Polymerase chain reaction for the detection of *Toxoplasma gondii* within aqueous humor of experimentally-inoculated cats

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Abstract

The purpose of this study was to determine the temporal appearance of *T. gondii* in aqueous humor of cats orally inoculated with *T. gondii* using polymerase chain reaction (PCR) for the detection of the B1 gene. Serum and aqueous humor were collected from five SPF cats prior to oral inoculation with *T. gondii* and days 7, 14, 21, 28, 42, 84, 140, 147, 154, 161, 168, and 182 after inoculation. Cats were inoculated orally with *T. gondii* tissue cysts on day 0 and day 140. *T. gondii*-specific IgM and IgG were measured in serum and aqueous humor from the cats at each sample date. *T. gondii* B1 gene PCR was performed on all the aqueous humor samples and the amplified DNA was detected by Southern blotting. Chorioretinitis developed in three out of the five cats, but anterior uveitis was not detected. All cats developed *T. gondii*-specific IgG titers in serum, and had *T. gondii*-specific IgG C-values >1 in both eyes at varying times during the study. *T. gondii* was detected by PCR and Southern blotting in aqueous humor in both eyes of all cats at times varying from days 14–84 after primary inoculation and days 14–42 after challenge inoculation. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: *Toxoplasma gondii*; DNA; Polymerase chain reaction; Chorioretinitis; Aqueous humor
1. Introduction

Inflammation of the iris, choroid, or ciliary body (uveitis), is common in cats and can result in lens luxations, secondary glaucoma, and enucleation of the affected eye (Davidson et al., 1991; Peiffer and Wilcock, 1991; Chavkin et al., 1992). It has been hypothesized that *T. gondii* is a common cause of feline uveitis. Until recently, this hypothesis was supported primarily by epidemiologic studies (Chavkin et al., 1992; Lappin et al., 1992a), documentation of *T. gondii*-specific antibody production in aqueous humor (Lappin et al., 1992b; Chavkin et al., 1994), and clinical improvement of uveitis after administration of anti-*Toxoplasma* drugs (Lappin et al., 1989, 1992b).

*T. gondii* can be detected in the aqueous humor of some people with toxoplasmic uveitis by PCR and in conjunction with demonstration of ocular antibody production is optimal for the diagnosis of human ocular toxoplasmosis (Aouizerate et al., 1991, 1993; Chan et al., 1994). In an earlier study, *T. gondii* was detected by PCR in the aqueous humor in 8 of 43 (18.6%) client-owned cats with uveitis and in 2 of 23 (8.7%) healthy cats proving that *T. gondii* enters the aqueous humor of some naturally exposed cats (Lappin et al., 1996a). However, since there was only a single sample collection from each of the cats, the duration of *T. gondii* presence in aqueous humor is currently unknown.

The purpose of the present study was to determine the temporal appearance of *T. gondii* in aqueous humor of cats orally inoculated with *T. gondii* using PCR for the detection of the B1 gene.

2. Materials and methods

Adult (11–13 month old) specific pathogen free cats were purchased (Liberty Laboratories, NJ) and individually housed in stainless steel cages. These cats had no detectable *T. gondii*-specific IgM or IgG antibodies in serum at a 1:64 dilution prior to inoculation with *T. gondii* (Lappin et al., 1992b). Approximately 1000 tissue cysts of the Mozart strain of *T. gondii* were administered orally to each of the five cats after food was withheld overnight.

