

Short Communication

## Seroprevalence of *Toxoplasma gondii* in White-Tailed Deer (*Odocoileus virginianus*) and Free-Roaming Cats (*Felis catus*) Across a Suburban to Urban Gradient in Northeastern Ohio

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**Abstract:** Felids serve as the definitive host of *Toxoplasma gondii* contaminating environments with oocysts. White-tailed deer (WTD; *Odocoileus virginianus*) are used as sentinel species for contaminated environments as well as a potential source for human foodborne infection with *T. gondii*. Here we determine the seroprevalence of *T. gondii* in a WTD and felid population, and examine those risk factors that increase exposure to the parasite. Serum samples from 444 WTD and 200 free-roaming cats (*Felis catus*) from urban and suburban reservations were tested for *T. gondii* antibodies using the modified agglutination test (MAT, cut-off 1:25). Antibodies to *T. gondii* were found in 261 (58.8%) of 444 WTD, with 164 (66.1%) of 248 from urban and 97 (49.5%) of 196 from suburban regions. Significant risk factors for seroprevalence included increasing age ( $P < 0.0001$ ), reservation type ( $P < 0.0001$ ), and household densities within reservation ( $P < 0.0001$ ). Antibodies to *T. gondii* were found in 103 (51.5%) of 200 cats, with seroprevalences of 79 (51%) of 155 and 24 (53.3%) of 45 from areas surrounding urban and suburban reservations, respectively. Seroprevalence did not differ by age, gender, or reservation among the cats' sample. Results indicate WTD are exposed by horizontal transmission, and this occurs more frequently in urban environments. The difference between urban and suburban cat densities is the most likely the reason for an increased seroprevalence in urban WTD. These data have public health implications for individuals living near or visiting urban areas where outdoor cats are abundant as well as those individuals who may consume WTD venison.

**Keywords:** seroprevalence, *Toxoplasma gondii*, white-tailed deer, free-roaming cats, urbanization

## INTRODUCTION

*Toxoplasma gondii* is a protozoan parasite that can infect all warm-blooded species (Dubey 2010). Although typically asymptomatic, toxoplasmosis is a significant veterinary and

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public health disease, particularly among pregnant and immunocompromised populations (Dubey and Jones 2008). Approximately 14% of the United States population is seropositive for this disease, with an estimated one million new cases diagnosed each year (Jones et al. 2007; Jones and Holland 2010). Exposure in humans and animals is most commonly via environmental contamination with oocysts or by the consumption of raw/undercooked meat contaminated with tissue cysts (Dubey and Jones 2008).

Felids, both domestic and wild, play a critical role in the epidemiology of *T. gondii* because they serve as the definitive hosts, fulfilling all the requirements needed for the parasite to sexually reproduce and complete its lifecycle (Dubey 1998). Domestic cats (*Felis catus*) are often infected at less than 1 year of age where they can contaminate the environment shedding millions of oocysts per day for 1–2 weeks (Dubey 2001). Free-roaming cats, those that are allowed free access to the outdoors, are more likely to be exposed and infected, thereby contributing more frequently to environmental contamination than domestic indoor cats (Dubey 1973). Although a domestic transmission cycle of *T. gondii* exists between cats and small rodents, numerous reports indicate wildlife are frequently exposed and infected with *T. gondii* (Dubey and Jones 2008).

Among the many wildlife species exposed to and infected with *T. gondii*, white-tailed deer (*Odocoileus virginianus*; WTD) are of particular concern. WTD are well-adapted to urban environments which may lead to more frequent exposure to environments contaminated with cat feces. Furthermore, *T. gondii* serostatus in WTD has significant public health implications as WTD can serve as sentinels for environmental contamination (Schaefer et al. 2013). Infected WTD can harbor viable tissue cysts that have been shown to pass to humans via consumption of undercooked venison (Dubey et al. 2004; Sacks et al. 1983).

