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PREVALENCE OF ENCYSTED *TOXOPLASMA GONDII* IN RAPTORS FROM ALABAMA

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ABSTRACT: Little is known about the prevalence of encysted *Toxoplasma gondii* in wild birds. We examined the hearts and breast muscles from 101 raptors for encysted *T. gondii*. All of the raptors had been submitted for necropsy to the State Veterinary Diagnostic Laboratory, Auburn, Alabama. Tissues were digested in acid-pepsin solution and inoculated into groups of 3–5 laboratory mice. *Toxoplasma gondii* was isolated from 27 of 101 (26.7%) raptors: 8 of 12 (66.7%) red-shouldered hawks (*Buteo lineatus*), 13 of 27 (41.1%) red-tailed hawks (*Buteo jamaicensis*), 1 of 4 (25%) Cooper's hawks (*Accipiter cooperi*), 1 of 5 (20%) great horned owls (*Bubo virginianus*), 4 of 15 (26.7%) barred owls (*Strix varia*), and 1 of 3 (33.3%) kestrels (*Falco sparverius*). *Toxoplasma gondii* was not isolated from 3 broad-winged hawks (*Buteo platypterus*), 3 sharp-shinned hawks (*Accipiter striatus*), 6 barn owls (*Tyto alba*), 9 screech owls (*Asio otus*), a Mississippi kite (*Ictinia misisippiensis*), 2 golden eagles (*Aquila chrysaetos*), a bald eagle (*Haliaeetus leucocephalus*), 4 ospreys (*Pandion haliaetus*), 4 turkey vultures (*Cathartes aura*), or 2 black vultures (*Coragyps atratus*). No significant difference ($P > 0.05$) in prevalence was detected based on sex using chi-square analysis. Chi-square analysis of the data demonstrated that adult raptors had encysted stages of *T. gondii* significantly ($P < 0.05$) more often than did immature raptors.

Little is known about the prevalence or importance of *Toxoplasma gondii* infections in raptors. It is logical to assume that raptors become infected with *T. gondii* by consuming infected prey. Therefore, the prevalence of *T. gondii* in raptors should reflect the prevalence in their prey. Oocyst-induced infections probably are less important in raptors because of their general lack of ground feeding and thereby decreased exposure to oocysts.

Experimental studies indicate that red-tailed hawks (*Buteo jamaicensis*), great horned owls (*Bubo virginianus*), barred owls (*Strix varia*), and screech owls (*Asio otus*) were susceptible to tissue cyst-induced infections with *T. gondii* isolates that are pathogenic for mice (Lindsay et al., 1991; Dubey et al., 1992). Clinical signs were not observed in these birds, but *T. gondii* was isolated from their tissues, and antibodies were demonstrated in their sera using the modified direct agglutination test (MAT) (Dubey and Desmonts, 1987) on *T. gondii* tachyzoites (Lindsay et al., 1991; Dubey et al., 1992). Miller et al. (1972) inoculated a sparrow hawk (*Falco sparverius*) with *T. gondii* but did not recover *T. gondii* from its tissues.

The purpose of the present study was to examine the prevalence of encysted *T. gondii* in raptors in Alabama.

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MATERIALS AND METHODS

Raptors examined in this study had been submitted to the State Veterinary Diagnostic Laboratory, Auburn, Alabama, for necropsy. One hundred one birds were examined for encysted *T. gondii*. Ninety-seven were patients or were submissions to the Auburn University Southeastern Raptor Rehabilitation Center (AUSRRC), College of Veterinary Medicine, 2 birds were from a wildlife rescue service, 1 was submitted by a game warden, and 1 was a long-term resident (about 28 yr) of a zoo. One of the birds had been at AUSRRC for about 10 yr; the period of hospitalization for the other birds at AUSRRC was not known. A complete examination was done on most birds, and appropriate tissues (always a small portion of the heart and breast muscles) were fixed in 10% neutral-buffered formalin solution for examination of hematoxylin and eosin-stained tissue sections. The remainder of the heart and a portion of breast muscles were refrigerated and used for examination for encysted *T. gondii*. The sex and approximate age (nestling, immature, adult) were recorded for most birds at necropsy.

