Seroprevalence of *Toxoplasma gondii* antibodies in clinically ill cats in the United States

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Objective—To determine regional seroprevalence estimates of *Toxoplasma gondii*-specific IgM and IgG in clinically ill cats throughout the United States.

Sample Population—Sera from 12,628 clinically ill, client-owned cats.

Procedure—*Toxoplasma gondii*-specific IgM and IgG antibodies were detected by use of ELISAs. Sera from clinically ill cats previously submitted for *T gondii* antibody testing were sequentially selected from our serum bank and the sample submission paperwork reviewed. The country was divided into 12 geographic regions. Overall prevalence as well as prevalence for each region, age group, season, sex (male vs female), and breed (domestic shorthair vs other) was calculated. Data were analyzed by logistic regression analysis.

Results—Overall, 31.6% of the cats were seropositive for *T gondii*-specific IgM, IgG, or both. Percentage of cats seropositive for *T gondii* antibodies ranged from 16.1% (southwestern United States) to 43.5% (northeastern United States). As age increased, odds of positive *T gondii* antibody assay results (IgM alone, IgG alone, and any combination of IgM or IgG) increased. Males were more likely than females to be seropositive for *T gondii* antibodies (IgG alone and any combination of IgM or IgG). Domestic shorthair cats were more likely than other breeds to be seropositive for *T gondii* antibodies (IgM alone, IgG alone, and any combination of IgM or IgG).

Conclusions and Clinical Relevance—*Toxoplasma gondii*-specific antibodies are common in serum samples of clinically ill cats from all regions of the United States. Seroprevalence increases as cats age and is higher in male and domestic shorthair cats, compared with females and other breeds. (*Am J Vet Res* 2005; 66:874–877)

Toxoplasma gondii is a protozoan parasite with worldwide distribution. Felids are the only host known to shed oocysts into the environment after the completion of a sexual phase in the gastrointestinal tract.[1,2] After sporulation in the environment, sporulated oocysts are infective to many species including people. In immunocompromised people, toxoplasmosis can result in severe clinical illness. For example, up to 30% of people with acquired immune deficiency syndrome will develop toxoplasmic encephalitis.[3] If a woman is infected for the first time during gestation, *T gondii* can cause stillbirth, CNS disease, and ocular disease in the fetus.[4,5]

Most cats exposed to *T gondii* have no noticeable clinical signs of infection. When clinical toxoplasmosis occurs, the most severe clinical signs of disease are observed in kittens or otherwise immunocompromised cats; however, disease can also occur in some presumably immunocompetent adult cats. The clinical signs most commonly attributed to feline toxoplasmosis are fever, ocular inflammation, hyperpnea, dyspnea, anorexia, lethargy, icterus, abdominal discomfort, and CNS disturbances.[1,2,6,7] A variety of tests have been used to detect antibodies against *T gondii* in feline serum.[1,2,8-14] Most experimentally inoculated cats become seropositive for antibodies against *T gondii* just as the oocyst shedding period is ending. *Toxoplasma gondii* may live in the tissues for the life of the cat, resulting in the maintenance of seropositivity for *T gondii*. Thus, it is generally assumed that seropositive cats are infected. Because serum antibodies can be detected in healthy and clinically ill cats, the positive predictive value of antibody testing is not 100%. However, serum *T gondii*-specific IgM titers are more common in cats with clinical signs of disease than in healthy cats and so may correlate better with disease than serum concentrations of IgG antibodies against *T gondii*.[1,2,15,16]

The estimated seroprevalence of antibodies against *T gondii* in felids is approximately 30% to 40% worldwide.[1,2] Studies conducted on the seroprevalence of antibodies against *T gondii* in felids in the United States have generally been in individual states or regions and used different assays.[1,2] The purpose of the study reported here was to determine regional seroprevalence estimates of *T gondii*-specific IgM and IgG in clinically ill cats throughout the United States.