Fecal samples were collected daily for analysis for oocysts by use of sugar flotation (Dubey and Beattie, 1988) until 14 days post-oocyst shedding, then monthly for the remainder of the study. At week 20 (day 140) after primary inoculation (PI), the cats were inoculated orally with 1000 *T. gondii* tissue cysts of the same strain as described (challenge inoculation, CI). Feces were examined for oocysts daily as described for 14 days after CI. In order to collect blood and aqueous humor samples, cats were premedicated subcutaneously with 0.04 mg/kg of atropine sulfate and 0.05 mg/kg of acepromazine maleate. General anesthesia was induced by intravenous injection of thiopental sodium to effect. While under anesthesia, blood samples (3.0 ml) were collected by jugular venipuncture and aqueous humor samples (0.25 ml) were collected from both eyes by anterior chamber paracentesis using a 27-gauge needle introduced at the temporal limbus. Serum was separated by centrifugation, aqueous humor was placed in 1.5 ml EDTA-containing tubes, and both were stored at −70°C until assayed. Samples
were collected from each cat prior to inoculation with *T. gondii* and on days 7, 14, 21, 28, 42, 84, 140, 147, 154, 161, 168, and 182 after PI. Eyes were examined prior to inoculation, 1–2 times weekly for the first 4 weeks after PI, weekly for 4–6 weeks after PI, then monthly until CI. After CI, the eyes were examined on weeks 1 and 2, then every other week for the remainder of the study.

*T. gondii*-specific IgM and IgG ELISA were performed on each serum and aqueous humor sample (Lappin et al., 1992b). When *T. gondii*-specific IgM or IgG antibodies were detected in aqueous humor, total IgG or total IgM was quantified in serum and aqueous humor by ELISA and the IgM or IgG C-values were calculated as follows: 
\[(T. gondii\)-specific IgM or IgG in aqueous humor/ *T. gondii*-specific IgM or IgG in serum) \times (Total IgM or IgG in serum/Total IgM or IgG in aqueous humor).\] 
C-values >1 were considered suggestive of local production of *T. gondii*-specific antibodies in aqueous humor (Lappin et al., 1992b).

*T. gondii* B1 gene PCR was performed as previously described, except that Thermus thermophilus DNA polymerase (Tth polymerase) was used at 1U per reaction instead of Taq polymerase (Lappin et al., 1996a). The primers amplified a 133 bp sequence of the *T. gondii* B1 gene (van de Ven et al., 1991). Results were evaluated by gel electrophoresis in a 2% agarose gel stained with ethidium bromide. To confirm results, all samples were Southern blotted as previously described (Lappin et al., 1996a). Sensitivity of the *T. gondii* B1 gene PCR utilizing Tth polymerase was evaluated by digesting serial dilutions of tachyzoites of two different strains of *T. gondii* in aqueous humor from specific pathogen free cats utilizing a previously published protocol (Lappin et al., 1996a). Based on the titration experiments, the *T. gondii* B1 gene PCR employed here could detect 1–10 tachyzoites diluted in aqueous humor.

### 3. Results

All the five cats shed oocysts on days 3 to 13 after PI and no cats shed oocysts after CI. Clinical signs of systemic disease were not detected. On days 10, 11, and 21 PI, cats one, two, and three, respectively, developed chorioretinitis. All ocular lesions were inactive by day 31 and no other lesions were noted for the duration of the study. Ophthalmic evidence of anterior uveitis was not detected during the study. One cat (No. 2) was lost to the study due to an anesthetic complication on day 28 post CI.

*T. gondii*-specific IgM was not detected in aqueous humor of any cat during the study. *T. gondii*-specific IgG was first detected in serum from 3 of 5 cats on day 21 PI; all five cats were positive by day 42 PI. *T. gondii*-specific IgG C-values >1 were detected in both eyes of all the cats at some point in the study with the earliest positive results occurring on day 28 PI (Table 1).

A *T. gondii* PCR product was not detected from any aqueous humor sample by gel electrophoresis. Following Southern blotting, aqueous humor from both eyes of all the cats was positive for *T. gondii*, on multiple days PI with the earliest positives detected on day 14 PI (Table 1). All five cats were negative for *T. gondii* by PCR on day 0 and day 7 after challenge inoculation. All cats were positive for *T. gondii* in aqueous humor by PCR on at least one test date post CI. Cat two was the only cat that did not have *T. gondii*
detected in both eyes on the same test date. Toxoplasma gondii was detected in aqueous humor before local production of IgG was demonstrated in three/five cats. Of the 33 aqueous humor samples with T. gondii in aqueous humor, 20 had IgG C-values >1 concurrently (Table 1).

