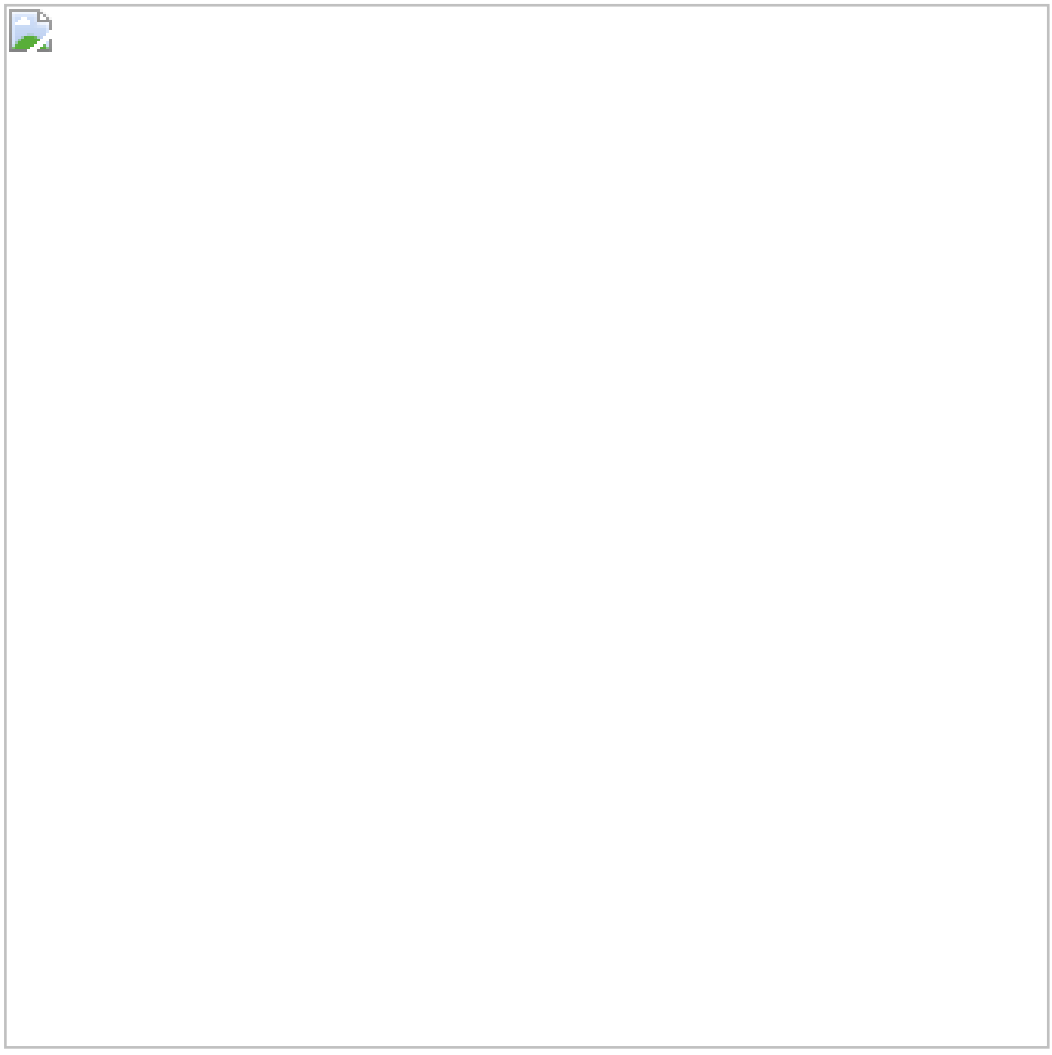

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Prevalence of *Toxoplasma gondii* IgM and IgG positive cats in Los Angeles County, California

Roubina Honarchian, Tracy Yen, Eva Ganz and Gie Kang

Abstract

Objectives The objective of this study was to determine the prevalence of *Toxoplasma gondii* IgM and IgG positive cats in Los Angeles County, California. *T gondii* antibodies are common in sera from cats in most reported studies around the world. Although the majority of infected cats never develop clinical disease, development of acute infection and recrudescence of latent infection secondary to immunosuppression has been reported. Knowledge of the serologic status of *T gondii* may be important when considering immunosuppressive treatments.

