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Antibody Prevalence and Isolation of Viable *Toxoplasma gondii* from Raptors in the Southeastern USA

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ABSTRACT: Raptors are good indicators of the prevalence of *Toxoplasma gondii* in the environment because they prey on small mammals and birds. These prey species are a major source of infection in domestic cats (*Felis catus*), which shed the environmentally resistant oocysts. We assessed *T. gondii* infection in 281 opportunistically available raptors at a rehabilitation facility between 2012 and 2014. Antibodies to *T. gondii* were assayed by a modified agglutination test (cutoff 1:25) and found in serum of 22/71 Red-tailed Hawks (*Buteo jamaicensis*), 25/54 Barred Owls (*Strix varia*), 9/41 Red-shouldered Hawks (*Buteo lineatus*), 13/28 Great Horned Owls (*Bubo virginianus*), 6/20 Broad-winged Hawks (*Buteo platypterus*), 2/16 Eastern Screech Owls (*Megascops asio*), 12/13 Bald Eagles (*Haliaeetus leucocephalus*), 6/12 Cooper's Hawks (*Accipiter cooperii*), 1/8 Black Vultures (*Coragyps atratus*), and 1/1 Golden Eagle (*Aquila chrysaetos*). Antibodies were not detected in 5 Barn Owls (*Tyto alba*), 3 American Kestrels (*Falco sparverius*), 1 Mississippi Kite (*Ictinia mississippiensis*), and 1 Osprey (*Pandion haliaetus*). Viable *T. gondii* was isolated from the tissues of 1 antibody-positive Barred Owl and identified as a strain having type II alleles at all 10 loci tested, except one (ToxoDB polymerase chain reaction–restriction fragment length polymorphism genotype 3). Type II strain is the most common strain in the US. Results of this study indicate a high prevalence of *T. gondii* in some raptor species and the first reported genotyping from a Barred Owl.

Key words: Genotype, isolation, raptor, serology, *Toxoplasma gondii*, type II.

Infection with the protozoan *Toxoplasma gondii* is prevalent worldwide in humans and animals, and it can cause serious disease in many species. Felids, such as domestic cats (*Felis catus*), are the only definitive hosts for *T. gondii*. All other hosts, including raptors, are considered intermediate hosts. Ingestion of infected small mammals and birds is considered the most important source of *T. gondii* infection for domestic cats (Dubey 2010). A cat can excrete millions of environmentally resistant oocysts, which are the primary source of contamination in the environment. In humans, the ingestion of raw or undercooked meat and consumption of food and water contaminated with oocysts are the two major modes of transmission postnatally. Surveys for *T. gondii* infection in intermediate hosts, such as rodents, are labor intensive, and they often can be a human health risk due to the variety of zoonotic diseases for which rodents are reservoirs (Dubey 2010). Consumption rates of small mammals and birds by raptors vary depending on species, with some raptors, such as the Common Kestrel, consuming over 500 small mammals and birds in a breeding season as a pair (Geng et al. 2009). Raptors are therefore considered a good indicator of the prevalence of *T. gondii* due to their consumption of other intermediate hosts of *T. gondii*.

We tested raptor species submitted to the Southeastern Raptor Rehabilitation Center (SERRC) from the southeastern US for *T. gondii* infection. No raptor was used for the sole purpose of this project. The study was approved by the Institutional Animal Care and Use Committee of Auburn University, and permits were obtained from the US Fish and Wildlife Service.

We obtained serum samples from 281 wild raptors of 14 species brought to the SERRC between August 2012 and August 2014. All birds were either housed in an aviary or small holding pen (mew), or they were patients residing in the SERRC Hospital. Birds listed as critical or unstable were not used.

Birds were restrained according to standard raptor-handling procedures at SERRC, outlined in Graham and Heatley (2007). We collected 100–500 μL of whole blood from the jugular or ulnar vein using a 26-gauge needle and 1-mL syringe. The blood sample was allowed to clot (in a refrigerator at 4 C), centrifuged, and stored at –20 C until testing. Any raptor that died naturally at the facility or was euthanized due to poor prognosis was necropsied, and whole hearts and whole brains or heads of the raptors were collected and refrigerated for shipping overnight (usually within 2 d of collection). The tissue samples were shipped cold, along with stored sera, to the US Department of Agriculture, Animal Parasitic Diseases Laboratory in Beltsville, Maryland, for *T. gondii* testing.

Raptor sera were diluted twofold serially from 1:25 to 1:200 and tested for antibodies against *T. gondii* by the modified agglutination test (MAT) as described by Dubey (2010). Serum with a titer ≥25 was considered positive. Though no sensitivity and specificity data are available specifically for raptors, this MAT test has been validated in chickens and is considered the most useful serologic test for *T. gondii* in many bird species (Dubey 2002; Dubey et al. 2015).

Myocardium or brain tissue of opportunistically available bird carcasses that were MAT positive were bioassayed in mice for isolation of *T. gondii*. The myocardium (30 g) was homogenized, digested in acidic pepsin, and washed with saline. Brain tissue was homogenized in saline, washed without pepsin digestion, and mixed with heart digest; aliquots of these mixed homogenates were inoculated subcutaneously into two to four outbreed Swiss Webster mice and one gamma interferon gene knockout (KO) mouse (Dubey 2010). Mice were bled on day 48 postinoculation (PI), and a 1:25 dilution of serum was tested for *T. gondii* antibodies by MAT. Mice were killed 50 d PI, and tissue imprints of lungs and brains of inoculated mice that died were examined for *T. gondii* tachyzoites or tissue cysts (Dubey 2010). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue cysts were found in tissues, or when antibodies to *T. gondii* were demonstrable in their serum.

