Effect of oral administration of cyclosporine on *Toxoplasma gondii* infection status of cats

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OBJECTIVE
To evaluate whether anti-inflammatory doses of cyclosporine activate *Toxoplasma gondii* in chronically infected cats or potentiate infection in cats exposed for the first time.

ANIMALS
30 *T gondii*-negative cats.

PROCEDURES
Cats were assigned to 1 of 3 groups (10 cats/group). Group 1 (control) cats were administered a placebo for 126 days; group 2 cats were administered a placebo for 84 days, followed by cyclosporine at 7.5 mg/kg/d, PO, for 42 days; and group 3 cats were administered cyclosporine at 7.5 mg/kg/d, PO, for 126 days. Cats were orally inoculated with *T gondii* on day 42. Results for fecal flotations, PCR assays, and histologic examinations and IgM and IgG titers were analyzed. Cyclosporine concentrations were measured on selected days.

RESULTS
All cats were infected by *T gondii* and developed signs of self-limiting gastrointestinal tract infection. Group 3 had the highest incidence and severity of CNS and pulmonary histopathologic findings typical of toxoplasmosis. One cat in group 3 died of systemic toxoplasmosis; that cat had a cyclosporine concentration of 1,690 ng/mL. Group 2 cats infected with *T gondii* before cyclosporine administration did not have repeated oocyst shedding. Group 3 cats shed fewer oocysts for a shorter time than did control cats of group 1.

CONCLUSIONS AND CLINICAL RELEVANCE
Oral administration of cyclosporine in accordance with the protocol for this study did not potentiate the enteroepithelial phase of *T gondii* infection. Cats with high cyclosporine blood concentrations at the time of primary *T gondii* infection may be at risk of developing systemic toxoplasmosis. (Am J Vet Res 2015;76:351–357)

Cyclosporine A has potent effects on the immune system. Rapidly reversible immunosuppression is induced primarily by blocking the transcription of genes encoding IL-2 and other cytokines. Absence of IL-2 synthesis prevents activation and proliferation of CD4+ lymphocytes and secondary synthesis of other cytokines, including IL-4 and interferon-γ. Classically, high doses of cyclosporine have been administered to cats to lessen the chance for organ rejection in those undergoing renal transplantation. At lower doses, cyclosporine has been used in the treatment of inflammatory bowel disease, stomatitis, and dermatitis in cats that are refractory to other drugs and most recently has been approved at a dose of 7 mg/kg for the treatment of feline allergic hypersensitivity dermatitis. When administered at high doses, the drug has been associated with activation of a variety of chronic infectious diseases or worsening of disease if primary exposure occurred while the cat was receiving immunosuppressive dosages.

*Toxoplasma gondii* is one of the most common zoonotic parasites of cats. In a study of 12,628 cats in the United States, 31.6% were reported to be infected. The sexual cycle of *T gondii* is completed in the intestines of cats. After a 1- to 5-day sporulation period, oocysts passed in feces are infectious to most vertebrate hosts, including humans. Administration of immunosuppressive doses of glucocorticoids can induce repeated oocyst shedding in some cats. However, when anti-inflammatory doses of glucocorticoids were administered to cats with acute or chronic toxoplasmosis in a previous study, repeated oocyst shedding was not detected. In another study, cats with and without FIV were inoculated with *T gondii*. The results suggest that oocyst shedding was similar between groups, and both groups of cats failed to shed oocysts when reinfected with *T gondii* months after the primary infection. Immunity against *T gondii* is based in part on a strong T-helper 1–lymphocyte response, including active production of interferon-γ. Thus, toxoplasmosis has been reported in some cats treated with cyclosporine. However, it is currently unknown whether anti-inflammatory doses of cyclosporine activate *T gondii* in chronically infected cats or potentiate *T gondii* infection in cats infected for...
Materials and Methods

