

## EXPERIMENTAL INFECTION OF *PEROMYSCUS CALIFORNICUS* WITH *TOXOPLASMA GONDII*

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**ABSTRACT:** Eight female *Peromyscus californicus* were infected with 10<sup>2</sup> or 10<sup>4</sup> *Toxoplasma gondii* culture-derived tachyzoites (Type II or X) isolated from southern sea otters. All but 2 mice survived infection and developed antibodies to *T. gondii*. The 2 fatally infected mice were inoculated with 10<sup>4</sup> tachyzoites of the Type X strain. Parasite detection by immunohistochemistry (IHC) and DNA amplification with 2 polymerase chain reaction (PCR) methods was compared for brain, heart, lung, liver, spleen, biceps muscle, and tongue, at a mean of 41 days postinfection. Parasites were detected most commonly by IHC in spleen (8/8) and brain (6/8). DNA amplification by PCR was most successful from brain, heart, and spleen.

Like most species of warm-blooded vertebrates, rodents are intermediate hosts for *Toxoplasma gondii*, a protozoan parasite that was first detected in the spleen, liver, and blood of an African rodent, the gundi, *Ctenodactylus gundi* (Nicolle and Manceaux, 1908). *Toxoplasma gondii* undergoes sexual reproduction in the intestinal epithelium of felids, resulting in the production of millions of oocysts that are shed in their feces (Joynson and Wreghitt, 2001). Rodents become infected by ingesting soil, vegetation, or water contaminated with *T. gondii* oocysts. In experimentally infected mice, *T. gondii* sporozoites excyst from the oocyst within 30 min of ingestion, and they enter enterocytes of the small intestine, later transforming to motile stages (tachyzoites) within the cells of the lamina propria (Dubey, Speer et al., 1997). Within 48–72 hr, tachyzoites disseminate to the mesenteric lymph nodes, and they can be found in many organs, including the heart, lung, spleen, and brain ≤6 day post-infection (PI) (Dubey, Speer et al., 1997). As host immunity increases, organisms form slowly dividing bradyzoites within thick-walled tissue cysts that are protected from the host's immune system and persist for long periods. Tissue cysts may be detectable as early as 8 days PI in the brains of mice (Dubey, Speer et al., 1997). Cysts in the brain are long-lived in some hosts, such as mice, but may eventually deteriorate in the brains of rats (Ferguson et al., 1994; Dubey, 1996; Guerrero and Chinchilla, 1996). Rats are more resistant to *T. gondii* than mice, and they may be capable of eliminating the parasites from their tissues because of differences in their immune response to *T. gondii* infection (Chinchilla et al., 1981; Pettersen, 1988; Dubey, 1996). Congenital transmission during primary infection occurs in both rats and mice, but vertical transmission in subsequent pregnancies is rare in rats, whereas it occurs with greater frequency in mice (Beverley, 1959; Remington et al., 1961; Dubey, Shen et al., 1997; Owen and Trees, 1998; Freyre et al., 2003; Marshall et al., 2004).

Mice and rats also differ in their susceptibility to strains of

*T. gondii*. Genotyping has distinguished 3 major strains, i.e., types I, II, and III (Howe and Sibley, 1995). Although rats and voles are relatively resistant to *T. gondii* infection (Chinchilla et al., 1981; Dubey and Frenkel, 1998; Sedlak et al., 2001), murine susceptibility to toxoplasmosis is parasite strain-dependent (Araujo et al., 1976; Suzuki et al., 1995; Dubey et al., 2003). Mice are highly susceptible to Type I strains, whereas Type II and III strains are usually avirulent (Suzuki et al., 1995; Dubey et al., 2003). Atypical *T. gondii* strains have also been reported (Darde, 1996; Grigg and Suzuki, 2003; Ajzenberg et al., 2004), including a Type X strain that predominates in sea otter stranding near Morro Bay, California (Miller et al., 2004; Conrad et al., 2005).

