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Risk Factors for *Toxoplasma gondii* Exposure in Semiaquatic Mammals in a Freshwater Ecosystem

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ABSTRACT: We assessed risk factors for *Toxoplasma gondii* exposure in semiaquatic mammals in east-central Illinois, US. This agricultural region has extensive drainage systems that could potentially transport *T. gondii* oocysts into the watershed. We used muskrats (*Ondatra zibethicus*) and American mink (*Neovison vison*) as sentinels of watershed contamination. We predicted individuals from larger subwatersheds would more likely be antibody-positive for *T. gondii*, as they were exposed to drainage from larger areas. We also evaluated amount of urban land cover within the subwatershed, proximity to farmsteads, and age of individuals in competing models of *T. gondii* infection. Antibodies to *T. gondii* were assayed in animal sera by modified agglutination tests (titer 25 or higher) and detected in 18 (60%) of 30 muskrats and 20 (77%) of 26 mink. Infection rates were ≥1.7 times higher than those typical for mammals in upland habitats in this region. Subwatershed size and age class were important predictors of *T. gondii* infection in muskrats (R^2=0.35). Models incorporating urban land cover and proximity to farmsteads had little support. None of our models of antibody prevalence in mink were well supported, possibly because mink are less strictly associated with riparian habitats. Because ~91% of our study area is devoted to agricultural production and urbanization, transport of *T. gondii* into freshwater ecosystems is likely facilitated by modified drainage practices common in these areas.

Key words: American mink, muskrat, *Toxoplasma gondii*, urbanization, watershed health, wildlife diseases.

Toxoplasma gondii is a protozoal parasite of endothermic vertebrates and a significant risk to human and wildlife health (Dubey 2010; Torey and Yolken 2013). Felids are the definitive host and are required to complete the parasite's sexual cycle. Infected felids shed *T. gondii* oocysts in feces, which have variable persistence times depending on environment and temperature (Dubey 1998). Infection occurs via ingestion of oocysts or ingestion of bradyzoites in tissues of terminal hosts.

Waterborne transmission of *T. gondii* is a growing concern. Contaminated runoff has been implicated in outbreaks in humans (Jones and Dubey 2010) and may negatively affect the survival of southern sea otters (*Enhydra lutris nereis*; Conrad et al. 2005). Direct testing for *T. gondii* in aquatic ecosystems is technically difficult (Jones and Dubey 2010) and often requires sampling sentinel species (Conrad et al. 2005).

High densities of domestic and free-ranging cats are associated with farmsteads (Wiegel et al. 1999) and urban areas (VanWormer et al. 2013) and contribute *T. gondii* oocysts to the environment. In east-central Illinois, most of the land is modified for row-crop agriculture, including extensive subsurface tile drainage and ditches. These drainage systems could potentially collect and transport *T. gondii* oocysts, although the viability of this transport mechanism has not been assessed. Similarly, run-off from urban areas could transport *T. gondii* into riparian systems (Miller et al. 2002).

We used muskrat (*Ondatra zibethicus*) and American mink (*Neovison vison*) as sentinels for *T. gondii* in a freshwater ecosystem. Both species are semiaquatic and associated with riparian habitats. We evaluated five predictors of prevalence: watershed, subwatershed size, proximity to farmsteads, area of urbanized landscape

within the subwatershed, and age class. If runoff from the surrounding landscape transports *T. gondii* oocysts, *T. gondii* antibody prevalence should be related positively to subwatershed size. Urbanized areas and farmsteads may contribute *T. gondii* from domestic and feral cats (Weigel et al. 1999; VanWormer et al. 2013), but their relative contribution to riparian habitats is unknown. We predicted antibody prevalence would be higher in older individuals because of longer, lifetime exposure to *T. gondii*.

We conducted this study in Champaign County, Illinois, US (40°12′N, 88°26′W; 258,337 ha). Eighty-five percent of the landscape is developed for corn-soybean production and 6% is urbanized. Most of the landscape (52–82%) is drained via subsurface tiles (David et al. 2010). Consequently, precipitation runoff is channeled by subsurface conduits into nearby streams and agricultural ditches. Bobcats (*Lynx rufus*) are rare and mountain lions (*Puma concolor*) were extirpated by 1870. Thus, domestic and feral cats are the primary definitive host of *T. gondii*. On average, there are six antibody-positive cats per farm in Illinois (Weigel et al. 1999).

