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PREVALENCE OF ANTIBODY TO *TOXOPLASMA GONDII* IN TERRESTRIAL WILDLIFE IN A NATURAL AREA

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ABSTRACT: We conducted a cross-sectional study from 2008 to 2009 to evaluate the occurrence of feral and wild cats and the risk of *Toxoplasma gondii* infection in terrestrial wildlife in a natural area in Illinois, USA. Felids are definitive hosts for *T. gondii* and cats are a key component of rural and urban transmission of *T. gondii*. We selected four forest sites within the interior of the park and four edge sites within 300 m of human buildings. Feline and wildlife occurrence in the natural area was determined with the use of scent stations, motion-detection cameras, and overnight live trapping. Based on scent stations and trapping, feral cats used building sites more than forest sites (scent stations: $P=0.010$; trapping: $P=0.083$). Prevalence of *T. gondii* antibodies was determined with the use of the indirect immunofluorescent antibody test (IFAT) with a titer of 1:25 considered positive; *T. gondii* antibodies were detected in wildlife at all sites. Wildlife species were classified as having a large home range (LHR) or a small home range (SHR), based on published estimates and using a cutoff of 100 ha. Small–home-range mammals had a higher prevalence of antibody to *T. gondii* (odds ratio [OR]=4.2; $P=0.018$) at sites with a high frequency of cat occurrence (defined as ≥9 cat occurrences across three detection methods); this finding indicates that feral cats are the most likely source of environmental contamination. Overall, the prevalence of antibody to *T. gondii* among LHR mammals was significantly higher than the prevalence among SHR mammals (OR=7.1; $P<0.001$). Small–home-range mammals are an essential part of *T. gondii*–antibody prevalence studies and can be used as sentinels for risk of disease exposure to humans and wildlife in natural areas. This study improves our understanding of ecologic drivers behind the occurrence of spatial variation of *T. gondii* within a natural area.

Key words: Feral cat, Illinois, natural area, opossum, raccoon, *Toxoplasma gondii*, white-footed mice, wildlife.

INTRODUCTION

Feral cats (*Felis catus*) utilize various habitats, including natural areas on suburban community edges (Schmidt et al., 2007), national parks (Roelke et al., 2008), nature preserves (Rouys and Theuerkauf, 2003), small forest patches, forest edges (Kays and DeWan, 2004), farms, and roadsides (Warner, 1985). Some owners allow their cats to roam free (Coleman and Temple, 1993), which may lead to negative effects on humans (Ruiz et al., 1973), wildlife (Suzán and Ceballos, 2005), and the ecosystem (Robertson, 2008). Domestic cats benefit from some dependent interaction with humans for shelter and food (Weber and Dailly, 1998), but despite supplemental feeding, free-roaming cats are opportunistic hunters and consume available garbage, cat food, carrion, and prey species (Slater, 2004).

Negative impacts of domestic cats (owned, semiferal, or feral) on wildlife populations in natural areas (Lepczyk et al., 2004) could be compounded by their ability to spread diseases. Feral cats have a higher prevalence of parasitic and viral infections compared to pet cats (Nutter et al., 2004) and can serve as definitive hosts for *Toxoplasma gondii*, a parasite that sexually reproduces exclusively in felids (Dubey et al., 1970).

Toxoplasma gondii–infected felids shed parasite oocysts in their feces, contaminating water, soil, and the environment

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(Dubey and Beattie, 1988). Infection in felids and intermediate hosts, including humans, is acquired by ingesting uncooked or undercooked meat or carcasses contaminated with *T. gondii* cysts or by consuming contaminated food, water, or soil. Toxoplasmosis in humans is associated with birth defects, encephalitis, blindness, ophthalmitis, multisystem infections, death, personality shifts, decreased intelligence, and schizophrenia (McAllister, 2005). An experimental vaccine capable of preventing cats from shedding *T. gondii* oocysts was highly efficacious but had practical limitations under field conditions on free-ranging cat populations (Mateus-Pinilla et al., 1999).