Animals

Thirty 1- to 2-year-old domestic shorthair cats (15 males and 15 females) that had negative results when tested for T gondii were enrolled in the study. These laboratory purpose-bred cats were healthy as determined on the basis of results for a comprehensive physical examination (including neurologic assessment and ophthalmic examination) performed by a veterinarian and review of results of fecal examination for parasites and laboratory evaluations (hematologic evaluation, coagulation profile, clinical biochemistry analysis, and urinalysis). Cats had negative results when tested for FeLV antigen and antibodies against FIV as determined with a commercially available kit. Cats were allowed to acclimate for approximately 2 weeks prior to the start of the study; cats were housed individually in cages with perches, litter pans, and enrichment toys in 2 environmentally controlled rooms (room temperature, 17.8°C to 28.9°C; relative humidity, 50% to 70%; and 12 hours of light to 12 hours of darkness). Each cat was uniquely identified by an ear tattoo. A commercially available diet and municipal tap water were available ad libitum, except during designated periods. Procedures were reviewed and approved by an institutional animal care and use committee and were in compliance with Novartis Animal Health animal welfare guidelines, the USDA Animal Welfare Act (2009), and the Public Health Service Policy on Humane Care and Use of Laboratory Animals (1986).

Study design

This study was conducted in accordance with a masked, randomized parallel design, with cat as the experimental unit. Food was withheld from cats every night beginning on day –8. Cats were assigned (on the basis of sex and body weight) by use of a random number generator to 3 treatment groups on day –1. There were 10 cats/group (5 males and 5 females). Cats of each of the 3 groups were housed in both of the environmentally controlled rooms.

Group 1 (control group) cats received a placebo orally on days 0 to 125 (total, 126 days). Group 2 cats received the placebo orally on days 0 to 83 (total, 84 days) and cyclosporine (7.5 mg/kg/d, PO) on days 84 to 125 (total, 42 days). Group 3 cats received cyclosporine (7.5 mg/kg/d, PO) on days 0 to 125 (total, 126 days). The placebo or cyclosporine was administered orally via syringe (volume measured to the nearest 0.01 mL) at approximately the same time each morning by investigators who were aware of the treatment group for each cat but were not involved in any other assessments. Dose of the placebo or cyclosporine was based on the most recent weekly body weight for each cat (measured to the nearest 0.01 kg). All cats were offered food at least 10 minutes before dose administration; the first day of administration was designated as day 0.

T gondii inoculation

All cats were inoculated orally with T gondii on day 42. Cats were inoculated with the Mozart strain of T gondii cultivated from mice. This strain initially had been isolated from the eye of a cat with chronic ocular toxoplasmosis and was found to induce oocyst shedding in cats. Young adult, female CF-1 mice previously inoculated SC with a serial passage of T gondii tissue cysts were shipped by air freight to our research facility. On day 42, some of the mice were euthanized (anesthetized with isoflurane, which was followed by cervical dislocation). The brain of each mouse was removed, macerated with scissors, passed repeatedly through an 18-gauge needle, and pooled in sterile saline (0.9% NaCl) solution for use as the inoculum for the cats. Mean tissue cyst count (mean of two 50-μL aliquots of the pooled inoculum) was 54, which was equivalent to approximately 10³ cysts/mL. All cats were inoculated orally with 1 mL of the inoculum.

After all cats were inoculated, an aliquot (0.1 mL) of the remaining inoculum was administered SC into 5 CF-1 mice, which were then shipped by air freight to a reference laboratory for viability testing and treatment with trimethoprim-sulfamethoxazole for 10 days. These mice subsequently were euthanized as previously described. The brain of each of these mice was removed and histologically examined; T gondii cysts were identified in the brain tissues, which confirmed the inoculum contained living T gondii.

