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Seroprevalence of Antibodies to *Toxoplasma gondii* in the Pennsylvania Bobcat (*Lynx rufus rufus*)

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ABSTRACT: From 2000 to 2002 bobcat blood samples were collected, in association with the Pennsylvania Game Commission, during the recently reactivated bobcat hunting and trapping season. Sex, age, and county/township data were recorded for each animal. Blood was tested for antibodies to *Toxoplasma gondii* using the modified agglutination test. In the 2-yr study, 131 bobcat samples were collected in 14 Pennsylvania counties and 109 (83%) of these had antibodies to *T. gondii* (titer \geq 25). A two-way Chi-Square test (95% confidence interval) yielded no significance differences in antibody prevalence between males (83%) and females (88%) or adults (83%) and juveniles (77%). All 14 counties had at least one bobcat with antibodies to *T. gondii*.

Key words: Blood collection strips, bobcat, *Lynx rufus*, modified agglutination test, seroprevalence, *Toxoplasma gondii*.

Toxoplasma gondii is an intracellular protozoan that infects animals and humans. The life cycle of *T. gondii* involves asexual reproduction in intermediate hosts and both asexual and sexual reproduction in the definitive host (Felidae). The infectious product of sexual reproduction, the oocyst, is shed in the feces of infected felids during the acute phase of infection that can persist for 2 wk, during which thousands of oocysts can be produced from a single infected animal (Dubey and Beattie, 1988). Intermediate hosts become infected by consuming the oocyst and/or consuming another infected host. Humans can act as an intermediate host, but infection is rarely fatal unless the person is immunocompromised (Dubey and Beattie, 1988).

Excluding feral cats, the bobcat (*Lynx rufus rufus*) is the only wild felid in Pennsylvania. Therefore, the bobcat is

a potentially important component for the propagation and perpetuation of *T. gondii* in Pennsylvania's sylvatic cycle and, consequently, a good indicator of the prevalence of *T. gondii* in the wild. For these reasons, we evaluated the prevalence of antibodies to *T. gondii* in bobcats.

Pennsylvania Game Commission (PGC) personnel collected blood samples from bobcats harvested during 2000–2001 and 2001–2002. Harvesting was restricted to Furbearer Management Zones 2 and 3, which included 20 counties in north-central and northeastern Pennsylvania. The PGC also collected blood samples from road-killed bobcats. Nobuto blood collecting strips Type I (Toyo Roshi Kaisha, Ltd; distributed by Advantec MFS, Inc., Dublin, California, USA) were mailed to field personnel along with an information packet, which included the reasons for conducting the study and directions for properly acquiring, drying, and mailing the Nobuto strips back to the Indiana University of Pennsylvania (IUP) for further processing and analysis.

Each bobcat was examined within 4 days after death by the PGC. Blood was taken immediately by using Nobuto strips if the carcass was fresh. Bobcats were frozen if they could not be examined immediately, then later thawed and blood samples taken. Sex, age (cementum-annulus method of age determination), location, and tag number were recorded for all animals. The strips were refrigerated until they could be further processed.

Nobuto strips were cut into three or four segments at IUP and placed into a 1.5 ml centrifuge tube. One milliliter of

phosphate buffered saline (pH 7.4; Sigma Diagnostics, St. Louis, Missouri, USA) was added to each tube, diluting the blood 1:25. After 2 hr, the supernatant was removed, centrifuged, and stored at 4 C until tested for antibodies to *T. gondii* using the modified agglutination test (MAT) as described (Dubey and Desmonts, 1987). Antibody testing was performed at the Animal Parasitic Diseases Laboratory, US Department of Agriculture, Beltsville, Maryland, USA. Blood samples were further diluted to provide 1:50 and 1:500 dilutions, and a 1:25 dilution was used as the positive threshold dilution as described (Dubey and Beattie, 1988). A two-way Chi-Square test with a 95% confidence interval was used to compare antibody prevalence differences in sex and age groups. A *P* value ≤ 0.05 was considered significant.

One hundred thirty-one blood samples were collected in two consecutive years, 2000–2001 and 2001–2002, encompassing two harvesting seasons and 109 (83%) of these samples had antibodies to *T. gondii* at a titer ≥ 25 . Animals with antibodies to *T. gondii* were 83% (52/63) males, 88% (53/60) females, 88% (81/92) adults, and 77% (23/30) juveniles, 50% (4/8) unknown sex, and 56% (5/9) unknown age. Although the prevalence of antibodies was higher in females than males and higher in adults than juveniles, these differences were not statistically significant. All 14 counties from which bobcats were sampled had at least one animal with antibodies to *T. gondii* (Table 1).

This is the highest prevalence of antibodies to *T. gondii*, with a sample size of this magnitude, reported for *Lynx rufus*. Four previous studies with >50 bobcat blood samples used serum from whole blood and indirect hemagglutination test (IHA), while this study used blood reconstituted from Nobuto blood collecting strips and MAT. They also used different positive dilution thresholds than this study. Based on Dubey and Beattie (1988), a 1:25 dilution was chosen as the

TABLE 1. Prevalence of antibodies to *Toxoplasma gondii* in *Lynx rufus rufus* in Pennsylvania by county.

