-fetal transmission in reinfected rats during the chronic infection.

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MATERIALS AND METHODS

ANIMALS

Female Wistar rats and female Holtzman rats three months old were used for mating. Swiss Webster mice were used for inoculation of rat fetal tissues (bioassay). All animals used in the experiment were obtained from CEBIO-Federal University of Minas Gerais (Belo Horizonte, Brazil) and were tested for the presence of *T. gondii* antibodies.

STRAINS OF *T. GONDII*

The N and P strains of *T. gondii* were used for the infection of the female rats. The N strain, isolated from a rabbit by Nobrega *et al.* (1952) is highly virulent for mice, causing 100 % of mortality between ten and 15 days after the infection of these animals with one tachyzoite by the intraperitoneal route. This strain is maintained through successive passages of tachyzoites by the intraperitoneal route in mice every 48-72 hours. The P strain was isolated from a dog by Jamra & Vieira (1991) and is mildly virulent for mice, with 100 % survival for 180 days after the infection of mice with ten cysts by the oral route. This strain is maintained by the oral inoculation of cysts in mice every six months.

MATING AND INFECTION OF FEMALE RATS

Three female rats of each strain were kept in separate cages with one *T. gondii* negative male of the respective strain during seven days for mating. After the period of mating, the female rats were housed in individual cages and grouped according to the inoculum. The infection of the animals was made on the 14th day after the beginning of mating, which corresponds to the period between the 7th and 14th days of pregnancy. Eight groups of animals (six rats per group)

were infected with *T. gondii* as shown in Table I. One animal from group W1, W6, H1 and two animals from group W4, H2 did not give birth.

REINFECTION OF THE FEMALE RATS

The rats of the groups W3, W4, W5, W6, H1 and H2 were mated again between 60 and 80 days after primary infection and received the same inoculum between the 7th and 14th days of pregnancy by the same route of infection used initially.

INVESTIGATION OF *T. GONDII* INFECTION IN FEMALE RATS

The rats were bled from the orbital sinus before the infection and before the reinfection (30 or 60 days after primary infection) and their sera were tested for the presence of *T. gondii*-specific IgG antibodies by the Enzyme-Linked Immunosorbent Assay (ELISA) (Voller *et al.*, 1976) and Indirect Immunofluorescence Assay (IFA) (Camargo, 1964). The rats were examined for *T. gondii* infection by microscopy of brain tissue cysts or by intraperitoneal subinoculation of brain into healthy mice.

BIOSSAY

For evidence of congenital transmission of pups that were born of infected rats with *T. gondii* during pregnancy the following protocol was used. After birth, the pups were killed between 0 and 12 days age and from each pup the lungs, heart and liver were removed. Tissues were homogenized in 1 ml of phosphate buffered saline (PBS) pH 7.2. One aliquots of 0.5 ml of homogenate of tissues of each pup was intraperitoneally inoculated into one uninfected mouse. Thirty days after inoculation, the surviving recipient mice were bled from the orbital sinus and the sera obtained were tested for the presence of antibodies to *T. gondii*.

Groups	Number of female rats	Inoculum	Number of female rats that transmitted the parasite (%)	Total of pups	Number of infected pups
W1	05	10 ⁶ N strain tachyzoites-intraperitoneal route	0 (0)	51	0 (0)
W2	06	10 ⁶ N strain tachyzoites-subcutaneous route	0 (0)	63	0 (0)
W3	06	10 ² P strain cysts-subcutaneous route	03 (50)	57	13 (22.8)
W4	04	10 ² P strain cysts-oral route	02 (50)	35	04 (11.4)
W5	06	8 × 10 ⁶ N strain tachyzoites-intraperitoneal route	0 (0)	50	0 (0)
W6	05	1.2 × 10 ³ P strain cysts-oral route	04 (80)	52	11 (21.2)
H1	05	10 ⁶ N strain tachyzoites-intraperitoneal route	0 (0)	43	0 (0)
H2	04	10 ² P strain cysts-oral route	01 (25)	35	01 (2.9)

Table I. – Experimental congenital toxoplasmosis in Wistar and Holtzman rats in acute stage of the infection inoculated with tachyzoites (N strain) and cysts (P strain) of *Toxoplasma gondii* between the 7th and 14th days of pregnancy.

by ELISA and IFA. Moreover, the brains of these mice were removed for microscopic examination to verify the presence of cysts. Mice were considered *T. gondii* positive when tachyzoites were found in the peritoneal fluid, cysts were seen in the brain or antibodies were sought in their sera.

LACTOGENIC TRANSMISSION OF *T. GONDII*

To study transmission of *T. gondii* through milk, seven Wistar rats and three Holtzman rats serologically negative to *T. gondii* were inoculated immediately after the delivery with 1.2×10^3 and 10^2 cysts of the P strain by the oral route, respectively. The pups suckled normally within a period of ten to 15 days. After this period, they were sacrificed and their lungs, heart and liver were homogenized in 1 ml of PBS (pH 7.2) and bioassayed in mice. The methodology used for detection of the parasite was the same used in the congenital transmission experiments.

STATISTICAL ANALYSIS

The significance of observed differences between groups of rats was assessed by the chi-square test ($p = 0.05$).

