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Susceptibility to Vertical Transmission of *Toxoplasma gondii* is Temporally Dependent on the Preconceptional Infection in *Calomys callosus*

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Abstract

Toxoplasma gondii is an obligate intracellular parasite that causes a variety of clinical syndromes, but the infection is more severe in immunocompromised individuals and in cases of congenital toxoplasmosis. This study aimed to verify if the susceptibility to vertical transmission of *Toxoplasma gondii* is temporally dependent on the preconceptional infection in *Calomys callosus*. Twelve *C. callosus* females were infected with 20 cysts of *T. gondii* ME49 strain and divided into three groups of four animals that were mated after approximately 10 days (group 1), 30 days (group 2), and 50 days (group 3) of infection. The animals were sacrificed from the 17th to 20th day of pregnancy, when placentas and embryos were collected for morphological and immunohistochemical studies, mouse bioassay for evaluating seroconversion and PCR for detecting parasite DNA. Serum samples from *C. callosus* females and mice used in bioassay were analysed for the detection of IgG antibodies to *T. gondii* by ELISA. Detection of *T. gondii* was observed by mouse bioassay and PCR in placentas and embryos from *C. callosus* females infected around 10 days pre-conception. However, only placentas, but not embryos, from females infected around 30 and 50 days pre-conception showed positivity for parasite DNA and seroconversion by mouse bioassay. In conclusion, this study model shows that vertical transmission of *T. gondii* may take place when maternal infection occurs within one month before conception, thus demonstrating the time of preconceptional seroconversion that rule out a risk of congenital toxoplasmosis.

Keywords: *Toxoplasma gondii*; *Calomys callosus*; Congenital toxoplasmosis; Preconceptional infection; Maternal–fetal interface

1. Introduction

Toxoplasmosis is an infection caused by the protozoan parasite *Toxoplasma gondii* and is usually asymptomatic in immunocompetent subjects. However, when the infection occurs in immunocompromised patients or during pregnancy, it can lead to serious disorders as encephalitis or congenital toxoplasmosis, respectively [1]. The vertical transmission is not obligatory and occurs in 20–50% of maternal primary infection by *T. gondii* during pregnancy [2] with a more frequent involvement of the strains I and II in humans [3].

Maternal infection prior to conception normally excludes the risk of fetal infection and congenital toxoplasmosis, which usually follows *T. gondii* infection acquired in early pregnancy [1]. However, preconceptional infection can occasionally lead to implication for the fetus in immunodeficient women [4] and in immunologically competent women with clinical signs as cervical adenopathies [5,6]. Accordingly, two unusual cases of congenital toxoplasmosis were reported, one occurring after preconceptional infection with cervical adenopathies and the other occurring after maternal infection at the very end of pregnancy with maternal seronegativity at delivery [7]. In

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the infection acquired at the very end of pregnancy, with the mother still seronegative at delivery, the rate of transmission from mother to fetus is around 70% during this period [8]. Thus, the risk of congenital toxoplasmosis can be better evaluated when the maternal seroconversion and the time of primary infection is more accurately determined to lead to an efficient therapeutic strategy. If the infection is confirmed, anti-parasitic treatment during pregnancy may prevent fetal infection and serious damages, such as chorioretinitis or neurological defects [1].

Calomys callosus, a rodent of the family Cricetidae widely distributed in Central Brazil, has been reported as important natural reservoir for *Trypanosoma cruzi* [9,10], but also susceptible to several infectious diseases as schistosomosis and leishmaniasis [11]. Our previous studies have demonstrated the high susceptibility of *C. callosus* to *T. gondii* infection, when the presence of the parasite was detected in several tissues, particularly liver, spleen, lung and brain [12]. Also, this rodent was demonstrated to be a suitable experimental model to study the dynamics of congenital toxoplasmosis, due to the ability of a highly virulent strain of *T. gondii* (RH strain) to infect trophoblast cells during the early blastocyst–endometrial relationship [13]. In another previous study, we demonstrated the vertical transmission of *T. gondii* in *C. callosus* acutely infected with the ME49 strain during pregnancy, but not in chronically infected animals. In addition, considering the sequence of events leading to the infection of the various organs, the placental trophoblast cells are infected later on during pregnancy, but were unable to completely stop the progression of *T. gondii* infection towards the fetal tissues [14].

Considering the possibility of vertical transmission of *T. gondii* when maternal seroconversion occurs a few weeks before conception and that so far this condition has not been kinetically studied in detail, the present study aimed to verify if the susceptibility to vertical transmission of *T. gondii* is temporally dependent on the preconceptional infection in *C. callosus*.

2. Materials and methods

2.1. Animals

C. callosus of the Canabrava strain came from a resident colony housed at the Institute of Tropical Medicine of São Paulo and were kindly provided by Dr Judith Kloetzel. The animals were kept under specific pathogen-free conditions on a 12-h light, 12-h dark cycle in a temperature-controlled room (25 ± 2 °C) with food and water *ad libitum*. All procedures were conducted according to institutional guidelines for animal ethics.