Small rodents play an important role in the life cycle of *T. gondii* on land, because they are thought to represent the main source of infection for domestic cats (*Felis catus*). *Toxoplasma gondii* infection in *Peromyscus* sp. has not been extensively studied, and there is a paucity of data on *T. gondii* infection in small mammals native to California (Dubey, 1983). To our knowledge, no studies have documented the outcome of experimental infection with *T. gondii* in *Peromyscus californicus*. *Peromyscus californicus* mice were selected for study because they are highly abundant in California ecosystems south of the San Francisco Bay (Jameson and Peeters, 1988), and they may be important in maintaining *T. gondii* infection in near shore-dwelling wild carnivores and domestic cats.

The aims of the experimental infection study were to (1) determine the susceptibility of *P. californicus* to *T. gondii* infection at 2 doses with 2 different strains; (2) compare serologic titers using anti-mouse and anti-*P. leucopus* secondary antibody in the indirect fluorescent antibody test (IFAT); (3) compare detection of *T. gondii* parasites by immunohistochemistry (IHC) to polymerase chain reaction (PCR) (2 methods) and tissue culture in experimentally infected *P. californicus*; and (4) determine which of 7 tissues (heart, brain, lung, liver, spleen, tongue, and biceps muscle) most frequently had detectable *T. gondii* parasites. The most sensitive method for parasite detection in the experimentally infected *P. californicus* was determined, so it could be applied to screen wild rodents from coastal California in future studies.

### MATERIALS AND METHODS

#### Experimental infection of *Peromyscus californicus*

Eight female *P. californicus* (*Peromyscus* Genetic Stock Center, Columbia, South Carolina) aged 8 to 12 wk and weighing 31–49 g were infected with *T. gondii* tachyzoites via s.c. inoculation at the scruff of

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the neck. The mice were randomly assigned to dose and strain type using the random number generator in MINITAB® Release 14.2 software (Minitab Inc., State College, Pennsylvania). Mice were bled via the retro-orbital sinus under anesthesia before inoculation, and their sera were tested for *T. gondii* antibodies by the IFAT to confirm negative serostatus before *T. gondii* inoculation. Four *P. californicus* mice experimentally infected with *Sarcocystis neurona* and housed in the same room served as negative controls. The *T. gondii* isolates were derived from naturally infected southern sea otters. Four of the mice were infected with Type II isolate #1340 and 4 with Type X isolate #1544 (Grigg et al., 2001; Miller et al., 2004). Two mice in each group were inoculated with a high dose ( $10^4$  tachyzoites) and 2 with a low dose ( $10^2$  tachyzoites). The mice were housed singly in a pathogen-free facility, and they had access to food and water ad libitum. The mice were bled from the retro-orbital sinus under anesthesia at 27 days postinoculation (DPI), and they were humanely killed by CO<sub>2</sub> asphyxiation and exsanguinated by intracardiac puncture at 41 DPI. All animal experiments were conducted with approval of the Institutional Animal Care and Use Committee at the University of California, Davis, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Seven tissues (brain, heart, lung, liver, spleen, tongue, and right biceps muscle) were harvested aseptically at necropsy. Excluding the brain, half of each tissue was placed in formalin for IHC, and the remainder was frozen at  $-20$  C for PCR. The brain was divided longitudinally into 3 approximately equal sections: 1 for IHC, 1 for PCR, and the third portion was inoculated into tissue culture, as described previously (Miller et al., 2001). Briefly, the brain tissue was homogenized using a 14-gauge needle, digested with 0.05% trypsin, and added directly to 1 ml of protozoan culture medium in a 25-cm<sup>2</sup> flask containing MA104 monkey kidney cells. The flask was rinsed after 2 hr, fresh medium was added, and the culture was incubated at 37 C for  $\leq 30$  days. Cultures were examined with an inverted microscope 2–3 times per week for the presence of *T. gondii* parasites.

