

Seroprevalence of *Toxoplasma gondii* in American Black Bears (*Ursus americanus*) of the Central Appalachians, USA

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ABSTRACT: We assessed *Toxoplasma gondii* seroprevalence in 53 free-ranging American black bears (*Ursus americanus*) in the Central Appalachian Mountains, US. Seroprevalence was 62% with no difference between males and females or between juvenile and adult bears. Wildlife agencies should consider warnings in hunter education programs to reduce the chances for human infection from this source.

The endoparasite *Toxoplasma gondii* is an important zoonotic protozoan pathogen that infects humans and many other warm-blooded vertebrates globally. The parasite is transmitted via ingestion of oocysts, from consumption of parasite-infected flesh, and congenitally in some animals (Dubey and Jones 2008). Domestic and wild felids are the definitive hosts and are the only documented taxa whereby sexual reproduction in the parasite occurs. However, asexual reproduction has been documented in many intermediate hosts, which are primarily infected from ingestion of oocysts (Dubey and Jones 2008; Ballash et al. 2015).

American black bears (*Ursus americanus*) are intermediate hosts of *T. gondii* (Bronson et al. 2014). Bear populations have recently expanded in range and numbers (Scheick and McCown 2014), with a corresponding increase in human-bear conflicts (Merkle et al. 2013). The raiding of garbage and consumption of pet foods bring bears into closer contact with human and domestic cat (*Felis domesticus*) sources of *T. gondii* (Bronson et al. 2014). American black bears were reintroduced to parts of the Central Appalachian Mountains, US, during the late 1990s. Currently, two reintroduced black bear populations inhabit the westernmost portion of the region, and comprise approximately 700 bears (Murphy et al. 2015, 2016). Despite several

years of black bear hunting in this region and its importance to human and bear health, prevalence of *T. gondii* in Central Appalachian Mountain bears was unknown. Therefore, we collected blood samples from 53 (26 female and 27 male) free-ranging black bears in these two populations during 2012–13 to investigate seroprevalence of *T. gondii*.

Bears were captured using modified Aldrich spring-activated snares, culvert traps, and via free-range darting and were immobilized using tiletamine-zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) administered at a dosage of 5–7 mg/kg of estimated body mass. We collected a 10-mL blood sample from the femoral artery of each bear and centrifuged samples ≤ 4 h after collection to separate serum and red blood cell fractions. Sera were screened for *T. gondii* antibodies by the Murray State University Breathitt Veterinary Center (Hopkinsville, Kentucky, USA) using a twofold serum dilution from 1:16 to 1:128 with a modified agglutination test kit (Eiken Toxotest MT, Chatsworth, California, USA). Samples with $\geq 1:16$ dilution were considered seropositive for *T. gondii*. We used a Wilcoxon rank-sum test to investigate for a difference in seroprevalence between male and female bears and a Jonckheere test to determine whether a relationship existed between age, based on cementum analysis, and titer. The small sample size of juvenile bears (i.e., ≤ 2 yr old; $n=9$) precluded analysis of titer differences between age classes. Statistical procedures were performed using Program R (R Core Team 2016). All animal handling procedures were approved by the Institutional Animal Care and Use Committee at the University of Kentucky (protocol 00626A2003).

Thirty-three of 53 bears (62%) were seropositive for *T. gondii*, including 17 of 26 females (65%) and 16 of 27 males (63%). Prevalence of antibodies at various dilutions were 1:16 ($n=5$; juvenile female=1, adult female=2, juvenile male=1, adult male=1), 1:32 ($n=8$; juvenile female=1, adult female=2, adult male=5), 1:64 ($n=13$; juvenile female=1, adult female=8, juvenile male=1, adult male=3), and 1:128 ($n=7$; adult female=2, juvenile male=2, adult male=3). We found no titer differences between males and females ($W=372.5$, $P=0.66$), and Jonckheere tests showed no relationship with age ($JT=361$, $P=0.42$).

Toxoplasma gondii has recently become the focus of increasing study regarding its effects on behavior of its hosts, including rodents and humans. Although little is known about its effects on wildlife species that serve as secondary hosts (Dubey and Jones 2008; Webster et al. 2013), *T. gondii* infection can cause bear mortality (Huffman and Roscoe 2014). More than one half of black bears in the two Central Appalachian Mountain populations we studied were seropositive for *T. gondii*, a number that, although lower than elsewhere in the eastern US (Bronson et al. 2014), is much higher than findings from some western US populations (Binninger et al. 1980). The apparent discrepancy between western and eastern black bear populations may be a consequence of higher human population densities in the east and associated exposure to common *T. gondii* sources, including ingestion of cat feces, livestock and wildlife carrion (e.g., white-tailed deer [*Odocoileus virginianus*]; Ballash et al. 2015), or soils contaminated by those materials.

We found no significant differences between male and female titers, which was not surprising given that bears of both sexes commonly engage in conflict behavior near human settlements and are thus likely to incur similar risks of infection from human-associated sources of *T. gondii*. Prior studies of black bear populations in Florida and Maryland, US, also found no sex-based differences (Chambers et al. 2012; Bronson et al. 2014); however, Nutter et al. (1998) found that females had

higher seroprevalence than males, with titers increasing with age. Nonetheless, infection of other wildlife species has been shown to increase in exurban and urban environments, likely because that is where domestic cat densities are highest (Ballash et al. 2015).

Consumption of bear meat may be a source of infection to humans (Tenter et al. 2000), which has been linked to schizophrenia and other behavioral changes (Webster et al. 2013). A survey of black bear hunters in North Carolina, US, suggested that nearly 20% of hunters did not adequately prepare bear meat to kill *T. gondii* oocysts (Nutter et al. 1998). Our findings indicate a relatively high prevalence of this parasite in the two westernmost bear populations of the Central Appalachian Mountains. We strongly encourage wildlife agencies to incorporate warnings about proper bear meat preparation into their hunter education programs to mitigate the chances of human infection from this source.

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