

Rat Model of Congenital Toxoplasmosis: Rate of Transmission of Three *Toxoplasma gondii* Strains to Fetuses and Protective Effect of a Chronic Infection

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The incidences of congenital toxoplasmosis in Fischer rats infected between the 8th and 12th days of pregnancy with three different strains of *Toxoplasma gondii* (RH, 76K, and Prugniaud) were 58.2, 35.2, and 62.8%, respectively. No infected fetuses were collected from rats previously infected with RH or Prugniaud strain parasites, even if the rats were reinfected during pregnancy. Since pups from chronically infected mothers are protected from congenital toxoplasmosis, rat infection could thus constitute a relevant model for immunological studies and vaccine design.

Toxoplasmosis is a widespread infection caused by the intracellular protozoan *Toxoplasma gondii* (8). This infection is generally mild or even totally inapparent in the human population except in immunosuppressed patients or fetuses infected in utero. Fetal infections by *T. gondii* can result in neurological sequelae, congenital malformations, and ocular disorders (7). The severity of human congenital toxoplasmosis and its importance as a public health concern are now recognized by many scientists and physicians (20). Congenital toxoplasmosis is also of considerable economic importance in the livestock industry, where sheep and goats are affected.

Mouse congenital toxoplasmosis was described as early as 1950 (4). Since that time, this animal model has been frequently used for experimental studies (13, 15, 27) and for vaccination trials (21), even though it had been reported that congenital infection can occur during chronic infection (23) and that several generations can be congenitally infected (2). In contrast, Roberts and Alexander (24) recently demonstrated that fetuses from chronically infected BALB/c mice are protected against congenital infection. Surprisingly, there are only limited data concerning congenital toxoplasmosis in the rat (9, 23). However, *T. gondii* infection of rats has several features that make it a potentially interesting model for the study of human toxoplasmosis: for instance, euthymic rats are resistant to *T. gondii* infection (16) as humans are, whereas immunocompromised rats such as nude rats are highly susceptible (26). In our laboratory, we have previously shown that the passive transfer of sera or lymph node cells from immune euthymic rats significantly protects these nude rats (5, 10, 11).

The purpose of the present work was to develop an experimental model of rat congenital toxoplasmosis by analyzing the rate of maternofetal transmission of the parasite after infection with different *T. gondii* strains during rat pregnancy and the protective role of chronic infection.

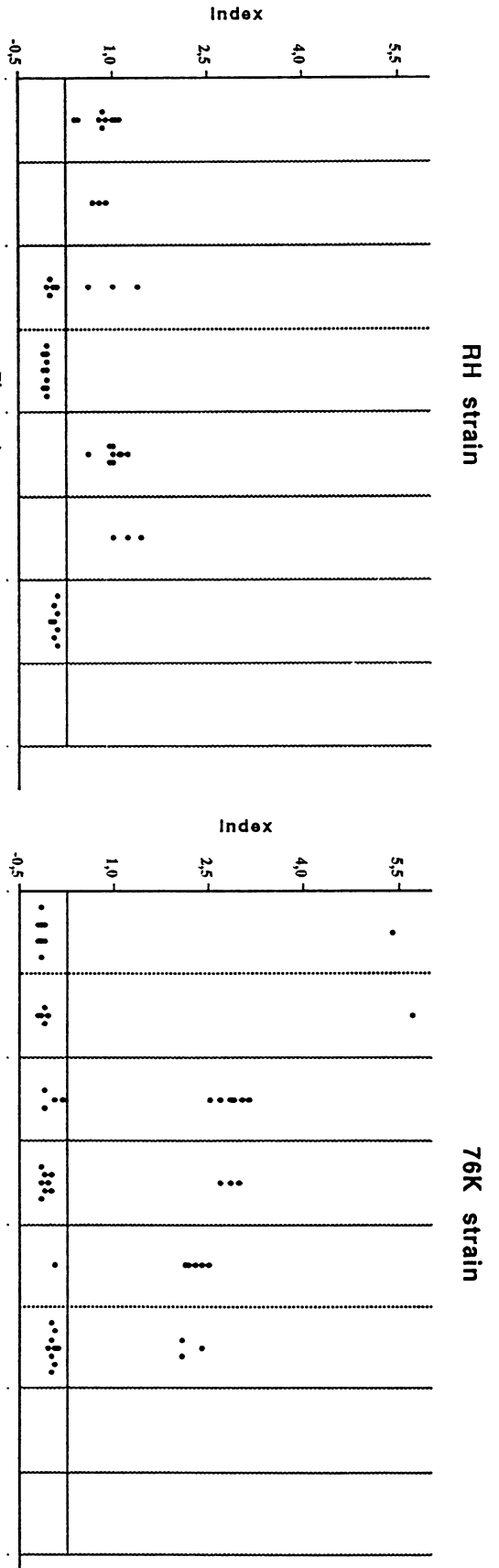
Virgin female Fischer rats from the animal facilities of

