Evaluation of vertical transmission of *Toxoplasma gondii* in *Calomys callosus* model after reinfection with heterologous and virulent strain

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**A B S T R A C T**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite that causes a variety of clinical syndromes, but the infection is severe in immunocompromised individuals and during pregnancy due to the possibility of transplacental transmission of the parasite causing congenital toxoplasmosis. Vertical transmission of the parasite usually occurs when females are primarily infected during pregnancy. *Calomys callosus* is resistant to *T. gondii* ME49 strain, which presents a moderate virulence and congenital disease occurs only during the acute phase of infection. The aim of this study was to determine whether vertical transmission occurs when females of *C. callosus* chronically infected with ME49 strain of *T. gondii* are reinfected with a highly virulent strain (RH, type I). Females were infected with cysts of the ME49 strain. On the 1st day of pregnancy, animals were reinfected with tachyzoites of the RH strain. In the 19th day of pregnancy, placentas and embryos were processed for morphological analysis, immunohistochemistry and for detection of the parasite by PCR and mouse bioassay. Morphological and immunohistochemical analyses revealed the presence of parasites only in placental tissues. Mouse bioassay results showed seroconversion only in mice that were inoculated with placental tissues. Also, *T. gondii* DNA was detected only in placental samples. Congenital toxoplasmosis does not occur in *C. callosus* females chronically infected with the moderately virulent ME49 strain of *T. gondii* and reinfected with the highly virulent RH strain, thus indicating that primary *T. gondii* infection before pregnancy leads to an effective long-term immunity preventing transplacental transmission to the fetus.

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**1. Introduction**

*Toxoplasma gondii* is an obligate intracellular protozoan parasite and an important opportunistic pathogen for humans and animals because it infects a wide range of hosts [1]. *T. gondii* infection can cause severe life-threatening disease in immunocompromised patients and congenital toxoplasmosis in newborns [2]. Tissue pathology associated with *T. gondii* infection results from parasite-induced destruction of host cells, and it can be related to strain virulence [3]. *T. gondii* is distributed in nature as a heterogeneous population that can be defined in genetic profiles consisting of three predominant clonal lineages (I, II and III) [4]. Infection in human as well as in animals, may occur with all genotypes, although the level of virulence varies between the genotypes [3,4]. Type I strains are virulent for the mouse, and infection with a single parasite results in the death of the animals. In contrast, types II and III are relatively avirulent in mice, frequently resulting in chronic infections. Recombinant and atypical strains have also been reported [3–5], particularly in Brazil where it was recently identified a higher genetic diversity than previously recognized [6].

Vertical transmission generally occurs when a woman is primarily infected with *T. gondii* during pregnancy [7], although rare exceptions have been reported in which women were infected just before pregnancy [8]. In addition, reactivation of an infection acquired before pregnancy can lead to congenital toxoplasmosis in immunocompromised women [9], whereas the transplacental transmission of *T. gondii* after maternal reinfection during pregnancy was already reported in immunocompetent women [10]. The risk of congenital infection is lower when maternal infection takes place in the first trimester (10–15%) and higher when infection occurs during the third trimester (60–90%) of pregnancy [7]. The vertical transmission is not obligatory, being observed in 20%–50%.
of maternal primary infection by *T. gondii* during pregnancy [11], mostly by types I and II strains in humans [2].

During the acute phase of infection, fastly-replicating tachyzoites convert into the latent or chronic phase with slowly-replicating bradyzoites in tissue cysts [12]. It has been accepted for years that primary *T. gondii* infection leads to life-long immunity preventing reinfection [13,14]. This view has been questioned by several authors using immunocompetent murine models [13–15]. These studies found that protection can be breached after reinfection with parasites belonging to different genotypes.

Congenital toxoplasmosis has been described in the literature in a variety of experimental models [16,17]. *Calomys callosus*, a rodent of the family Cricetidae widely distributed in Central Brazil, has been used in our previous studies, demonstrating its high susceptibility to *T. gondii* infection [18]. Also, this rodent was shown 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 [17]. In another study, we demonstrated the vertical transmission of *T. gondii* in *C. callosus* acutely infected with the relatively avirulent ME49 strain during pregnancy, but not in chronically infected animals [19]. Recently, we reported 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 [20].