4. Discussion

Detection of T. gondii DNA in aqueous humor of all cats of this study after primary and challenge inoculation confirms our previous study which showed that T. gondii infects the eyes of some naturally exposed cats with and without uveitis (Lappin et al., 1996a). The PCR used here detected 1 to 10 tachyzoites in aqueous humor, antigen ELISA and lymphoblast transformation studies for detection of T. gondii antigen in aqueous humor had sensitivity limits of 5–20 ng of protein (Lappin et al., 1992b) and 600 ng of protein (Lin, 1994), respectively. Thus, PCR should be used in studies of aqueous humor in cases with suspected ocular toxoplasmosis. However, since T. gondii can be detected transiently by PCR in aqueous humor of some healthy cats, PCR results cannot be used alone to document clinical ocular toxoplasmosis.

T. gondii was detected in aqueous humor prior to local antibody production in all three cats with chorioretinitis suggesting that the technique is more sensitive in early stages of infection for the detection of ocular toxoplasmosis. T. gondii-specific IgG C-values >1

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<tr>
<th>Day PI/CI</th>
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Day PI/CI=Days after primary inoculation with T. gondii and days after challenge inoculation with T. gondii.
\(^{a}\)Cats developing chorioretinitis.
\(^{b}\)PCR positive.
OS: Left Eye; OD: Right eye
NA=This cat was lost to the study to causes unrelated to T. gondii just prior to the collections marked NA.
Numerical data represents T. gondii-specific IgG C-values; if no number is listed, T. gondii-specific antibody was not detected in aqueous humor.
were detected concurrently or before the organism was detected in cats four and five. It is likely that the organism was present and stimulated local antibody production in these two cats as well, but that PCR was negative. Aqueous humor samples may be negative by PCR due to low organism numbers present within the eye at the time of sampling. It is possible that the sample size of the aqueous humor taken for PCR would have precluded detection if only a few organisms were present within the eye. It is also possible that the organism was not in the ocular fluids at the time the fluid was sampled. Results described herein confirm murine studies that indicated that *T. gondii* organisms remain free in solution only for short periods (Lai et al., 1986; McMenamin et al., 1986). In addition, the organism may be present and replicating in ocular tissues, but may not be in aqueous humor leading to negative PCR findings. Detection of the organism at the time chorioretinitis was developing suggests that intraocular tachyzoite replication may play a role in causing ocular toxoplasmosis.

*T. gondii*-specific IgG C-values >1 have been detected in aqueous humor of other experimentally inoculated cats after challenge inoculation (Chavkin et al., 1994). Some of those cats also developed chorioretinitis suggesting that the organism disseminated to the eye again or was activated by repeat exposure. *T. gondii* was detected in the aqueous humor by PCR in all five cats described here after challenge inoculation documenting that the organism was present within the eye. *T. gondii* parasitemia can be detected by PCR in previously infected cats after challenge inoculation (Burney D.P., Ph.D. dissertation, Colorado State University). This suggests that the detection of the organism in aqueous humor from the cats described here may have been from repeat dissemination to the eye. Alternately, the organism may have been detected within aqueous humor after challenge from reactivation of the previous ocular infection. Inoculation of previously infected cats with Freund’s adjuvant, alone or in combination with soluble *T. gondii* antigens, can induce production of *T. gondii*-specific IgG in aqueous humor (Lappin et al., 1996b). It is possible specific or nonspecific immune stimulation can activate quiescent *T. gondii* in ocular tissues resulting in positive PCR results and an anamnestic immune response by the resident intraocular lymphocyte population. Lastly, detection of *T. gondii* in aqueous humor following challenge in this study may have been related to the relatively large dose of *T. gondii* used.

5. Conclusions

Results of this study add to the evidence that *T. gondii* infects the eyes of cats, that *T. gondii* DNA can be detected in aqueous humor of clinically normal cats (Lappin et al., 1996a), and that PCR techniques should be combined with antibody detection when assessment of aqueous humor is used to aid in the diagnosis of ocular toxoplasmosis.

References