The main objectives of this study were to determine the seroprevalence of *T. gondii* in WTD and free-roaming cats in northeastern Ohio. Previous studies have identified serostatus in cats and WTD in other areas of the USA, however we aim to build on those studies by identifying significant risk factors resulting in seropositivity. Additionally, we focused particularly on understanding how urbanization can influence serostatus in free-roaming cats and WTD populations and how the serostatus of cats may influence serostatus of WTD. We hypothesize that older deer and cats from more urban environments will have an increased likelihood of being seropositive because of increased annual exposure to highly contaminated environments.

## MATERIALS AND METHODS

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### Study Site and Sample Collection

Sample collection took place at the Cleveland Metroparks in Northeastern Ohio, USA. WTD were harvested nightly between January 11 and March 5, 2010 as part of a deer population management program. Six reservations within the Metroparks were assigned for deer management efforts; two suburban reservations (Brecksville and North Chagrin) and four urban reservations (Rocky River, Mill Stream Run, Bedford and Bradley Woods) (Table 1). Reservations were classified as suburban or urban based on the 2012 ACS 5-year estimate Census data (<http://factfinder2.census.gov>) of the number of households within a half mile buffer around each reservation. Reservations were classified as urban if there was greater than one unit per acre and suburban if they contained less than 1 U per acre but greater than 1 U per 10 acres (Theobald 2001).

Authorized sharpshooters conducted all harvesting efforts from roadside and tree stands overlooking bait stations. All harvested deer were transported to a centralized processing station. Once at the processing station, the deer were weighed, aged, sexed, and field dressed. Age of deer was estimated by dental characteristics and for this study the deer were divided into three age groups; fawn (<1 year), yearling (1–2 years), and adults (>2 years) (Cain and Wallace 2003). During field dressing, blood samples were collected from the jugular vein. Clotted blood was centrifuged and serum was separated and immediately put on ice. The sera were transported nightly to Cleveland Metroparks Zoo where they were stored at  $-80^{\circ}\text{C}$  prior to antibody analysis. The samples were transported with cold packs to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, MD for serological testing.

A sample of free-roaming cats was collected in conjunction with a local spay/neuter and return program in the Greater Cleveland area in 2011. Cats included in this study were cats housed outdoors, fed and cared for by the individuals bringing them to the program. Serum samples from a total of 200 cats were tested as part of this study. Samples were collected from 100 cats from address east of the Cuyahoga River, and 100 cats from west of the Cuyahoga River, all within the Greater Cleveland Area. One cat per address was selected from the cats brought to the program, until 100 samples were collected from the east and west side regions.

**Table 1.** Household Densities Per Reservation

Reservation	Households	Area of buffer area (acre)	Households/acre
Rocky River Reservation	24,741	8,132.8	3.04*
Mill Stream Run Reservation	12,973	7,322.5	1.77*
Bradley Woods Reservation	4,011	2,290.3	1.75*
Bedford Reservation	7,872	6,072.9	1.30*
North Chagrin Reservation	2,552	3,621.3	0.70
Brecksville Reservation	2,604	5,116.8	0.51

Reservations considered urban ( $\geq 1$  household/acre) are marked with (\*). Suburban reservations are those with household densities of  $< 1$  to  $\geq 0.1$  households/acre.

Upon arrival, each cats was sexed, aged, and blood samples were collected from the jugular vein as part of the health screening protocol prior to spay/neuter surgery. Cats were divided into three age groups; kitten ( $< 1$  year), yearling (1–2 years), and adults ( $> 2$  years) in order to maintain a consistent analytical approach between WTD and cat exposure data. Clotted blood was centrifuged and serum was separated and frozen in a zero-degree freezer until they could be transported to Cleveland Metroparks Zoo. Once the sera were transported to the Cleveland Metroparks Zoo, they were stored at  $-80^{\circ}\text{C}$  until transport for antibody analysis. The samples were transported with cold packs to the APDL, Beltsville, MD for serological testing. Free-roaming cats were assigned to the reservation that was closest to the address where their caretaker lived. It was assumed that cats would not cross the Cuyahoga River.