In vivo observations

All cats were observed by a trained technician twice each day throughout the study period to detect morbidity, death, or injury and for evidence of food and water consumption. All cats were observed daily for signs of depression, coughing, dyspnea, ocular abnormalities, and CNS abnormalities on days 42 (prior to inoculation with T gondii cysts) to 127. A veterinarian was available to further investigate abnormalities when necessary. Baseline values for body weight and feed consumption were established during the acclimation period. During treatment administration, body weight was recorded and feed consumption was calculated on a weekly basis. A veterinarian performed a physical examination, including neurologic assessment, on each cat twice during the acclimation period and on days 13, 55, and 97. Each examination involved, at a minimum, assessment of general health, behavior, rectal temperature, mucous membranes, integument, equilibrium and coordination, body condition, and the cardiovascular, respiratory, renal, urogenital, gastrointestinal, musculoskeletal, and reproductive systems. Ophthalmic examinations were performed on all cats by a board-certified veterinary ophthalmologist.
prior to inoculation with *T. gondii* as well as 15 and 80 days after inoculation. Urine and blood samples for hematologic evaluation, coagulation testing, and clinical biochemical analysis were collected from all cats twice during the acclimation period (once during the first week of acclimation and once during the second week of acclimation prior to assignment to a group) and on days 0, 14, 28, 41, 56, 70, 84, 98, 112, and 125.

*T. gondii* oocyst shedding

Feces were collected from each cat twice during the acclimation period (once during the first week of acclimation and once during the second week of acclimation prior to assignment to a group) and then daily on days 42 (prior to *T. gondii* inoculation) through 126. Fecal samples were individually packaged, labeled, and shipped by overnight air freight on cold packs to the reference laboratory. After fecal samples arrived at the reference laboratory, they were processed immediately or stored at 4°C and processed within 2 days after arrival. Fecal samples were subjected to sugar solution centrifugation, and oocyst numbers on the entire cover slip were counted by the same investigator (AVS) throughout the study. For statistical analysis, a score of 0 to 5 was assigned as follows: 0 = 0 oocysts, 1 = 1 to 50 oocysts, 2 = 51 to 100 oocysts, 3 = 101 to 200 oocysts, 4 = 201 to 500 oocysts, and 5 = > 500 oocysts. All fecal samples assessed prior to *T. gondii* inoculation of each cat had negative results when tested for *T. gondii* oocysts and other enteric parasite eggs, cysts, or oocysts.

**Anti-*T. gondii* IgM and IgG ELISA**

Serum samples for ELISAs were obtained from each cat at the same times as those for clinicopathologic testing and shipped by overnight air freight on cold packs to the reference laboratory. After serum samples arrived at the reference laboratory, they were stored at 4°C and assayed within several working days or stored at −70°C for subsequent assay in batches. All anti-*T. gondii* IgM and IgG assays were performed by trained technicians, who used the standard operating procedures for the laboratory. Results were recorded as negative or as the reciprocal titer for statistical analysis. All cats had negative results for anti-*T. gondii* IgM and IgG prior to *T. gondii* inoculation.

**T. gondii** PCR assay

Blood samples were collected at the same times that serum samples were obtained for clinicopathologic testing. Blood samples were placed into tubes containing EDTA and shipped by overnight air freight on cold packs to the reference laboratory. After blood samples arrived at the reference laboratory, they were stored at 4°C until DNA was extracted and assayed in batches by use of a PCR assay that amplified *T. gondii* DNA. All *T. gondii* PCR assays were performed by trained technicians who used the standard operating procedures for the laboratory. Results were recorded as positive or negative for statistical analysis. All cats had negative results for PCR assays on samples obtained before *T. gondii* inoculation.

### Necropsy and histologic evaluation

Cats were euthanized on study day 126 or 127 by IV administration of an overdose of sodium pentobarbital solution followed by exsanguination. Gross postmortem examination was conducted, and the full complement of organs and tissues were collected under the supervision of a veterinary pathologist. Organs were removed, examined, weighed (paired organs were weighed together), and, where indicated, placed in 10% neutral-buffered formalin for fixation. Eyes were fixed in modified Davidson fixative, and lungs were infused with formalin. Representative samples of organs and tissues as well as any gross lesions were prepared and processed in accordance with standard histologic methods. Paraffin sections were stained with H&E, and slides were initially examined by a veterinary pathologist at a commercial laboratory. Subsequently, slides of lung and CNS tissues with the most severe changes were reviewed separately by another veterinary pathologist who had extensive experience with *T. gondii* infection. Both pathologists were unaware of the treatments administered or the group assignment for each cat.