| County | No. of samples | Percent positive ($\geq 1:25$) | No. with titers of: | | |
|------------|----------------|----------------------------------|---------------------|----|------------|
| | | | 25 | 50 | ≥ 500 |
| Bradford | 10 | 90 | 0 | 6 | 3 |
| Cameron | 4 | 50 | 0 | 1 | 1 |
| Centre | 1 | 100 | 0 | 1 | 0 |
| Clearfield | 10 | 90 | 0 | 7 | 2 |
| Clinton | 10 | 80 | 0 | 8 | 0 |
| Columbia | 1 | 100 | 0 | 0 | 1 |
| Elk | 7 | 86 | 0 | 6 | 0 |
| Forest | 1 | 100 | 0 | 1 | 0 |
| Luzerne | 3 | 67 | 1 | 1 | 0 |
| Lycoming | 19 | 84 | 2 | 8 | 6 |
| Mckean | 8 | 88 | 0 | 7 | 0 |
| Potter | 20 | 85 | 2 | 13 | 2 |
| Sullivan | 9 | 100 | 2 | 5 | 2 |
| Tioga | 17 | 82 | 0 | 11 | 3 |
| Unknown | 11 | 64 | 1 | 4 | 2 |
| Total | 131 | 83 | 8 | 79 | 22 |

positive dilution threshold for this study, whereas Oertley and Walls (1980) used a positive threshold of 1:16 and Franti et al. (1976), Riemann et al. (1978), and Kikuchi et al. (2004) used 1:64. Because dilutions of 1:25, 1:50, and 1:500 were used in this study, there can be no direct comparison with their data. However, comparing similar dilutions, the number of bobcats with antibodies to *T. gondii* at 1:25 (83%) is four times that reported by Oertley and Walls (1980) at 1:16 (18%). If the number of animals positive for antibodies to *T. gondii* at the 1:50 dilution (77%) is compared with animals positive at 1:64 in the studies of Franti et al. (1976; 69%), Riemann et al. (1978; 61%), and Kikuchi et al. (2004; 50%), the prevalence in this study is still higher. These differences could result from sensitivity of MAT compared with IHA or discrepancies in titer comparisons, or they could simply indicate different levels of *T. gondii* infection from different geographical locations (Dubey and Beattie, 1988). Although other studies have been conducted, a meaningful comparison would be difficult because of their smaller sample size (Walton and Walls, 1964; Franti et al.,

TABLE 2. Number of harvested deer (*Odocoileus virginianus*) and bear (*Ursus americanus*) per sampled county in 2001 and the number of theoretically *T. gondii* infected gut piles.

| County | No. harvested deer ^a | No. harvested bear ^a | No. of theoretically infected | | Total theoretically infected gut piles |
|------------|---------------------------------|---------------------------------|-------------------------------|---------------------------|--|
| | | | Deer (60%) ^b | Bear (79.8%) ^c | |
| Bradford | 11,467 | 47 | 6,880 | 38 | 6,919 |
| Cameron | 1,183 | 126 | 710 | 101 | 811 |
| Centre | 6,566 | 151 | 3,940 | 120 | 4,061 |
| Clearfield | 8,781 | 130 | 5,269 | 104 | 5,373 |
| Clinton | 2,494 | 267 | 1,496 | 213 | 1,711 |
| Columbia | 5,343 | 36 | 3,206 | 29 | 3,235 |
| Elk | 2,861 | 109 | 1,717 | 87 | 1,805 |
| Forest | 4,695 | 81 | 2,817 | 65 | 2,882 |
| Luzerne | 5,671 | 95 | 3,403 | 76 | 3,479 |
| Lycoming | 6,040 | 242 | 3,624 | 193 | 3,818 |
| Mckean | 6,859 | 107 | 4,115 | 85 | 4,202 |
| Potter | 6,567 | 117 | 3,940 | 93 | 3,735 |
| Sullivan | 2,215 | 55 | 1,329 | 44 | 1,373 |
| Tioga | 7,328 | 133 | 4,397 | 106 | 4,504 |
| Total | 78,070 | 1,696 | 46,843 | 1,354 | 48,208 |

^a Numbers of harvested deer/bear are from Pennsylvania Game Commission.

^b Percent seroprevalence to *Toxoplasma gondii* in deer (*Odocoileus virginianus*) is from Humphreys et al. (1995).

^c Percent seroprevalence to *Toxoplasma gondii* in bear (*Ursus americanus*) is from Briscoe et al. (1993).

1975; Riemann et al., 1975; Marchiondo et al., 1976; Burrige et al., 1979; Dubey et al., 1987; Heidt et al., 1988; Labelle et al., 2001; Dubey et al., 2004; Riley et al., 2004).

Higher prevalence of antibodies to *T. gondii* in adults than juveniles is similar to other studies (Riemann et al., 1978; Labelle et al., 2001), with the exception of the study Oertly and Walls (1980). Differences such as decreases in the rate of vertical transmission and/or environmental prevalence of the parasite may account for the incongruity between this study and that of Oertly and Walls (1980). There was no statistical difference in prevalence of antibodies to *T. gondii* between males and females, which is in accordance with previous studies (Riemann et al., 1978; Oertly and Walls, 1980; Labelle et al., 2001).

The high prevalence of antibodies to *T. gondii* in the Pennsylvania bobcat is probably a reflection of the abundant source of infected intermediate hosts. With a seroprevalence of 60% and 79.8%, respectively, white-tailed deer,

and to a lesser extent bear, may represent a major reservoir of infection (Briscoe et al., 1993; Humphreys et al., 1995; McLean et al., 2005). In part, this may be due to the copious supply of potentially infected carrion (gut piles) left after harvest (Table 2). Raccoons (48.3%) may represent yet another possible source of infection (Dubey et al., 1992). Further research is needed to determine whether other reservoirs, such as lagomorphs and/or rodents, exist in Pennsylvania.

It can be inferred that in Pennsylvania the bobcat plays a major role in the dissemination of *T. gondii* impacting other wildlife and subsequently humans. The ramifications of *T. gondii* infection (or sequelae to infection) on the Pennsylvania bobcat population are not well understood.

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