Toxoplasmosis in rats (*Rattus norvegicus*): congenital transmission to first and second generation offspring and isolation of *Toxoplasma gondii* from seronegative rats

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(*Received 27 November 1996; revised 2 January 1997; accepted 6 January 1997*)

SUMMARY

To study congenital transmission of *Toxoplasma gondii* during acute and chronic infections, 4 pregnant Sprague-Dawley rats were each fed 10000 oocysts of the VEG strain. *Toxoplasma gondii* was recovered from 33, 55, 83 and 57% of rats (F1) when dams were inoculated at 6, 9, 12 or 15 days of gestation, respectively. Progeny of 15 congenitally infected female rats were examined for *T. gondii*. *Toxoplasma gondii* was recovered from tissues of 1 of 155 rats (F2) born to congenitally infected dams. A total of 4 (F2) females were mated; 0 of 40 (F3) rats born to them were infected. None of the acutely infected 4 dams that had given birth to congenitally infected litters produced congenitally infected offspring during the second pregnancy. Thus, unlike mice, evidence for repeated congenital transmission of *T. gondii* in the rat was found in $< 1\%$ of cases. Of the 16 congenitally *T. gondii* infected pups with demonstrable tissue cysts, 5 were seronegative ($< 1:4$) in the Sabin-Feldman dye test and 5 were seronegative ($< 1:20$) in the modified agglutination test by the use of whole formalinized tachyzoites and mercaptoethanol.

Key words: *Toxoplasma gondii*, rat, *Rattus norvegicus*, congenital transmission.

INTRODUCTION

Congenital transmission of *Toxoplasma gondii* in rats may be of epidemiological significance because rats are considered reservoirs of infection for pigs and cats. Recently, Webster (1994) found a high serological prevalence of *T. gondii* in rats from England and she proposed the hypothesis that *T. gondii* can be maintained in the environment without contamination by oocysts from infected cats. This hypothesis is also based on the assumption that *T. gondii* can be vertically transmitted repeatedly in the rat. The paper cited in support of the vertical transmission refers to mice (Beverley, 1959), and showed repeated vertical transmission through successive generations in mice and repeated transmission from the same dam. However, there is overwhelming evidence (reviewed by Dubey & Frenkel, 1997) that in *Rattus norvegicus*, with which Webster worked, *T. gondii* is rarely transmitted during the chronic stage of infection (Hellbrügge, 1957; Thiermann, 1957; Remington, Jacobs & Melton, 1961; Dubey & Shen, 1991; Zenner *et al.* 1993). We are also not aware of any published study showing that congenitally infected rats can regularly produce congenitally infected offspring.

The main objective of the present investigation was to study repeated and successive congenital transmission of *T. gondii* in rats.

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MATERIALS AND METHODS

Primary T. gondii *infection in pregnant rats*

Four (nos 1162–1165) pregnant Sprague–Dawley, 74 or 81-day-old rats (*R. norvegicus*) weighing approximately 200g were obtained from Taconic Farms, Germantown, New York. Pregnant rats were each inoculated by a stomach tube with 10000 mouse-infective oocysts of the VEG strain of *T. gondii* (Dubey *et al.* 1996) on days 6, 9, 12 or 15 of pregnancy. The same pool of oocysts had been bioassayed in rats and mice as reported (Dubey, 1996). For 7 days after oral oocyst inoculation, all bedding and faeces from rat cages were incinerated to kill any oocysts that might have passed unexcysted in faeces (Dubey & Frenkel, 1973). Thereafter, the pregnant rats were transferred to clean cages and housed individually.

Bioassay of F1 rat tissues for T. gondii

Toxoplasma gondii infection in newborn rat pups was detected by a bioassay in mice. The male pups were killed on the 4th or 5th day of age. Female rat pups

https://doi.org/10.1017/S0031182097008950 Published online by Cambridge University Press

Table 1. Congenital toxoplasmosis in rat pups in relation to gestational age of dams at the time of oocyst feeding

Dam			Pups (F1)			
No.	Days of gestation	Days to delivery	No. born	No. killed	Day killed	No. infected with *T. gondii*
1163	6	16	15	9 M	4	0
				2 F	35	1
				3 F* (nos 1797–1799)	81	3
				1 F* (no. 1800)	134	1
						Total 5 (33%)
1165	9	13	9	5 M	4	1
				1 F* (no. 1793)	85	1
				1 F (no. 1792)	89	1
				2 F* (nos 1791 and 1794)	137	2
						Total 5 (55%)
1162	12	10	12	5 M	4	3
				3 F	35	3
				2 F* (nos 1776 and 1777)	87	2
				1 F* (no. 1779)	88	1
				1 F* (no. 1787)	140	1
						Total 10 (83%)
1164	15	7	14	6 M	5	0
				4 F	35	4
				1 F* (no. 1788)	89	1
				1 F* (no. 1789)	90	1
				1 F* (no. 1790)	94	1
				1 F* (no. 1987)	142	1
						Total 8 (57%)

* Used for second generation congenital transmission.