Methods *T gondii* IgM and IgG antibody titers were measured in 225 cats. Sera from owned cats tested at a multispecialty veterinary hospital were included both retrospectively and prospectively (n = 125). Sera from feral cats tested through a collaborating humane society were included prospectively (n = 100).

Results Of the 13 (5.8%) cats with IgM titers, 10 were positive at the minimal cut-off titer (1:64), one cat was clinically ill and none were currently positive for IgG antibodies, suggesting false-positive results for nine cats, giving an adjusted IgM prevalence rate of 1.8% (95% CI 0.7–4.5). A total of five (2.2%) cats were positive for IgG antibodies and no cat was positive for both antibodies.

Conclusions and relevance Most studies of *T gondii* antibodies in cat sera from California have shown higher prevalence rates, suggesting the cats in this municipality have a low risk of exposure. The study emphasizes that serological test results do not necessarily correlate to the presence of clinical illness.

Keywords: Toxoplasma gondii; *T gondii*; IgM; IgG; prevalence; Los Angeles

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Introduction

Toxoplasma gondii is an important worldwide protozoan.[1,2] This agent continues to be a major public health concern as it infects both humans and animals. Since only cats complete the sexual phase resulting in the passage of oocysts into the environment, this species is often focused on when *T gondii* is discussed.[3] Although *T gondii* infection is seen in domestic animals, wild animals and humans, infection is usually subclinical.[1,2] Cell-mediated immunity is the main immune defense in the control of *T gondii* and some immunosuppressive therapies may place some cats at greater risk for development of clinical illness.[4–8]

The reasons why some animals develop clinical disease and others do not are multifactorial and not completely understood. In one study of cats experimentally inoculated with *T gondii* and then administered cyclosporin alone, clinical toxoplasmosis was not induced.[7] In addition, cats that were administered cyclosporin before *T gondii* unexpectedly shed less oocysts in feces than control cats, likely since this drug is also an antimicrobial agent.[7]

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Many veterinary clinicians who need to prescribe immunosuppressive treatments in the management of diseases in cats are concerned about reactivation of *T gondii*. The understanding of the prevalence of *T gondii* in the regional feline population can be beneficial when assessing risk in patients. Prevalence rates of *T gondii* varies with lifestyle, sex and age.[1,2,9] Older male cats fed raw food diets or that hunt are commonly associated with increased rates of *T gondii* antibody prevalence. Prevalence in urban vs rural environments and between owned and feral cats may or may not differ.[3,9] Prevalence can vary significantly in different countries, cities and even within a city.[2,4,9]

Once a cat is exposed to *T gondii*, IgM antibodies are generally detected first, followed by seroconversion to IgG in healthy cats.[1] Results from IgM assays are commonly false negatives due to the short duration and can be false positives in both indirect fluorescent antibody (IFA) and ELISA in approximately 2–5% of specific pathogen-free cats when assessed at the 1:64 dilution. Thus a 1:128 dilution has been used as the cut-off in some field studies.[1,10] Once cats have developed IgG antibodies, most maintain persistent tissue infections. Thus, the presence of IgG antibodies is generally used to estimate true infection rates.

Multiple studies about *T gondii* antibody prevalence have been performed around the world, including in California.[2,9,10] However, it can be difficult to determine whether the data are from large municipalities or a mixture with suburban or rural areas. Thus, the primary aim of the present study was to report feline *T gondii* seroprevalence rates in Los Angeles, California to serve as an example of an urban environment.

Materials and methods

Sera from 125 client-owned cats (Metropolitan Animal Specialty Hospital) and 100 feral cats (Pasadena Humane Society) were tested for *T gondii* antibodies over the course of the study. To the authors' knowledge, all 225 were currently living in Los Angeles County at the time of sample collection, but where the cats had lived previously was unknown.

All sera were tested for IgM and IgG antibodies in 1/3 tests at either IDEXX Laboratories or Antech Laboratories, with the results reported as separate titers. At IDEXX Laboratory, some samples were assayed by IFA (IDEXX IgM/IgG titers by IFA) and others by ELISA (IDEXX toxoplasmosis split titer ELISA Test). At Antech Laboratory, all samples were assayed by ELISA (IgG and IgM 2 panel ELISA). While complete information is unknown for each of these assays, at least some of the ELISA tested samples have the quality control performed at Colorado State University (https://www.dlab.colostate.edu). A titer of 1:64 was considered positive in the ELISA and a titer of 1:25 was considered positive in the IFA.