Toxoplasma gondii can modify the behavior of infected animals (Berdoy et al., 1995) and can infect most warm-blooded animals (Dubey and Beattie, 1988). The parasite has been identified as a cause of death in sea otters (*Enhydra lutris*; Miller et al., 2002), wombats (*Vombatus ursinus*), koalas (*Phascolarctos cinereus*; Canfield et al., 1990), bobcats (*Lynx rufus*; Dubey et al., 1987), woodchucks (*Marmota monax*), and American red squirrels (*Tamiasciurus hudsonicus*; Bangari et al., 2007). Although *T. gondii* is unable to reproduce sexually in nonfelids, asexual stages in infected tissues of intermediate hosts serve as a source of parasite transmission (Miller et al., 1972) to other animals by carnivorism, which helps maintain enzootic *T. gondii* in the food chain (Dubey et al., 1999).

In a fragmented habitat, where few pristine natural areas exist, evaluating and maintaining the health of the ecosystem and its wildlife remains a priority for conservation biology. Additionally, *T. gondii* can have a negative health effect on native fauna. The main objective of this study was to evaluate the occurrence of bobcats (*Lynx rufus*) and feral cats in a natural area and to evaluate *T. gondii* prevalence in terrestrial wildlife in relation to the frequency of feral cat occurrence, home range, and proximity to human buildings.

MATERIALS AND METHODS

Study area and site selection

We conducted a cross-sectional study of the prevalence of antibody to *T. gondii* in wildlife in the Robert Allerton Park (RAP) natural area, located along 4 km of the Sangamon River, 7 km southwest of the city of Monticello, Piatt County, Illinois, USA (39°59′37.23″ N, 88°39′5.1402″ W). Encompassing >6 km^2 of river corridor of upland and bottomland forests, sculpture gardens, meadows, and prairies, RAP is the largest natural area in this highly fragmented region. It is surrounded by intensively farmed agricultural lands and a few buildings and barns on the edge of the park. We selected eight trapping sites within the study area. Because previous findings suggested that free-ranging cats stay within 300 m of buildings (Horn, 2009) and human interactions result in highly localized free-roaming cat densities (Schmidt et al., 2007), we selected four sites on the park edge within 300 m of human buildings (building sites: 1–4) and four sites in the park interior, where potential bobcat sightings had been reported (forest sites: A–D; Fig. 1).

Wildlife live capture

Sites were visited every 3 wk between the spring and fall of 2008 and 2009. Each night of trapping consisted of 40 each of model 108 and 103 tomahawk traps (Tomahawk Live Trap, Tomahawk, Wisconsin, USA) at each site. Two overnight live-trappings were conducted per visit to the site. Three building sites and three forest sites were visited three times each and the remaining forest and building site were visited two times (44 nights of trapping) in 2008. In 2009, all eight sites were visited four times (64 nights of trapping).

Mice were trapped during the summer of 2009. Each of the eight sites was trapped at least three nights (one forest site had an additional night—25 nights of trapping). Forty Sherman traps were placed within a 100×4-m grid and 40 were placed along buildings or trails depending on the site.

Wildlife processing

Medium mammals (all trapped mammals larger than mice) were immobilized with the use of a combination of ketamine and xylazine (Nielsen, 1999; Kreeger et al., 2002). Species, sex, and age (adult, subadult, or juvenile) were recorded. Blood was collected from the cephalic, ventral coccygeal (Virginia opossum; *Didelphis virginiana*), marginal ear (eastern cotton-

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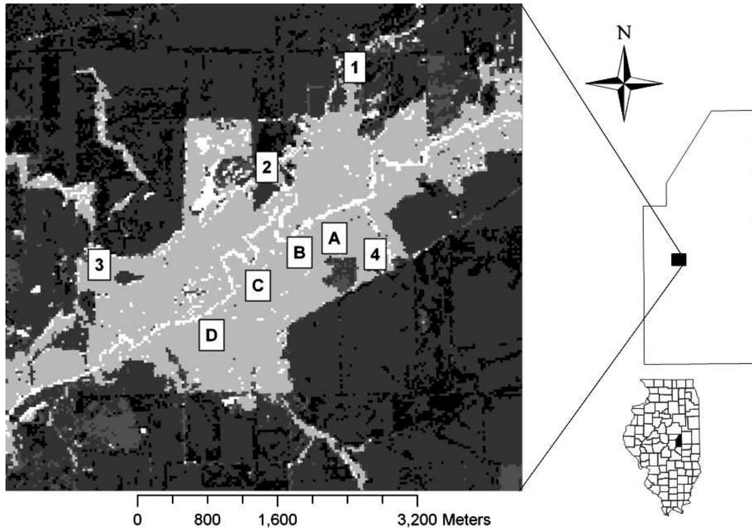

