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Short communication

Toxoplasma gondii: A study of oocyst re-shedding in domestic cats

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ABSTRACT

The aim of the present study was to evaluate the re-shedding of *T. gondii* oocysts in cats fed tissue cysts of homologous and heterologous strains 12, 24 and 36 months after the first infection. Thirteen cats were used in the present study and were divided into four groups: G1 (n = 2), G2 (n = 3), G3 (n = 5), and G4 (n = 3). G1, G3 and G4 cats were infected with brain cysts of ME49 and G2 with TgDoveBr8, both genotype II strains of *T. gondii*. The G1 and G2 cats were re-infected after twelve months with brain cysts of VEG strain (genotype III), and G3 cats were re-infected with TgDoveBr1 (genotype II). The G3 cats were re-infected a third time after 24 months from the second infection, and the G4 cats were re-infected 36 months after the initial infection with cysts of the VEG strain. The cats' feces were evaluated using fecal flotation and genotyped with PCR-RFLP. The serological responses for IgM, IgA and IgG were determined by ELISA. All cats shed oocysts after the initial infection. Only one G1 cat shed oocysts when re-infected after twelve months with the VEG strain. No G2 cats excreted oocysts after the second infection with VEG. G3 cats, when re-infected after twelve months with the TgDoveBr1 strain, did not shed oocysts. However, when challenged after a third time with the VEG strain, three out of four cats shed oocysts. In the G4 group, when re-infected after thirty-six months with the VEG strain, two out of three cats shed oocysts. All oocyst samples were genotyped and characterized as the same genotype from the inoculum. Protection against oocyst re-excretion occurred in 90%, 25%, and 33.4% of cats after 12, 24, and 36 months from the initial infection, respectively. Therefore, the environmental contamination by oocysts from re-infected adult cats is only 30% lower than from kittens. In conclusion, the excretion of *T. gondii* oocysts was higher in experimentally re-infected cats throughout the years, especially when a heterologous strain was used.

1. Introduction

Toxoplasma gondii is an obligatory intracellular protozoan that can infect all warm-blooded animals, birds and reptiles, including human beings (Tenter et al., 2000). However, felids are the only definitive host of *T. gondii* (Frenkel et al., 1970). Cats are considered a key component in *T. gondii* transmission, and domestic cats are important because of their direct contact with humans and because they are carnivores (Dubey, 1995). Those animals after primary infection are able to shed millions of oocysts through their feces, which contaminate the environment and, after sporulation, are infective for animals and humans (Dubey, 1995).

After the first oocyst excretion, cats usually develop immunity to the re-excretion of oocysts when challenged with homologous or heterologous strains of *T. gondii* (Dubey and Frenkel, 1974; Dubey, 1995). The long-lasting immunity to oocyst shedding in domestic cats was not known until Dubey (1995), who showed that four out of nine cats re-excreted *T. gondii* oocysts 77 months after the initial infection with tissue cysts. To our knowledge, this is the only study to investigate the long-lasting immunity in cats against *T. gondii*, and the author observed that the number of oocysts eliminated during the second infection was lower than the first.

Thus, in the present work, we describe the immunity to shedding of *T. gondii* oocysts by domestic cats infected with homologous and heterologous strains 12, 24 and 36 months after the initial infection.

2. Materials and methods

2.1. Ethics committee

The present study was approved by the Institutional Ethics Committee in Animal Use (CEUA, protocol number 51/07 and 102/12).

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2.2. Toxoplasma gondii strains

Four strains of *T. gondii* were used in this experiment: three genotype II strains, ME49, TgDoveBr1 and TgDoveBr8 strains (Lunde and Jacobs, 1983; Barros et al., 2014) and one genotype III strain, VEG (Dubey, 1996). Oocysts obtained from a previous study (Zulpo et al., 2012) were used to orally infect ten mice with 50 sporulated oocysts. Mice were euthanized 60 days after being infected, and their brain cysts were counted (Igarashi et al., 2008) and prepared for challenge (≅ 800 brain cysts/cat).

2.3. Cats and previous monitoring

The animals used in this experiment were selected from kittens that were abandoned at the university campus. Cats were monitored for 2 months prior to the beginning of the experiment. All cats were serum negative (titer < 16) for *T. gondii* as measured with an indirect immunofluorescence assay (Camargo, 1974), and the absence of *T. gondii* oocysts was confirmed by fecal examination.