For the 125 client-owned cats, data collected from medical records included the *T gondii* test performed and results, breed, age, sex, living environment, clinical signs (if any), the presenting service, and the results of a complete blood cell count and serum biochemical panel. Cats were categorized as either 'allergic' or 'sick'. The allergic population included cats that were presented to the MASH Dermatology service and diagnosed by the authors (EG, TY, RH) as having allergic disease. These cats were screened for *T gondii* antibodies before the initiation of ciclosporin (CsA) therapy. All cats in the allergic category were apparently healthy, aside from their allergic disease. Sick cats were tested for *T gondii* antibodies after exhibiting clinical signs consistent with systemic toxoplasmosis. Clinical signs included (but were not limited to) seizures, abnormal mentation, pulmonary nodules, diarrhea, lethargy, anorexia, weight loss, leukopenia and difficulty walking/paresis.

The 100 feral cats were from throughout Los Angeles County and presented by various organizations for vaccination and sterilization through a trap–neuter–return program. Informed consent was granted by the veterinarian overseeing the program. After sedation for their surgical procedure, 2–3 ml of blood was collected from cats via venipuncture into a serum separator tube and the blood submitted to IDEXX Laboratories for the IgM and IgG ELISA assay. Cats were assigned a number from 1 to 3 based on approximate age (1 = approximately 3 months to 1 year, 2 = approximately 1–3 years, 3 = approximately 3–5 years). Cats suspected to be aged <12 weeks were excluded from the study. Breed and sex were recorded.

Statistical analysis

An initial review of the serologic test results was performed and due to the low prevalence rates for both *T gondii* IgM and IgG antibody titers, the data are presented descriptively. Estimated prevalence rates and 95% Cis were calculated using SAS version 9.4 (SAS Institute) and Wilson score binomial confidence interval.

Results

T gondii IgG (Table 1) was detected at 1:64 or greater in 4/225 cats, with one additional cat that was positive by IFA at 1:25 for an estimated prevalence rate of 2.2% (5/224; 95% CI 1–4). *T gondii* IgM was detected at 1:64 or greater in 13/225 (5.8%) cats (Table 2). However, for 9/13 cats, the titer was at the low-end cut-off of 1:64; each of these cats was considered healthy and current IgG titers were not present. Thus, these titers are likely false positives and were excluded, giving an estimated IgM prevalence rate of 1.8% (95% CI 0.7–4.5).

Of the 13 cats with positive IgM titers, one was feral and the other 12 were client-owned. Of the 12 client-owned cats, four were classified as sick and eight as allergic.

Of the four sick cats, two were euthanized. The other two were prescribed clindamycin. Re-check examinations

Table 1 IgG positive cats

Population	Patient ID	Age (years)	Sex	Breed	Living environment	Condition	Titer magnitude	Test
Hospital	M19	14	MN	DSH	Unknown	Sick	1:800	IFA
Hospital	M22	4	MN	DMH	Indoor/outdoor	Allergic	1:512	ELISA
Hospital	M40	0.17	MI	DSH	Unknown	Sick	⩾1:12,800	IFA
Hospital	M55	3	MN	DSH	Indoor	Allergic	1:25	IFA
Trap–neuter–return		2	MN	DSH	Outdoor	Healthy	1:64	ELISA

A titer of 1:64 was considered positive in the ELISA and a titer of 1:25 was considered positive in the IFA
DMH = domestic mediumhair; DSH = domestic shorthair; IFA = indirect fluorescent antibody; MI = male intact; MN = male neutered

Table 2 IgM positive cats

Population	Patient ID	Age (years)	Sex	Breed	Living environment	Condition	Titer magnitude	Test
Hospital	M4	1	MN	DSH	Indoor	Allergic	1:64	ELISA
Hospital	M7	5	FS	DSH	Indoor	Allergic	1:64	ELISA
Hospital	M28	8	FS	DSH	Indoor/outdoor	Allergic	1:64	ELISA
Hospital	M32	13	MN	DSH	Outdoor	Allergic	1:64	ELISA
Hospital	M35	10	MN	DSH	Indoor	Allergic	1:64	ELISA
Hospital	M49	14	MN	DSH	Unknown	Sick	1:800	IFA
Hospital	M54	1	FS	Sphynx	Unknown	Allergic	1:64	ELISA
Hospital	M62	11	MN	DSH	Unknown	Sick	1:100	IFA
Hospital	M79	1	MN	DSH	Unknown	Sick	1:256	ELISA
Hospital	M88	4	MN	DSH	Indoor/outdoor	Sick	1:64	ELISA
Hospital	M108	12	MN	DSH	Unknown	Allergic	1:64	ELISA
Hospital	M119	12	MN	DSH	Unknown	Allergic	1:64	ELISA
Trap–neuter–return		1	FI	DSH	Outdoor	Healthy	1:64	ELISA