### Serology

Serum was diluted in phosphate-buffered saline (PBS), pH 7.4 (Diamedix, Miami, Florida), in 2-fold serial dilutions ranging from 1:40 to the endpoint. Titer was characterized as the inverse of the dilution of the last well with distinctive fluorescence outlining formalin-fixed tachyzoites. Ten microliters of serum (or pericardial fluid for fatally infected mice) was incubated at 37 C for 1 hr on 12-well slides (Erie Scientific, St. Louis, Missouri) coated with Formalin-fixed *T. gondii* tachyzoites derived from tissue culture, as described previously (Miller et al., 2001). Slides were washed 3 times for 5 min each in PBS, and 10  $\mu$ l of fluorescein isothiocyanate-labeled goat anti-mouse (Jackson ImmunoResearch Laboratories, West Grove, Pennsylvania) or goat anti-*P. leucopus* (Kirkegaard & Perry Laboratories Inc., Gaithersburg, Maryland) IgG secondary antibody, both diluted 1:100, was added to detect the presence of antibodies bound to *T. gondii* tachyzoites. Each sample was tested using both types of secondary antibody. After addition of the appropriate secondary antibody, slides were incubated for 1 hr at 37 C, washed 3 times in PBS, 10  $\mu$ l of buffered glycerol, pH 8.5, was added to each well, and the slides were examined at  $\times 200$  on an immunofluorescent microscope (Carl Zeiss Optical, Inc., Chester, Virginia). Positive controls consisted of sera from experimentally-infected Swiss Webster mice. Negative controls were sera from mice bled prior to *T. gondii* infection.

### Immunohistochemistry

Murine tissues were prepared for IHC as described previously (Miller et al., 2001; Arkush et al., 2003). Available tissues were sliced into 5- $\mu$ m-thick sections, paraffin-embedded, and immunohistochemically stained with polyclonal rabbit antibodies to *T. gondii*. All slides were examined microscopically at  $\times 40$  and  $\times 100$  by a pathologist for the presence of *T. gondii* tachyzoites or bradyzoites.

### TaqMan PCR for amplification of *T. gondii* DNA from *P. californicus* tissues

**Primers and internal probe:** For each target gene, 2 primers and an internal, fluorescent labeled TaqMan probe (5' end, reporter dye FAM [6-carboxyfluorescein], 3' end, quencher dye TAMRA [6-carboxytetra-

methylrhodamine]) were designed using Primer Express software (Applied Biosystems, Foster City, California). One of the primers of each system spans an exon–exon junction (if available in the sequence; see Table I) to restrict the TaqMan PCR specificity to cDNA without detecting genomic DNA contamination in the total RNA samples. TaqMan PCR systems were validated as described previously (Leutenegger et al., 1999), using 10-fold dilutions of cDNA testing positive for the target genes. The dilutions were analyzed in triplicate and a standard curve was plotted against the dilutions. The slope of the standard curve was used to calculate amplification efficiencies using the formula  $E = 10^{1/s} - 1$ .

**Sample collection and automated nucleic acid preparation:** Tissue biopsies weighing 100 mg were collected in 500  $\mu$ l of stabilization solution (nucleic acid purification lysis buffer; Applied Biosystems) and stored at  $-20$  C until processed. Proteinase K and 2, 4-mm diameter stainless steel grinding beads (SPEX CertiPrep, Metuchen, New Jersey) were added and the tissues homogenized in a GenoGrinder2000 (SPEX CertiPrep) for 2 min at 1,000 strokes per min. Protein digest was done at 56 C for 30 min followed by a 30-min period at  $-20$  C to reduce foam and precipitate RNA. Total RNA was extracted from the tissue lysates using a 6100 automated nucleic acid workstation (Applied Biosystems) according to the manufacturer's instructions.