Table 2. Toxoplasmosis in congenitally infected rats (F1) born from dams infected during pregnancy

Rat no. (F1)	MAT *T. gondii* antibody titres		Day killed	Tissue cysts in brain	Bioassay for *T. gondii*
	Day weaned	Titre			
1797	18	⩾ 5000	81	N	4*
1798	18	⩾ 5000	81	N	4
1799	18	⩾ 5000	81	N	4
1800	18	⩾ 5000	134	N	4
1791	21	500	137	N	4
1792	21	500	89	+	N.D.
1793	21	500	85	N	4
1794	21	500	137	N	4
1776	24	500	87	+	N.D.
1777	24	500	87	N	4
1778	24	500	140	N	4
1779	24	500	88	+	N.D.
1787	26	500	142	+	4
1788	26	500	89	+	N.D.
1789	26	500	90	N	4
1790	26	500	94	N	4

N, No tissue cysts seen; +, tissue cysts seen. N.D., Not done.
*No. of mice positive for *T. gondii* of 4 mice inoculated.

were killed at different periods after birth. Portions of brain, heart, lung and liver of each neonate were pooled, ground in a mortar with a pestle, suspended in antibiotic saline solution (0·9% NaCl with 1000 units of penicillin and 100 μg of streptomycin/ml of saline) and inoculated subcutaneously into 2–4 Swiss

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Table 3. Congenital transmission of *Toxoplasma gondii* to pups (F2) from congenitally infected dams (F1)

Dam		Pups (F2)		
No.	Age (days)	No. born (male + female)	Day killed	No. infected with *T. gondii*/ No. bioassayed
1797	69	10 (6 + 4)	9	0/6
			13	0/4
1798	69	13 (5 + 8)	6	0/5
			13	0/8
1799	76	12 (7 + 5)	7	0/12
1800	71	10 (2 + 8)	5	0/2
			37	0/6
			65	0/2
1776	74	11 (6 + 5)	0	0/6
			13	0/5
1777	85	11 (6 + 5)	3	0/11
1778	69	5 (2 + 3)	5	0/2
			43	0/3
1779	77	11 (4 + 7)	7	0/4
			11	0/7
1787	76	13 (7 + 6)	7	0/7
			39	0/6
1788	88	11 (5 + 6)	2	0/11
1789	82	16 (9 + 7)	5	0/9
			9	0/7
1791	68	11 (5 + 6)	3	1/5
			38	0/6
1792	72	1	16	0/1
1793	71	10 (7 + 3)	10	0/7
			14	0/3
1794	69	10 (5 + 5)	2	0/5
			37	0/5

Webster female mice. For bioassay of weaned rats ($\geqslant$ 18 days old), only brain tissue was used for bioassay in mice. The mice were obtained from Taconic Farms, Germantown, New York.

The inoculated mice were examined for *T. gondii* infection. Imprints of lungs and brain were made from mice that died before 2 months and these were examined for *T. gondii* stages. Survivors were bled 2 months p.i. and a 1:50 dilution of serum of each mouse was tested for *T. gondii* antibodies using the modified agglutination test (MAT) as described (Dubey & Desmonts, 1987). The brain of each mouse (irrespective of serological results) was examined microscopically for tissue cysts (Dubey & Beattie, 1988). Mice were considered infected when *T. gondii* was found in their tissues.

Bioassay of F2 generation rats for T. gondii

A total of 16 female F1 rats (4 from each litter) were mated with *T. gondii*-negative male rats. Each pregnant rat was housed individually. Male rat pups were bioassayed within 10 days of age and female rat pups were bioassayed for *T. gondii* between 5 and 65 days of age, 28 rats had been weaned before bioassay.

Bioassay of F3 generation rats for T. gondii

A total of 4 grand-daughters from infected dams were mated with *T. gondii*-free males and their progeny were bioassayed for *T. gondii*.

Repeat congenital infection from chronically infected dams

The original 4 dams (nos 1162–1165) that had produced congenitally infected rats after they had been fed oocysts during first pregnancy were mated with *T. gondii*-free males a second time and their offspring were bioassayed for *T. gondii*.

Serological examination of rats for T. gondii

Rats were bled from the orbital sinus under anaesthesia and their serum was initially screened for MAT antibodies using dilutions of 1:25, 1:50, 1:500, and 1:5000, as described by Dubey & Desmonts (1987). Some sera with low-level antibodies were tested by the Sabin–Feldman dye test and MAT performed as described by Desmonts & Remington (1980). Some rat sera were also tested using the latex agglutination test kit (Toxo Test-MT, Eiken Chemical Co., CA, USA) and an indirect haemagglutination test kit (TPM-Test, Wampole Laboratories, Cranbury, NJ, USA). For the latex agglutination test (LAT), sera were diluted 2-fold from 1:8 to 1:512 and for the indirect haemagglutination test (IHAT), sera were diluted 2-fold from 1:32 to 1:512; in both tests a titre of $<$ 1:64 was considered negative.