Stained tissue sections from each bird were examined for *T. gondii* tissue cysts by light microscopy. Fresh tissues were examined by acid-pepsin digestion (Jacobs et al., 1960) and mouse inoculation for encysted *T. gondii* bradyzoites. The entire heart sample was combined with breast muscle to obtain up to 20 g of tissue for digestion. Samples that contained 10–20 g of tissue were digested at 37 C in a shaking water bath for 30 min, whereas samples that contained less than 10 g were digested for 15 min. Samples were washed in 0.85% (w/v) saline and resuspended in 4–5 ml of Hanks' balanced salt solution that contained 1,000 U/ml penicillin G and 1,000 μg/ml streptomycin sulfate (GIBCO, Grand Island, New York). The entire digestate was injected subcutaneously into groups of 3–5 outbred, female, 18–25-g, Hsd:ICR mice (Harlan Sprague Dawley, Inc., Indianapolis, Indiana).

TABLE I. Sex, age, amount of tissue examined, and prevalence of encysted *Toxoplasma gondii* in various species of hawks.

Species	Sex*	Age†	Tissue‡	Brain§	IFA‖	Result#
Buteo jamaicensis						
1	M	A	20.0	0/4	0/4	Neg
2	F	A	20.0	1/5	2/5	Pos
3	F	ND	20.0	0/4	0/4	Neg
4	M	ND	19.0	2/4	4/4	Pos
5	ND	I	14.0	4/5	5/5	Pos
6	F	ND	20.0	0/4	0/4	Neg
7	M	A	16.2	0/4	0/4	Neg
8	M	I	20.0	0/4	0/4	Neg
9	M	A	20.0	2/4	4/4	Pos
10	F	A	20.0	0/5	0/5	Neg
11	F	ND	20.0	0/5	0/5	Neg
12	F	I	7.8	0/4	0/4	Neg
13	F	A	20.0	2/4	4/4	Pos
14	F	A	20.0	4/4	4/4	Pos
15	F	A	20.0	0/4	0/4	Neg
16	F	I	17.5	0/4	0/4	Neg
17	M	ND	15.7	4/4	4/4	Pos
18	F	ND	20.0	5/5	1/1¶	Pos
19	M	A	20.0	5/5	5/5	Pos
20	M	ND	20.0	3/4	4/4	Pos
21	F	A	20.0	2/4	4/4	Pos
22	M	I	20.0	0/4	0/4	Neg
23	F	ND	20.0	0/4	0/4	Neg
24	M	I	20.0	3/4	4/4	Pos
25	ND	I	7.0	0/4	0/4	Neg
26	F	I	20.0	0/4	0/4	Neg
27	F	ND	19.1	0/4	0/4	Neg
Buteo lineatus						
1	M	A	20.0	4/5	5/5	Pos
2	F	A	11.9	2/4	4/4	Pos
3	M	A	8.5	5/5	5/5	Pos
4	F	ND	18.3	5/5	5/5	Pos
5	F	A	15.7	0/4	0/4	Neg
6	M	A	20.0	5/5	5/5	Pos
7	F	ND	20.0	4/5	4/5	Pos
8	M	A	11.8	4/4	3/3¶	Pos
9	F	A	20.0	3/4	4/4	Pos
10	F	ND	9.3	0/4	0/4	Neg
11	M	I	13.7	0/4	0/4	Neg
12	F	ND	19.0	0/4	0/4	Neg
Buteo platypterus						
1	M	A	14.1	0/5	0/5	Neg
2	F	I	6.3	0/4	0/4	Neg
3	M	ND	7.4	0/4	0/4	Neg
Accipiter cooperi						
1	F	I	20.0	0/4	0/4	Neg
2	F	ND	18.0	0/5	0/5	Neg
3	F	A	20.0	0/4	0/4	Neg
4	F	A	20.0	4/4	4/4	Pos
Accipiter striatus						
1	F	A	9.4	0/4	0/4	Neg
2	F	A	18.4	0/4	0/4	Neg
3	F	ND	5.2	0/4	0/4	Neg

* M, male; F, female; ND, not determined.
† A, adult; I, immature; ND, not determined.
‡ Grams of tissue digested and inoculated into mice.
§ Number of mice positive for *T. gondii* tissue cysts/number examined.
‖ Number of mice positive for *T. gondii* antibodies/number examined.
Neg, hawk negative for *T. gondii*; Pos, hawk positive for *T. gondii*.
¶ Some mice in this group died of toxoplasmosis.

Four to six weeks postinoculation, mice were bled from the retroorbital plexus, and the serum was examined in an indirect immunofluorescent antibody test (Lindsay et al., 1990) for IgG antibodies to *T. gondii*. Mice were killed and squash preparations from their brains were examined for *T. gondii* tissue cysts by light microscopy as described (Lindsay et al., 1991). Chi-square analysis was used to test for significant differences ($P < 0.05$) in *T. gondii* prevalence based on sex or age of the raptors.

