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Congenital Transmission of Experimental Chronic Toxoplasmosis in Rats

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ABSTRACT: A 10% transplacental transmission rate was observed in litters from 89 Wistar rats chronically infected with *Toxoplasma gondii,* as judging from bioassays. The rats had been fed *T. gondii* 2 mo prior to mating. Six of 7 isolates of *T. gondii* were transplacentally transmitted. The frequency of transmission did not appear to be affected by the strain of *T. gondii* or the size of the inoculum.

Recently, rats have been proposed as a model for congenital toxoplasmosis of man (Dubey and Shen, 1991; Zenner et al., 1993). One advantage of the rat model is the low transmission rate of *Toxoplasma gondii* from chronically infected rats (Dubey et al., 1997). However, transmission of *T. gondii* was reported in 2% of rats (Thiermann, 1957) and 25% of rats (Remington et al., 1961) inoculated intraperitoneally before pregnancy. The purpose of the present study was to investigate transmission of *T. gondii* in a large number of rats after oral inoculation of *T. gondii* bradyzoites or oocysts before pregnancy.

Groups of 6–10 rats were orally inoculated with tissue cysts of different strains of *T. gondii.* In 1 instance, oocysts were used as inoculum. After 2 mo, the animals were bred. Immediately after delivery, the newborn rats were examined for *T. gondii* infection by bioassay. After 1 mo, anti-*T. gondii* antibodies were determined in the sera of inoculated mice.

Two hundred-gram Wistar rats and 20-g CF-1 mice were used. Both mice and rats were free of *T. gondii* infection as judging from negative results in the direct agglutination (DA) test for *T. gondii* of Desmonts and Remington (1980), using 1:64 as the threshold titer indicative of *T. gondii* infection.

Groups of 6–7 females and 2–3 males were caged together overnight. The next morning, vaginal smears of the rats were made and examined for spermatozoa. Females that had copulated were caged individually. The procedure was repeated until the desired number of copulated females was obtained. Eighty-nine pregnant rats were studied.

The strains of *T. gondii* used in the present study are characterized in Table I. To obtain *T. gondii* tissue cysts, mice were inoculated subcutaneously with approximately 1,000 tachyzoites and 30 mg% sulfamerazine was administered in drinking water for days 3–18 after inoculation when pathogenic strains of *T. gondii* were used. Mice that survived 30–60 days after infection were used as donors of brain cysts. To obtain *T. gondii* oocysts, a recently weaned cat with a negative DA reaction at a titer of 1:64 was used. The cat received brain and the carcass of a mouse with a chronic *T. gondii* infection. Fecal specimens were collected 4–7 days after inoculation, and oocysts were separated by sugar flotation (Frenkel, 1977). Oocysts were incubated in 2% sulfuric acid at room temperature with agitation for 4–7 days. After sporulation, oocysts were stored at 4 C until used. Tissue cysts were counted in 4 × 25-μl aliquots between a slide and a coverslip and diluted in 0.9% NaCl if necessary. The dose was 2×10^2 to 10^4 cysts per rat by mouth. Oocyst suspensions were neutralized with a solution of NaOH, counted in a hemocytometer, and diluted in water as necessary. The dose was 10^4 oocysts per rat by mouth.

Tissues of newborn 1–3-day-old rats were inoculated into mice; 2 mice per litter were used. Tissues (brain, liver, lungs) from half of the litters with 8 or more newborn rats, or tissues from all the fetuses of litters with less than 8 fetuses, were inoculated into mice. Touch smears were made from brain, liver, and lungs of mice that died after inoculation. Mice that sur-

TABLE I. Transmission of congenital toxoplasmosis in Wistar rats fed 2 × 10^2–10^3 *Toxoplasma gondii* cysts, based on bioassay of fetuses.

Experiment no.	Strain of *T. gondii*	Isolation data	Mouse pathogenicity*	No. litters infected/total no. litters†
1	KSU	Germany, 1960 (Masihi et al., 1984)	Low	0/8
2	KSU			1/10
3	KSU‡			0/9
4	ME-49	U.S., 1960 (Dubey et al., 1995)	Low	0/8
5	ME-49			2/6
6	T-265	U.S., 1960 (Jacobs et al., 1960)	High	1/7
7	Hopa-Hopa	Uruguay, 1993§	Intermediate	3/7
8	Elg	France, 1960‖	High	0/9
9	Pruignaud	France, 1964 (Martrou, 1965)	Intermediate	1/7
10	Pruignaud			0/9
11	Castells	Uruguay, 1993§	Intermediate	1/9
Total				9/89 = 10.1%

* High mouse pathogenicity: One zoite kills a mouse. Low pathogenicity: Mice with chronic infection can be obtained without medicating with sulfonamides. Intermediate pathogenicity: 1 zoite does not kill a mouse, but it is necessary to medicate mice infected with higher doses with sulfonamides to prevent deaths.
† Number of mother rats.
‡ Oocysts were fed.
§ Isolated by the authors from cat feces (Hopa-Hopa) and from sheep abortion (Castells).
‖ Isolated from the brain of an AIDS patient with toxoplasmic encephalitis (M. L. Dardé, pers. comm.)

vived 30 days after inoculation were bled, and 1:64 dilution of their sera was examined for DA antibodies.

To detect any association between congenital transmission, the *T. gondii* strains, and doses used, the chi-square association test was employed at a significance level of $\alpha = 0.05$.

Toxoplasma gondii was detected by bioassay in 9 of 89 litters (10%) from rats inoculated with 6 of 7 strains of *T. gondii* (Table I). Significant associations between congenital transmission and the strains and doses of *T. gondii* used were not detected.

