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Prevalence of Antibodies to *Toxoplasma gondii* in Woodchucks across an Urban–rural Gradient

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ABSTRACT: Increasing urbanization has important consequences for wildlife, including the potential for higher prevalence of diseases within "urban adapter" species exposed to spillover from domestic animals. We investigated whether prevalence of antibodies to *Toxoplasma gondii* in woodchucks (*Marmota monax*) was related to urbanization in a Midwestern landscape. We collected serum samples from adult woodchucks captured across an urban–rural gradient in Illinois, USA in May–November 2007. We used an indirect fluorescent antibody test (IFAT) on the serum samples to detect *T. gondii* antibodies. Five of 35 (14.3%) sera from woodchucks had detectable *T. gondii* antibodies. Prevalence was related positively to urbanization. All positive samples were from individuals inhabiting areas in which urban land cover exceeded 70%. Urban woodchucks are likely exposed to high levels of *T. gondii* oocysts in the environment due to habitat overlap with the definitive hosts for the parasite, domestic and feral cats, which reach high densities in urban areas.

Key words: *Marmota monax*, *Toxoplasma gondii*, urbanization, woodchuck.

Urbanization is increasing worldwide with important consequences for biodiversity conservation. Habitat loss due to urbanization often reduces species richness and shifts community composition toward "urban adapter" species (McKinney, 2002). Prevalence of wildlife diseases also can change across urbanization gradients (e.g., Riley et al., 2004) with diseases that affect urban adapters sometimes increasing in urban areas (Bradley and Altizer, 2006). Emerging infectious diseases of wildlife in urban environments have been related to "spillover" transmission dynamics from domestic animals to wildlife populations (Daszak et al., 2000). The impact of these diseases in urban adapter species and their role in the overall transmission cycles of diseases remain unknown for many pathogens.

Toxoplasma gondii is a protozoal parasite of zoonotic importance capable of causing toxoplasmosis in humans, livestock (especially pigs and sheep), and wildlife (Dubey and Jones, 2008). The definitive hosts of the parasite are felids, including wild and domestic cats, which shed *T. gondii* oocysts in feces (Dubey, 2009). Many species of mammals and birds can be infected directly through ingestion of oocyst-contaminated food, water, or soil, or by consumption of infected tissues from intermediate hosts (Dubey and Jones, 2008). Because domestic and feral cats (*Felis catus*) play a key role in the transmission dynamics of *T. gondii* (Sumner and Ackland, 1999; Dubey and Jones, 2008), spillover from cats to wildlife could produce higher prevalence of *T. gondii* infections in wildlife species in urban areas (Riley et al., 2004; Bradley and Altizer, 2006).

Our objective was to conduct a serosurvey for *T. gondii* infection in woodchucks (*Marmota monax*) across an urban–rural gradient in a Midwestern landscape. Woodchucks are herbivores considered to be urban adapters (McKinney, 2002). Woodchucks are susceptible to *T. gondii* infections (Stewart et al., 1995) and toxoplasmosis (Bangari et al., 2007), but prevalence of infection in relation to urbanization has not been evaluated.

Our study area (700 km^2) represented an urbanization gradient centered on Champaign–Urbana (40°6′N, 88°13′W) in east-central Illinois (Watson, 2009). Champaign–Urbana is a medium-sized, growing community located within an

intensive region of row crop agriculture (Mankin and Warner, 1997). The urban end of the gradient is characterized by mostly impervious land cover with high densities of buildings (441 buildings/km^2) and people, whereas the rural end consists of mostly agricultural land cover (corn and soybeans) with few homesteads (five buildings/km^2) and people. The urbanization level for each woodchuck was quantified as the amount of urban land cover within a 500-m buffer centered on the individual's home range (Watson, 2009). Urban land cover included developed areas and urban open space (i.e., parks and lawns) classified from digital orthophotographs.

We live-trapped woodchucks across the urbanization gradient from May to November 2007 using baited Tomahawk traps (Model 207; Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) and following procedures approved by the University of Illinois' Institutional Animal Care and Use Committee. Captured adult woodchucks ($\geq$1 yr old as determined by weight and pelage; Kwiecinski, 1998) were transported to the Veterinary Teaching Hospital at the University of Illinois, anesthetized with medetomidine (0.5 mg/kg), and fitted with an intraperitoneal radio transmitter for a concurrent study of movements and survival (Watson, 2009). Juveniles were released at their capture sites and not sampled for parasites. We collected blood from 35 adult woodchucks. Serum was preserved at −80 C until analyzed. Serologic diagnostic testing was done as part of a broader surveillance of wildlife diseases in the region. We released adult woodchucks at their original capture sites after they had fully recovered from surgeries.