Institut Pasteur de Lille (Lille, France) were 16 to 20 weeks old when used for reproduction. Male Fischer rats (15 to 40 weeks old) and female OF1 mice (8 to 10 weeks old) obtained from IFFA-CREDO laboratories (L'Arbresle, France) were used for mating and for inoculation of rat fetal tissues, respectively. All animals were tested for the absence of toxoplasmosis by study of their sera by an enzyme-linked immunosorbent assay (ELISA) (6) before experimentation. Three female rats were caged with one male at day 0 for 4 days and infected 12 days after, which corresponds to the period between the 8th and 12th days of pregnancy. The virulent RH strain of *T. gondii* (25) and the 76K strain (17) were maintained in our laboratory and used for infection as described previously (12). The Prugniaud strain of *T. gondii* (19) was used as described for the 76K strain. Fetuses were delivered via laparotomy 2 or 3 days before parturition. After euthanasia, fetuses were thoroughly washed with distilled water in order to avoid possible contamination with circulating parasites from maternal blood. They were then homogenized in a Potter's tube in 4 ml of RPMI 1640 medium, and 0.5-ml aliquots of each fetus homogenate were intraperitoneally inoculated into one uninfected mouse. Six weeks later, the sera of mice that survived inoculation were tested for the presence of antibody to *T. gondii* by ELISA (6), and their brains were removed for microscopic examination. Mice that did not survive from 8 days onwards after subinoculation were considered infected, since none of the uninfected mice died in these experiments. Comparison of the results obtained by ELISA and those obtained by the detection of brain cysts was carried out in a series of mice subinoculated with fetuses from litters infected with 76K or Prugniaud strains cysts. The detection of cysts confirmed the value of the ELISA (data not shown).

Maternofetal transmission of parasites after infection with three *Toxoplasma* strains via two different routes of inoculation was investigated: group A consisted of seven pregnant rats intraperitoneally infected with 8×10^6 RH strain tachyzoites, group B contained eight rats perorally infected with 1,200 cysts of the Prugniaud strain, and group C contained six rats perorally infected with 1,200 cysts of the 76K strain. The corresponding controls were five noninfected rats and