**RT-reaction and real-time TaqMan PCR:** Complementary DNA (cDNA) was synthesized from DNase-digested total RNA using 100 units of SuperScript III, 600 ng of random hexadeoxyribonucleotide primers (random hexamer primer), 10 U of RNaseOut (RNase inhibitor), and 1 mM dNTPs (all Invitrogen, Carlsbad, California) in a final volume of 40  $\mu$ l. The reverse transcription reaction proceeded for 120 min at 50 C, and it was terminated by heating for 5 min to 95 C. Samples were cooled on ice, and 60  $\mu$ l of water was added to each well. Each PCR reaction contained 20 $\times$  primer and probes for the respective TaqMan system, with a final concentration of 400 nM for each primer and 80 nM for the TaqMan probe. The commercially available PCR mastermix (TaqMan Universal PCR Mastermix; Applied Biosystems) contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM MgCl<sub>2</sub>, 2.5 mM deoxynucleotide triphosphates, 0.625 U of AmpliTaq Gold DNA polymerase per reaction, 0.25 U of AmpErase micrograms per reaction, and 5  $\mu$ l of the diluted cDNA sample in a final volume of 12  $\mu$ l. The samples were placed in 384-well plates and amplified in an automated fluorometer (ABI PRISM 7900 HTA FAST; Applied Biosystems). ABI's standard amplification conditions were used: 2 min at 50 C, 10 min at 95 C, 40 cycles of 15 sec at 95 C, and 60 sec at 60 C. Fluorescent signals were collected during the annealing temperature, and CT values were extracted with a threshold of 0.04 and baseline values of 3–15. For stronger signals, the baseline was adjusted manually to 3–10. Rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene (catalog no. RN99999916-s1; Applied Biosystems) was amplified to verify the extraction protocol and to normalize the target gene CT values. Samples with negative GAPDH values were repeated.

**Relative quantitation of gene transcription:** Final quantitation was done using the comparative CT method (User Bulletin #21 Applied Biosystems), and results are reported as relative transcription or the n-fold difference relative to a calibrator cDNA, i.e., lowest target gene transcription. In brief, the housekeeping gene GAPDH was used to normalize the CT values of the target genes ( $\Delta$ CT). The  $\Delta$ CT was calibrated against the weakest signal within each target gene. The linear amount of target molecules, relative to the calibrator, was calculated by  $2^{-\Delta\Delta CT}$ . Therefore, all gene transcription is expressed as an n-fold difference relative to the calibrator.

### Nested PCR for amplification of the *T. gondii* B1 gene from *P. californicus* tissues

**Extraction of DNA from tissues:** DNA was extracted from murine tissues using the DNeasy® extraction kit (QIAGEN, Valencia, California). Tissue weighing 20 mg was suspended in 180  $\mu$ l of ATL buffer and treated with 40  $\mu$ l of proteinase K. After vortexing, samples were incubated at 55 C for 2–3 hr or overnight. The remainder of the protocol followed the manufacturer's instructions, with the following modification for the final step. Before the final centrifugation in the DNeasy mini spin column, 75  $\mu$ l of 95 C PCR-grade water was added to the spin column. Then, the samples were incubated for 1 min at room temperature and centrifuged at 14,000 rpm in an Eppendorf microcentrifuge for 2 min to elute the DNA.

TABLE I. Nucleotide sequence of PCR primers and TaqMan probes used to detect *Toxoplasma gondii* B1 gene, *T. gondii* 18S small subunit ribosomal RNA (ssRNA), or murine GAPDH.

Target	Primer	Primer sequence	Length of PCR product (bp)	Probe	Probe sequence
<i>T. gondii</i> B1 gene (TaqMan)	ToxB-41f	5'-TCGAAGCTGAGATGCTCAAAGTC-3'	129	ToxB-69p	5'-FAM-ACCGGAGATGCACCCGCA-TAMRA-3'
	ToxB-169r	5'-AATCCACGTCCTGGGAAGAACTC-3'			
	Tox18-213f	5'-CCGGTGGTCTCAGGTGAT-3'	120	Tox18-249p	5'-FAM-ATCGGTTGACTTCGGTCTGGGAC-TAMRA-3'
	Tox18-332r	5'-TGCCACGGTAGTCCAAATACAGTA-3'			
Rat GAPDH		Proprietary, Ref #NM 017008	GAPDH.200p	5'-FAM-GAAACCCATCACCACTTCCAGGAG-TAMRA-3'	
<i>T. gondii</i> B1 gene (conventional, nested)	Burg-T1	5'-GGAACTGCATCCGTTTCATGAG-3'	193 (T1 + T4)	Not applicable	
	Burg-T4	5'-TCTTTAAAGCGTTCGTGGTC-3'	133 (T2 + T4)	Not applicable	
	Burg-T2	5'-TGCATAGGTGGCAGTCACTG-3'		Not applicable	