RESULTS

The frequency of congenital toxoplasmosis in Wistar and Holtzman rats infected with the N and P strains is summarized in Table I. None of the pups born to dams inoculated with N strain (Groups W1, W2, W5 and H1) was infected with *T. gondii*. However, after infection with the P strain (Groups W3, W4, W6 and H2), it was observed that nine of the fifteen Wistar rats and one of the four Holtzman rats transmitted *T. gondii* to their pups. The frequency of congenital transmission in the groups W3, W4, W6 and H2 was 22.8 %, 11.4 %, 21.2 % and 2.9 %, respectively. The results obtained demonstrated differences in the frequency of congenital toxoplasmosis in Wistar rats (Group W4) compared to Holtzman rats (Group H2) inoculated by the same route and with the same quantity of parasites. In the same way there were observed differences in the frequency of congenital toxoplasmosis in rats inoculated by the subcutaneous route (Group W3) compared with the oral route (Group W4) and in rats infected with 1.2×10^3 cysts (Group W6) and 10^2 cysts (Group W4). Nevertheless, these differences were not statistically significant.

None of the rats reinfected during the second pregnancy transmitted the parasite to their pups.

ELISA (Mean absorbance) and IFA ($\geq 1: 2.048$) results revealed that all the female rats were infected after the first inoculum. Tissue cysts were observed in the brain

of female rats inoculated with the P strain, but not with the N strain.

There was no transmission of *T. gondii* through milk in any of the 50 pups of Wistar rats inoculated with 1.2×10^3 cysts of the P strain by the oral route, like in 23 pups of Holtzman rats inoculated with 10^2 cysts of the P strain by the oral route.

DISCUSSION

In pregnant rats inoculated with tachyzoites of the N strain (highly virulent for mice), no case of congenital transmission was observed, whatever the rat strain (Wistar or Holtzman), the quantity of parasites (10^6 or 8×10^6 tachyzoites) and the route of inoculation (intraperitoneal or subcutaneous). In literature, there are no reports of strains of *T. gondii* that do not produce congenital infection in rats that received the primoinfection during pregnancy. Thiermann (1957), Remington *et al.* (1961) and Zenner *et al.* (1993) observed rats that received the primoinfection during pregnancy with tachyzoites of *T. gondii* strains, which have similar behavior to N strain, transmitted the parasite to their pups. Zenner *et al.* (1993) obtained 58.2 % of transmission using 8×10^6 RH strain tachyzoites, while in the present work, with the use of 10^6 or 8×10^6 tachyzoites of N strain, no case of congenital transmission was observed. It is possible that some pups sacrificed later might have been infected by the congenital route with N strain, however the infection was already eliminated at the moment of the bioassay. *T. gondii* was not isolated from brain of rats infected with the virulent N strain, although antibody response suggests active infection. Cystogenesis was not observed, indicating cystogenic incapacity of N strain in Wistar and Holtzman rats.

The congenital transmission of the parasite occurred in all the primoinfected groups, whose rats were infected with cysts of the P strain during pregnancy. The transmission occurred either in Wistar rats or in Holtzman rats, whatever of the route of inoculation (subcutaneous or oral) and the number of parasites (10^2 or 1.2×10^3 cysts). In the present work it was used the same quantity of cysts of the P strain as determined by Zenner *et al.* (1993) with 76K and Prugniaud strains. However, these authors observed a rate of transmission of 35.2 % and 62.8 % respectively compared to 21.2 % in Wistar rats. Zenner *et al.* (1993) observed that all the rats infected with 76K and Prugniaud strains transmitted the parasite to their pups, while only 80 % of the rats inoculated with 1.2×10^3 cysts of the P strain were able to transmit the parasite. Probably, the 76K and Prugniaud strains are more invasive than the P strain, decreasing the time of spreading until reaching

the fetal tissues. Also, it is probable that different strains of rats such as Fischer rats used by Zenner *et al.* (1993) show different susceptibility to *T. gondii* considering the low frequency of congenital transmission observed in Holtzman and Wistar rats. Probably, if, in our work, the brain had been included in the pool of organs used in the bioassay, the frequency of congenital transmission could have been higher. It may be possible that the pups that were sacrificed 12 days after birth had brain cysts and rare parasites in the lung, heart and liver already. Bioassay using fetus homogenate according to Zenner *et al.* (1993) will probably increase the rate of transmission observed in our experiments.

No rats of the groups W3, W4, W5, W6, H1 and H2 reinfected during the second pregnancy transmitted the parasite to their pups. Similarly to this study, Thiermann (1957) with the Santiago and St./P1 strains, Remington *et al.* (1961) with the RH and S6 strains, Dubey & Shen (1991) and Zenner *et al.* (1993) did not find congenital transmission of the parasite from chronically infected rats either. However, Thiermann (1957) with the C.M strain, Remington *et al.* (1958) with RH strain, Remington *et al.* (1961) with the Beverley strain and Dubey *et al.* (1997) observed a low rate of congenital transmission in pups born from chronically infected mothers (for review, see Dubey & Frenkel, 1998).

Johnson (1997) recently hypothesized that life cycle of *T. gondii* can be maintained solely by vertical route. However, in our studies, vertical or lactogenic transmission of the virulent N strain was not observed in Wistar and Holtzman rats. These results suggest that the natural life for the virulent strains of *T. gondii* hypothesized by Johnson (1997) is not applicable for all the rats or *T. gondii* lineage.

The results obtained permit to conclude that congenital transmission depends on the strain of *T. gondii* used to infect female Wistar and Holtzman rats and the period of the maternal infection. Therefore, this experimental model can be used to evaluate the methods of antenatal diagnosis and test chemotherapeutic agents and vaccines.

ACKNOWLEDGEMENTS

We thank José Luiz de Faria and Rosalida Estevam Nazar Lopes for technical assistance. This study was supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

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Reçu le 28 juillet 1998

Accepté le 29 octobre 1998