The aim of the present work was to detect transplacental transmission of *T. gondii* during the chronic stage of the infection in rats, and so a rather large number of rats was used; tissues of the whole litter as a pool were bioassayed, although this method did not allow us to know if 1 or more fetuses are infected in the litters.

Variable rates of transplacental transmission of *T. gondii* during chronic infection have been reported by other investigators. Thiermann (1957) found 2% transmission in 50 litters born to rats inoculated with the Santiago strain of *T. gondii* 36–405 days before delivery. Remington et al. (1961) reported congenital transmission from 3 of 12 rats intraperitoneally inoculated with cysts of the Beverley strain several months before pregnancy. Dubey and Shen (1991), Zenner et al. (1993), and Dubey et al. (1997) did not find transmission of *T. gondii* from chronically infected rats. However, we obtained transmission in 1 out of 16 litters. These results may be due to the strain of rat used, dose and stage of *T. gondii* used, and duration of infection in rats before pregnancy. In the present study, transmission of *T. gondii* was independent of the inoculum size used.

The overall transplacental transmission of *T. gondii* of 10% (Table I) should be considered when planning experiments of immunization/challenge of pregnant rats with *T. gondii* by forming control groups of rats inoculated 2 mo before conception with live *T. gondii* that will be not challenged later in pregnancy.

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Prevalence of *Sarcocystis kirkpatricki* Sarcocysts in the Central Nervous System and Striated Muscles of Raccoons from the Eastern United States

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ABSTRACT: A retrospective survey of 760 raccoons (*Procyon lotor*) revealed 9 animals with sarcocysts of *Sarcocystis kirkpatricki* in their brains. Six of the raccoons also had the organisms in their skeletal muscles, and 1 had them in the heart muscle. No age or gender predisposition was seen. Seven of the raccoons had concurrent viral diseases (canine distemper or rabies), suggesting that concurrent viral infections in raccoons may facilitate infection of brain tissue with *S. kirkpatricki.*

Four species of *Sarcocystis* have been reported in striated muscles (skeletal and cardiac) of raccoons (*Procyon lotor*) (Kirkpatrick et al., 1987; Snyder et al., 1990; Stolte et al., 1996). However, in North America only *Sarcocystis kirkpatricki* has been documented in the muscles. Although the prevalence of infection of this parasite appears to be variable, up to 50% of raccoons in a study from the northeastern U.S.A. were found to be infected in either the heart, tongue, diaphragm, masseter muscle, or the esophagus (Kirkpatrick et al., 1987). Snyder et al. (1990) named this organism *S. kirkpatricki* and reported a prevalence of 66% in raccoons from Illinois. Neither of these studies reported *Sarcocystis* in the brain of these raccoons. Subsequently, there has been a single report of *S. kirkpatricki* in the cerebellum of a raccoon with dual infections of *Toxoplasma gondii* and canine distemper (Dubey et al., 1992). In the present communication, we provide additional evidence of infection by *S. kirkpatricki* in the central nervous system of raccoons.

Necropsy examination records and formalin-fixed tissue submissions of raccoons at the Laboratory of Large Animal Pathology, University of Pennsylvania, were reviewed for the past 10 yr (1985–1995). A majority of the raccoons were obtained for laboratory and field evaluation of an oral vaccinia–rabies glycoproptein (V-RG) recombinant virus vaccine (Rupprecht et al., 1992). All cases with a record of the presence of *S. kirkpatricki* in the brain were reevaluated, and the hematoxylin and eosin (H&E)-stained tissue sections were reexamined for the presence of these parasites in the brain, proximal cervical spinal cord, heart, diaphragm, tongue, masseter muscle, and esophagus. Initially, for each case 3 coronal sections of the brain (cerebrum, cerebellum, brain stem) were examined and in 2 cases (nos. 2 and 8, Table I), the whole brain was sectioned at 3–4-mm-wide coronal sections; all blocks were paraffin embedded and examined by light microscopy.

During the 10-yr period (1985–1995), 760 raccoons were examined from the eastern U.S.A. These revealed a prevalence of

TABLE I. Geographical location, age, gender, final diagnosis, and presence of *Sarcocystis kirkpatricki* in various tissues of 9 raccoons.*

Host no.	Sex	Age	Location	Presence of *S. kirkpatricki* in: Heart	Tongue	Diaphragm	Masseter muscle	Esophagus	Cerebrum	Cerebellum	Brainstem	Spinal cord	Diagnosis
1	F	A	PA	−	−	−	−	−	+	+	−	−	None
2	M	SA	PA	+	+	+	+	−	−	+	−	−	Rabies
3	M	A	OH	−	+	+	+	−	−	+†	−	−	Rabies
4	M	A	OH	−	−	−	−	−	−	+	−	−	Rabies
5	F	SA	PA	−	+	+	+	−	−	+	−	−	CD
6	F	A	PA	−	−	−	−	−	−	+	−	−	None
7	F	A	PA	−	+	+	+	+	−	+	−	−	CD
8	M	SA	PA	−	+	+	+	+	−	+‡	−	−	CD + toxo
9	M	SA	NJ	−	+	+	+	+	−	+	−	−	Encephalitis

* F = female; M = male; A = adult; SA = subadult; +/− = positive/negative for *S. kirpatricki*; spinal cord = proximal cervical spinal cord; CD = canine distemper; Toxo = toxoplasmosis.
† Examination of the whole brain revealed no additional *S. kirkpatricki.*
‡ Examination of the whole brain revealed 4 additional *S. kirkpatricki* in the cerebellum.