### Serological Testing

The modified agglutination test (MAT) was used to detect *T. gondii* antibodies as described by Dubey and Desmonts (1987). Deer sera were diluted twofold from 1:25 to 1:3,200. *T. gondii* seroprevalence was reported as a titer score. Titers of 1:25 or greater were considered a seropositive result as defined in previous studies (Dubey 2010).

### Statistical Analysis

Differences in the likelihood of being seropositive for deer and cats were estimated using multilevel logistic regression mixed models with SAS Proc Glimmix (SAS v. 9.3, SAS Institute, Cary, NC). Predictor variables included in the logistic regression models for both species were individual age, gender, reservation, and household density nested within reservation. The Tukey–Kramer method was used to calculate multiple pair-wise comparisons of least-square

means. Adjusted odds ratios and their 95% confidence intervals are presented for each predictor variable.

## RESULTS

### White-Tailed Deer

Antibodies to *T. gondii* were found in 261 (58.8%) of 444 WTD sampled with titers ranging from 1:25 to 1:3,200. Seroprevalence of *T. gondii* was 35.8% (54/151) in fawns, 71.3% (72/101) in yearlings, and 70.3% (135/192) in adults (Table 2). Overall, seropositivity increased significantly with age when adjusting for gender and reservation ( $P < 0.0001$ ;  $f = 24.58$ ;  $df = 2,435$ ). The adjusted odds ratio (and its 95% confidence intervals) of seropositive yearlings to fawns was 5.13 (2.56–10.20;  $P = < 0.0001$ ,  $t = -5.55$ ;  $df = 435$ ), of adults to fawns was 5.05 (2.77–9.17;  $P = < 0.0001$ ;  $t = -6.35$ ;  $df = 435$ ) and of adults to yearlings was 0.99 (0.50–1.94;  $P = 1$ ;  $t = 0.05$ ;  $df = 435$ ). Seroprevalence was lower in males (55.5%) than in females (60.9%). No association between seropositivity and gender was observed after adjusting for age group and reservation ( $P = 0.79$ ;  $F = 0.07$ ,  $df = 1,435$ ).

Seroprevalence of WTD was 64.4% (208/323) and 43.8% (53/121) in urban and suburban reservations, respectively (Table 2). The odds of deer being seropositive were 2.98 times those of deer from urban reservations compared to suburban reservations (95% CI 1.87–4.76;  $P < 0.0001$ ;  $t = -4.61$ ;  $df = 435$ ) when adjusted for age and gender. Household densities within reservations was a significant predictor of serostatus in WTD ( $P = < 0.0001$ ,  $F = 6.18$ ,  $df = 5,435$ ). Analysis of pair-wise comparisons of reservation household densities showed reservations with the greatest household densities (Rocky River and Mill Stream Run Reservations) had significantly higher

**Table 2.** WTD and Free-Roaming Cat Seroprevalence by Age and Reservation; Number Seropositive/Number Tested (Percent Positive)

	Urban reservations	Suburban reservations	Total
<b>WTD</b>			
Fawns	48/116 (41.4)	6/35 (17.1)	54/151 (35.8)
Yearlings	56/70 (80.0)	16/31 (51.6)	72/101 (71.3)
Adults	104/137 (75.9)	31/55 (56.3)	135/192 (70.3)
Total	208/323 (64.4)	53/121 (43.8)	261/444 (58.8)
<b>Free-roaming cats</b>			
Kittens	0/2 (0)	0/3 (0)	0/5 (0)
Yearlings	11/29 (37.9)	5/7 (71.4)	16/36 (44.4)
Adult	68/124 (54.8)	19/35 (54.3)	87/159 (54.7)
Total	79/155 (51.0)	24/45 (53.3)	103/200 (51.5)

proportions of seropositive WTD when compared to the suburban reservations. Interestingly, the reservation with the most urbanization (Rocky River) also had significantly higher proportions of seropositive WTD when compared to another urban reservation (Bradley Woods) (Table 3).