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Prugniaud strain

Control

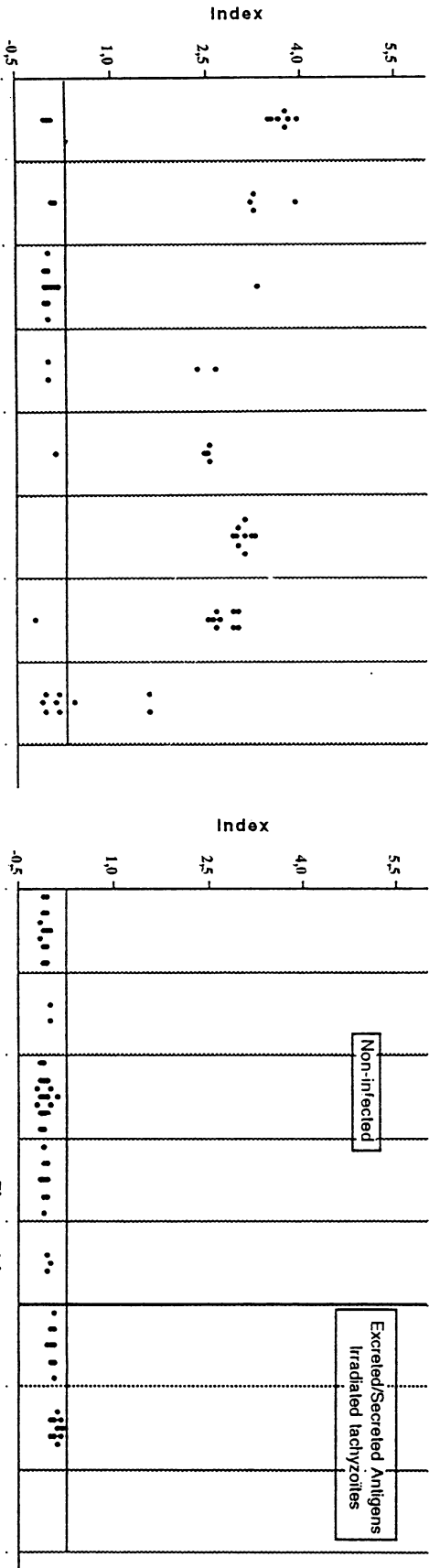


FIG. 1. Antibody responses of surviving mice subinoculated with a homogenate of rat fetuses in the case of a mother primoinfection between the 8th and 12th days of pregnancy. (a) Intrapertoneal inoculum of 8×10^6 RH strain tachyzoites; (b) 1,200 cysts of the Prugniaud strain per os; (c) 1,200 cysts of the 76K strain per os; (d) controls. Each column shows the results for one litter; each point represents the ELISA index of one mouse subinoculated with a single fetus. The index was calculated from the optical density (OD) of a test mouse serum and the OD of two reference sera (one negative and one weakly positive) according to the following formula: $\text{index} = (\text{OD of tested serum} - \text{OD of negative serum}) / (\text{OD of positive serum} - \text{OD of negative serum})$. The mean value of indexes from 32 negative sera was considered positive when its index was higher than this mean value plus five times the standard deviation ($P < 0.001$ by the Student distribution). Bars shows thresholds of positivity.

two rats injected intravenously for 2 h and then 10 min before euthanasia with a mixture of 0.25 ml of excreted-secreted antigens (5) and 8×10^6 RH strain tachyzoites irradiated with 100 Gy for 10 min with an X-ray generator (Philips RT 100, filter 1.7 AI, 100 kV, 8 mA). These latter animals were used to evaluate a possible transplacental passage of antigens to the fetuses, which could have led to false positivity in the ELISA in subinoculated mice.

Results of ELISA of the mouse sera are shown in Fig. 1. After infection with the RH strain (Fig. 1a), 32 (58.2%) of 55 fetuses were found positive, with two litters completely uninfected. In the case of Prugniaud strain (Fig. 1b), all litters were infected, and 44 (62.8%) of 70 fetuses were positive. After infection with the 76K strain, 19 (35.2%) of 54 fetuses were positive, and all litters were also infected (Fig. 1c). All 64 fetuses from control pregnant rats, either not infected or transferred with excreted-secreted antigens and irradiated tachyzoites before delivery, were negative (Fig. 1d).

To investigate the degree of variability in our model, we did similar infections with the Prugniaud strain in two other groups of pregnant rats. The results presented in Table 1 showed parasite transmission in 55 of 74 fetuses and 43 of 59 fetuses; all litters were infected. These results, including those of the first group, were not significantly different according to the chi-square test ($\alpha = 0.05$).