A titer of 1:64 was considered positive in the ELISA and a titer of 1:25 was considered positive in the IFA
DSH = domestic shorthair; FI = female intact; FS = female spayed; IFA = indirect fluorescent antibody; MN = male neutered

and titers were recommended for both; however, the cats were lost to follow-up.

Of the eight allergic cats that were IgM seropositive, four (with 1:64 titers) were neither re-tested nor treated. One cat was not treated but was re-tested 7 months later; re-check titers were negative for both IgM and IgG. Another cat was treated with clindamycin but never re-tested. Two cats were treated with a course of clindamycin and were re-tested. Both cats had negative IgM and IgG titers on re-check.

Of the five cats that were IgG positive, one was feral and four were client-owned. Of the four client-owned cats, two were sick. One sick cat was euthanized. The other was prescribed clindamycin and lost to follow-up. The other two were allergic and were started on CsA without treatment with any anti-*T gondii* medications. One cat was prescribed CsA at a 7.3 mg/kg PO q24h and the other cat was started at a dose of 6.1 mg/kg PO q24h, which was later increased to a dose of 7.3 mg/kg PO q24h. CsA was initiated in both these cats as it was thought that a positive IgG did not indicate active infection and risk was low. Both were doing well at the time of writing this study. Neither were retested for *T gondii* antibodies nor developed any clinical signs of illness.

Discussion

Antibody titers alone should not be considered a reflection of true infection. Suspicion of true infection should increase with concurrent clinical signs, high IgM titers, rising consecutive titers and/or the occurrence of seroconversion of IgM to IgG antibodies. Although this was a small sample, seroconversion from IgM to IgG did not occur with any with low titers (1:64) that were re-tested, supporting our suspicion of false positives. In addition, one cat with a low titer (1:64) was negative for both IgG and IgM when re-tested without any treatments.

It is important to remember that seropositivity is only one part of the clinical picture. Without identification of the tachyzoite, the diagnosis of clinical infection should be made in the light of many factors. This includes clinical signs, response to treatment, the degree of seropositivity, trends of re-check titers and seroconversion from IgM to IgG. One low positive titer, as seen with many of the cats in this study, does not likely represent true infection or clinical disease and false positives should be considered.

Neither of the cats with positive *T gondii* IgG titers that were administered cyclosporine without pre-treatment with anti-*T gondii* drugs developed clinical toxoplasmosis. These results are similar to those reported in an experimental study where cats exposed to *T gondii* 42 days prior did not develop systemic disease when administered cyclosporine alone.[7] These results suggest that it may be difficult to reactivate *T gondii* when cyclosporine is administered to otherwise healthy cats. Many cases of reactivated *T gondii* infection reported were cats that were administered other drugs concurrently, such as prednisolone. In experimentally infected cats, those administered cyclosporine with high trough concentrations before *T gondii* infection were most likely to become ill. Thus, cats on CsA or other immunosuppressive therapies should not be fed undercooked meat or allowed to hunt to lessen the risk of exposure after starting cyclosporine therapy.

The present study has some limitations. These include a small sample size and potentially inappropriate representation of feral cats in Los Angeles. The feral cats in this study were younger colony members being trapped for sterilization. These younger cats may not have had enough time for exposure to the protozoan (in contrast with older colony members), which may have skewed results. Studies done in major US cities with larger sample sizes and samples from feral cats of all ages may provide more accurate prevalence rates.

Conclusions

It appears that exposure to *T gondii* may be low in urban US communities, such as Los Angeles. In comparison with other prevalence studies in cats from rural or mixed regions throughout the USA and California, the prevalence rates found in this study were quite low. This can be because cats living in major cities are less likely to hunt, more likely to live indoors, be fed quality/commercial cat foods and are generally better cared for. Feral cats living in urban communities are also likely to be fed commercial cat food by a colony caretaker, making hunting for food and exposure to *T gondii* less likely.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognized high standards ('best practice") of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS Open Reports*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner of legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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