### Cats

A total of 200 cats were sampled, 45 (23%) near suburban reservations and 155 (77%) near urban reservations. The seroprevalence among cats was 51.5% (103/200); 0% (0/5) in kittens, 44.4% (16/36) in yearlings, and 54.7% (87/159) in adults. Although there was an increase in seropositivity as the cats aged, this trend was not significant after controlling for gender and reservation ( $P = 0.57$ ,  $F = 0.56$ ,  $df = 2,191$ ). Gender was not a significant predictor of seropositivity ( $P = 0.61$ ;  $F = 0.26$ ;  $df = 1,191$ ) as the seroprevalence among male cats (51.2%; 43/84) was nearly identical to female cats (51.7%; 60/116).

Seroprevalence of cats found in proximity to urban reservations was 51% (79/155) and to suburban reservations was 53.3% (24/45). There was no significant difference in the odds of being seropositive among cats from urban reservations compared to suburban reservations ( $P = 0.55$ ,  $t = 0.6$ ,  $df = 195$ ). In addition, household densities within reservations did not significantly predict serostatus among the sampled cats ( $P = 0.20$ ;  $F = 1.48$ ;  $df = 5,191$ ).

## DISCUSSION

Overall seroprevalence of *T. gondii* antibodies in the sampled WTD population was estimated to be 58.8%. Similar

estimates for WTD were found in Iowa (53.5%, 64.2%), Pennsylvania (60%), Mississippi (46.5%), and Ohio (44%) using the same MAT serological assay (Humphreys et al. 1995; Crist et al. 1999; Dubey et al. 2004, 2008, 2009b). A significant positive association between age and seroprevalence was observed within the deer population in which individuals over 1 year of age were more likely to be seropositive and therefore potentially infected. This finding is consistent with other studies that investigated the effect of age on seropositivity in hunter-killed WTD samples (Vanek et al. 1996; Schaefer et al. 2013). The significant increase in seropositivity with increasing age indicates that exposure early on in life (less than 1 year old) may not be as common as in yearlings and adults. The trend indicates horizontal transmission (i.e., via ingestion of oocysts from contaminated environments) as the main mode of exposure. This is further supported by a study by Dubey et al. (2014) which noted that vertical transmission among WTD is considered a rare event.

Free-roaming cats sampled in this study had a seroprevalence of 51.5%. Interestingly, this seroprevalence was quite similar to the reported deer seroprevalence (58.8%). Other studies in the USA have reported a variety of seroprevalences among cats, ranging from 1 to 84.7% (Bevins et al. 2012; Fredebaugh et al. 2011; Dubey et al. 2009a). In other locations of Ohio, a seroprevalence of 48% has been noted among domestic cats (Dubey et al. 2002). The seroprevalence reported in this study fit within the national seroprevalences reported for cats and are consistent with the previous reports from other locations in Ohio. The wide range of seroprevalence among the multiple studies may be attributed to the type of cat (outdoor vs. indoor), age distribution, serologic test used, and geographical

**Table 3.** Logistic Regression of Risk Factors for Seropositivity to *T. gondii* for WTD with Tukey–Kramer Pair-wise Comparison of Household Density Within Reservations

