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SEROLOGICAL AND PARASITOLOGICAL PREVALENCE OF *TOXOPLASMA GONDII* IN WILD BIRDS FROM COLORADO

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ABSTRACT: Ground-feeding birds are considered important in the epidemiology of *Toxoplasma gondii* because they serve as indicators of soil contamination by oocysts, and birds of prey are indicators of *T. gondii* prevalence in rodents and other small mammals. Cats excrete environmentally resistant oocysts after consuming tissues of *T. gondii*-infected birds. In the present study, sera and tissues from 382 wild birds from Colorado were tested for *T. gondii* infection. Antibodies to *T. gondii* were found in 38 birds with the use of the modified agglutination test (MAT, 1:25 titer). Tissues (brains, hearts) of 84 birds were bioassayed in mice. Viable *T. gondii* was isolated from 1 of 1 barn owl (*Tyto alba*), 1 of 5 American kestrels (*Falco sparverius*), 1 of 7 ferruginous hawks (*Buteo regalis*), 1 of 4 rough-legged hawks (*Buteo lagopus*), 2 of 13 Swainson's hawks (*Buteo swainsoni*), and 1 of 25 red-tailed hawks (*Buteo jamaicensis*). This is the first time *T. gondii* has been isolated from the barn owl, ferruginous hawk, rough-legged hawk, and Swainson's hawk.

The protozoan *Toxoplasma gondii* infects virtually all warm-blooded animals, including humans, livestock, and marine mammals (Dubey, 2009). In the United States, various surveys have found that 10–50% of the adult human population has been exposed to this parasite (Dubey and Jones, 2008). Infection in western North America is considered lower than in the east (Feldman and Miller, 1956; Feldman, 1965), probably related to oocyst survival in dry, colder areas.

Toxoplasma gondii infection in birds is considered important epidemiologically because ground-foraging birds can indicate soil contamination with oocysts; raptors, which prey on small mammals, can indicate infections in local rodent populations, which also represent a source of infection for cats. For example, owls consume as many as 140 rodents per month (Kirkpatrick et al., 1990).

Little is known of *T. gondii* infection in Colorado. In the present article we surveyed *T. gondii* infection in different avian species in Colorado.

MATERIALS AND METHODS

Samples from 382 birds of 29 species were collected in 2008–2010 (Table I), and transported on blue ice to the Animal Parasitic Diseases Laboratory (APDL), Beltsville, Maryland, where all testing was done. Birds were either killed by hunters, or during animal damage management activities, and were kept cool until sampling, which was conducted within 24 hr. All instruments were cleaned and disinfected between necropsies of individual birds to prevent cross-contamination. Blood samples were collected from the heart and chest cavities with serum-separator tubes, and then refrigerated for up to 24 hr before centrifugation to allow for complete clotting. Tissues (brains, hearts, or both) from 84 birds were bioassayed in mice (Table I). Sera from animals were tested for antibodies to *T. gondii* by the modified agglutination test (MAT) as described by Dubey and Desmonts (1987).

Tissues (brain and heart pooled) were homogenized, digested in acidic pepsin, and washed, and aliquots of homogenates were inoculated subcutaneously into 2–5 outbred Swiss Webster (SW) mice (Dubey, 2009) and/or 1–2 gamma-interferon gene knock-out (KO) mice (Jackson Laboratories, Bar Harbor, Maine) (Dubey and Lindsay, 1998). Tissue imprints of lungs and brains of inoculated mice that died were examined for *T. gondii* tachyzoites or tissue cysts. Survivors were bled 45 days postinoculation (PI) and a 1:25 dilution of serum was tested for *T. gondii* antibodies by MAT. Mice were killed 46 days PI and brains of all mice were examined for tissue cysts as described (Dubey, 2009). The inoculated mice were considered infected with *T. gondii* when tachyzoites or tissue

cysts were found in tissues. Tissues from raptor species (hawks, falcons, owl, and vulture) were bioassayed when available; otherwise, only tissues from birds that were sero-positive to *T. gondii* were bioassayed. Some raptors were captured and relocated, and so only serum samples were available for *T. gondii* testing.

RESULTS

Antibodies to *T. gondii* were found in several avian species; for some species, this is the first such finding in the United States (mourning dove, western meadowlark, western kingbird, horned lark, rough-legged hawk, ferruginous hawk, and Swainson's hawk) (Table I).

Viable *T. gondii* organisms were isolated from 1 of 1 barn owl, 1 of 5 American kestrels, 1 of 4 rough-legged hawks, 1 of 7 ferruginous hawks, 2 of 13 Swainson's hawks, and 1 of 25 red-tailed hawks, but not from other bird species (Table II). All isolates of *T. gondii* from this study were non-virulent for SW out-bred mice.