Following euthanasia and necropsy of each mouse, portions of brain and lung tissue were collected, packaged separately, and shipped frozen to the reference laboratory where they were stored at −70°C. The tissues subsequently were thawed, and a representative 25-mg sample was collected for DNA extraction and analysis with the *T. gondii* PCR assay.

### Statistical analysis

Cat was the experimental unit. Continuous variables measured only once (eg, organ weight) during the study were analyzed by use of an ANOVA. The model included treatment, sex, and the sex-by-treatment interaction as fixed effects. Continuous outcomes measured multiple times during the study (eg, clinicopathologic variables) were analyzed by use of a repeated-measures ANCOVA, with the classification variables nested within block by sex (random effect), and, where indicated, the sex-by-treatment interaction as fixed effects. Continuous outcomes measured multiple times during the study (eg, clinicopathologic variables) were analyzed by use of a repeated-measures ANCOVA, with the classification variables nested within block by sex (random effect), treatment, time, and sex and corresponding 2- and 3-way interactions. The pretreatment value or the mean of the 2 pretreatment values closest to onset of placebo or cyclosporine administration was used as a covariate. Depending on the significance of interactions, treatments were compared in a pairwise manner overall or for each day on the basis of linear contrasts. All analyses were performed with the aid of commercially available software.

For the 84 days after *T. gondii* inoculation, the total number of days *T. gondii* oocysts were detected (fecal score > 0) was calculated for each cat, and group means were compared by use of a 1-way ANOVA. For the 84 days after *T. gondii* inoculation, differences in the numbers of *T. gondii* oocysts shed among groups.
and differences in the magnitude of anti-\textit{T. gondii} IgG titers over time were assessed by use of a repeated-measures ANOVA. Numbers of cats with positive results for IgM in serum or for results of PCR assay of \textit{T. gondii} DNA in blood were too small for statistical evaluation and thus were reported descriptively.

To determine whether the presence of inflammation detected during histologic evaluation of tissues was related to treatment group, each tissue was scored as positive (any inflammation) or negative (no inflammation) and compared among groups of cats by means of a Fisher exact test (2 tailed). For statistical analyses, values of $P \leq 0.05$ were considered significant.

**Results**

**Clinical findings**

One cat in group 3 died of disseminated toxoplasmosis on day 64 (22 days after inoculation with \textit{T. gondii}). Three days prior to death, the cat was febrile and lethargic, had labored breathing, and appeared dehydrated. The cyclosporine concentration in blood samples obtained from this cat for the last sample collected before death (day 56 [14 days after inoculation with \textit{T. gondii}]) was 1,690 ng/mL; the other group 3 cats had cyclosporine concentrations that ranged from 307 to 951 ng/mL (mean ± SD, 626 ± 259 ng/mL) in blood samples obtained on that same day. In addition, 1 cat in group 3 developed protracted vomiting and weight loss and was euthanized on day 84 (42 days after \textit{T. gondii} inoculation); the cyclosporine concentration was 419 ng/mL in a sample obtained immediately prior to euthanasia. Necropsy findings for that cat were consistent with pancreatitis, and \textit{T. gondii} oocysts were reported in the pancreatic duct epithelial cells.