RESULTS

Toxoplasma gondii was not observed in H&E-stained tissue sections from any of the raptors examined. *Toxoplasma gondii* was isolated in mice inoculated with tissues from 27 of the 101 (26.7%) raptors examined (Tables I–III). Specifically, *T. gondii* was recovered from 8 of 12 (66.7%) red-shouldered hawks, 12 of 27 (44.4%) red-tailed hawks, 1 of 4 (25%) Cooper's hawks, 1 of 5 (20%) great horned owls, 4 of 15 (26.7%) barred owls, and 1 of 3 (33.3%) kestrels. *Toxoplasma gondii* was not isolated from 3 broad-winged hawks, 3 sharp-shinned hawks, 6 barn owls, 9 screech owls, a Mississippi kite, 2 golden eagles, a bald eagle, 4 ospreys, 4 turkey vultures, or 2 black vultures. Sex, approximate age, and amount of tissue assayed are given in Tables I–III.

The sex was determined for 94 of the 101 birds. Thirteen of 42 (31.0%) male and 12 of 52 (23.1%) female raptors were positive for encysted *T. gondii*. No significant difference ($P > 0.05$) was present in prevalence of infection based on sex. The ages were determined for 67 birds. Fifteen of 46 (32.6%) adults, 2 of 20 (10.0%) immature, and 0 of 1 nestling were positive for encysted *T. gondii*. Significant differences ($P < 0.05$) were present in prevalence of infection between adult and immature birds. The nestling was omitted from analysis because it was the only nestling examined.

Three (11.1%) of the 27 *T. gondii* isolates were pathogenic for mice. One of 4, 1 of 4, and 4 of 5 mice died after inoculation with the tissues of a kestrel, red-shouldered hawk, or red-tailed hawk, respectively. All surviving mice in these 3 groups were positive for *T. gondii* antibodies and tissue cysts.

DISCUSSION

In the present study, we used a murine bioassay technique to demonstrate *T. gondii* in avian hosts. We used mouse inoculation because sera from avian species may not react in sero-

TABLE II. Sex, age, amount of tissue examined, and prevalence of encysted *Toxoplasma gondii* in various species of owls.

Species	Sex*	Age†	Tissue‡	Brain¶	IFA‖	Result#
Asio otus						
1	F	A	5.4	0/5	0/5	Neg
2	F	A	4.2	0/5	0/5	Neg
3	M	A	1.8	0/5	0/5	Neg
4	M	A	5.0	0/4	0/4	Neg
5	F	ND	4.4	0/4	0/4	Neg
6	F	A	12.5	0/4	0/4	Neg
7	F	I	6.1	0/4	0/4	Neg
8	M	ND	4.5	0/4	0/4	Neg
9	M	ND	3.9	0/4	0/4	Neg
Strix varia						
1	ND	ND	4.2	1/4	1/4	Pos
2	M	A	17.7	4/4	4/4	Pos
3	M	A	17.4	0/5	0/5	Neg
4	M	A	20.0	0/5	0/5	Neg
5	F	ND	9.2	0/5	0/5	Neg
6	F	A	10.0	0/4	0/4	Neg
7	F	ND	20.0	0/4	0/4	Neg
8¶	M	A	19.7	0/4	0/4	Neg
9	M	A	20.0	4/4	4/4	Pos
10	M	A	19.5	0/4	0/4	Neg
11	F	A	14.7	0/4	0/4	Neg
12	F	I	20.0	0/3	0/3	Neg
13	M	A	17.7	0/4	0/4	Neg
14	M	ND	11.5	0/4	0/4	Neg
15	F	ND	13.5	1/4	1/4	Pos
Bubo virginianus						
1	ND	NES	5.0	0/4	0/4	Neg
2	M	ND	20.0	0/5	0/5	Neg
3	M	ND	20.0	5/5	5/5	Pos
4	F	A	18.3	0/4	0/4	Neg
5	M	ND	20.0	0/4	0/4	Neg
Tyto alba						
1	ND	ND	12.7	0/4	0/4	Neg
2	M	ND	7.2	0/5	0/5	Neg
3	F	I	17.0	0/4	0/4	Neg
4	M	A	6.3	0/4	0/4	Neg
5	M	A	8.5	0/4	0/4	Neg
6	F	A	7.0	0/4	0/4	Neg

* M, male; F, female; ND, not determined.
† A, adult; I, immature; NES, nestling; ND, not determined.
‡ Grams of tissue digested and inoculated into mice.
§ Number of mice positive for *T. gondii* tissue cysts/number examined.
‖ Number of mice positive for *T. gondii* antibodies/number examined.
Neg, owl negative for *T. gondii*; Pos, owl positive for *T. gondii*.
¶ This owl had been at a zoo for about 28 yr.