The indirect fluorescent antibody test (IFAT) was used to detect serum antibodies to *T. gondii* (Miller et al., 2001; Dabritz et al., 2007). We prepared IFAT slides with methanol-fixed tachyzoites of the strain ME-49 as antigens. A fluorescein isothiocyanate-conjugated (FITC) antirat IgG (H+L) (Lockhart et al., 1997; Kirkegaard & Perry Laboratories, Gaithersburg, Maryland, USA) diluted at 1:50 in phosphate buffer saline was used as the secondary antibody for woodchuck sera, and FITC antimouse IgG (H+L; Bethyl Laboratories, Inc., Montgomery, Texas, USA) was used on mouse control sera. We used negative and positive control serum from bioassay-known *T. gondii* negative and positive mice (Dubey and Beattie, 1988), and serum from two woodchucks that tested negative and positive. Repeatability and agreement of test results from woodchuck positive and negative control sera with mouse control sera were evaluated. Sera reacting at dilutions $\geq$1:25 were considered seropositive for *T. gondii* antibodies (Stewart et al., 1995).

We detected *T. gondii* antibodies in 5 of 35 (14.3%) woodchucks. There was 100% agreement and repeatability in positive and negative results of control mouse sera with the woodchuck control sera. Overall prevalence of antibodies to *T. gondii* in woodchucks from our study (14.3%) was similar to prevalence estimated for woodchucks in Pennsylvania (9.4%; Stewart et al., 1995). In our study, prevalence of *T. gondii* antibodies did not differ between the sexes (Fisher's exact test; $P=1.00$). Antibodies were found in 2 of 15 (13.3%) males and 3 of 20 (15.0%) females. We used logistic regression to test for an association between seropositivity and percentage of urban land cover. Because of our small sample size, we considered $P\leq0.10$ as significant. Prevalence of *T. gondii* antibodies in woodchucks was related positively to urbanization (likelihood ratio test; $P=0.097$, $\chi^2=2.74$, df=1). The five individuals that were positive occurred in areas in which urban land cover was >70% (Fig. 1).

Urban woodchucks were more likely to have *T. gondii* antibodies than were rural woodchucks. This pattern probably reflected greater densities of domestic and feral cats in urban areas, which shed oocysts into the environment that are subsequently

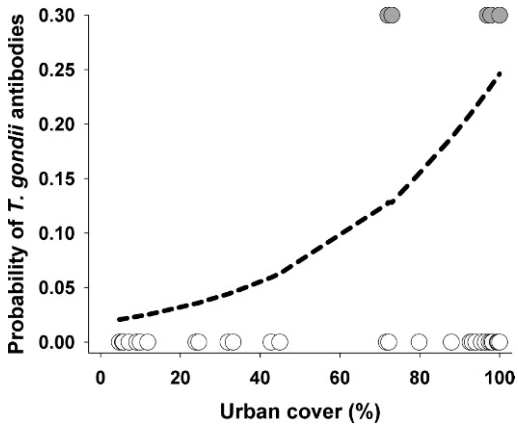

FIGURE 1. Probability that *Toxoplasma gondii* antibodies occurred in woodchucks (*Marmota monax*) across an urbanization gradient in central Illinois, USA. Dashed line is a prediction curve from a logistic regression model. Shaded circles represent antibody-positive woodchucks, and open circles represent antibody-negative woodchucks.

ingested by woodchucks. Foraging and other activities by woodchucks are restricted around their burrows (>90% within 6 m; Ouellet and Ferron, 1988). Cats use woodchuck burrows (Lehrer, pers. obs.), and cats could be attracted to soil disturbances created by woodchucks as potential latrine sites. Hence, woodchuck burrows might function as hotspots for transmission of the parasite. Increased prevalence of *T. gondii* in urban woodchucks also could be related to higher incidence of the parasite in urban cats compared to rural cats, but we do not have such data for our gradient. Prevalence of *T. gondii* in domestic cats did not differ between suburban and rural areas in Melbourne, Australia (Sumner and Ackland, 1999). In Hungary, antibody prevalence of *T. gondii* was lower for urban cats than for rural cats (Hornok et al., 2008) in a region in which rural cats occurred in villages (unlike our study area).

Woodchucks are herbivorous urban adapters that should benefit from abundant food resources and reduced natural predators in urban areas. Countering these benefits could be costs from diseases such as toxoplasmosis that spill over from domestic animals and reduce body condition, cause neurological impairment, and possibly increase predation risk (Berdoy et al., 2000; Bangari et al., 2007). Further studies are needed to document prevalence patterns of *T. gondii* in relation to urbanization for a broader sample of wildlife species, and to develop a better understanding of transmission mechanisms and consequences for biodiversity conservation and human health.

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