2.2. Parasites

Cysts of *T. gondii* ME49 strain were obtained from brains of *C. callosus* infected 30–45 days earlier with 20 cysts via oral route. The brains were removed, washed in sterile 0.01 M phosphate-buffered saline (PBS) pH 7.2, and homogenised with a syringe and a 25×7 gauge needle. The brain preparations were further washed by centrifugation at $1000 \times g$ for 10 min in PBS and cysts were counted under light microscopy (10× magnification).

2.3. Experimental groups

Twelve *C. callosus* virgin females aged 2–3 months were perorally infected with 20 cysts of *T. gondii* ME49 strain and then divided into three groups of four animals that were mated with males after different times of infection as follows: group 1 (8–13 days after infection), group 2 (28–35 days after infection), and group 3 (42–57 days after infection). The presence of a vaginal plug was considered 1st day of pregnancy (dop).

The animals were euthanised from the 17th to 20th dop, when placentas and embryos were collected for morphological and immunohistochemical assays, mouse bioassay and polymerase chain reaction (PCR) for the detection of *Toxoplasma*. Blood samples were collected at 1st dop and when animals were euthanised (17th to 20th dop) to determine the levels of IgG antibodies to *T. gondii* by immunoenzymatic assays (ELISA).

2.4. Morphological and immunohistochemical assays

For conventional light microscopy, specimens were fixed by immersion in 10% formalin in 0.1 M phosphate buffer (pH 7.4), dehydrated, and embedded in methacrylate resin. Sections measuring 2 μm in thickness were stained with 0.25% toluidine blue and then examined in a photomicroscopy (Reichert-Jung-Polyvar, Lab. Optica Robert Koch, Austria).

For immunolocalisation of the parasites, specimens fixed were dehydrated and embedded in paraffin. Sections measuring 4 μm in thickness were placed on glass slides and processed as previously described [14]. Briefly, samples were first incubated (10 min at room temperature) with 5% acid acetic to block endogenous alkaline phosphatase and then (30 min at 37 °C) with 2% normal goat serum to block non-specific binding sites. Next, samples were incubated (12 h at 4 °C) with rabbit anti-*T. gondii* serum and then (30 min at 37 °C) with biotinylated goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO, USA). The reaction was amplified by using the ABC system (Biomeda, Foster City, CA, USA) and developed with fast red-naphthol (Sigma). Samples were counterstained with Mayer's haematoxylin and examined in photomicroscopy.

2.5. Detection of T. gondii in placentas and embryo tissues

Detection of *T. gondii* was first evaluated by mouse bioassay as described elsewhere [15], with minor modifications. Placentas and embryo tissues (liver and brain) were homogenised in PBS and separately inoculated in mice Swiss by intraperitoneal route, in duplicate. After 30–45 days of inoculation, blood samples from mice were collected and sera obtained were analysed for the detection of IgG antibodies to *T. gondii* in ELISA.

The presence of *T. gondii* DNA was investigated by PCR as previously described [16]. Briefly, placentas and embryo tissues were treated with 100 μg/ml proteinase K (Invitrogen Life Technologies, São Paulo, Brazil) in buffer containing 10 mM Tris–HCl, 0.1 M EDTA, 0.5% SDS, pH 8.0, at 50 °C during 3 h. Samples were then submitted to phenol/chloroform/isoamyl alcohol (25:24:1, pH 8.0) extraction and DNA was precipitated from the aqueous phase by treatment with 2.5 volumes of cold ethanol. Qualitative PCR detecting the 35-copy B1 gene of *T. gondii* was performed using the primers 5′TCTTCCCAGAGGTGGATTTC-3′ (sense, nucleotides 151–171) and 5′CTCGACAATACGCTGCTTG-3′ (antisense, nucleotides 682–663) (Invitrogen Life Technologies, São Paulo, Brazil), which should amplify a fragment of 531 bp. After initial incubation for 3 min at 95 °C, samples were subjected to 38 cycles of denaturing at 94 °C for 1 min, annealing for 1.2 min at 62 °C, and extension for 2 min at 72 °C [17]. PCR products were analysed by 1% agarose gel containing 0.5 μg/ml of ethidium bromide and visualised under UV illumination.

2.6. ELISA and ELISA-avidity

A conventional ELISA to detect IgG antibodies to *T. gondii* was carried out as previously described [14] with some modifications in order to confirm the pre-conceptional seroconversion as indicator of infection of *C. callosus* females. Polystyrene microtitre plates were coated overnight at 4 °C with *T. gondii* soluble antigen at 10 μg/ml in carbonate buffer 0.06 M (pH 9.6). Plates