**DNA amplification:** A hemi-nested conventional PCR for the *T. gondii* B1 gene was used (Burg et al., 1989). The product of the first round of amplification (primers T1 and T4) is 193 bp, and the second round of amplification (primers T2 and T4) results in a 133-bp product. Each 50- $\mu$ l reaction contained 1 $\times$  PCR buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, and 400 nM forward and reverse primers (Invitrogen), 1 U of Taq polymerase (Applied Biosystems), and 5  $\mu$ l of DNA template. Thirty cycles of amplification were performed in a GeneAMP 2400 thermocycler (Perkin-Elmer, Norwalk, Connecticut) for both inner and outer reactions. Each reaction consisted of 3 min of pre-PCR incubation at 94 C followed by 30 cycles of 1-min denaturation at 94 C, 15 sec of annealing at 60 C, and a 45-sec extension at 72 C. The post-PCR extension step continued for 4 min at 72 C. When the reaction was complete, samples were maintained at 4 C.

**Visualization of DNA by gel electrophoresis:** Ten microliters of the PCR product was electrophoretically separated on a 2% agarose gel stained with ethidium bromide and visualized under UV light. Product size was verified against a 100-bp ladder on each gel. Positive controls consisted of brain tissue from *P. californicus* experimentally infected with *T. gondii* tachyzoites and culture-derived *T. gondii* tachyzoites. Negative controls consisted of sterile deionized water and brain tissue from *P. californicus* experimentally infected with *S. neurona*.

#### Data analysis

Outcomes (dead or alive) were compared using Fisher's exact test (2-sided) for strain (Type II or X) and dose (100 or 10,000 tachyzoites). Serologic titer values were natural log-transformed, and their distribution was tested for normality by generating a probability plot and evaluating the Anderson-Darling statistic. Serologic titers for anti-*Peromyscus* and anti-mouse secondary antibody were compared at the 27-day time point with the Student's *t*-test. If there was no statistically significant difference in titers for the 2 types of antibody, results of both tests were incorporated into *t*-tests to evaluate the difference in mean titer for *T. gondii* Type II versus X, and high (10,000 tachyzoites) versus low (100 tachyzoites) dose. Statistical analyses were done in SAS, version 9.1 (SAS Institute, Cary, North Carolina) and MINITAB Release 14.2, and were values considered significant if the *P* value was <0.05.

## RESULTS

Both *P. californicus* mice inoculated with 10<sup>4</sup> tachyzoites of the Type X strain of *T. gondii* died, the first at 15 DPI and the second at 22 DPI. The first mouse exhibited mild respiratory distress 2–4 hr before death, and the second mouse had no observable clinical signs. All 6 surviving *T. gondii*-infected mice had no clinical signs. Results of serology, parasite isolation, IHC, and 2 PCR methods for detection of parasites in 7 tissues are summarized in Table II. All surviving mice had detectable antibodies to *T. gondii* in the IFAT by 27 DPI, and they developed high IFAT titers of 81,920 by 7 wk PI. Both mice that died also had antibody titers detectable by IFAT at the time of death. None of 4 *S. neurona*-infected mice housed in the same room developed *T. gondii* antibodies.

