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RAPID POINT-OF-CARE TESTING FOR DETECTION OF ANTIBODIES TO *TOXOPLASMA GONDII* IN BLACK VULTURES AND RING-BILLED GULLS FROM PENNSYLVANIA

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ABSTRACT

Toxoplasma gondii is a zoonotic protozoan parasite that infects most warm-blooded animals, including birds. Scavenging birds are epidemiologically important hosts because they can serve as indicators of environmental *T. gondii* levels. A rapid point-of-care (POC) test that detects antibodies to *T. gondii* in humans is commercially available. In this research, we assessed the ability of the human POC test to detect anti–*T. gondii* antibodies in 106 black vultures (*Coragyps atratus*) and 23 ring-billed gulls (*Larus delawarensis*) from Pennsylvania, USA. Serum samples were tested with the POC test and compared to the modified agglutination test (MAT) in a blinded study. Overall, anti–*T. gondii* antibodies were detected in 2.8% (3/106) of black vultures and 60.9% (14/23) of ring-billed gulls by the POC test. One false-positive POC test occurred in a black vulture that was negative by MAT. False-negative results were obtained in 2 black vultures and 4 ring-billed gulls that had MAT titers of 1:25 or 1:50. The sensitivity and specificity of the POC for both black vultures and ring-billed gulls combined were 95.7% and 95.5%, respectively. This is the first study using human POC tests to detect antibodies to *T. gondii* in birds. Further study of the rapid test as a screening tool for serological surveillance of *T. gondii* in birds is warranted.

Toxoplasma gondii is a protozoan parasite that infects animals including humans around the world. Cats, both wild and domestic species, are the only definitive hosts and excrete oocysts in their feces that contaminate the environment. Nearly all warm-blooded animals, including birds, can be intermediate hosts of *T. gondii* and become infected by ingesting oocysts or by consuming infected animal tissues (Dubey, 2010). In either case, *T. gondii* develops into tissue cysts in the avian host. Consumption of infected small mammals and birds is the most important source of infection for domestic cats (Dubey, 2010). Humans also can acquire *T. gondii* infection by eating undercooked birds (Dubey, 2002), but poultry (e.g., chickens or turkeys) are usually thoroughly cooked, thereby reducing the risk of transmission (Almeria and Dubey, 2021). In humans, most *T. gondii* infections are asymptomatic, but immunosuppressed patients and congenitally infected children can develop severe clinical toxoplasmosis (Almeria and Dubey, 2021).

Birds are susceptible to *T. gondii* infections, with fatal toxoplasmosis reported in many avian species (Dubey, 2002). Birds are considered useful biological sentinels for the presence of environmental *T. gondii* because they ingest oocysts contaminating both the ground and water, and they consume infected prey animals (Dubey et al., 2021). Scavenging birds, such as vultures and gulls, routinely feed on refuse and carrion, which may play an epidemiological role in *T. gondii* infections. Raptors, such as black vultures (*Coragyps atratus*), scavenge potentially infected mammal and bird carcasses (Love et al., 2016). Ring-billed gulls (*Larus delawarensis*) are opportunistic scavengers that may be exposed by consuming oocysts in sewage and runoff water, ingesting intermediate hosts, or scavenging refuse near humans. A previous study from the eastern United States indicated that the *T. gondii* seroprevalence ranged from 3.8% to 75% among carnivorous birds using the modified agglutination test (MAT; Ammar et al., 2021).

Many serological tests have been used to detect antibodies to *T. gondii*, and the MAT has been used extensively in studies of toxoplasmosis in animals (Dubey et al., 2010). Not all serological tests used in mammals, however, give accurate results when using

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 vultures (*Coragyps atratus*) and ring-billed gulls (*Larus delawarensis)* from Pennsylvania.*

Category	Number of birds	No. of positive/ no. tested by POC	Percent positive no. tested by POC	No. of positive/ no. tested by MAT	Percent positive by MAT
Total	129	17/129	13.2	22/129	17.1
Black vultures	106	3/106	2.8	4/10	63.8
Sex					
Male	60	3/60	5	4/60	6.7
Female	46	0/46	0	0/60	0
Ring-billed gulls	23	14/46	60.9	18/23	78.3
Sex					
Male	11	9/11	81.8	11/11	100
Female	7	4/7	57.1	6/7	85.7
Undetermined	5	1/5	20	1/5	20

* The POC test was 95.7% sensitive and 95.5% specific overall in these experiments compared to MAT as gold standard for comparison.

avian serum samples (Dubey, 2002). Among all of the serological tests available to detect antibodies to *T. gondii*, the MAT is considered the best choice for all bird species (Dubey, 2002).