Materials and Methods

Serum samples—Serum samples used in this study came from a laboratory[a] that supplies *T gondii* serologic testing to veterinarians in the United States. Sera were refrigerated upon receipt and assayed with current adaptations of previously reported ELISAs for the detection of *T gondii*-specific IgM and IgG within 72 hours of reaching the laboratory.[11,12] Standardized controls were applied to each assay, and quality control for the assays is performed continually.[a] In the detection of *T gondii*-specific IgM and IgG by use of the ELISAs, titers ≥ 1:64 were considered positive and titers < 1:64 were considered negative. Analytic sensitivity and specificity of both ELISAs were determined by comparison to the results of *T gondii*-specific IgM and IgG western blot immunoassay[9,10] and are believed to be > 98%. These assays are more sensitive and specific than other commercially available tests.[8,11] We sequentially selected results from 12,628 cats for which sera were submitted from various regions in the United States between May 4, 1998, and December 28, 2001, because of suspicion of clinical toxo-

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plasmosis. The primary clinical complaints were CNS disease, ophthalmologic disease, fever, anorexia, weight loss, lethargy, respiratory disease, gastrointestinal tract disease, and hematologic and serum biochemical abnormalities. Information on age, breed, sex, month at collection, geographic location, and clinical signs was obtained from the sample submission form, if recorded. We then attempted to exclude all healthy cats from the analysis.

Geographic regions—To determine the regional seroprevalence, the United States was divided into 12 areas: Northwest (Oregon, Idaho, and Washington), Pacific (California and Nevada), Southwest (Arizona, New Mexico, and Utah), Rocky Mountains (Montana, Wyoming, and Colorado), Midwest (Minnesota, Wisconsin, Michigan, Iowa, Illinois, Indiana, and Ohio), Great Plains (North Dakota, South Dakota, Nebraska, and Kansas), Southeast (Georgia, Florida, South Carolina, and North Carolina), Mid-south (Missouri, Oklahoma, and Arkansas), Gulf Coast (Texas, Alabama, Louisiana, and Mississippi), Mid-atlantic (Tennessee, Kentucky, Virginia, West Virginia, and Maryland), and Northeast (Pennsylvania, New Jersey, New York, Maine, Delaware, Rhode Island, Connecticut, Massachusetts, Vermont, and New Hampshire). No serum samples were assayed from Alaska. Hawaii was evaluated individually. Because there were large variations in numbers of serum samples submitted per month, results were grouped by season as follows: December, January, and February; March, April, and May; June, July, and August; and September, October, and November.

Statistical analysis—All data and laboratory information were entered into a spreadsheet. The proportional odds assumption was tested ($P = 0.646$), and logistic regression[b] was used to evaluate the effects of age, season, sex, breed, and region on the immunoglobulin status of cats. Regional information was limited to 3,640 observations; as a result, regional effects were evaluated in a model containing only that term. Otherwise, the model included age, season, sex, and breed. For prevalence for *T gondii*-specific IgM only and IgG only, results were scored as positive or negative and a binary logit model was used. Serum samples that were positive for *T gondii*-specific IgM and IgG were scored as 2, serum samples that were positive for either *T gondii*-specific IgM or IgG were scored as 1, and serum samples that were negative for *T gondii*-specific IgM and IgG were scored as 0; a cumulative logit model was used to determine differences between prevalence. Values of $P < 0.05$ were deemed significant.

Results

All possible combinations of serologic results were detected (**Table 1**). Overall, 31.6% of serum samples had positive ELISA results for *T gondii*-specific IgM or IgG. The estimated seroprevalence of either *T gondii*-specific IgM or IgG in clinically ill cats ranged from 16.1% for the Southwest region to 43.5% in the Northeast region (**Figure 1**). The estimated seroprevalence of either *T gondii*-specific IgM or IgG in clinically ill cats in Hawaii was 59.2%. Significant differences were found among regions for prevalence of serum samples that had positive ELISA results for *T gondii*-specific IgG alone and *T gondii*-specific IgM alone. As age increased, odds of detecting *T gondii*-specific IgM

Table 1—*Toxoplasma gondii* estimated seroprevalence in clinically ill cats in the United States.

Class	No. positive	Prevalence (%)	95% confidence limits (%)
IgM positive only	1,925	15.2	14.6(15.9)
IgG positive only	2,842	22.5	21.8(23.2
IgM and IgG positive	777	6.2	5.7(6.6)
IgM or IgG positive	3,990	31.6	30.8(32.4)
Negative to IgM and IgG	8,636	68.4	67.6(69.2)

The total number of serum samples assayed was 12,628.