RESULTS

Toxoplasma gondii was found in rat pups (F1) born to all 4 dams fed oocysts, irrespective of gestational age of the dam at the time of oocyst feeding (Table 1). The highest rate of congenital infection (83%) was in pups born to dam no. 1162 inoculated at 12 days gestational age.

All 16 female F1 pups (4 from each litter) saved for the F2 generation transmission experiment were found to be infected with *T. gondii* (Table 2). *Toxoplasma gondii* tissue cysts were seen in brain squashes of cerebrum of 5 F1 rats and the remaining 11 rats were positive for *T. gondii* by mouse bioassay.

Toxoplasma gondii was found by mouse bioassay in 1 of 155 F2 rat pups born to 15 congenitally infected dams (Table 3); both mice inoculated with tissues of a male rat killed the third day of birth were infected with *T. gondii*. The 16th dam (no. 1790) ate her 5 pups.

Toxoplasma gondii was not found in tissues of any of the 40 rat pups (F3) born to 4 (F2) generation

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Table 4. Attempted congenital transmission from daughters (F2) of congenitally infected dams (F1)

Dam (F2) *T. gondii* antibodies (MAT)					Pups (F3)		
No.	Day	Titre	Day	Titre	No. born	Day killed	Biossay for *T. gondii*
3954	31	400	63	< 25	11	3	0/11*
3955	31	400	63	< 25	5	1	0/5
3958	39	25	53	< 25	15	5	0/15
3959	31	< 25	N.D.	N.D.	9	1	0/9

* No. of pups positive for *T. gondii*/No. bioassayed.

Table 5. Attempted transmission of *Toxoplasma gondii* from chronically infected dams during a second pregnancy

Dam no.	Day after oocyst inoculation	Pups		
		No. born	Day killed*	Bioassay†
1162	81	13	2	0/13
1163	95	16	8	0/16
1164	82	6	1	0/6
1165	82	6	1	0/6

* Day after birth.
† No. positive for *T. gondii*/No. bioassayed.

Table 6. *Toxoplasma gondii* antibody reciprocal titres in sera of congenitally infected rats using different serological tests

Rat no.	Age (days)	MAT*	Dye test	LAT	IHAT
1776	85	32	8	8	< 32
1777	85	640	64	64	< 32
1778	85	⩾ 2560	⩾ 512	16	< 32
1779	85	16	16	< 8	< 32
1787	87	320	256	64	< 32
1788	87	8	32	256	32
1789	87	320	< 4	256	256
1790	87	⩾ 2560	⩾ 512	28	< 32
1791	82	320	128	16	< 32
1792	82	64	16	32	< 32
1793	82	160	< 4	64	< 32
1794	82	160	64	32	< 32
1797	79	4	< 4	256	128
1798	79	16	< 4	128	64
1799	79	8	< 4	< 8	< 32
1800	79	⩾ 2560	⩾ 512	256	128

* According to method of Desmonts & Remington (1980).

dams (Table 4). *Toxoplasma gondii* was not found in 51 pups born to the original 4 dams (nos 1162–1165) from a second pregnancy (Table 5).

Of the 16 (F1) congenitally infected female rats serologically tested, MAT antibodies (⩾ 1:500) were found in the sera of all rats bled on the day (days 18–26) of weaning (Table 2). The MAT antibody titres had declined in 15 of the 16 female congenitally infected rats by 87 days of age. Because *T. gondii* tissue cysts were seen microscopically in brains of 2 rats (no. 1776 and 1788, Table 2) that had no detectable antibody by MAT in a 1:25 dilution of serum, further serological examinations were performed (Table 6). Five rat sera were found to be negative by the dye test (1:4 dilution) and 3 of these were also seronegative by MAT (⩽ 1:16). By LAT, 7 rats had titres of < 1:64. By IHAT, only 4 rats had titres of ⩾ 1:64. One rat (no. 1799) was negative by all serological tests.

All *T. gondii*-infected rats appeared clinically normal.

DISCUSSION

Results of the present study confirm earlier observations (Dubey & Shen, 1991; Zenner *et al*. 1993) that *T. gondii* is efficiently transmitted congenitally in pups born to dams infected orally with oocysts, during pregnancy. The strain of *T. gondii* is probably not important for the high efficiency of congenital transmission because the VEG strain used in the present study is different from the CT-1 and GT-1 strains used by Dubey & Shen (1991) and the 76K strain and Prugniaud strain used by Zenner *et al*. (1993). Compared with the CT-1 and GT-1 strains, which are highly pathogenic for mice, the VEG strain is of relatively low pathogenicity for mice (Dubey, 1996).