We sampled mink and muskrats for *T. gondii* antibody prevalence in three watersheds (Kaskaskia, 4,064 km^2; Vermillion, 3,726 km^2; Embarras, 6,324 km^2) from 2007–12. Individuals were live-trapped in streams and ditches using baited traps affixed to floating platforms. We transported animals to a sterile surgical laboratory immediately after capture, placed them under surgical anesthesia (see Ahlers et al. 2010), determined age class (subadult or adult), and collected blood samples (1.5 mL) via cranial venae cavae. We used a modified agglutination test (MAT) to measure specific *T. gondii* antibodies. Sera were diluted 1:25 before evaluation and reactive sera were considered antibody positive. Although MAT has not been evaluated specifically for muskrats and mink, extensive testing across taxa indicates that a titer of 25 indicates *T. gondii* exposure (Dubey 2010). All procedures were approved by the Animal Care and Use Committee at the University of Illinois.

We assessed differences in antibody prevalence among watersheds for both species. Although sampling occurred within the three large watersheds, subwatershed size for each mink or muskrat sample was the total surface area drained (km^2) from the head of the watershed downstream to the capture site. Thus, a unique subwatershed size was calculated for each individual. We calculated subwatershed sizes using watershed and raster elevation layers in ArcMap v9.2 (Environmental Systems Research Institute, Inc. Redlands, California, USA). We quantified the percentage land cover by urban areas (high, medium, and low-density urbanization combined) within individual subwatersheds and measured the Euclidian distance (m) from capture sites to the nearest farmstead.

We used logistic regression to model *T. gondii* antibody prevalence as a function of landscape factors (watershed, subwatershed area, urbanization, distance to nearest farmstead) and age class. We used Akaike's Information Criterion corrected for small sample sizes (AIC_c) to rank 31 models for each species that represented single and additive effects of all combinations of our variables plus an intercept-only model. We considered models with $\Delta AIC_c \leq 2$ competitive and evaluated the goodness-of-fit of our most-supported model with a Hosmer-Lemeshow test (Lemeshow and Hosmer 1982).

We tested serum samples from 30 muskrats (6 adults, 24 subadults) in the Kaskaskia ($n=17$) and Embarras ($n=13$) watersheds and 26 mink (15 adults, 11 subadults) in the Kaskaskia ($n=11$), Vermillion ($n=5$), and Embarras ($n=10$) watersheds. Sixty percent of muskrats (18/30; 95% confidence interval [CI]: 43–78%) and 77% of mink (20/26; 95% CI: 61–93%) were antibody positive. In the Kaskaskia watershed, 65% (11/17) of muskrats and 81% (9/11) of mink were antibody positive.

TABLE 1. Ranking of models predicting *Toxoplasma gondii* antibody prevalence in muskrats (*Ondatra zibethicus*) and American mink (*Neovison vison*) in a freshwater ecosystem. We examined the support of 31 models for each species that included the effects of landscape-level factors and age. Only models with $\Delta AIC_c \leq 2$ are presented along with the Intercept Only model for both species.

	Parameter[a] and model[b]			
	K	ΔAIC_c	−2 log-like	ω_i
Muskrats				
Subwatershed	2	0.00	29.13	0.25
Subwatershed + Age	3	0.63	26.73	0.18
Intercept Only	1	8.95	40.38	0.00
Mink				
Intercept Only	1	0.00	28.09	0.15
WatershedID	2	0.73	23.89	0.11
Subwatershed + WatershedID	3	0.99	21.35	0.09
Subwatershed	2	1.21	26.94	0.08
DistFarm	2	1.67	27.41	0.07

[a] *K* = number of parameters including the intercept term; ΔAIC_c = difference in model AIC_c (Akaike's Information Criterion corrected for small sample sizes) and the lowest model AIC_c of the candidate set; −2 log-like is used for assessing model fit; ω_i = model weight.

[b] Covariates include: WatershedID = Kaskaskia, Embarras, or Vermillion watershed; Subwatershed = subwatershed drainage area; DistFarm = Euclidian distance between sampling point and nearest farmstead; Age = subadult or adult.

In the Embarras watershed, 54% (7/13) of muskrats and 60% (6/10) of mink were antibody positive. All five mink sampled in the Vermillion watershed were antibody positive.

Subwatershed area and age class were the only factors included in the two most-supported models explaining *T. gondii* antibody prevalence in muskrats (model-fit statistics for our second-ranked model: Hosmer-Lemeshow test, $\chi^2=3.82$, df=7, $P=0.80$; $R^2=0.35$; Table 1). Muskrats captured in larger subwatersheds had a higher probability of being antibody positive ($\beta=0.0387$, SE=0.0144; odds ratio [OR]=1.039, 95% CI=1.010–1.069; Fig. 1). Antibody prevalence in muskrats was higher among adults (67%; 4/6) than in subadults (58%; 14/24); however, this effect was weak (OR=1.172, 95% CI=0.112–12.237). In contrast, none of our models of mink antibody prevalence were well supported; the intercept-only model was our top-ranked model ($\omega_i=0.15$, Table 1).