Considering that a primary *T. gondii* infection can provide protective immunity against reinfection, but the risk of congenital toxoplasmosis during pregnancy is unclear when the infection occurs among parasites with different genotypes, the present

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**Fig. 1.** Photomicrographs of placenta and embryo tissues from *Calomys callosus* chronically infected with ME49 strain of *T. gondii* and reinfected with RH strain after different days of primary infection (doi). Group I, after 48–53 doi; group II, after 58–63 doi; group III, after 73–78 doi; and group IV, after 88–93 doi. Staining by toluidine blue shows the presence of parasites in (a) placenta labyrinth cells (asterisk) from females of group II and (b) placenta decidua (arrowhead) from females of group IV. Immunohistochemical staining using alkaline phosphatase and fast red-naphthol showing the presence of parasites (arrows) in (c) placenta labyrinth zone from females of group I; (d) placenta labyrinth zone from females of group II; (e) placenta decidua from females of group III; and absence of parasites in (f) embryo liver cells from group III. Counterstaining by Mayer’s haematoxylin. Bar scale: (a, c–f): 14 μm; (b): 35 μm.
study aimed to verify if the reinfection with the highly virulent T. gondii RH strain may cause vertical transmission of the parasite in C. callosus chronically infected with the relatively avirulent ME49 strain.

2. Materials and methods

2.1. Animals

Calomys callosus and Swiss mice were kept under standard conditions on a 12 h light, 12 h dark cycle in a temperature-controlled room (25 ± 2°C) with ad libitum food and water in the Bioterism Center and Animal Experimentation, Federal University of Uberlandia, Brazil. Animal experiments and procedures were conducted according to institutional guidelines for ethics in animal experimentation.

2.2. Parasites

Cysts of T. gondii ME49 strain were obtained from brains of C. callosus after 30–45 days of infection as described previously [20]. Briefly, brains were removed, homogenized, washed in sterile phosphate-buffered saline (PBS, pH 7.2) at 1000 × g for 10 min and cysts were counted under light microscopy (10x magnification).

The reinfection was performed with an intraperitoneal inoculum of 100 tachyzoites of the highly virulent RH strain at the 19th dpo, when placentas and embryos were collected for morphological and molecular analysis. The presence of vaginal plug was considered as first day of pregnancy (dop).

Blood samples were collected from all animals at the 1st dop before reinfection with RH strain for analysis of antibodies to T. gondii. The animals were euthanized on the 19th dop, when placentas and embryos were collected for morphological and immunohistochemical assays, mouse bioassays and polymerase chain reaction (PCR) for the detection of genomic DNA from T. gondii.

2.3. Experimental groups

Sixteen C. callosus virgin females, aged 2–3 months, were perorally infected with 20 cysts of T. gondii ME49 strain and then divided into four groups (n = 4) and placed with males after different days of primary infection (doi), as follows: group I (48–53 doi), group II (58–63 doi), group III (73–78 doi) and group IV (88–93 doi).

The reinfected females became seropositive for T. gondii, regardless of the group. In addition, T. gondii-specific IgG antibodies were detected in all mice that were inoculated only with placentas from infected C. callosus, regardless of the group. In Table 1, Swiss mice were inoculated either with placenta or embryo tissues from C. callosus females reinfeet with T. gondii RH strain after different days of primary infection with ME49 strain. Mouse seroconversion was analyzed after 15, 30 or 45 days of inoculation by ELISA.

To confirm preconceptional seroconversion of female C. callosus, as well as the seroconversion of Swiss mice inoculated in bioassay experiments as an indicator of T. gondii infection, an indirect ELISA to detect serum IgG antibodies to T. gondii was carried out as previously described [20].

2.6. Seroconversion assay

For all groups, placentas of T. gondii RH strain-reinfected C. callosus showed a normal appearance, but parasites, when present, were noted in basal deciduas and in the labyrinth zone (Fig. 1a and b). Immunohistochemical assay confirmed the presence of T. gondii in the decidua and labyrinth cells (Fig. 1c–e). No parasites were found in embryo tissues (Fig. 1f).

3. Results

3.1. Morphological and immunohistochemical assays after T. gondii reinfection

After infection with ME49 strain and before conception and reinfection, all C. callosus females became seropositive for T. gondii, as determined by ELISA. In addition, T. gondii-specific IgG antibodies were detected in all mice that were inoculated only with placentas from infected C. callosus, regardless of the group. In addition, when inoculated with embryos, no seroconversion determined by the absence of antibodies to T. gondii.