No cat in the study developed anterior uveitis. On day 57, ophthalmic examinations revealed 2 cats of group 2 with punctate tapetal scars and 2 cats (1 cat of group 1 and the other of group 3) with punctate nontapetal scars. On day 122, 2 cats of group 2 and 1 cat of group 1 had punctate tapetal scars and 1 cat of group 3 had retinal edema of the left eye and a nontapetal retinal scar in the right eye. Clinical signs that could occur with \textit{T. gondii} intestinal tract infection, including bloody feces, lethargy, and vomiting or regurgitation, were seen in all cats of all 3 groups but resolved within 6 weeks after inoculation. These clinical signs were more severe and lasted the longest in group 3 cats. There were no significant differences in hematologic findings among cats of the 3 groups. The activated partial thromboplastin time was prolonged (40 to 60 seconds) in cats administered cyclosporine, compared with the result for control cats. In addition, cholesterol, glucose, total protein, and globulin concentrations were slightly higher than reference ranges in cyclosporine-treated cats. No treatment-related effects on urine variables were detected. There were no differences in mean body weight among groups; however, several cats in each group lost weight after \textit{T. gondii} inoculation. Most cats returned to preinoculation body weights by day 70 (28 days after \textit{T. gondii} inoculation). In addition, several cats in all 3 groups had decreased food consumption following inoculation, with their appetites returning to preinoculation levels by day 56 (14 days after \textit{T. gondii} inoculation).

**\textit{T. gondii} oocyst shedding duration**

The range of days on which oocysts were first detected, the range of total days on which oocysts were detected, and the mean number of days on which oocysts were detected were summarized (Table 1). Mean values differed significantly ($P = 0.045$) among groups. Cats of groups 1 and 2 shed oocysts for a significantly longer period than did cats of group 3, and group 2 cats shed oocysts for a significantly longer period than did group 1 cats. Of the 27 cats from which fecal samples were available throughout the entire 84-day \textit{T. gondii} postinoculation period, 25 had completed oocyst shedding by day 57 (15 days after inoculation). Oocysts were detected in feces of 1 cat from group 1 on days 63, 64, and 66 (21, 22, and 24 days after inoculation). For 1 cat of group 3, oocysts were detected in feces on days 49 to 52 (7 to 10 days after inoculation), were not detected in feces from days 53 to 78 (11 to 36 days after inoculation), and then were detected in feces on days 79 and 80 (37 and 38 days after inoculation). \textit{Toxoplasma gondii} oocyst shedding was not detected in any group 2 cat after initiation of cyclosporine administration on day 84 (42 days after \textit{T. gondii} inoculation).

**Numbers of \textit{T. gondii} oocysts shed**

Analysis of the oocyst shedding scores over time revealed a significant group-by-time interaction. Overall group effects were detected on days 47 to 55 (5 to 13 days after \textit{T. gondii} inoculation; Figure 1). The oocyst shedding score for group 3 was significantly lower than that for groups 1 and 2 on days 47 to 53 (5 to 11 days after \textit{T. gondii} inoculation); however, the oocyst shedding score for group 1 was significantly lower than that for group 2 on days 48 to 53 (6 to 11 days after \textit{T. gondii} inoculation).

**Table 1—Shedding of \textit{Toxoplasma gondii} oocysts for 3 groups of cats (10 cats/group).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Period when oocysts first detected*</th>
<th>Oocyst shedding period (No. of days)</th>
<th>Mean duration of oocyst detection (No. of days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47–53 (5–11)</td>
<td>4–9</td>
<td>5.9*</td>
</tr>
<tr>
<td>2</td>
<td>47–50 (5–8)</td>
<td>3–9</td>
<td>6.5*</td>
</tr>
<tr>
<td>3</td>
<td>48–52 (6–10)</td>
<td>2–7</td>
<td>4.1*</td>
</tr>
</tbody>
</table>

*Results represent the number of days in the study (number of days after oral administration of \textit{T. gondii}).

Values with different superscript letters differ significantly ($P \leq 0.05$).
separately by 2 veterinary pathologists, cyclosporine in tissue PCR assay of results for histologic examination and DNA in blood. *T gondii* inoculation), 3 cats in each and IgG but had *T gondii* IgM titers. This cat was seronegative for anti–*T gondii* IgM and IgG prior to *T gondii* inoculation. On that same day (day 56 [14 days after *T gondii* inoculation]), this cat was seronegative for anti–*T gondii* IgM and IgG titers and shortly before the cat died of disseminated toxoplasmosis.