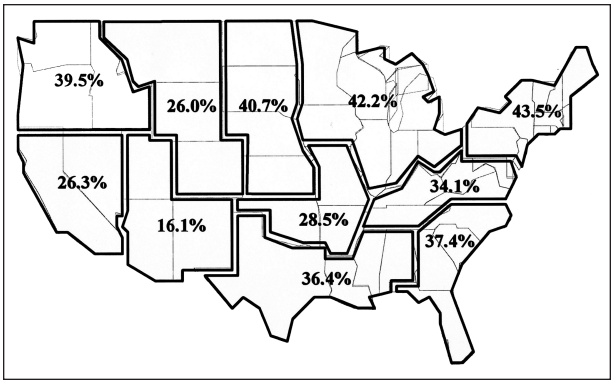

Figure 1—United States regional *Toxoplasma gondii* seroprevalence in clinically ill cats. Results were based on the presence of *T gondii*-specific IgG of IgM in serum. Information was available from 3,640 serum samples.

Table 2—Influence of age, breed, sex, and season on estimated *T gondii* seroprevalence in clinically ill cats in the United States.

Class*	Age	Season	Sex	Breed
IgM				
P value	< 0.001	0.524	0.068	0.004
Odds ratio	1.072	1.017	0.893	0.828
95% confidence limits	1.059, 1.086	0.966, 1.071	0.792, 1.008	0.728, 0.942
IgG				
P value	< 0.001	0.649	0.001	< 0.001
Odds ratio	1.086	1.011	0.841	0.683
95% confidence limits	1.074, 1.097	0.966, 1.058	0.757, 0.935	0.609, 0.766
Combined				
P value	< 0.001	0.708	0.001	< 0.001
Odds ratio	1.088	1.008	0.852	0.727
95% confidence limits	1.078, 1.099	0.968, 1.049	0.777, 0.936	0.658, 0.803

*Information was available for 8,683 cases. Logistic regression analysis was conducted on the IgM results (positive or negative); IgG results (positive or negative); and combined results of cats positive to either IgG or IgM (score = 1), positive to both (score = 2), or negative to both (score = 0).

alone, *T gondii*-specific IgG alone, or any combination of *T gondii*-specific IgM or IgG also significantly increased (**Table 2**). Males were significantly more likely than females to be seropositive for *T gondii*-specific IgG alone and any combination of *T gondii*-specific IgM or IgG. Domestic short hair cats were more likely than purebred cats to be seropositive for *T gondii*-specific IgM alone, *T gondii*-specific IgG alone, or any combination of *T gondii*-specific IgG or IgM. Season was not significantly associated with estimated seroprevalence of either *T gondii*-specific IgM or IgG in clinically ill cats.

Discussion

Because sensitivity and specificity of any assay are never 100%, seroprevalence determined in our study are only estimates and so the true prevalence may vary slightly. Results of our study indicate that cats in the United States are commonly exposed to *T gondii*. The overall seroprevalence of 31.6% is greater than that reported for cats in Japan[17] and Brazil[18] but less than that of Melbourne, Australia,[19] which have a seroprevalence of 8%, 26.3%, and 39%, respectively. However, it is impossible to directly compare the data among studies because of differences in sample size, assay method, and geographic location. Our results for cats are similar to those for humans, which have a mean estimated seroprevalence of antibodies against *T gondii* of approximately 30%.[1] In our study, only results from cats with clinical signs previously reported to be associated with *T gondii* infection were included and the veterinarians suspected toxoplasmosis. Thus, the seroprevalence may be higher than would be expected for a general population of cats.