The Fisher's exact test for survival (or not) versus *T. gondii* strain or dose was not significant, as expected for this small sample size. The log transformation of the 4-wk serologic titer fit a normal distribution. Tests for differences in geometric mean titer between anti-*Peromyscus* and anti-mouse antibody were not significantly different, so both values were incorporated into *t*-test comparisons of titers for *T. gondii* strain and dose. Neither test yielded statistically significant results (*P* = 0.56 for both tests).

*Toxoplasma gondii* parasites were seen in 6 of 8 cultures prepared from brain material of infected mice (Table II). One of the 2 mice that were negative in tissue culture received the lower dose (10<sup>2</sup> tachyzoites) of Type II, and its brain was negative on IHC, despite having a titer of 81,920. The other mouse

TABLE II. Identification of *Toxoplasma gondii* in experimentally infected *Peromyscus californicus* mice based on antibody detection, in vitro parasite isolation, IHC, and DNA amplification by TaqMan or conventional nested PCR.

ID	Dose	Type*	IFAT with anti- <i>P. leucopus</i>		IFAT with anti-mouse		Brain culture	Detection of parasites (IHC/TaqMan PCR/conventional nested PCR)						
			Titer†	Titer	Titer	Titer		Brain	Heart	Lung	Liver	Spleen	Tongue	Biceps
1‡	10 <sup>2</sup>	X	81,920	81,920	20,480	40,960	+	+/+/+	-/+/+	-/+/+	-/+/+	+/+/+	-/+/+	-/-/-
2	10 <sup>2</sup>	X	20,480	81,920	20,480	20,480	+	+/+/+	-/+/+	+/-/-	-/-/-	+/-/+	-/+/+	-/-/-
4	10 <sup>4</sup>	X	5,120	ND#	2,560	ND	-	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	-/+/+	-/+/+
5	10 <sup>4</sup>	X	160	ND	40	ND	+	+/ND/ND	+/ND/ND	+/ND/ND	+/ND/ND	+/ND/ND	+/ND/ND	+/ND/ND
3	10 <sup>2</sup>	II	5,120	81,920	320	81,920	+	+/+/+	-/+/+	+/+/+	-/+/+	+/+/+	-/+/+	-/+/+
7	10 <sup>2</sup>	II	640	81,920	80	81,920	-	-/+/+	-/+/+	-/+/+	-/+/+	+/+/+	-/+/+	-/+/+
8¶	10 <sup>4</sup>	II	40,960	81,920	2,560	81,920	+	-/+/+	-/+/+	-/+/+	-/+/+	+/+/+	+/+/+	-/+/+
9¶	10 <sup>4</sup>	II	40,960	81,920	2,560	81,920	+	+/+/+	+/+/+	+/+/+	-/+/+	+/+/+	-/+/+	-/+/+

\* *T. gondii* type II and X tachyzoites isolated in vitro from the brain tissue of naturally infected southern sea otters (*Enhydra lutris nereis*).

† Titer is the reciprocal of endpoint dilution.

‡ Mice were killed 41 DPI unless noted otherwise.

§ IHC result (positive or negative)/TaqMan *BI* PCR result (positive or negative)/*BI* nested PCR result (positive or negative).

|| Mice 4 and 5 died 22 DPI and 15 DPI, respectively. They were refrigerated at 4 C and necropsied 3 days postmortem. Pericardial fluid was collected at necropsy for antibody testing.

# Not done.

¶ Blood from mice 8 and 9 was accidentally pooled.