Risk Factor	Adjusted Odds Ratio	95% CI	Significance ( <i>P</i> )
Age class			
Fawn	1		
Yearling	5.13	2.56–10.20	<0.0001
Adult	5.05	2.77–9.17	<0.0001
Gender			
Female	1		
Male	0.94	0.06–1.47	0.79
Reservation			
Suburban	1		
Urban	2.98	1.87–4.76	<0.0001
Household density			
RR vs. MSR	1.20	0.45–3.18	0.995
RR vs. BW	3.57	1.01–12.63	0.047
RR vs. Bedford	1.93	0.66–5.62	0.490
RR vs. Brecksville	4.39	1.45–13.28	0.002
RR vs. NC	4.51	1.44–14.10	0.003
MSR vs. BW	2.98	0.95–9.38	0.072
MSR vs. Bedford	1.61	0.64–4.10	0.684
MSR vs. Brecksville	3.66	1.39–9.69	0.002
MSR vs. NC	3.77	1.37–10.38	0.003
BW vs. Bedford	0.54	0.16–1.84	0.706
BW vs. Brecksville	1.23	0.35–4.30	0.997
BW vs. NC	1.26	0.35–4.57	0.995
Bedford vs. Brecksville	2.27	0.79–6.56	0.233
Bedford vs. NC	2.33	0.78–6.97	0.233
Brecksville vs. NC	1.03	0.34–3.13	1.000

RR Rocky River, MSR Mill Stream Run, BW Bradley Woods, NC North Chagrin.

location of the sample population (Dubey 2010). Although our study did not find age to be a significant predictor for seroprevalence, it should be noted that seroprevalence did increase with age. This trend is supported by other studies, indicating horizontal transmission via contaminated environments and/or infected prey animals are major routes of exposure in cats (Afonso et al. 2006; Dubey et al. 2009a).

WTD were significantly more likely to have antibodies to *T. gondii* when they were harvested from urban reservations with high household densities compared to suburban reservations with lower household densities. Urbanization has been identified as a significant risk factor for *T. gondii* exposure in other species including sea otters and woodchucks, an urban adapter species similar to deer (Conrad et al. 2005; Lehrer et al. 2010). The current study

contrasts with a previous study that did not identify a significant direct correlation between human population densities and WTD seroprevalences to *T. gondii* (Schaefer et al. 2013). Our study differed from the previous study because we used a culled deer sample and household densities as a measure of urbanization. Most importantly, the current study compared deer from multiple types of habitats, urban and suburban, in contrast with Schaefer et al. (2013) the WTD collected solely from rural habitats. An earlier study (Crist et al. 1999) examined serostatus of WTD from different counties in Ohio, finding a lower seroprevalence in deer from a more urbanized county (Franklin County) as compared to a more rural county (Hocking County). The authors noted surprise at this finding, having expected to find a higher seroprevalence in

deer from the more urban county. This study did not evaluate the urban characteristics of the areas from which the deer were sampled. In addition, the study was comparative at the county level, in contrast to the present study which examines seroprevalence in deer from an urban to suburban gradient across adjacent counties. The contrast in findings of the Crist study and the present study may also be explained by different sampling techniques (hunter-killed deer versus sharpshooters) or the overall sample size difference between the two studies.

Free-roaming cats from urban and suburban populations had similar *T. gondii* seroprevalence. There is a lack of data on the serostatus of *T. gondii* in cats from urban and suburban habitats in the United States. However, one study from Hungary noted rural and suburban cats had a higher incidence of exposure when compared to urban cats (Hornok et al. 2008). Similarly, in other regions of Ohio a higher seroprevalence of *T. gondii* in rural free-roaming cats was noted when compared to urban cats (Dubey et al. 2002). Serostatus in cats is often correlated to the amount of time spent outdoors, cohabitation with rodents, and amount of hunting/predation (Afonso et al. 2006; Coelho et al. 2011; Opsteegh et al. 2012). The lack of significant differences among seroprevalence between the two populations of cats may indicate consumption of prey as the most significant mode of transmission when compared to ingestion of oocysts from the environment. The density and seroprevalence among prey species has not been studied in Northeast Ohio and may provide evidence for why serostatus is similar in both populations of cats. Alternatively, in urban environments, where environmental contamination is higher, transmission in cats may solely be by ingestion of oocysts from the environment. Whereas in suburban environments, where contamination is lower, transmission may be a combination of ingestion of oocysts from the environment and tissue cysts from prey species, leading to similar seroprevalences among cats from urban and suburban habitats.