FIGURE 1. Location of Robert Allerton Park study area in Piatt County, Illinois and location of study sites (building sites: 1–4 and forest sites: A–D) within the study area.

tail; *Sylvilagus floridanus*) or saphenous veins. Recaptured animals were processed if >3 wk had passed since their previous sample. Wildlife were passive integrated transponder (PIT) tagged for future identification (Biomark, Inc., Boise, Idaho, USA), allowed to recover from sedation, and released at the specific site of capture. Feral cats were digitally photographed for future identification.

Captured mice were ear tagged (National Band and Tag Company, Newport, Kentucky, USA) and restrained (Hem et al., 1998). Species, sex, and age were recorded. A blood sample was collected from the saphenous vein (Abatan et al., 2008) with the use of a Sarstedt® Microvette CB300Z capillary tube (Sarstedt Inc., Newton, North Carolina, USA).

Wildlife survey

To quantify presence of felids and wildlife at RAP, we used scent stations, motion-detection cameras, and live-trapping (described above). Two scent stations were located at each of the eight sites and consisted of a cleared plot 1 m in diameter covered with a sand–mineral oil combination and a fatty-acid scent disk (Howard et al., 2002). Three times per week, scent stations were checked, the number of animal tracks of each species counted, and the station reset. Weekly between March and November of 2008 and 2009, three motion-detection cameras were placed at study sites and rotated to new sites the following week.

Serology

Blood samples from medium-sized mammals (excluding squirrels and chipmunks) were centrifuged at 1,800 × G for >10 min (Bush et al., 2001). Blood samples from mice, squirrels, and chipmunks were centrifuged at 10,000 × G for 5 min per manufacturer's instructions (Sarstedt Inc.). To detect *Toxoplasma gondii* antibodies, a modified indirect immunofluorescent antibody test (IFAT) was performed (Dabritz et al., 2008).

Slides with 12 5-mm wells (Thermo Fisher Scientific, Portsmouth, New Hampshire, USA) were prepared in the laboratory with the use of a modified technique from Miller et al. (2001). Tachyzoites were used as antigen at a concentration of 10,000 tachyzoites/µl and fixed in methanol instead of formaldehyde before air drying.

Serum samples were diluted with phosphate-buffered saline (PBS, pH 7.2) at a titer of 1:25, and 20 µl were placed in each well. Slides contained a positive and negative control from known *T. gondii*–negative and –positive mice or cats (feral cat samples only). After initial results from IFAT with the use of mouse controls, one negative and one positive sample each from raccoon (*Procyon lotor*) and opossum samples were used as controls for slides containing samples from those species. Slides were incubated for 30 min at 37 C and 5% CO_2. After each incubation, slides were rinsed with 1× IFA buffer rinse (4× IFA rinse

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buffer, pH 9.0, diluted 1:4 in distilled water). Buffer was placed on the slides and incubated for 10 min.

A 1:50 ratio of antianimal antibody and PBS dilutions were prepared and 20 μl added to each well. Antianimal antibodies were fluorescein isothiocyanate (FITC) labeled and included anti-raccoon immunoglobulin G (IgG), anti-cat IgG, anti-rat IgG for squirrels (*Sciurus* spp. and *Ictidomys tridecemlineatus*), chipmunks (*Tamias striatus*), and woodchucks (*Marmota monax*) (Lockhart et al., 1997), anti–*Peromyscus leucopus* IgG (Dabritz et al., 2008), anti-ferret IgG for skunks (*Mephitis mephitis*) and long-tailed weasels (*Mustela frenata*; Levin et al., 2002; Kirkegaard and Perry Laboratories, Gaithersburg, Maryland, USA), anti-mouse IgG for mouse controls and anti-rabbit IgG (Bethyl Laboratories, Montgomery, Texas, USA). Slides were incubated with the corresponding FITC-labeled antianimal antibody for 30 min followed by the addition of 1× IFA buffer to each well and 10-min incubation.