Immunochromatographic tests (ICT) are an alternative serological assay. Point of care (POC) tests are low-cost, rapid ICTs that yield results in minutes without the need for expensive laboratory infrastructure or training (Gomez et al., 2018). A POC test that detects *Toxoplasma*-specific IgG and IgM antibodies in humans is commercially available, but it has not been used previously in birds. Of note, birds have IgY, which is the avian homolog of mammalian IgG. In this study, we aimed to use a human POC test to detect antibodies to *T. gondii* in black vultures and ring-billed gulls from Pennsylvania and to add to the knowledge on exposure among scavenging birds.

For the present study, serum was collected from 106 black vultures (46 females; 60 males) and 23 ring-billed gulls (7 females; 11 males; 5 undetermined) from Pennsylvania. Black vulture samples were collected from June to November 2018 from Dauphin, Lancaster, and Philadelphia counties. Ring-billed gull samples were collected in July 2018 from Erie County. All birds included in this study were captured and dispatched, via gunshot, carbon dioxide, or cervical dislocation as per AVMA and USDA Wildlife Services Directive, as part of ongoing wildlife damage management activities by the USDA under an approved U.S. Fish and Wildlife Services permit (MBPER0015312). Blood samples were collected immediately after death was confirmed. Blood samples were centrifuged and serum was stored at −20 C until serologic testing was performed. The sex of birds was determined based on the identification of internal reproductive organs (testis or ovaries) at necropsy.

Black vulture and ring-billed gull serum samples were tested for antibodies to *T. gondii* using both a qualitative POC test and a quantitative MAT. The POC assay used in this study (Toxoplasma ICT IgG-IgM®; LDBiodiagnostics, Lyon, France) is an ICT test commercially available for humans and it detects *Toxoplasma*-specific IgG and IgM antibodies (Khan and Noordin, 2020). The POC tests were run according to the manufacturer's directions. Black vulture or ring-billed gull serum (15 µl) was aliquoted into the sample well of the ICT followed by 4 drops of eluent provided in the test kit. After 20–30 min, the test results were recorded. According to the test manual, after 20–30 min, a blue control line and another black line in the *Toxoplasma* test region indicated a positive result. A single blue line in the control region only was considered a negative test. The quantitative MAT test to detect antibodies to *T. gondii* was simultaneously performed using previously described methods (Desmonts and Remington, 1980). Bird sera were tested using 2-fold serial dilutions from 1:25 to 1:3,200. Serum samples with a MAT titer of 1:25 or higher were considered positive and a titer less than 1:25 was considered negative for *T. gondii*. To determine the accuracy of the POC test, sensitivity and specificity were calculated using standard formulas with the MAT as the reference test for comparison (Rosypal et al., 2014).

Data were analyzed in contingency tables to identify any associations between the seroprevalence of *T. gondii* and a gull's sex, the seroprevalence of *T. gondii* and a vulture's sex, and the seroprevalence of *T. gondii* in gulls and vultures. The chi-square test of independence was used when all expected cell counts in the contingency table were at least 5 and Fisher's exact test was used if at least 1 expected cell count in the contingency table was less than 5. The sample proportion of matching test results for vultures was very close to 1, and the sample size for gulls was small, so a 95% Wilson confidence interval was constructed to estimate the proportion of POC and MAT tests that matched for each species. All analyses were performed using IBM SPSS Statistics, Version 28).

Antibodies to *T. gondii* were detected by POC tests in 3 of 106 black vultures (2.8%) and in 14 of 23 (60.9%) ring-billed gulls from Pennsylvania (Table I). Positive MAT results were obtained in 4 of 106 (3.8%) black vultures and 18 of 23 (78.3%) ring-billed gulls. Fisher's exact test yielded $P < 0.001$, indicating that there was sufficient evidence at the 0.05 significance level of an association between seroprevalence of *T. gondii* in gulls and vultures. One false-positive result was obtained in a black vulture with an MAT titer of <1:25 and 2 false-negative POC tests occurred in black vultures with MAT titers of 1:50 and 1:25. All black vultures with antibodies to *T. gondii* by either the POC (3/60, 5.0%) or the MAT (4/60, 6.7%) were male. Four false-negative POC results were obtained in ring-billed gulls with MAT titers of 1:25 and 1:50. Ring-billed gulls with positive antibodies to *T. gondii* included 4 females by POC (4/7, 57.1%) and 6 females by MAT (6/7, 85.7%). Ring-billed gulls were positive in 9 males by POC (9/11, 81.8%) and in 11 males by MAT (11/11, 100%). One gull of unknown sex (1/5, 20.0%) was positive by both POC and MAT.

A single false-positive POC test was obtained in a black vulture that was negative by MAT. The false positive may have been caused by the detection of IgM antibodies by the POC that were not detectable by the IgG-specific MAT. False-negative results occurred in 2 black vultures and 4 ring-billed gulls. All birds with false-negative POC results had MAT titers of 1:25 or 1:50. The

overall sensitivity and specificity of the POC for both black vultures and ring-billed gulls combined were 95.7% and 95.5%, respectively. In black vultures only, the POC was 80% sensitive and 98.1% specific. The sensitivity was 100% and the specificity was 55.6% in ring-billed gulls.

