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USE OF A POINT OF CARE TEST TO DETERMINE THE PREVALENCE OF ANTIBODIES TO *TOXOPLASMA GONDII* IN BLACK BEARS FROM NORTH CAROLINA AND PENNSYLVANIA

Jennifer Strules[1], Tania Dawant[2], Katie Riese[2], Richard Gerhold[2], Justin Brown[3], Colleen Olfenbuttel[4], Christopher S. DePerno[1], Brian J. Hunt[5], and Alexa Rosypal von Dohlen[6]

[1] Department of Forestry and Environmental Resources, Fisheries, Wildlife, and Conservation Biology, North Carolina State University, Raleigh, North Carolina 27606.

[2] Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, Tennessee 37996.

[3] Department of Veterinary and Biomedical Sciences, Pennsylvania State University, University Park, Pennsylvania 16802.

[4] North Carolina Wildlife Resources Commission, Raleigh, North Carolina 27699.

[5] Department of Computer Sciences, Engineering and Mathematics, College of Science, Technology, Engineering and Mathematics, Johnson C. Smith University, Charlotte, North Carolina 28216.

[6] Department of Natural and Behavioral Sciences, College of Science, Technology, Engineering and Mathematics, Johnson C. Smith University, Charlotte, North Carolina 28216.

Correspondence should be sent to Dr. Alexa Rosypal von Dohlen at: *acrosypal@jcsu.edu*

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ABSTRACT

Toxoplasma gondii is an important protozoan parasite of humans and animals throughout the world. Black bears are among the animals with the highest seroprevalence of *T. gondii* in the United States. A rapid point of care (POC) test is commercially available to detect antibodies to *T. gondii* in humans. We evaluated the utility of the POC test to detect anti–*T. gondii* antibodies in 100 wild black bears from North Carolina (n = 50) and Pennsylvania (n = 50). In a blind study, sera were tested by the POC test, and results were compared to the modified agglutination test (MAT). Overall, anti–*T. gondii* antibodies were detected in 76% (76/100) black bears by both MAT and POC tests. One false positive and one false negative result in the POC test were obtained in bears from Pennsylvania. The sensitivity and specificity of the POC test were both 99% when compared to the MAT. Results from our study indicate the POC test could be a useful screening tool for serological surveillance of *T. gondii* in black bears.

Toxoplasma gondii is a zoonotic protozoan parasite with worldwide distribution. Wild and domestic felid species are the definitive hosts, and they excrete oocysts in their feces. Virtually all warm-blooded animals are intermediate hosts of *T. gondii*, and infections in humans occur primarily by ingesting oocysts or tissue cysts in infected meat, or vertically through the placenta (Dubey, 2010). Humans infected with *T. gondii* are typically asymptomatic, but severe toxoplasmosis can occur in immunosuppressed individuals and congenitally infected children (Almeria and Dubey, 2021). In animal hosts, *T. gondii* infections are usually subclinical (Dubey, 2010). *Toxoplasma gondii* exposure has been studied extensively in wildlife, and antibody levels in black bears (*Ursus americanus*) are among the highest in wild animal species (Dubey et al., 2021). Most serosurveys in black bears have been from Pennsylvania with an overall exposure rate of approximately 80% (Dubey et al., 2021). A study from North Carolina detected antibodies to *T. gondii* in 120 of 143 (84%) black bears (Nutter et al., 1998).

Multiple serologic assays have been used for *T. gondii* in black bears (reviewed by Dubey et al., 2021), but the modified agglutination test (MAT) is considered the most specific and efficient test in bears (Dubey et al., 2021). Point of care (POC) tests for *T. gondii* are immunochromatographic (ICT) antibody tests that are inexpensive and yield rapid results without expensive equipment or personnel training (Gomez et al., 2018). The POC tests are commercially available to detect antibodies to *T. gondii* in humans but have not been used in black bears. The purpose of the present study was to evaluate the ability of POC tests to detect antibodies to *T. gondii* in black bears from North Carolina and Pennsylvania when compared to the MAT.