The results of the present study also confirm earlier observations that there is no repeat congenital transmission of *T. gondii* during the chronic stage in rats (for review, see Dubey & Frenkel, 1997). In another study, reinoculation of 32 previously infected rats with the RH or Prugniaud *T. gondii* strain during a second pregnancy did not lead to congenital infection (Zenner *et al*. 1993). Even the administration of cortisone to chronically infected rats led to repeat congenital infection in only 1 of 192 rats (Thiermann, 1957).

The results of congenital transmission in rats are different to those obtained with mice or hamsters

https://doi.org/10.1017/S0031182097008950 Published online by Cambridge University Press

(Beverley, 1959; de Roever-Bonnet, 1969). In mice and hamsters, *T. gondii*-infected dams can produce several infected litters without reinfection. Congenitally infected offspring can again produce congenitally infected mice for up to 9 generations (Beverley, 1959). The experiments reported by Beverley (1959) were terminated prematurely before it was realized that congenitally infected mice can have low dye-test antibodies (considered negative) and because of a high rate of mortality in congenitally infected mice.

Jacobs (1964) proposed that lack of detectable dye test antibodies in congenitally infected mice might be due to immunological tolerance. Jacobs (1964) found evidence of congenital transmission in 16 of 88 litters born to mice inoculated with the Beverley strain of *T. gondii*: in 11 instances only 1 mouse from the litter was positive, in 4 instances 2 mice, and in 1 instance 3 mice. Of the 19 young mice with persistent dye-test antibodies, 18 were positive for *T. gondii* stages. Of 357 young mice without antibody, 4 (in 3 litters) had demonstrable *T. gondii*. Of 300 young mice injected with dead *T. gondii* antigen, 16 failed to produce antibodies, thus lending support to the immunological unresponsiveness of the young mice to *T. gondii* antigens (Jacobs, 1964).

The rate of congenital infection in rats from dams infected during pregnancy was higher than the rate of congenital transmission in mice. However, unlike mice there was no (or rare) congenital transmission of *T. gondii* during the chronic stage.

In the present study, at least 5 (from 3 litters) of 16 congenitally infected rats had antibody titres usually regarded as negative for *T. gondii* antibodies by the dye test yet they harboured viable *T. gondii* in their brains.

The MAT is generally more sensitive than the dye test for *T. gondii* infection and titres obtained by MAT are generally higher than those obtained using the dye test (Dubey & Beattie, 1988; Dubey *et al.* 1995*b*). In the MAT, sera are usually screened at a starting serum dilution of 1:20 or 1:25 (Desmonts & Remington, 1980; Dubey *et al.* 1995*b*) because the mercaptoethanol used in the test may not inactivate non-specific IgM in serum at lower dilutions (Dubey, Lappin & Thulliez, 1995*a*). Therefore, at least 5 of 16 congenitally infected rats in the present study would have been considered seronegative by the MAT because titres were $< 1:20$. In the present study some of the congenitally infected seronegative rats were killed before it was realized that they might be infected with *T. gondii*. Therefore, it was not possible to make extended serological observations in all congenitally infected rats.

The LAT and IHAT are generally less sensitive than the MAT, at least for pigs (Dubey *et al.* 1995*c*). In the present study, sera from 2 littermate pups, no. 1797 and no. 1798, were seropositive by both LAT and IHAT ($\geqslant 1:64$) but seronegative by the dye test ($< 1:4$) and MAT ($< 1:25$). These results may be because these serological tests measure different antibodies, although tachyzoites of the same strain of *T. gondii* (RH strain) are used as antigen in all of these tests. The antigen consists of live tachyzoites for the dye test, formalin-fixed whole tachyzoites for the MAT, and soluble antigens for IHAT and LAT.

What proportion of mice or other rodents in nature carry viable *T. gondii* in the absence of detectable antibodies is not known but it is of epidemiological significance. In a recent survey, *T. gondii* was isolated from tissues of 5 of 7 serologically negative (in both dye test and MAT) mice trapped in and around swine farms (Dubey *et al.* 1995*d*). In another instance *T. gondii* was isolated from tissues of 4 house mice trapped in a field around the horse stable where an acute outbreak of toxoplasmosis had occurred in humans (Dubey *et al.* 1981). It is likely that these seronegative *T. gondii*-infected mice were congenitally infected because in post-natally acquired infections in mice, *T. gondii* was not found in thousands of seronegative mice used for bioassay (Dubey *et al.* 1995*b*). *Toxoplasma gondii* has also been isolated occasionally from seronegative rats (Eyles, 1952). The serological diagnosis of toxoplasmosis in congenitally infected rats needs further research.

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