The group 3 cat that died of disseminated toxoplasmosis was seronegative for antibodies against *T gondii*. The remaining cats developed detectable IgG titers; 20 of 28 cats and 28 of 28 cats with serum available for testing were seropositive by days 70 and 84 (28 and 42 days after *T gondii* inoculation), respectively. Repeated-measures analysis revealed that the group-by-time interaction was significant. Overall group effects were detected only on day 126 (84 days after *T gondii* inoculation), whereby IgG titers detectable IgG titers, and all of the 27 cats that completed the 84-day study period after inoculation with *T gondii* shed oocysts. Cats with previous *T gondii* infection generally are seropositive, rarely shed oocysts, and, if oocysts are shed, shed them in small numbers. Thus, the cats in the present study likely were *T gondii* negative prior to inoculation. The detection of shedding of *T gondii* oocysts and development of anti–*T gondii* IgG titers confirmed that the organisms in the inoculum were alive. The Mozart strain of *T gondii* has been used in 2 other studies. The final inoculum dose of 10³ cysts/cat used in the present study was higher than that used previously and was chosen in an attempt to mimic the maximum dose of *T gondii* a naive cat would likely ingest in a field setting. This high dose of *T gondii* also maximized the likelihood that we would be able to detect any adverse effects.

A concern for the use of cyclosporine in cats is the potential for immune suppression induced by the drug to affect shedding of *T gondii* oocysts, which might increase the zoonotic risk. The purpose of administering the placebo for 84 days followed by cyclosporine at 7.5 mg/kg/d, PO, for 42 days in group 2 cats was to determine whether the dose and formulation of cyclosporine used could induce additional oocyst shedding in cats that had completed the *T gondii* oocyst shedding period. Shedding of *T gondii* oocysts was not detected in group 2 cats for the 27 days prior to initiation of cyclosporine administration, and oocysts were not detected in feces during the 42 days of cyclosporine administration. These results suggested that cyclosporine administration did not reactivate oocysts in 3 groups of cats (10 cats/group). Cats of group 1 (black bars) received a placebo for 126 days, cats of group 2 (gray bars) received a placebo for 84 days followed by cyclosporine (7.5 mg/kg/d, PO) for 42 days, and cats of group 3 (white bars) received cyclosporine (7.5 mg/kg/d, PO) for 126 days. All cats were orally inoculated with *T gondii* on day 42 of drug or placebo administration.

Anti–*T gondii* IgM and IgG titers

All cats had negative results when tested for anti–*T gondii* IgM and IgG prior to *T gondii* inoculation. Only 3 cats had IgM titers after *T gondii* inoculation: 1 cat of group 1 on day 98 (56 days after *T gondii* inoculation), 1 cat of group 3 on days 98 and 112 (56 and 70 days after *T gondii* inoculation), and another cat of group 3 on days 84, 98, and 112 (42, 56, and 70 days after *T gondii* inoculation).

The group 3 cat that died of disseminated toxoplasmosis was the most severe CNS and pulmonary disease were not detected and no *T gondii* organisms were observed in tissues of cats that survived to day 126 (84 days after *T gondii* inoculation). *Toxoplasma gondii* DNA was not amplified from any of the lung tissues and was amplified only from brain tissue of 2 of 10 group 2 cats and 1 of 7 group 3 cats.

Discussion

Prior to *T gondii* inoculation, cats in the study reported here had negative results for antibodies specific against *T gondii* in serum and for *T gondii* oocysts in feces. All cats, except for 1, developed detectable IgG titers, and all of the 27 cats that completed the 84-day study period after inoculation with *T gondii* shed oocysts. Cats with previous *T gondii* infection generally are seropositive, rarely shed oocysts, and, if oocysts are shed, shed them in small numbers. Thus, the cats in the present study likely were *T gondii* negative prior to inoculation. The detection of shedding of *T gondii* oocysts and development of anti–*T gondii* IgG titers confirmed that the organisms in the inoculum were alive. The Mozart strain of *T gondii* has been used in 2 other studies. The final inoculum dose of 10³ cysts/cat used in the present study was higher than that used previously and was chosen in an attempt to mimic the maximum dose of *T gondii* a naive cat would likely ingest in a field setting. This high dose of *T gondii* also maximized the likelihood that we would be able to detect any adverse effects.