For the present study, 100 wild black bears were sampled from North Carolina (n = 50) and Pennsylvania (n = 50). The age of black bears in this study was determined based on dentition and tooth histology of an extracted premolar tooth collected from each bear by Matson's Laboratory in Manhattan, Montana (Willey, 1974) and was categorized as juvenile (<2 yr old), or adult (≥2 yr old). The sex of black bears was determined based on external genitalia. All blood samples, in both Pennsylvania and North Carolina, were collected through venipuncture of the femoral vein while bears were anesthetized. Serum was separated

Table I. Sensitivity and specificity of the *Toxoplasma gondii* point of care (POC) test compared to the *T. gondii* modified agglutination test (MAT) to detect antibodies in black bears (*Ursus americanus*) from North Carolina and Pennsylvania.*

Category	No. of bears	No. of positive/no. tested by POC	Percent positive by POC	No. of positive/no. tested by MAT	Percent positive by MAT
Total	100	76/100	76	76/100	76
Sex					
Male	40	29/40	73	28/40	70
Female	60	47/60	78	48/60	80
North Carolina	50	33/17	66	33/17	66
Sex					
Male	19	13/19	68	13/19	68
Female	31	20/31	65	20/31	65
Age					
Juvenile	33	19/33	58	19/33	58
Adult	17	14/17	82	14/17	82
Pennsylvania†	50	43/7	86	43/7	86
Sex					
Male	21	16/21	76	15/21	71
Female	29	27/29	93	28/29	97

* The POC test was 99% sensitive and 99% specific in these experiments compared to MAT as gold standard for comparison.
† All black bears from Pennsylvania were adults.

by centrifugation and stored frozen at –20 C until further analysis. The time from sample collection until storage at –20 C was <2 days, and during that time, the samples were stored at 4 C. All samples were obtained legally and with appropriate state permits. Handling of bears was approved by the Institutional Animal Care and Use Committee at North Carolina State University (16-263-O and 19-723-O) and was consistent with the guidelines of the American Society of Mammalogists (Sikes et al., 2016).

Black bears in North Carolina were sampled from April 2019 to August 2021 in the city of Asheville (Buncombe County) using culvert-style traps on private properties and anesthetized. Black bears in North Carolina included 31 females and 19 males and 33 juveniles and 17 adults. Black bears in Pennsylvania were sampled from March 2017 to July 2018. Samples were collected from bears in multiple counties (Bradford, Cambria, Cameron, Centre, Clinton, Huntingdon, Mifflin, Monroe, Pike, Potter, Snyder, Somerset, Sullivan, Tioga, Union, Westmoreland, and Wyoming) and through various mechanisms, including summer research trapping (n = 31), winter den visits of collared sows (n = 17), a single bear euthanized for severe sarcoptic mange (n = 1), and a nuisance trap and relocation bear (n = 1). All black bears sampled in Pennsylvania were adults (≥2 yr old) and consisted of 29 females and 21 males.

Black bear sera were simultaneously tested for antibodies to *T. gondii* using a qualitative POC test and a quantitative MAT. The POC test used in this study (Toxoplasma ICT IgG-IgM®, LDBIO Diagnostic, Lyon, France) is a lateral flow ICT assay that is commercially available to simultaneously detect *Toxoplasma*-specific IgG and IgM antibodies in humans (Khan and Noordin, 2020). Serology was performed using POC tests according to the manufacturer's directions. Briefly, black bear serum (15 µl) was dispensed onto the sample well of the cassette and 4 drops of manufacturer-provided eluent were pipetted into the well. Test results were read in 20–30 min. According to the test procedure, after 20–30 min, the appearance of a blue control line and a second black line in the *Toxoplasma* test region was considered positive. A single blue line in the control region was interpreted as negative. Quantitative antibody testing was performed with the MAT using methods described by Chambers et al. (2012). Bear sera were screened for *T. gondii* antibodies using 2-fold serial dilutions from 1:25 to 1:3,200. Serum samples with titers less than 1:25 were considered negative, and a titer of 1:25 or higher was considered positive for *T. gondii* by MAT. The sensitivity and specificity of the POC tests were calculated with standard formulas using the MAT as the reference test for comparison (Rosypal et al., 2014).

The data were analyzed in contingency tables to identify any associations between the seroprevalence of *T. gondii* and a bear's state of origin, the seroprevalence of *T. gondii* and a bear's gender in North Carolina and Pennsylvania, and the seroprevalence of *T. gondii* and a bear's age in North Carolina. The Pennsylvania bears were all adults so the age analysis was not possible. The chi-square test of independence was used when all expected cell counts in the contingency table were at least five, and Fisher's exact test was used if at least one expected cell count in the contingency table was less than 5. The sample proportion was very close to one, so a 95% Wilson confidence interval was constructed to estimate the proportion of POC and MAT tests that match. All analyses were performed using IBM SPSS Statistics, Version 28 (IBM Corp., 2021).