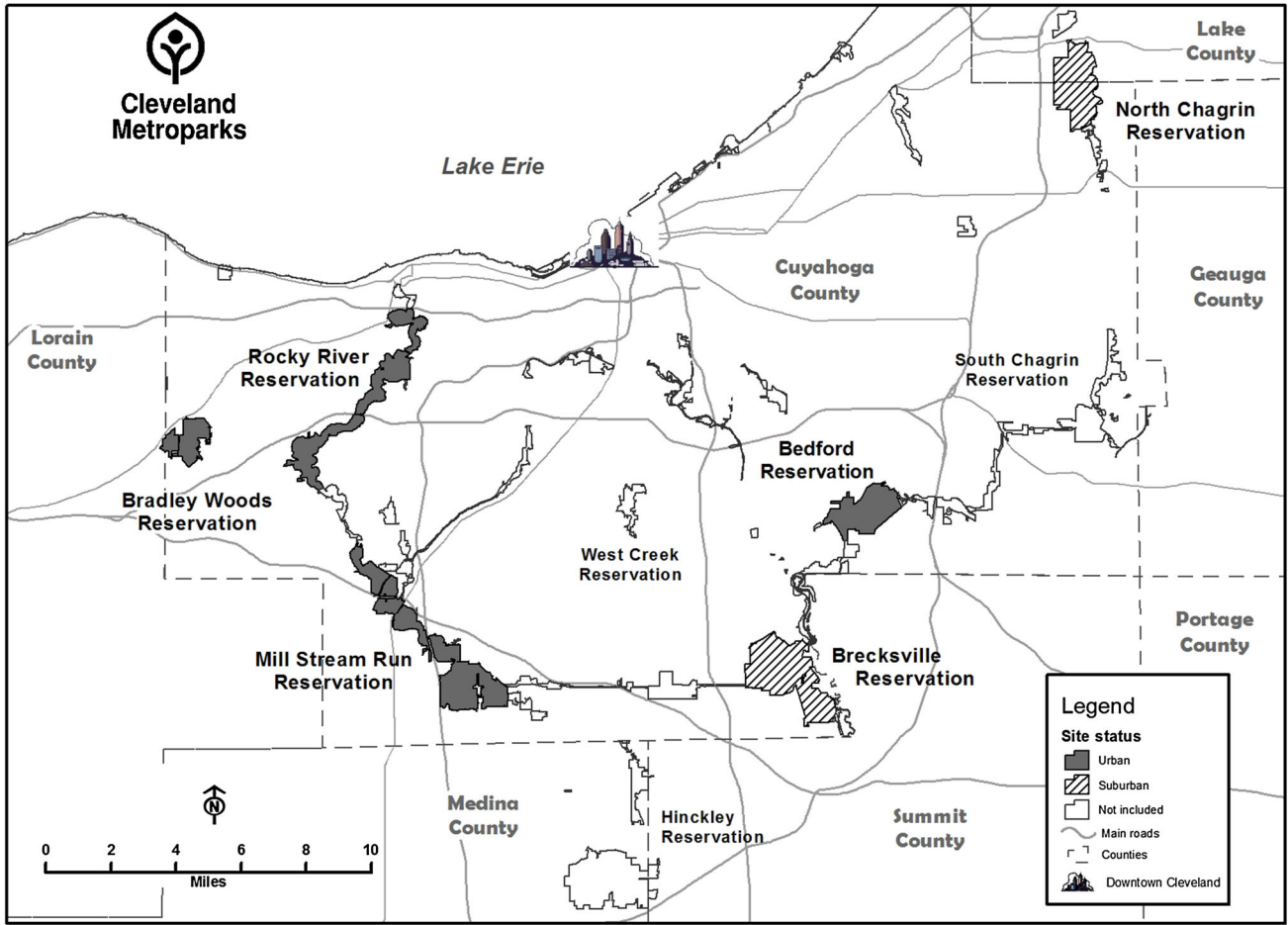
One explanation for an increase of seroprevalence in deer across the urban gradient is the increased risk of encountering environments contaminated with feces from free-roaming cats. In the current study, nearly 75% of the cats sampled were from areas in close proximity to urban reservations. Although the cats were not randomly sampled, Lepczyk et al. (2003) found similar results to those reported in this study, noting that feral cat quantities and densities were greater in urban environments when compared to suburban environments using random sampling.