FITC-labeled anti-opossum antibodies were not commercially available, but anti-opossum IgG antibody (Bethyl Laboratories) was produced in rabbit. Therefore, 20 μl of rabbit anti-opossum antibody were used as a secondary antibody for opossum samples, incubated for 30 min followed by 1× IFA buffer and 10-min incubation. Lastly, 20 μl of FITC-labeled anti-rabbit was used as a tertiary antibody for opossum samples, incubated for 30 min, rinsed with 1× IFA and a final 10-min incubation.

Slides were viewed under a fluorescence microscope. A sample was classified as positive if all edges around the tachyzoites glowed bright green, and was classified as negative if the entire edge of the tachyzoites did not fluoresce or if only partial edge florescence occurred. Only the last sample from resampled animals was used for data analysis. Repeatability of samples evaluated with mouse controls and a raccoon or opossum control was 100%.

Data analysis

All statistical analyses were performed with the use of SAS version 9.1 (Statistical Analysis System Institute Inc., Cary, North Carolina, USA) and results were considered significant at $P \leq 0.05$. To test the hypothesis that feral cats in RAP use building sites more frequently compared to forest sites, the number of cat visitations to building sites and forest sites was compared separately for each detection method (scent stations and number of individual cats trapped, except motion detection cameras due to low cat detection from this method) using negative binomial regression. For the trapping analysis, total number of trapping nights to each site was taken into account. A visitation based on the scent station method was defined as the occurrence of cat tracks at a scent station. Ratio of mean number of cat visitations or cats trapped was used to quantify the effect size.

To evaluate the hypothesis that the prevalence of *T. gondii* in wildlife is associated with home range size, animals were classified as having a large home range (LHR; >100 ha) or a small home range (SHR; ≤100 ha) and logistic regression was used. Animals included in the LHR group were raccoons (Beasley et al., 2007), skunk (Bixler and Gittleman, 2000), long-tailed weasel (Gehring and Swihart, 2004), and Virginia opossums (Gipson and Kamler, 2001). Animals classified as SHR included white-footed mice (*Peromyscus leucopus*; Hofman, 2008), eastern chipmunks (*Tamias striatus*; Blair, 1942), eastern cottontail (Bond et al., 2001), eastern grey squirrels (*Sciurus carolinensis*; Gurnell et al., 2001), woodchucks (Hayes, 1977), fox squirrel (*Sciurus niger*), thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), and meadow jumping mice (*Zapus hudsonius*).

To evaluate wildlife abundance at building or forest sites, total number of individual animals trapped at building and forest sites was compared using negative binomial regression. LHR and SHR mammal groups were evaluated separately and the total number of trapping nights to each site was accounted for in the analysis. The ratio of mean number of wildlife captured per trapping night was used to quantify the difference. Prevalence of *T. gondii* infection between building and forest sites was compared using logistic regression. Odds ratio (OR) of *T. gondii* prevalence was used to quantify the difference between the comparison groups.

The potential impact of infected cats on wildlife was assessed by comparing prevalence of *T. gondii* infection in wildlife at sites with at least one antibody-positive cat to sites with no cats trapped or only antibody-negative cats. Logistic regression was used to evaluate this association for LHR and SHR mammals. To evaluate the impact of the presence of feral cats on wildlife in relation to *T. gondii*–antibody prevalence further, we grouped the study sites into sites with higher and lower frequencies of cat occurrence based on an arbitrary cutoff of nine cat occurrences from the three detection methods. The antibody prevalence of *T. gondii* in SHR and LHR mammals was compared between high and low cat-occurrence sites with the use of logistic

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TABLE 1. Occurrence of feral cats within the Robert Allerton Park, Illinois, USA study area by building and forest sites. The data are based on individual cats trapped, number of cat visitations to scent stations, and individual cat pictures from motion-detection cameras. Total cat detection was used to classify sites as having a high or low frequency of cat occurrence.