A concern for the use of cyclosporine in cats is the potential for immune suppression induced by the drug to affect shedding of *T gondii* oocysts, which might increase the zoonotic risk. The purpose of administering the placebo for 84 days followed by cyclosporine at 7.5 mg/kg/d, PO, for 42 days in group 2 cats was to determine whether the dose and formulation of cyclosporine used could induce additional oocyst shedding in cats that had completed the *T gondii* oocyst shedding period. Shedding of *T gondii* oocysts was not detected in group 2 cats for the 27 days prior to initiation of cyclosporine administration, and oocysts were not detected in feces during the 42 days of cyclosporine administration. These results suggested that cyclosporine administration did not reactivate oocysts in the inoculum were alive. The Mozart strain of *T gondii* DNA was not amplified from any of the lung tissues and was amplified only from brain tissue of 2 of 10 group 2 cats and 1 of 7 group 3 cats.
cyst shedding. This finding is similar to that previously reported after the administration of anti-inflammatory doses of glucocorticoids.16 Thus, it is unlikely that administration of cyclosporine to cats previously infected with *T. gondii* will increase the risk that humans will encounter *T. gondii* oocysts.

The purpose of including group 3 was to determine whether the cyclosporine dose, formulation, and duration would lead to a prolonged *T. gondii* oocyst shedding period or lead to shedding of a greater number of *T. gondii* oocysts in treated cats. Neither group 1 nor 2 had been administered cyclosporine when *T. gondii* was inoculated into all 3 groups. The detection of a shorter period of oocyst shedding and lower oocyst scores in group 3 cats, compared with results for groups 1 and 2, suggested that *T. gondii*-naïve cats treated with cyclosporine likely will not increase the risk of *T. gondii* exposure to humans by increasing the duration of the *T. gondii* oocyst shedding period or the numbers of oocysts shed. The results also suggested that the previously described anti-*T. gondii* effect of cyclosporine28,29 may have influenced *T. gondii* oocyst shedding. Differences in the duration of oocyst shedding and oocyst scores also were detected between groups 1 and 2; the explanation for this is unclear because the groups were of similar sex and age. However, the range of days for oocyst shedding was small for all groups of cats; thus, this finding is unlikely to have biological relevance. Overall, oocyst shedding results for the 3 groups were tightly clustered and similar to those previously reported for this strain.24 In 1 cat of group 3, *T. gondii* oocysts were detected in feces on days 79 (37 days after *T. gondii* inoculation [22 oocysts]) and 80 (38 days after *T. gondii* inoculation [19 oocysts]), but these were small numbers of oocysts and likely to be epidemiologically unimportant, as described for other cats that repeated an oocyst shedding period.30 Oocyst shedding occurs prior to the detection of IgG antibodies in most cats, so the negative predictive value of the assay is > 100%.16,24 In addition, some cats positive for anti-*T. gondii* antibodies repeat oocyst shedding when exposed again to the organisms,30 which suggests that this antibody class cannot be used to predict resistance to shedding.

Other concerns for veterinarians prescribing anti-inflammatory doses of cyclosporine are whether the drug can induce sufficient immunosuppression to activate preexisting subclinical *T. gondii* infection or potentiate the pathogenesis of *T. gondii* infection if there is a primary exposure during cyclosporine treatment. Thus, another reason for including group 2 was to determine whether the dose and formulation of cyclosporine used could reactivate preexisting infection and result in measurable disease. Considering that severe polysystemic or substantial ocular disease was not detected in these cats, the results suggested that cyclosporine (at a dosage of 7.5 mg/kg/d, PO) did not reactivate quiescent *T. gondii* infection. This finding is similar to that previously reported after the administration of anti-inflammatory doses of glucocorticoids.16 Thus, it is unlikely that administration of cyclosporine to cats previously infected with *T. gondii* will develop clinical toxoplasmosis. In future studies, immune function variables (eg, interferon-γ production) important for maintaining *T. gondii* in tissues (eg, bradyzoites) should be evaluated.18