From these data, it seems that oral infections with cysts of the Prugniaud strain constitute the most promising model of congenital toxoplasmosis, since a constant transmission of about 70% was observed, with at least one or more fetuses infected per litter. Very recently, in a Sprague-Dawley rat model using the highly pathogenic CT-1 *Toxoplasma* strain, Dubey and Shen (9) reported results which differed depending on the parasite stage used for the infection but not on the day of infection (from days 7 to 15). The present study, carried out on a larger number of litters (67 litters) and using a different rat strain and other strains and stages of parasites (tachyzoites and cysts), confirms that the transmission rate strongly depends on the parasite (strain, stage, and route of inoculation) and probably also on the rat strain.

In addition, it was surprising that some mice subinoculated with fetuses infected with the highly virulent RH strain did not die but instead developed an antibody response. However, very recently, Lecomte et al. (18) presented evidence for RH cystogenesis in Fischer rats and the subsequent attenuation of RH pathogenicity in mice following a passage of parasites through rats.

The fact that rat placentas are independent can explain why fetuses from the same litter were not all infected. Similarly, in human congenital toxoplasmosis, infection of only one twin has been reported in cases of dizygotic twins (3).

We also considered the amount of postimplantation loss by counting the resorptions in the uterus, but without significant results. Numbers of fetuses per litter were not compared, since this number directly depends on the rat prolificity, which can be extremely variable (14). Moreover, it is well known that the number of resorptions in rodents is high; it can vary from 5 to 20% depending on rat strain and breeding conditions (1). The study of abortion rate in rat congenital toxoplasmosis would require experiments using a larger number of pregnant rats and could be done during a study of teratogenesis and fetal pathology.

As a further parallel to human congenital toxoplasmosis, female rats were infected either intraperitoneally with 8×10^6 RH strain tachyzoites or orally with 1,200 Prugniaud

TABLE 1. Mother-to-fetus transmission of *T. gondii*

Strain	No. of litters		No. of fetuses		% positive fetuses
	Total	Positive	Total	Positive ^a	
RH	7	5	55	32 (6)	58.2
76K	6	6	54	19 (0)	35.2
Prugniaud	8	8	70	44 (5)	62.8
	8	8	74	55 (6)	74.5
	6	6	59	43 (3)	72.9
RH					
Chronic infection	6	0	66	0 (0)	0
Chronic infection and then reinfection	7	0	66	0 (0)	0
Prugniaud					
Chronic infection	10	0	84	0 (0)	0
Chronic infection and then reinfection	9	0	90	0 (0)	0

^a The number of positive fetuses corresponds to the number of ELISA-positive mice combined with the number of mice having died from 8 days onward. Mice accidentally damaged when subinoculated (about three to five of more than 400) died immediately or within 2 to 3 days and were not taken into account.

strain cysts at 7 to 9 weeks before mating. After a check by ELISA showed that these animals were seropositive for *T. gondii*, female rats were mated. In the absence of reinfection during pregnancy, none of the resulting fetuses was infected (Table 1). These results are in agreement with those of Remington et al. (22, 23) with RH and S6 parasite strain and of Dubey and Shen (9) with the CT-1 strain, although exceptional cases of congenitally infected pups from mothers chronically infected with the Beverley strain have been described (23).

Female rats chronically infected as described above were respectively reinfected between the 8th and 12th days of pregnancy by 8×10^6 RH strain tachyzoites or 1,200 Prugniaud strain cysts. The rate of fetal infection fell from 58 and 70 to 0%. These data clearly demonstrate, with two different strains (RH and Prugniaud) and routes of infection (intraperitoneal and oral), that when chronically infected dams are reinfected during gestation, all pups are protected against congenital toxoplasmosis.

In conclusion, after 30 years of silence in the literature on congenital toxoplasmosis in rats, two groups have simultaneously shown promising results with this model (9, 29). In the present study, we have established for the first time to our knowledge that a chronic infection of rats prevents fetal infection after congenital challenge, as observed in the human disease. Thus, rats provide a new experimental model for human congenital toxoplasmosis and should prove useful not only for pathological studies but also for immunological studies and vaccine design. Concerning the latter point, preliminary data obtained in our laboratory (28) indicate that pups born from mothers immunized with *T. gondii* excreted-secreted antigens are significantly protected against congenital infection.

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