	Site	Trapped[a] (*n*)	Scent stations[b] (*n*)	Cameras[c] (*n*)	Total[d]	Cat occurrence[e]
Building	1	0	9	0	9	High
	2	13	28	3	44	High
	3	1	4	0	5	Low
	4	2	10	0	12	High
Forest	A	0	6	0	6	Low
	B	0	5	1	6	Low
	C	2	2	0	4	Low
	D	0	3	0	3	Low

[a] Number of individual cats trapped.

[b] Number of cat visitations to scent stations.

[c] Number of individual cat pictures captured by cameras.

[d] Sum of cat occurrence across all three detection methods.

[e] Frequency of cat occurrence based on a cutoff of nine (high ≥9; low <9).

regression. Exact tests were used if sparse sample size (expected count <5) was observed in any joint distribution of the variables under investigation. The OR of *T. gondii* infection was used to quantify the effect size.

The association between sex, age (adults versus subadults and juveniles combined), and prevalence of *T. gondii* was also evaluated separately for SHR and LHR mammals with the use of logistic regression. Logistic regression was used to compare the prevalence of *T. gondii* between raccoons and opossums only, because of small sample sizes for other animal species captured.

RESULTS

Feral cat and wildlife distribution in RAP

Feral cats were trapped at three of four building sites and one of four forest sites (Table 1). The mean number of feral cats trapped at building sites was greater than at forest sites (ratio=8.0 [95% confidence limits: 0.8, 82.8]; $P=0.083$). Additionally, based on scent-station data, feral cats were not only present at all eight sites, but were more common at building than at forest sites (ratio=3.2 [1.3, 7.7]; $P=0.010$). Motion-detection cameras detected feral cats at one building and one forest site (Table 1). However, bobcats were not detected in the park by any method. Five sites were classified as having a low frequency of cat occurrence and three sites as having a high frequency of cat occurrence based on a cutoff of nine cat occurrences across the three detection methods (Table 1). Of 317 individual LHR mammals trapped during the study, 237 were trapped at building sites (ratio=2.9 [1.6, 5.3]; $P<0.001$), and a similar number of SHR mammals were trapped at building and forest sites (ratio=1.2 [0.9, 1.7]; $P=0.208$).

Toxoplasma gondii–antibody prevalence

Among feral cats, 33% (6/18) were antibody positive (95% confidence limits [13%, 59%]). Feral cats from three building sites and one forest site were tested for *T. gondii* antibodies and only feral cats from building site 2 and forest site C were antibody positive (Table 2). Antibody prevalence of *T. gondii* in SHR mammals ranged from 0% to 15%, with an overall antibody prevalence of 8% (4%, 14%) at building sites combined (Table 2). Antibody prevalence at forest sites ranged from 0% to 9%, with an overall prevalence of 4% (1%, 9%) at forest sites combined (Table 2). The odds of infection with *T. gondii* in SHR mammals at building sites was greater than at forest sites (OR=2.3 [0.7, 4.2]; $P=0.174$). At building sites, the

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TABLE 2. Prevalence of *Toxoplasma gondii* antibodies in feral cats, large–home-range (LHR) mammals, and small–home-range (SHR) mammals by building and forest sites at Robert Allerton Park, Illinois, USA.

		Feral cat		SHR[a]		LHR[b]	
Location	Group site	*n*[c]	%[d]	*n*[c]	%[d]	*n*[c]	%[d]
Building	1	0	0	36	11	67	30
	2	13	38	22	5	110	26
	3	1	0	33	0	34	41
	4	2	0	34	15	26	35
Forest	A	0	0	22	9	20	35
	B	0	0	30	0	14	36
	C	2	50	34	3	20	25
	D	0	0	23	4	26	39

[a] Small–home-range (SHR) mammals=white-footed mouse (*Peromyscus leucopus*), eastern chipmunk (*Tamias striatus*), eastern cottontail (*Sylvilagus floridanus*), eastern grey squirrel (*Sciurus carolinensis*), woodchuck (*Marmota monax*), fox squirrel (*Sciurus niger*), thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), and meadow jumping mouse (*Zapus hudsonius*).