DISCUSSION

Raptors

Toxoplasma gondii was isolated from 7 of 60 raptors, including a rough-legged hawk, a barn owl, 2 Swainson's hawks (a first time for these hosts), an American kestrel, a ferruginous hawk, and a red-tailed hawk. Lindsay et al. (1993) attempted to isolate *T. gondii* from 101 raptors submitted to the State Veterinary Diagnostic Laboratory, Auburn, Alabama. They isolated viable *T. gondii* from 26.7% of 101 raptors, including 41.1% of 27 red tailed hawks, 1 of 3 kestrels, but none from 6 barn owls. Herein, we isolated viable *T. gondii* from only 11.8% of 51 raptors or carnivorous birds. The higher isolation success in their study compared to the present study may be because of the types of animals examined; i.e., 97 of the 101 raptors studied by Lindsay et al. (1993) were patients at a rehabilitation center, 1 was submitted by a game warden, and 1 bird was from a zoo; whether any of these 99 animals were exposed to *T. gondii* in captivity is unknown.

Raptors are generally resistant to clinical toxoplasmosis. Experimentally, owls fed *T. gondii* bradyzoites became infected, but remained clinically normal (Dubey et al., 1992). To our knowledge, there are no reports of clinical toxoplasmosis in the last 20 yr in the United States in any avian species surveyed in the present study (Dubey, 2009). In fact, only 1 case of fatal toxoplasmosis has been diagnosed in raptors; this was identified immunohistochemically in an immature bald eagle that was hospitalized at a pet hospital in Pennsylvania (Szabo et al., 2004). Unfortunately, unfixed tissues were not available for isolation of

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TABLE I. *Toxoplasma gondii* prevalence in avian species from Colorado.

Species	No. tested	No. seropositive (MAT titer)	No. bioassayed	Previous reports from the United States
American crow (<i>Corvus brachyrhynchos</i>)	2	0	2	
American kestrel (<i>Falco sparverius</i>)	6	0	5	Lindsay et al. (1993)
American wigeon (<i>Anas americana</i>)	7	0	0	
Barn owl (<i>Tyto alba</i>)	1	0	1	Kirkpatrick et al. (1990)
Blue-winged teal (<i>Anas discors</i>)	2	0	0	
Brown-headed cowbird (<i>Molothrus ater</i>)	1	0	0	Lehmann et al. (2003)
Bufflehead (<i>Bucephala albeola</i>)	7	0	0	
Canada goose (<i>Branta canadensis</i>)	6	0	0	Dubey et al. (2004, 2007)
European starling (<i>Sturnus vulgaris</i>)	27	4 (50, 200, 200, 200)	4	Lehmann et al. (2003)
Ferruginous hawk (<i>Buteo regalis</i>)	7	2 (25, 3200)	7	
Gadwall (<i>Anas strepera</i>)	1	0	0	
Great blue heron (<i>Ardea herodias</i>)	1	0	0	
Great horned owl (<i>Bubo virginianus</i>)	3	0	1	Lindsay et al. (1993)
Green-winged teal (<i>Anas crecca</i>)	1	0	0	
Horned lark (<i>Eremophila alpestris</i>)	9	3 (25, 50, 100)	0	
Killdeer (<i>Charadrius vociferus</i>)	4	0	0	
Mallard (<i>Anas platyrhynchos</i>)	12	0	0	
Mourning dove (<i>Zenaida macroura</i>)	30	2 (25, 200)	3	
Northern harrier (<i>Circus cyaneus</i>)	3	0	3	
Prairie falcon (<i>Falco mexicanus</i>)	1	0	0	
Redhead (<i>Aythya americana</i>)	1	0	0	
Red-tailed hawk (<i>Buteo jamaicensis</i>)	47	8 (25, 25, 25, 25, 100, 100, 100, 200)	25	Lindsay et al. (1993)
Ring-necked duck (<i>Aythya collaris</i>)	1	0	0	
Rock pigeon (<i>Columba livia</i>)	129	5 (50, 50, 200, 200, 400)	5	Kirkpatrick et al. (1990), Feldman and Sabin (1949), Manwell and Drobeck (1951), Gibson and Eyles (1957), Franti et al. (1975), Jacobs et al. (1952)
Rough-legged hawk (<i>Buteo lagopus</i>)	4	2 (25, 25)	4	
Swainson's hawk (<i>Buteo swainsoni</i>)	45	5 (25, 25, 50, 50, 200)	13	
Turkey vulture (<i>Cathartes aura</i>)	2	1 (50)	2	Franti et al. (1975)
Western kingbird (<i>Tyrannus verticalis</i>)	10	3 (25, 25, 100)	3	
Western meadowlark (<i>Sturnella neglecta</i>)	12	3 (100, 200, 200)	3	

viable *T. gondii*, which could serve to confirm this diagnosis. Worldwide reports of all wild avian species were summarized by Dubey (2002, 2008).