TABLE III. Sex, age, amount of tissue examined, and prevalence of encysted *Toxoplasma gondii* in miscellaneous species of raptors.

Species	Sex*	Age†	Tissue‡	Brain§	IFA‖	Result#
Falco sparverius						
1	F	A	20.0	4/4	3/3¶	Pos
2	M	ND	4.7	0/5	0/5	Neg
3	F	ND	3.3	0/4	0/4	Neg
Ictinia misisippiensis						
1	F	I	10.7	0/4	0/4	Neg
Pandion haliaetus						
1	M	A	20.0	0/5	0/5	Neg
2	ND	ND	20.0	0/4	0/4	Neg
3	ND	ND	20.0	0/5	0/5	Neg
4	M	I	20.0	0/5	0/5	Neg
Haliaeetus leucocephalus						
1	M	A	20.0	0/5	0/5	Neg
Aquila chrysaetos						
1	F	I	20.0	0/5	0/5	Neg
2	M	A	20.0	0/5	0/5	Neg
Cathartes aura						
1	F	I	20.0	0/5	0/5	Neg
2	F	ND	20.0	0/5	0/5	Neg
3	M	A	20.0	0/5	0/5	Neg
4	F	I	3.7	0/5	0/5	Neg
Coragyps atratus						
1	F	I	20.0	0/4	0/4	Neg
2	M	A	20.0	0/5	0/5	Neg**

* M, male; F, female; ND, not determined.
† A, adult; I, immature; NES, nestling; ND, not determined.
‡ Grams of tissue digested and inoculated into mice.
§ Number of mice positive for *T. gondii* tissue cysts/number examined.
‖ Number of mice positive for *T. gondii* antibodies/number examined.
Neg, raptor negative for *T. gondii*; Pos, raptor positive for *T. gondii*.
¶ One mouse in the group died 27 days postinoculation and was positive for *T. gondii* tissue cysts.
** This black vulture had been at a rehabilitation center for about 10 yr.

logical tests for *T. gondii* as does mammalian sera (Frenkel, 1981). Direct isolation of *T. gondii* from bird tissues precludes false-positive serological reactions. Some birds that contained *T. gondii* tissue cysts in locations other than the heart or breast muscles may have been missed. However, experimental studies have indicated that the heart and breast muscles are most often infected with *T. gondii* tissue cysts in raptors (Lindsay et al., 1991; Dubey et al., 1992).

Kirkpatrick et al. (1990) found that 11 of 66 (16.7%) adult and 18 of 124 (14.5%) nestling barn owls from New Jersey were positive for antibodies to *T. gondii* in the MAT on tachyzoites. The MAT has been validated in owls (Dubey et al., 1992), and the serological results probably are accurate. Interestingly, we did not isolate *T. gondii* from the tissues of the 6 barn owls.

Franti et al. (1975, 1976) found that 1 of 2 (50%) and 0 of 4 turkey vultures from California were positive for antibodies to *T. gondii* in the indirect hemagglutination test. We did not isolate *T. gondii* in the 4 turkey vultures.

Franti et al. (1976) did not detect antibodies to *T. gondii* in the sera from 2 golden eagles or a red-tailed hawk from California that they examined using the indirect hemagglutination test. We did not isolate *T. gondii* from the 2 golden eagles but did isolate *T. gondii* from 12 of the 27 red-tailed hawks.

The differences in prevalence of *T. gondii* observed in serologic surveys compared to isolation studies may represent differences in technique or numbers of animals examined by each method. However, they may reflect actual differences in prevalence. If differences in prevalence are accurate, differences in food sources of the raptors from various areas of the U.S.A. may account for them.

Male raptors were infected with *T. gondii* as often as female raptors, indicating that significant differences ($P > 0.05$) in susceptibility to or exposure to *T. gondii* based on sex were not present. Adult raptors were infected with *T. gondii* significantly ($P < 0.05$) more often than immature raptors. This probably reflects increased exposure to infected prey by adult raptors because of their age.

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