[b] Large–home-range (LHR) mammals=raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), long-tailed weasel (*Mustela frenata*), and Virginia opossum (*Didelphis virginiana*).

[c] Number of individual animals sampled.

[d] Prevalence of antibody to *T. gondii.*

antibody prevalence of *T. gondii* in LHR mammals, was 30% ([25%, 37%], Table 2), similar to 34% (24%, 45%) at forest sites ($P=0.574$). However, the overall antibody prevalence in LHR wildlife was 31% (26%, 37%), greater than 6% (3%, 10%) in SHR mammals (OR=7.1 [4.0, 12.9]; $P<0.001$).

Presence of an antibody-positive cat compared to an antibody-negative or no cats was not associated with *T. gondii*–antibody prevalence in LHR (OR=0.7 [0.4, 1.1]; $P=0.132$) or SHR mammals (OR=0.5 [0.1, 2.4]; $P=0.611$). However, there was a significantly higher *T. gondii*–antibody prevalence among SHR mammals at sites with a high frequency of cat occurrence compared with low frequency sites (OR=4.2; $P=0.018$, Table 3). In the LHR mammal group, there was no difference between sites with high and low cat occurrence (OR=0.7; $P=0.174$, Table 3).

Toxoplasma gondii–antibody prevalence in LHR mammals was higher among females than males ($P=0.004$, Table 4) and the odds of *T. gondii* infection was higher in adult mammals (OR=5.0;

TABLE 3. Association between *Toxoplasma gondii*–antibody prevalence in small–home-range (SHR) and large–home-range (LHR) mammals, and frequency of cat occurrence at Robert Allerton Park, Illinois, USA.

Mammal group	Variables	*n* (antibody prevalence)	Odds ratio	95% confidence interval	*P*
SHR[a]	High cat occurrence	92 (11%)	4.2	1.3–13.8	0.018
	Low cat occurrence[c]	142 (3%)			
LHR[b]	High cat occurrence	203 (29%)	0.7	0.4–1.2	0.174
	Low cat occurrence[c]	114 (35%)			

[a] Small–home-range mammals=white-footed mouse (*Peromyscus leucopus*), eastern chipmunk (*Tamias striatus*), eastern cottontail (*Sylvilagus floridanus*), eastern grey squirrel (*Sciurus carolinensis*), woodchuck (*Marmota monax*), fox squirrel (*Sciurus niger*), thirteen lined ground squirrel (*Ictidomys tridecemlineatus*), and meadow jumping mouse (*Zapus hudsonius*).

[b] Large–home-range mammals=raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), long-tailed weasel (*Mustela frenata*), and Virginia opossum (*Didelphis virginiana*).

[c] Reference group for computing odds ratio.

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TABLE 4. Association between *Toxoplasma gondii*–antibody prevalence, sex, and age in large–home-range (LHR) and small–home-range (SHR) mammals in the Robert Allerton Park, Illinois, USA study area.

Mammal group	Variables	*n* (antibody prevalence)	Odds ratio	95% confidence interval	*P*
LHR[a]	Male[c]	170 (24%)	2.1	1.3–3.3	0.004
	Female	147 (39%)			
	Adult	148 (49%)	5.0	3.0–8.4	<0.001
	Juvenile[c]	169 (16%)			
SHR[b]	Male[c]	142 (5%)	1.6	0.5–4.7	0.402
	Female	92 (8%)			
	Adult	144 (8%)	2.4	0.7–8.8	0.189
	Juvenile[c]	90 (3%)			

[a] Large–home-range mammals=raccoon (*Procyon lotor*), skunk (*Mephitis mephitis*), long-tailed weasel (*Mustela frenata*), and Virginia opossum (*Didelphis virginiana*).