Infection rates of *T. gondii* in mink and muskrats were 1.7 times greater than those reported for terrestrial mammals in this region (Lehrer et al. 2010; Fredebaugh et al. 2011; range 0–35%). Contaminated runoff has been implicated in high *T. gondii* infection rates for some marine mammals (Conrad et al. 2005; Dubey 2010) and likely contributes *T. gondii* oocysts into freshwater watersheds. Rates of *T. gondii* infection in mink in our study were similar to those reported by Smith

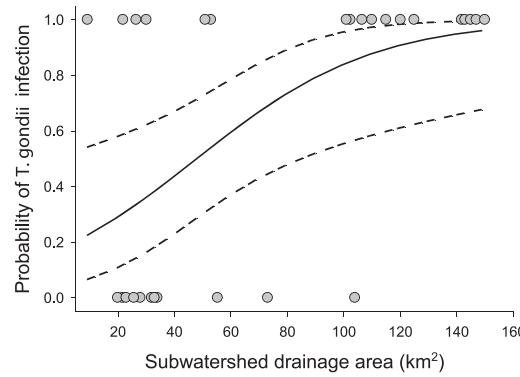

FIGURE 1. Probability of muskrats (*Ondatra zibethicus*) being antibody positive for *Toxoplasma gondii* as a function of subwatershed drainage area. Probabilities (solid line) and 95% confidence intervals (dashed line) are from the top-ranked logistic regression model (see Table 1). Circles indicate individuals within the watershed and if they were antibody positive (1) or negative (0) for *T. gondii* antibodies.

and Frenkel (1995) (66%, $n=29$, 1:8 titer, Sabin-Feldman dye test) and Sepúlveda et al. (2011) (70%, $n=30$, 1:32 titer, latex agglutination test). Infection rates in muskrats were much higher than those reported by Nezval and Literak (1994) (47.3%, $n=146$; 9.1%, $n=110$, 1:4 titer, Sabin-Feldman dye test), Hejlíček et al. (1997) (24%, $n=437$, 1:4 titer, Sabin-Feldman dye test), and Smith and Frenkel (1995) (17%, $n=42$, 1:8 titer, Sabin-Feldman dye test).

As predicted, muskrats captured in larger subwatersheds were more likely to be antibody positive than those in smaller subwatersheds. This effect is likely a result of contaminated runoff from the surrounding landscape increasing risk of exposure to *T. gondii* oocysts in riparian areas. Oocyst concentration in larger subwatersheds may occur if land-derived oocysts attach to organic aggregates (Shapiro et al. 2012) or biofilms found on submergent vegetation (Mazzillo et al. 2013) within the stream. Our inability to detect a similar effect in mink may be a result of interspecific differences in trophic and spatial ecologies or that few mink were negative for *T. gondii* antibodies. Mink are predators of aquatic and terrestrial prey, including muskrats, and use upland habitats more than do muskrats. Mink exposure to *T. gondii* may be enhanced by consuming infected vertebrates (Smith and Frenkel 1995). Thus, exposure to *T. gondii* for mink may be less tightly linked to subwatershed area. Muskrats, however, are mostly herbivorous and rarely leave the stream edge (Ahlers et al. 2010). Their exposure likely occurs through ingestion of oocysts while drinking, grooming, or foraging on submergent vegetation.

The weak difference in *T. gondii* antibody prevalence between adult and subadult muskrats is likely due to adults having longer, life-time exposure. However, a more-robust sample may be required to confirm this effect. Unlike Sepúlveda et al. (2011), we did not detect an age effect on *T. gondii* seropositivity in mink. Few mink in our study were antibody negative, and our sample might not have been large enough to detect an age effect.

Proximity to farmsteads and amount of urbanized land cover within the subwatersheds did not predict antibody prevalence for muskrat or mink. However, because ~91% of our study area is dedicated to urbanization and agricultural production, drainage practices associated with these land-use types are likely transporting *T. gondii* oocysts into freshwater habitats. Although we could not distinguish the relative contribution of oocysts from agricultural and urban areas, both have drainage systems that bypass natural wetlands and riparian zones that historically have impeded transport of *T. gondii* oocysts into watersheds (Shapiro et al. 2010). This study represents an important first step in understanding spatial patterns of water-borne transmission of *T. gondii*.

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