Furthermore, assuming equal cat ownership per household among the areas surrounding urban and suburban reservations, it can be concluded that as urbanization increases so would free-roaming cat numbers. The presence of cats has been established as a significant risk factor for exposure to *T. gondii* in other species of wildlife (Fredebaugh et al. 2011). The increased quantity of free-roaming cats located in proximity to urban environments is most likely a result of more abundant food resources, shelter, and cat ownership compared to suburban environments.

The amount of forest edge habitat may also play a role in transmission dynamics between cats and WTD. We noted that two urbanized reservations, Bradley Woods and Rocky River showed significantly different seroprevalences among WTD. One explanation is that Bradley Woods is a more heavily wooded reservation with limited forest edge habitat/buffer region when compared to Rocky River. Rocky River provides considerably more open and fragmented woods, offering abundant forest edge/buffer region habitat. Both WTD and free-roaming cats tend to prefer forest edge and open habitats which may lead to habitat overlap and increased exposure of WTD to cats (Kurta 1995; Crooks and Soule 1999).

It is important to consider bobcats as a potential source for environmental contamination with *T. gondii* oocysts. Bobcats have been shown to be exposed at high rates and a major contributor to fecal shedding across geographical gradients (Bevins et al. 2012). However, to our knowledge there is not a significant bobcat population in the Cleveland Metropark reservations.

Cat serum samples were collected from free-roaming cats and brought to spay/neuter and release program at a local animal shelter in the greater Cleveland area. Free-roaming cats were not specifically trapped within the participating reservations. Most reservations are within 25 km of each other with the farthest reservation distance being approximately 50 km. Free-roaming cats have been shown to travel linearly for up to 33.8 km and can have long-term home ranges encompassing 22.1 km<sup>2</sup> (Fitzgerald and Karl 1986; Edwards et al. 2001). Therefore, cats could travel between urban and suburban reservations depending on individual home ranges, thus influencing potential *T. gondii* exposure. Deer are not confined to the reservations, thus cats do not have to be within the reservation boundaries to be a source of exposure to deer. Deer home range varies from 1 to 5 km<sup>2</sup> depending on a number of variables including age, sex, and season (Walter et al. 2011). The reservations, the WTD were sampled from in this study



**Figure 1.** Map of study area within Cleveland Metroparks.

were all greater than 12 km<sup>2</sup>, suggesting WTD were most likely exposed in the same reservations they were collected. However, WTD have been shown to disperse large distances up to 50 km in forested habitats, complicating this assumption (Rosenberry et al. 1999). In addition, large dispersal distances of WTD may serve as a risk factor for *T. gondii* exposure as they encounter a diverse set of habitats that cats may populate. Road density and the abundance of resources may limit home ranges and dispersal distances of both cats and deer, however these factors have not been studied in Northeast Ohio (Fig. 1).

## CONCLUSION

WTD and free-roaming cats are primarily exposed via horizontal transmission. Urban environments seem to be more contaminated than suburban environments, presenting a greater potential risk of exposure to both animals and humans. It is likely that urban environments are more

contaminated than suburban habitats because of the increased density of free-roaming cats in these areas, not necessarily because of a higher incidence of infected cats in urbanized areas. Understanding transmission dynamics along urbanization gradients is necessary to limit exposure of cats and WTD, and consequently humans, to *T. gondii*.

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