The purpose of the administration of cyclosporine at 7.5 mg/kg/d, PO, for 126 days to group 3 cats was to determine whether the cyclosporine dose, formulation, and duration used in the study would lead to potentiated clinical illness in cats during an initial exposure to *T. gondii*. Clinical signs consistent with *T. gondii* infection were seen in all cats after inoculation; however, most signs resolved within 6 weeks. Cats of groups 1 and 2 did not develop severe polysystemic or ocular disease. One cat of group 3 died of disseminated toxoplasmosis on day 56 (22 days after *T. gondii* inoculation), and a second cat of group 3 was euthanized before day 84 (42 days after *T. gondii* inoculation). The last available sample for a third cat of group 3 that was found dead was obtained 10 days prior to its death; the cyclosporine concentration in that sample was approximately 1,000 ng/mL higher than the mean concentration for the other cats in group 3 at that same time and > 700 ng/mL higher than the next highest concentration. The increased cyclosporine concentration in this cat may explain the reason for disseminated toxoplasmosis inasmuch as the drug may have lessened Thelper 1-lymphocyte responses. However, *T. gondii* is a dangerous pathogen, and infection can result in the death of apparently healthy cats after primary exposure. Use of this strain at a lower dose reportedly resulted in death of 2 adult cats that were not administered cyclosporine24,27; however, both of those cats were pregnant. None of the other cats of group 2 or 3 that were administered cyclosporine had increasing IgG titers over 2 time points or the presence of *T. gondii* DNA in blood, both of which would have been indicative of *T. gondii* activation. However, the use of increasing IgG titers as evidence of *T. gondii* activation may not be accurate in cats treated with cyclosporine because titer increases could be blunted by the drug. Overall, it appears unlikely that the cyclosporine dose, formulation, and duration used in the present study will induce toxoplasmosis in most previously naïve cats. However, the finding emphasizes the importance that cats should not be allowed to hunt prey and should be fed processed foods to lessen risk of exposure to *T. gondii* and other potential pathogens when cats are receiving agents that have potential immunosuppressive effects.

In cats that survived to the completion of the study (day 126), necropsy revealed inflammation in some lung and CNS tissues. However, differences existed in scores assigned by the 2 pathologists. Because *T. gondii* organisms were not detected in the affected tissues, *T. gondii* DNA was detected only occasionally in the samples of lung and CNS tissues tested, and the cats were clinically normal at the end of the study, it
is unclear whether the inflammation was important or related to Toxoplasma gondii infection.

In the present study, cyclosporine increased the severity of Toxoplasma gondii infection in naive (seronegative) cats but not in cats previously exposed to Toxoplasma gondii infection (seropositive). Naïve cats may be at risk of developing clinical toxoplasmosis if they become infected while receiving cyclosporine treatment, and such an infection can be fatal. In the event infection with Toxoplasma gondii or any serious illness is suspected, cyclosporine administration should be stopped and appropriate treatment initiated. In this study, treatment with cyclosporine did not reactivate oocyst shedding in cats previously exposed to Toxoplasma gondii.

Acknowledgments

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Presented in part as an abstract at the American College of Veterinary Internal Medicine Forum, Denver, June 2011.

Footnotes


b. SNAP FelV/FIV Combo, IDEXX Laboratories, Portland, Me.

c. Lab Diet, certified feline diet No. 5003, PMI Nutrition International Inc, St. Louis, Mo.

d. Karo syrup, ACH Food Co Inc, Memphis, Tenn.

e. Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, Colo.

f. Experimental Pathology Laboratories Inc, Herndon, Va.

g. Dr. David Getzy, private consultant, Fort Collins, Colo.

h. SAS/STAT software, version 9.1, SAS Institute Inc, Cary, NC.

References


20. Undersecretary for marketing and regulatory programs. 7 CFR 2.22.