[b] Small–home-range mammals=white-footed mouse (*Peromyscus leucopus*), eastern chipmunk (*Tamias striatus*), eastern cottontail (*Sylvilagus floridanus*), eastern grey squirrel (*Sciurus carolinensis*), woodchuck (*Marmota monax*), fox squirrel (*Sciurus niger*), thirteen lined ground squirrel (*Ictidomys tridecemlineatus*), and meadow jumping mouse (*Zapus hudsonius*).

[c] Reference group for computing odds ratio.

P<0.0001). However, the direction of sex and age effect on the antibody prevalence of *T. gondii* in SHR mammals was not statistically significant although it followed that of LHR mammals (Table 4).

Among LHR mammals, antibody prevalence in raccoons and opossums was 29% (59/204; [23%, 36%]) and 35% (39/111; [26%, 45%]), respectively, but this difference was not statistically significant (OR=0.8 [0.5, 1.2]; *P*=0.256). One skunk and one of three rabbits had detectable *T. gondii* antibodies. One long-tailed weasel, three woodchucks, one grey squirrel, one fox squirrel, one thirteen-lined ground squirrel, five chipmunks, and one meadow jumping mouse were negative for *T. gondii* antibodies.

DISCUSSION

Feral cats were detected throughout the study area, and our results indicate that SHR mammals inhabiting sites with a high frequency of cat occurrence are at a significantly higher risk of infection with *T. gondii* compared to sites with low cat occurrence. To our knowledge, this is the first attempt to evaluate *T. gondii*–antibody prevalence in wildlife in relation to frequency of cat occurrence in a natural area. A high frequency of cat occurrence allows increased fecal deposition and a greater likelihood of contamination with *T. gondii* oocysts. Because the diet of most SHR mammals consists of seeds, plant material, and insects, SHR mammals must be infected through ingestion of oocysts or by vertical transmission (Owen and Trees, 1998). The oocyst stage of the parasite is only present in feces from recently infected felids (Hutchison, 1965); thus feral cat habitat use is an integral part of SHR-mammal *T. gondii* infection. Small–home-range mammals are likely to be good sentinels for risk of *T. gondii* exposure to humans and wildlife in a natural area.

The higher prevalence of *T. gondii* infection in SHR mammals at building sites compared to forest sites can be explained in part by the high frequency of cat occurrence at most building sites. Our findings are similar to those from a study where woodchucks in urbanized areas had a higher prevalence of *T. gondii* compared to woodchucks living in rural areas (Lehrer et al., 2010). Our findings further support the notion that anthropogenic influences by way of feral cats in natural areas could influence wildlife through the spread of *T. gondii*.

This study demonstrates that there is *T. gondii* environmental contamination at

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small local scales in this natural area. Because oocysts can survive in the environment for up to 1 yr (Dubey and Beattie, 1988), one positive cat could contaminate a natural area with *T. gondii* oocysts, increasing the risk of exposure to wildlife. Although environmental detection of oocysts is difficult and was not evaluated in this study, the Sangamon River crosses the middle of the park; so it is possible that LHR mammals could have acquired infection from consumption of contaminated water.

Although cats were not trapped in each area, all eight sites had evidence of feral cats based on the scent-station data; this highlights the value of multiple detection methods to locate elusive animals. Feral cat tracks and scat have been detected throughout nature reserves, even in remote areas (Rouys and Theuerkauf, 2003), which is in accordance with our findings that feral cats use the interior of natural areas. Not only did we detect feral cats in the forest, but the localized habitat use, predominantly herbivorous diet, and prevalence of *T. gondii* infection in SHR mammals suggest feral cats were the source of *T. gondii*–oocyst contamination in RAP.

Cats used areas around buildings more frequently than forest areas, as evidenced by our multiple detection results. Cat dependence on humans, even in a natural area, for shelter, prey, and supplemental food (Liberg, 1984) may explain this finding. Some areas in the park located close to buildings have picnic areas and trash cans that may act as animal attractants and serve as a food source. Large–home-range mammals exhibit a similar abundance at areas with buildings and a presumed dependence on human food supplies and shelter (Prange et al., 2003). These results imply that, for urban adaptive species such as raccoons, opossums, and feral cats, buildings in natural areas may increase contact rates between wildlife and feral cats, potentially increasing interspecies disease transmission.