Antibodies to *T. gondii* were detected by POC tests and MAT in 76 (76%) of the 100 black bears from North Carolina and Pennsylvania with one false positive and one false negative result in the POC test (Table I). The POC and MAT test results matched in 98 (98%) of 100 samples, and the 95% Wilson confidence interval indicated that the proportion of tests that match is captured between 93% and 99.45%. Seventy-six (76%) of the 100 bear samples were positive by POC tests, including 33 (66%) of 50 black bears from North Carolina and 43 (86%) of 50 black bears

from Pennsylvania. Positive MAT results were detected in 76 (76%) of 100 bear samples with titers ranging from 1:100 to >1:3,200. Thirty-three (66%) of 50 black bears from North Carolina and 43 (86%) of 50 black bears from Pennsylvania had detectable antibodies by MAT. The chi-square test of independence yielded $\chi^2 = 5.482$ ($P = 0.019$) indicating that there was sufficient evidence at the 0.05 significance level of an association between seroprevalence of *T. gondii* and a bear's state of origin.

The overall seroprevalence in male bears was 70% (28/40) and 80% (48/60) in females. In North Carolina bears, antibodies were detected in 64.5% (20/31) of females and 68.4% (13/19) of males. The chi-square test of independence yielded $\chi^2 = 0.080$ ($P = 0.777$) indicating that there is not sufficient evidence of an association at the 0.05 significance level between the seroprevalence of *T. gondii* and a bear's gender in North Carolina. Among Pennsylvania bears, antibodies were detectable in 96.6% (28/29) of females and 71.4% (15/21) of males. Fisher's exact test yielded $P = 0.033$, indicating that there was sufficient evidence at the 0.05 significance level of an association between seroprevalence of *T. gondii* and a bear's gender in Pennsylvania.

Antibodies to *T. gondii* were detected in 82.4% (14/17) of adult and 57.6% (19/33) of subadult bears from North Carolina. The chi-square test of independence yielded $\chi^2 = 3.070$ ($P = 0.080$) indicating that there was not sufficient evidence of an association at the 0.05 significance level between the seroprevalence of *T. gondii* and a bear's age in North Carolina.

A single false positive POC test was obtained in a black bear from Pennsylvania that was negative by MAT. The false positive was possibly due to the detection of IgM antibodies by the POC that were not detectable by the IgG-specific MAT. One false negative result occurred in a bear from Pennsylvania that had a titer of 1:1,600 by MAT. The false negative test may have been the result of the prozone effect. This phenomenon occurs when an excess accumulation of antigens or antibodies leads to false negative reactions in rapid diagnostic tests (Gillet et al., 2009). The sensitivity and specificity of the POC test were both 99%.

Toxoplasmosis is an important disease of humans and animals with worldwide distribution. Infected humans are typically asymptomatic, but immunosuppressed individuals and congenitally infected children can suffer severe disease. Infections in black bears are typically subclinical, and, unlike in humans, transplacental transmission is not known to occur (Dubey et al., 2016). Many studies have investigated the seroprevalence of *T. gondii* in black bears and showed that they have consistently high antibody levels (reviewed by Dubey et al., 2021). In Pennsylvania, antibodies to *T. gondii* were detected in 80% (535/665) of hunter-killed black bears collected between 1989 and 1992 (Briscoe et al., 1993) and in 87.6% (206/235) of adult black bears trapped between 2015 and 2016 (Dubey et al., 2016). A study from North Carolina detected antibodies to *T. gondii* in 84% (120/143) of hunter-harvested black bears (Nutter et al., 1998). Studies of free-ranging black bears found a seroprevalence of 85.4% (70/82) in Maryland (Bronson et al., 2014), 74% (52/70) in Tennessee (Ammar et al., 2020), and 44.8% (13/29) in Florida (Chambers et al., 2012). All of these previous studies used the MAT, which is the preferred test to detect antibodies to *T. gondii* in black bears (Dubey et al., 2021). The high seroprevalence in the present study is similar to previous reports from the United States, including in North Carolina and Pennsylvania.

The POC test used in this research is commercially available to detect antibodies to *T. gondii* in humans, but it has not been validated using black bear serum. The POC tests are qualitative and simultaneously detect IgG and IgM anti-*Toxoplasma* antibodies. We compared POC results to MAT, and we determined 99% sensitivity and specificity compared to the reference test. To our knowledge, this is the first serological study using the *Toxoplasma* POC test in black bears. The POC tests are simple to use, give rapid results in minutes, and are easier to perform than MAT. Results from this study indicate these tests deserve further study to validate their value as a serological screening tool in black bears.

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