The presence of serum antibodies against *T gondii* only proves current or previous infection, not clinical disease as a result of infection.[8] Thus, veterinarians need to take care interpreting the results of serologic tests for *T gondii* for individual cats. A positive serologic assay result for *T gondii*-specific IgM or IgG keeps the agent on the differential list, and a negative serologic assay result for *T gondii*-specific IgM or IgG makes the diagnosis less likely. If a cat that is seropositive for antibodies against *T gondii* has clinical signs of disease associated with the infection and another cause for the clinical syndrome cannot be found, it is prudent to treat with an appropriate antiprotozoal drug.[2,8] If a positive response occurs, it is possible the cat had clinical toxoplasmosis. In a previous study,[12] we have shown that serum *T gondii*-specific IgM titers are detectable for approximately 12 weeks after experimental inoculation; therefore, positive serologic assay results generally denote recent or active infection. In a previous study[15] of 188 cats in Georgia in which similar methods were used, the seroprevalence of sick and healthy cats for antibodies against *T gondii* were 57% and 49.4%, respectively. Positive serologic assay results for *T gondii*-specific IgM are found for many cats with presumed clinical toxoplasmosis.[16,20,21] Because *T gondii*-specific IgM is usually not detected in healthy cats but is commonly detected in clinically ill cats, including 21.4% of the clinically ill cats of our study, it has been proposed that positive serologic assay results for *T gondii*-specific IgM have a better positive predictive value for clinical toxoplasmosis than *T gondii*-specific IgG in cats.[2,8] Because sera from healthy cats are only rarely evaluated by our laboratory, only clinically ill cats were assessed in our study. Thus, we cannot use the data of our study to further assess the predictive values of *T gondii*-specific IgM or IgG for clinical toxoplasmosis.

Feline sera positive for *T gondii*-specific IgG or IgM were detected in all regions, and a significant difference was found between regions of the United States. Hawaii had the highest prevalence of 59.2%; however, the sample size from this state was small (n = 49). Variation in prevalence may reflect the survivability of *T gondii* oocysts in the environment.[2,22] Oocysts generally survive in warm humid environments for longer periods than in hot, dry, or extremely cold environments. Cats are infected by ingestion of sporulated oocysts in the environment, oocysts carried by transport hosts, or tissue cysts. The longer the organism survives in the environment, the more likely it is for a cat to be exposed directly or through contact with intermediate or transport hosts. Numbers of available intermediate or transport hosts in a region may also influence overall prevalence. Lastly, cats fed uncooked or undercooked meats would also be expected to have an increased risk of exposure.

As in previous studies,[15,19,23] it was shown that the seroprevalence of *T gondii*-specific antibodies increases with age, which is likely to relate to an increased chance of exposure over time. No known sex-linked or breed-associated predisposition to *T gondii* infection exists.[15,24-26] In our study, a higher prevalence was detected in male cats, compared with female cats, and in domestic shorthair cats when compared with other breeds. These results suggest that a greater number of male cats and domestic shorthair cats were allowed to go outdoors or were fed undercooked meat diets. Unfortunately, housing and dietary histories were usually not available on the laboratory submission sheets; therefore, these factors could not be assessed further in our study.

Humans and cats are infected with *T gondii* transplacentally by ingestion of sporulated oocysts or by ingestion of tissue cysts in undercooked meats.[1,2,27] Cats are generally not considered to be a direct *T gondii* risk to humans because oocysts are unsporulated and not infectious when passed and because cats are generally fastidious and do not leave feces on their bodies.[2,27] However, human infection from ingestion of sporulated *T gondii* oocysts from a contaminated environment has been proven.[2,27] Results of our study indicate that infection of cats is common in the United States. Each of the seropositive cats probably shed millions of oocysts into the environment. Efforts should be made to keep cats from becoming infected with *T gondii* in an attempt to decrease contamination of the environment. Cats should be housed indoors, should be fed only cooked foods, and should not be allowed to hunt and efforts should be made to control potential transport hosts.[27]

Although cats that are seropositive for antibodies against *T gondii* generally have the organism in tissues,

it is rare for seropositive cats to repeat oocyst shedding.[2,27] Seropositive cats have failed to repeat oocyst shedding when administered clinical doses of glucocorticoids, when coinfected with FeLV or FIV, and when repeat inoculation was administered within 56 weeks of the first infection.[2,27] Repeated oocyst shedding has been detected in some *T gondii*-infected cats after superinfection with *Cystoisospora felis* and administration of prednisolone (10 to 80 mg/kg) and in previously infected cats housed in isolation for years before reinfection.[2,27] However, in each of these instances, only small numbers of oocysts were shed transiently. In contrast, most seronegative cats shed millions of oocysts after exposure to *T gondii*. Thus, seropositive cats are likely to be less of a public health risk than seronegative cats. Because people generally do not acquire toxoplasmosis from touching cats, serologically screening healthy cats is of minimal benefit and is generally not recommended.[27]

a. Special Serology and Endocrinology Laboratory, Veterinary Diagnostic Laboratory, Colorado State University, Fort Collins, Colo.
b. LOGISTIC procedure in SAS, SAS Institute, Cary, NC.

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