Ground-foraging birds

In the present study, we found antibodies to *T. gondii* in 5 (3.9%) of 129 rock pigeons and 4 (15%) of 27 European starlings. Infection in these animals is considered to reflect contamination of soil with *T. gondii* oocysts. Before the discovery of the *T. gondii* oocysts in 1970, pigeons were surveyed for *T. gondii* infection in

the United States for their possible role in transmission to humans. Viable parasites were isolated from 1% of 100 (Feldman and Sabin, 1949), 2% of 50 (Manwell and Drobeck, 1951), 5% of 80 (Jacobs et al., 1952), and 6% of 16 (Gibson and Eyles, 1957) pigeons from the eastern parts of the United States. In these studies, antibodies to *T. gondii* were found in 6–10% of pigeons tested by the Sabin–Feldman dye test. The dye test does not detect *T. gondii* antibodies in many species of birds, including chickens, but the dye test works well with pigeon sera (Frenkel, 1981; Dubey, 2009). Kirkpatrick et al. (1990) reported *T. gondii* antibodies in 5.9% of 34 pigeons tested by the MAT. Unlike the

TABLE II. Details of isolation of *Toxoplasma gondii* in avian species from Colorado.

Species and identification number	Date killed (month, year)	Location	Modified agglutination test	Bioassay*	Strain designation
American kestrel (<i>Falco sparverius</i>), CT-268	April 2009	Aurora, Colorado	<25	4/4	TgFsCoUs1
Barn owl (<i>Tyto alba</i>), CT-092	May 2008	Aurora, Colorado	<25	1/2	TgTaCoUs1
Ferruginous hawk (<i>Buteo regalis</i>), FEHA-2	January 2010	Denver, Colorado	3,200	1/4	TgBrCoUs1
Rough-legged hawk (<i>Buteo lagopus</i>), RLHA-2	May 2010	Denver, Colorado	25	3/5	TgBICoUs1
Swainson's hawk (<i>Buteo swainsoni</i>), CT-339	May 2010	Denver, Colorado	<25	1/4	TgBsCoUs1
Swainson's hawk (<i>Buteo swainsoni</i>), CT-341	May 2010	Denver, Colorado	50	1/4	TgBsCoUs2
Red-tailed hawk (<i>Buteo jamaicensis</i>), CT-358	June 2010	Denver, Colorado	200	1/4	TgBjCoUs1

* Number of mice infected/No. inoculated.

dye test, the MAT detects *T. gondii* antibodies in all hosts of *T. gondii* (Dubey, 2009). Therefore, the MAT was used for the present study. We are not aware of any recent *T. gondii* surveys in birds, except that reported by Lehmann et al. (2003). In that survey 105 birds with endemic toxoplasmosis were trapped over 6 days on a Massachusetts pig farm in April 2002. All birds were bioassayed at APDL; *T. gondii* was isolated from a sero-negative European starling (*Sturnus vulgaris*). The highest sero-prevalence (MAT, 1:10) was in 8 of 18 American robins (*Turdus migratorius*) and 5 of 7 grackles (*Quiscalus quiscula*). Of the other 80 birds belonging to 12 species, only 2 (1 house sparrow, 1 northern cardinal) were sero-positive (Lehmann et al., 2003). Unlike the study on the pig farm, the present study was conducted during a 2-yr period in suburban and metropolitan areas, and without an identifiable outbreak of toxoplasmosis.

In the present study, we used serology as an indicator of likely *T. gondii* infection in birds. We bioassayed all raptor tissues for *T. gondii* infection, but we pre-screened all non-raptors serologically first, and only bioassayed sero-positive birds. However, we did isolate *T. gondii* from a barn owl with no detectable antibody (at 1:25 serum dilution). Moreover, in a prior study, *T. gondii* was isolated from a sero-negative European starling (*Sturnus vulgaris*) (Lehmann et al., 2003), and *T. gondii* has been occasionally isolated from domestic chickens with a low MAT titer of 1:5 and 1:10 (Dubey, 2009). Thus, isolation in our study may have been higher if all birds were bioassayed, irrespective of their serological status.

Prevalence in other hosts in Colorado

Overall, the serological prevalence of *T. gondii* in Colorado birds was low, and is in keeping with the view that the prevalence of *T. gondii* in the western United States is low (Feldman and Miller, 1956; Feldman, 1965), although there are few prevalence studies specific to Colorado. Hill et al. (2000) reported *T. gondii* antibodies in 23.6% of 206 cats from Fort Collins surveyed in 1993–1995; in 19.7% of 129 client-owned cats, and 29.8% of 77 cats from humane shelters. This sero-prevalence was generally lower than sero-prevalence in cats in eastern United States, although no strict comparisons can be made with the type of data analyzed (Vollaire et al., 2005). We are not aware of *T. gondii* surveys in livestock from Colorado. In a national meat survey, neither antibodies to *T. gondii*, nor viable *T. gondii* organisms were found in any of 74 pork, 74 chicken, and 74 beef samples collected from meat stores in Fort Collins, Colorado in September 2002 (Dubey et al., 2005). In another national study, Denver had the lowest prevalence of toxoplasmosis reported from 9 metropolitan areas throughout the United States (Jones et al., 1996). These results suggest a low prevalence of *T. gondii* in Colorado, which is further supported by this study.

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