with negative tissue culture results died at 22 DPI after receiving the higher dose (10<sup>4</sup> tachyzoites) of Type X, but it had parasites detectable by IHC in its brain. The second fatally infected mouse had positive results for both tissue culture and IHC. *Toxoplasma gondii* DNA was detected by PCR in at least 4 tissues from the 6 surviving *T. gondii*-infected mice. The TaqMan *BI* gene PCR detected parasites in 7/7 samples from brain, heart, and biceps muscle; 6/7 samples from lung, liver, and spleen; and 5/7 tongue samples (Table II). The *BI* gene hemi-nested PCR detected parasites in 7/7 spleen samples, 6/7 samples from brain and tongue, 5/7 samples from heart and lung, 4/7 liver samples, and 2/7 biceps muscle samples (Table II). All mice had parasites detectable by IHC in their spleens, 6/8 in the brain, 5/8 in the lung, and 3/8 in the heart (Table II). The area of the brain where organisms were most frequently observed was the cerebrum (6/8). Tissues where parasites were less likely to be observed by IHC or were not seen included liver and tongue (2/8) and biceps muscle (0/8). For the 3 techniques combined, 21 (95%) of 22 spleen and 19 (86%) of 22 brain samples tested positive for *T. gondii* parasites.

## DISCUSSION

Six of 8 experimentally infected *P. californicus* mice survived *T. gondii* infection with no apparent harmful sequelae. Both mice that developed fatal infections were infected with *T. gondii* Type X at the higher dose. These results suggest that *T. gondii* Type X may be more pathogenic for *P. californicus*, but our small sample size was insufficient to test this hypothesis. Assuming all (99% for purposes of calculation) of mice infected with Type II survived and 50% or 75% of mice infected with Type X survived, then a sample size of 12 or 30 mice allocated to each strain, respectively, would be required to detect statistically significant differences between strain lethality (MINITAB Release 14.2). Infection with *T. gondii* Type X has been associated with cause-specific mortality due to toxoplasmic me-

ningoencephalitis in southern sea otters, although this finding was also not statistically significant (Miller et al., 2004).

Serologic titers did not differ significantly for the 2 types of secondary antibody, although at the 27-day time point the anti-*Peromyscus* secondary antibody tended to yield higher titers, differing by up to 4 dilutions (Table II). For 95% confidence and 80% power, a sample size of 43 per group would be needed to detect a mean difference between titers of 640 and 2,560; and a sample size of 20 per group to detect a mean difference between titers of 640 and 5,120 (MINITAB Release 14.2). Funding for this project was insufficient to achieve this sample size.

The hemi-nested conventional PCR and IHC detected *T. gondii* parasites most frequently in spleen tissue, whereas TaqMan PCR was most sensitive in brain, heart, and biceps muscle. Our results suggest that brain and spleen samples are most likely to yield positive results in field studies of *P. californicus*. An earlier study of *T. gondii* tissue tropism in oocyst-inoculated laboratory mice, using mouse bioassay to detect the presence of *T. gondii* parasites from 4 different strains, found the brain to be the most frequently parasitized organ 62 to 130 days PI (Dubey, 1997). In the current study, IHC demonstrated that *T. gondii* parasites were most likely to be detected in the cerebrum. A recent study (Vyas et al., 2007) showed more specifically that *T. gondii* tissue cysts in rat and murine brain are most abundant in the amygdalae, structures located in temporal lobe of the cerebrum. Our hemi-nested PCR was most successful for spleen samples, but an earlier study (Dubey, 1997) detected *T. gondii* infection only in spleens from mice infected with the VEG (Type II) strain. However, mice in the earlier study were maintained for ≥20 day longer than the *P. californicus* mice in the present study, and the parasites may have been cleared from the spleen by the time bioassay was performed. Alternatively, PCR may be more sensitive than mouse bioassay for spleen, because PCR assay from the *BI* gene has an analytic sensitivity of 2 tachyzoites in 200 μl of serum (Haf-

id et al., 2001). A study in Manchester, U.K. (Marshall et al., 2004), where PCR methods were used to detect *T. gondii* infection in house mice, found an unusually high proportion (59%) of mice infected with *T. gondii*. The Manchester study demonstrates that PCR is a useful tool for field investigations. Although IHC methods are capable of confirming infection with *T. gondii* because they use *T. gondii*-specific cyst wall antibodies, PCR techniques will continue to enhance the value of field studies. In addition to being highly sensitive, PCR enables DNA sequencing so that *T. gondii* genotypes can be identified.

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