The proportion of results in the sample of vultures where the MAT and POC tests matched was approximately 0.9717 (103/106 with 2 false negatives and 1 false positive). The 95% Wilson confidence interval indicated that the proportion of all MAT and POC tests that match was captured between 0.9201 and 0.9903. The seroprevalence of *T. gondii* in female vultures was 0 (0/46) and the seroprevalence of *T. gondii* in male vultures was approximately 0.0667 (4/60). Fisher's exact test yielded $P = 0.131$, indicating that there was not sufficient evidence at the 0.05 significance level of an association between seroprevalence of *T. gondii* and a vulture's sex.

The proportion of results in the sample of gulls where the MAT and POC tests matched was approximately 0.8261 (19/23 with 4 false negatives). The 95% Wilson confidence interval indicated that the proportion of all MAT and POC tests that match was captured between 0.6286 and 0.9302. The margin of error was larger than desired because the sample size of gulls was small; however, the confidence interval indicated that the test results matched more than half of the time, implying a positive association. The seroprevalence of *T. gondii* in female gulls was approximately 0.857 (6/7) and the seroprevalence of *T. gondii* in male gulls was 1.00 (11/11). Fisher's exact test yielded $P = 0.389$, indicating that there was not sufficient evidence at the 0.05 significance level of an association between seroprevalence of *T. gondii* and a gull's sex.

Toxoplasma gondii infections commonly occur in animals, including humans, worldwide. Toxoplasmosis in humans is usually asymptomatic, but congenital infections in children and infections in immunocompromised hosts can lead to severe disease. Wild birds play an important role in the epidemiology of *T. gondii*. Raptors feed on potentially infected prey and some avian species can serve as sentinels of oocyst contamination on the ground and in water (Love et al., 2016; Gamble et al., 2019; Dubey et al., 2021). The seroprevalence of *T. gondii* has been investigated in wild bird species and these studies primarily used the MAT (Dubey, 2002; Dubey et al., 2021).

Raptors are typically considered resistant to overt disease associated with toxoplasmosis, although serological surveys indicate they are frequently exposed to *T. gondii* (Dubey, 2002; Dubey et al., 2021). Previous studies using the MAT showed that 1 of 8 (12.5%) of black vultures from the southeastern United States had antibodies to *T. gondii* (Love et al., 2016) and 4 of 104 (3.8%) black vultures from Pennsylvania were seropositive (Ammar et al., 2021). Black vultures are carnivorous scavengers that feed on dead animals. Previous research has suggested that vultures' acidic stomachs and intestinal microbiota protect them from pathogens in carrion and may also defend against *Toxoplasma* infections (Waite and Taylor, 2015; Ammar et al., 2021). The low seroprevalence among black vultures in the present study is similar to previous reports from the United States (Love et al., 2016; Ammar et al., 2021).

Gulls are opportunistic scavengers that live close to humans. Gulls may be exposed to *T. gondii* by ingesting oocysts in water, food, and sewage or by consumption of intermediate hosts (Cabezon et al., 2016; Gamble et al., 2019). A study of seagull chicks in Spain indicated that 21% of chicks were seropositive by MAT (Cabezon et al., 2016). Burridge et al. (1979) found that 2 of 13 (15.3%) ring-billed gulls from Florida had antibodies to *T. gondii* by the indirect hemagglutination test. The 60.8% seroprevalence detected in ring-billed gulls in this work is higher than in previous reports. In addition, vertical transmission of *T. gondii* occurs in mammals and can lead to spontaneous abortion and congenital toxoplasmosis. A study of yellow-legged gulls in the Mediterranean region suggested that vertical transmission may also be possible in wild birds (Gamble et al., 2019). The anthropogenic habitat and feeding behavior of gulls make them useful for monitoring environmental and public health risks to humans.

The rapid, point-of-care test used in the present study is sold commercially to detect *Toxoplasma*-specific IgG and IgM antibodies simultaneously in humans, but it has not been used previously in birds. The POC results were compared to MAT data revealing that the overall sensitivity and specificity for the POC for both black vultures and ring-billed gulls combined were 95.7% and 95.5%, respectively, when compared to the reference test. Ours is the first serological study using the *Toxoplasma* POC test in birds. The POC tests are easy to use, provide results in minutes, and are less complicated to perform than MAT. They could be particularly useful in clinical settings such as rehabilitation centers and zoos for detecting birds with suspected *T. gondii* infections as an inexpensive, easy alternative to direct efforts for MAT testing. In conclusion, our results suggest that *Toxoplasma* POC tests deserve further investigation as a potential rapid serological screening test for birds.

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