African green monkey kidney fibroblast (CV-1) cells were used for in vitro cultivation of *T. gondii*. Tissues of mice found positive for *T. gondii* after bioassay were homogenized in aqueous antibiotics (1,000 U penicillin, 100 μg streptomycin/mL saline) and seeded into African green monkey kidney fibroblast cell culture flasks. Tachyzoites from successful cultures were harvested from the medium for DNA isolation and cryopreserved in liquid nitrogen for future studies (Dubey 2010).

Toxoplasma gondii DNA was extracted from cell culture–derived tachyzoites using DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions. DNA quantification and quality were determined by a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA). We performed PCR–restriction fragment length polymorphism (PCR-RFLP) genotyping using the genetic markers SAG1, SAG2 (5′-3′SAG2, and alt.SAG2), SAG3, BTUB, GRA6, c22-8, c29-2, L358, PK1, and Apico (Su et al. 2010). Appropriate positive and negative controls were included in all batches.

Prevalence of antibody to *T. gondii* varied from 0% (in four species) to 100% (in one species), with a total prevalence in all raptors assayed of 34.5% (Table 1). Viable *T. gondii* was isolated from the tissues of a MAT-positive (1:50) Barred Owl (*Strix varia*), while no viable *T. gondii* was isolated from bioassays of the following antibody-positive raptors: four Red-tailed Hawks, four Red-shouldered Hawks, three Broad-winged Hawks, and one Eastern Screech Owl. All four Swiss Webster mice inoculated with Barred Owl tissues remained asymptomatic, and tissue cysts were found in their brains when euthanized 50 d PI. The KO mouse inoculated with tissues of this owl died 19 d PI, and tachyzoites were found in its lungs. *Toxoplasma gondii* was successfully cultivated in cell culture seeded with tachyzoites from the lung homogenate of the KO mouse. The Barred Owl isolate is

TABLE 1. Prevalence of antibody to *Toxoplasma gondii* in raptors (as demonstrated by modified agglutination test [MAT]) from the southeastern USA housed at a rehabilitation facility, 2012–14.

Raptor species[a]	MAT titer				No. tested	MAT positive (%)	Bioassays in mice (isolated)
	25	50	100	≥200			
Red-tailed Hawk (*Buteo jamaicensis*)	11	4	2	5	71	22 (31)	4 (0)
Barred Owl (*Strix varia*)	6	4	5	10	54	25 (46)	1 (1)
Red-shouldered Hawk (*Buteo lineatus*)	0	4	3	2	41	9 (22)	4 (0)
Great Horned Owl (*Bubo virginianus*)	2	8	1	2	28	13 (46)	0
Broad-winged Hawk (*Buteo platypterus*)	2	2	0	2	20	6 (30)	3 (0)
Eastern Screech Owl (*Megascops asio*)	1	1	0	0	16	2 (12)	1 (0)
Bald Eagle (*Haliaeetus leucocephalus*)	0	3	6	3	13	12 (92)	0
Cooper's Hawk (*Accipiter cooperii*)	0	6	0	0	12	6 (50)	0
Black Vulture (*Coragyps atratus*)	1	0	0	0	8	1 (12)	0
Golden Eagle (*Aquila chrysaetos*)	0	0	1	0	1	1 (100)	0

[a] Serologically negative (MAT<25) raptors (species) [total no. tested]: Barn Owl (*Tyto alba*) [5], American Kestrel (*Falco sparverius*) [3], Osprey (*Pandion haliaetus*) [1], and Mississippi Kite (*Ictinia mississippiensis*) [1].

designated TgSaUS1. The PCR-RFLP from DNA derived from cell-cultured tachyzoites revealed type II alleles at all loci tested, except type I at Apico (ToxoDB PCR-RFLP genotype 3). Analysis of samples from raptors submitted to rehabilitation centers is not random sampling, and it may not represent true prevalence.

Although *T. gondii* has been isolated from other raptors in the US (Lindsay et al. 1993; Dubey et al. 2010, 2011), to our knowledge this is the first from a Barred Owl. The Barred Owl is an opportunistic predator, and although it preys on many small mammals and birds, its main prey is the meadow vole (*Microtus pennsylvanicus*).

Historically, *T. gondii* was considered to be clonal with low genetic diversity and grouped into types I, II, and III. However, recent studies have revealed greater genetic diversity, particularly in isolates from domestic animals in Brazil and wildlife in the US (Shwab et al. 2014). It is not known why only a small percentage of antibody-positive adult humans or other animals develop clinical signs, or whether the severity of toxoplasmosis in immunocompetent hosts is due to the parasite strain, host variability, or other factors (Dubey 2010).

Opportunities for genetic characterization of *T. gondii* isolated from indicator species, such as raptors, allow for further understanding of the spatial prevalence of certain *T. gondii* strains. Type II strains (ToxoDB 1 or 3) are the most prevalent genotypes in Europe, and they are also abundant in North America (Shwab et al. 2014). Although we obtained only one isolate, it was genotyped as a type II strain, which is common in higher animals in the US.

Raptors are considered resistant to clinical toxoplasmosis. Serologic surveys indicate exposure to *T. gondii* is common, and viable *T. gondii* has been isolated from several species of raptors (Dubey 2010; Dubey et al. 2011). In previous studies, experimentally induced infections in raptors were asymptomatic, and clinical disease was rare (Dubey et al. 1992; Dubey 2010). In another study in the southeastern US, Lindsay et al. (1993) isolated viable *T. gondii* from 26.7% of 101 raptors, including four of 15 Barred Owls. In this study, we estimated the prevalence of antibody to *T. gondii* in raptors in the southeastern US, and we confirmed a type II strain of *T. gondii* from a Barred Owl.

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