2.4. Infection of cats

Thirteen domestic short-haired cats (*Felis catus*) of both sexes, between 3 and 6 months of age, were randomly divided into four groups: G1 (n = 2), G2 (n = 3), G3 (n = 5), and G4 (n = 3). The initial infection of the G1, G3 and G4 cats was performed with tissue cysts of ME49, and the G2 cats were infected with TgDoveBr8. One year later, the G1 and G2 cats were re-infected with VEG, and the G3 cats were re-infected with TgDoveBr1. Finally, after 36 months of the experiment, the G3 and G4 cats received another inoculum with the VEG strain (Table 1).

All infections were performed with ≅800 tissue cysts (contained in a volume of 2 mL), administered via stomach tube. After the infection, each animal was injected with 5 mL of saline. This procedure was performed with all animals anesthetized with tiletamine plus zolazepam (Zoletil, Virbac-Brazil, 3.15 mg/kg/IM). The G^1 and G^2 groups were accompanied for 15 months and the G^3 and G^4 groups were accompanied for 39 months of the experiment. During the oocyst shedding period 20 days after infections, the cats were housed in individual cages, but outside of this period, they were housed collectively in cages. The cats received only commercial feed and water ad libitum.

2.5. Oocyst shedding

Feces from each cat were collected daily from the 1st day after the challenge until the 20th day and were examined microscopically for oocysts, as described by (Garcia et al., 2007).

2.6. Enzyme-linked immunosorbent assay (ELISA) - IgM, IgA and IgG

ELISAs for IgG and IgM were performed as described by Garcia et al. (2007) while for IgA, the assay was performed as in Zulpo et al. (2012). Optimal dilutions were established by using checkerboard titrations with dilutions of sera and conjugates. Proteins from membrane tachyzoites of *T. gondii* were used as antigens (Garcia et al., 2004) to coat the flat-bottom 96-well polystyrene microtitration plates (Nunc-Immuno Plate, MaxiSorp, Denmark).

2.7. Genotyping of tissue cysts and oocysts

Samples of tissue cysts used in the inoculum and feces (a pool from each group) were used to perform the genetic characterization of *T. gondii*. Tissue cysts and feces underwent DNA extraction using a commercial kit following the manufacturer's instructions (NucleoSpin® Tissue, Macherey-Nagel, Germany). Genotyping was performed using multilocus PCR-RFLP with 11 genetic markers (SAG1, 5′-3′SAG2, alt.SAG2, SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1 and Apico) as previously described (Su et al., 2010).

2.8. Statistical comparisons

A Wilcoxon-Mann-Whitney *U* test was used to test for significant differences in OOPG (oocysts per gram of feces) from ME49 and TgDoveBr8 at initial infection. A $p \leq 0.05$ was considered statistically significant. The prevention against oocyst elimination in cat feces was calculated by estimating the preventable fraction (PF) as previously described (Garcia et al., 2007) with modifications: PF = (P2-P1)/P2, where P2 is the total number of oocysts shed at initial infection and P1 is the total number of oocysts shed at the second or third infection.

Table 1
Oocyst shedding of cats infected with homologous and/or heterologous strain of *Toxoplasma gondii*.

Groups	Cat numbers	Age in months	Sex	Infections (months)					
				0		12		36	
				Strain (type)	OOPG	Strain (type)	OOPG/%PF[b]	Strain (type)	OOPG/%PF[b]
G1	61	5	M	ME49 (II)	14,250	VEG (III)	0/100	ND	
	65	5	M		213,250		118,750/ 44.3		
	31	6	M	TgDoveBr8 (II)	1,402,500	VEG (III)	0/100	ND	
G2	32	6	F		24,500		0/100		
	34	6	M		485,000		0/100		
	1	4	F	ME49 (II)	25,500	TgDoveBr1 (II)	0/100	VEG (III)	0/100
	2	4	F		2000		0/100		3750/nc[c]
G3	4	5	M		1,527,000		0/100		617,500/59.6
	11[a]	4	M		12,500		0/100		[a]
	23	6	M		415,000		0/100		215,000/48.2
	18	6	M	ME49 (II)	25,500	ND		VEG (III)	3750/85.3
G4	4D	3	F		304,000				0/100
	5E	3	M		3,966,500				55,000/ 98.6
Number of positive cats (%)					13(100)		1(10)		5(7)

OOPG = Total of oocyst per gram of feces during 20 days of evaluation; ND = Not done

[a] Animal 11 died by noninfectious causes before third infection (36 months).

[b] Individual preventable fraction (%) PF = (P2-P1)/P2.

[c] PF was not calculated because animal excreted more oocysts than previously.