Additionally, the distribution of LHR mammals and feral cats in RAP may be explained by "mesopredator release" dynamics (Crooks and Soule, 1999). Coyote presence in the interior of the park could displace raccoons, opossums, and feral cats, but at building sites, these animals are released from coyote predation pressure (Crooks and Soule, 1999). Conclusions on mesopredator release in RAP require further data collection and study design to test this hypothesis.

Higher antibody prevalence in LHR mammals compared to SHR mammals may be related to life-history characteristics associated with species in each group. Movement throughout the park exposes the LHR mammals to more infected areas. Furthermore, the LHR mammals are omnivores that consume more animal tissue compared to SHR mammals, potentially exposing them to *T. gondii* tissue cysts in intermediate hosts. Another differing characteristic between the LHR and SHR groups is their life span; because LHR mammals tend to live longer than the SHR mammals, their lifetime chance of becoming infected is greater.

Among white-footed mice, we found a 6% prevalence of *T. gondii* antibodies, which was high, compared to other studies (Dubey et al., 1995; Smith and Frenkel, 1995; DeFeo et al., 2002). The findings that short-lived mice were antibody-positive implies an active infection and recent *T. gondii* contamination in RAP even if vertical transmission occurs. The high prevalence among SHR mammals suggests that wildlife within this isolated natural area could be impacted by the presence of cats in RAP.

Although not an initial objective of the study, higher antibody prevalence was found in adult LHR and SHR mammals compared to juveniles. These findings are similar to other studies on the antibody prevalence of *T. gondii* in cats (Dubey et al., 1995), rodents (Dabritz et al., 2008), and sea otters (Miller et al., 2002). Older animals have more time and potential to

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encounter *T. gondii*–oocyst-infected environmental areas than do younger animals. In contrast to other studies, we found that female LHR and SHR mammals had a higher antibody prevalence than did males. Other studies suggest that males have larger home ranges and a higher *T. gondii*–antibody prevalence (Miller et al., 2002), but, because of reproductive changes, there are enhanced energy requirements for females (Harder et al., 1996), which may result in greater food intake and increased risk of infection.

Some false-positive reactions may have occurred with the *T. gondii* IFAT. The test has not been validated for each of the wild-animal species we investigated, and the occurrence of cross-reacting antibodies is possible. However, a significant problem with cross reactions is unlikely, because the IFAT has been used successfully in a large number of animal species. A particular advantage of IFAT, in comparison with modified agglutination and enzyme-linked immunosorbent assays, is the requirement to visualize the reaction pattern of the entire pellicle of individual organisms (Paré et al., 1995). This allows investigators to eliminate cross reactions caused by apical complex antigens that are conserved among closely related organisms (Taylor et al., 1990). Validation of serologic tests is a common problem of wildlife studies. When possible, it is desirable to employ multiple tests to attempt to determine the true infection status of each animal, including such laboratory methods as culture, polymerase chain reaction (PCR), and bioassay. We only obtained serum specimens because animals were live-trapped, then released; culture, PCR, and bioassay tests of sera would have been extremely insensitive for the detection of latent *T. gondii* infections, which seldom manifest parasitemia.

Management implications

This work provides insight into how feral cats use natural areas and how cat presence may influence wildlife. This information is necessary for establishing successful feral cat control strategies. The overall implications for pathogen transmission between wildlife and feral cats remain understudied. Collaborative studies of feral cat populations are necessary to aid in further refinement of management strategies that could benefit wildlife and human health.

Educating the public on how to care for animals (including keeping cats indoors to decrease the health risks for wildlife, cats, and humans) and implementing regulations on free-roaming cats are desirable. Persistent monitoring of cat populations and continued study of the effects of cat diseases in wildlife populations are needed (Stoskopf and Nutter, 2004). Further research and support of scientific programs are required to provide useful information to the public, cat owners, and wildlife biologists for creation and implementation of effective toxoplasmosis control strategies.

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