Table 1

<table>
<thead>
<tr>
<th>Groups of C. callosus*</th>
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* A total of 64 Swiss mice were used for seroconversion assays, with two mouse per tissue sample (placenta and embryo tissue) or four mice per pregnant C. callosus female, totaling 16 mice per group of pregnant C. callosus females. No seroconversion determined by the absence of antibodies to T. gondii.
contrast, seroconversion was not detected in any animals inoculated with only embryo tissues from all groups (Table 1).

Similar results were obtained by PCR and T. gondii DNA was detected in placentas from all females, but not in embryo tissues (Fig. 2).

4. Discussion

Calomys callosus are resistant to ME49 strain of T. gondii and congenital transmission occurs only during the acute phase of the infection [19,20]. A previous study in C. callosus showed that the time between infection and maternal resistance to vertical transmission of T. gondii is approximately 60 days [20]. However, there is limited information concerning the time of acquisition of maternal resistance to T. gondii infection when animals are reinfected with different genotypes during the chronic phase of the infection. In this study, our data demonstrated that reinfection with the highly virulent RH strain (type I) was unable to cause vertical transmission in C. callosus chronically infected with the moderately virulent ME49 strain (type II).

The morphological organization of the placenta from rodents consists of three regions: decidual layer, junctional zone and labyrinth zone [24,25]. In the present study, the placentas of C. callosus showed the same regions, with no morphological change in the placental architecture observed in the tissues of females from all four experimental groups.

The immunohistochemical analysis of placental tissues of C. callosus demonstrated the presence of parasites in the decidual layer and specifically in the labyrinth. The area of the labyrinth is bathed directly by maternal blood, which facilitates the contact of the cells of this region with the parasite [19]. Conversely, no parasites were seen in the junctional zone of the placenta. This can be explained by the fact that, in this region, T. gondii invasion occurs later because certain cells at this place, for example, spongiotrophoblasts, are isolated from maternal blood by trophoblastic giant cells.

In the present study, seroconversion to T. gondii occurred only in mice inoculated with homogenized placentas, regardless of the group. Although the parasite was able to reach the placentas in all experimental groups later on, no embryo tissues became infected at any time point, indicating the putative role of the placenta as an efficient barrier. These findings were confirmed by the absence of parasites carried out by immunohistochemical analysis from embryonic tissues. Accordingly, PCR analysis confirmed the absence of vertical transmission of T. gondii in the present study, since the detection of DNA was observed only in samples of placental tissue.

The hypothesis accepted for years indicating that primary T. gondii infection leads to life-long immunity preventing vertical transmission of the parasite has been recently questioned. Reinfection of chronically infected rats with homologous T. gondii strains was able to avoid the vertical transmission of the parasite, while reinfection with heterologous challenge showed a considerable amount of parasites in embryonic tissues [22]. It was also demonstrated that the transmission of the parasite in cases of reinfection seems to be possible only when the strains have different genotypes [14]. It should be emphasized, however, that cases of reinfection with T. gondii involving an immunocompetent pregnant woman are exceptional, but show that the presence of residual specific antibodies is not always indicative of protection against a new infection [26].

Thus, T. gondii reinfection with a different strain is a rare event as evidenced by few reports available in the literature. In the present study, however, we observed that reinfection with a different T. gondii genotype was able to prevent vertical transmission of the parasite in the C. callosus model, suggesting that primary infection with the ME49 strain prevents congenital toxoplasmosis even when a highly virulent type I strain as RH strain was used for reinfection. Atypical genotypes have been described in South America with significant genetic differences from Eurasia, Africa and North America populations [27]. It has been reported that the immunity against European T. gondii strains may not protect against reinfection by atypical strains as well as the immunity against South American not protect against reinfection by European T. gondii strains [26].

In conclusion, we showed that congenital toxoplasmosis does not occur in C. callosus females previously infected with the moderately avirulent ME49 strain of T. gondii and reinfected with the highly virulent RH strain, thus indicating that primary T. gondii infection leads to an effective long-term immunity preventing the occurrence of vertical transmission of the parasite in this model.

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