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## Toxoplasmosis in Wild Turkeys: A Case Report and Serologic Survey

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**ABSTRACT:** Toxoplasmosis was diagnosed in a free-ranging wild turkey (*Meleagris gallopavo*) from West Virginia (USA) in June 1993. Gross findings included emaciation, splenomegaly, multifocal necrotizing hepatitis and splenitis, and crusting dermatitis on the head and neck. Histologically, multifocal necrosis with mononuclear inflammation was present in kidney, liver, spleen, heart, lungs, and pancreas. *Toxoplasma gondii* was confirmed in sections of liver by avidin-biotin immunohistochemical analysis. Subsequently, a retrospective serosurvey of wild turkeys for *T. gondii* antibodies was conducted using turkey sera collected between 1984 and 1989. An antibody prevalence of 10% was detected in 130 birds from 21 locations in the southeastern United States. While wild turkeys in the Southeast have *T. gondii* antibodies, this is only the second natural case of fatal toxoplasmosis reported; it appears that wild turkeys infrequently develop clinical disease when infected with *T. gondii*.

**Key words:** Toxoplasmosis, *Toxoplasma gondii*, wild turkey, *Meleagris gallopavo*, serologic survey, case report.

Toxoplasmosis, a systemic infection with the protozoan parasite *Toxoplasma gondii*, occurs in a wide variety of warm-blooded species, including domestic and wild mammals, humans, poultry, and wild birds. Clinical toxoplasmosis in chickens and turkeys seldom is observed (Siim et al., 1963; Dubey and Beattie, 1988; Dubey et al., 1993). Antibody prevalence studies performed in the past on turkeys and other species of birds have been hampered by insensitive or inconsistent testing procedures (Frenkel, 1981; Dubey et al., 1993). Infections and antibody titers have been documented in free-ranging wild turkeys (*Meleagris gallopavo*) with improved isolation procedures and serological tests (Lindsay et al., 1994).

Since 1975, Southeastern Cooperative Wildlife Disease Study (SCWDS) diag-

nosticians have necropsied over 250 wild turkeys from member southeastern states, including Alabama, Arkansas, Florida, Georgia, Louisiana, Missouri, North Carolina, South Carolina, Tennessee, Virginia, and West Virginia (USA) (Davidson et al., 1985; SCWDS, College of Veterinary Medicine, University of Georgia, Athens, Georgia). Forrester (1992) documented disease conditions in an additional 76 birds from Florida (USA). Only one case of fatal toxoplasmosis has been described (Howarth and Rodenroth, 1985). Here, we report a second case of toxoplasmosis in a wild turkey. We also conducted a retrospective serological survey using banked sera to better document the prevalence of *T. gondii* antibodies in free-ranging wild turkeys in the southeastern United States.

An ill adult male wild turkey was found in a field in Pocohantas County, West Virginia (80°00'W, 38°07'30"N) in June 1993. It was weak and easily approached by the landowner who reported the bird to the West Virginia Department of Natural Resources. The bird was alive when captured but died shortly thereafter. The carcass was chilled and shipped overnight to SCWDS for examination.

This bird was thin, weighing approximately 4.5 kg, and had an extensive crusting dermatitis around the head and neck. Pectoral musculature was atrophied and serous atrophy of fat was noted on the heart. Multiple white pinpoint foci were scattered across the hepatic and splenic capsular surfaces. The spleen was mildly enlarged (15 × 25 mm) and had pale nodules approximately 1 to 2 mm in diameter throughout the parenchyma. The kidneys were large and friable. Numerous nema-

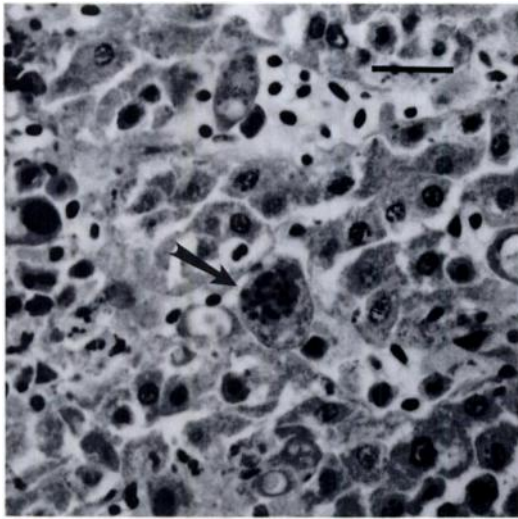


FIGURE 1. Photomicrograph of a turkey liver section with numerous intracellular tachyzoites of *Toxoplasma gondii*. H&E. Bar = 12.5  $\mu$ m.

todes, resembling *Heterakis gallinarum*, were found in the ceca.

Specimens of brain, liver, lung, spleen, kidney, crop, proventriculus, large and small intestine, pancreas, skin from several sites on the head and neck, and testicle were placed in 10% buffered formalin, embedded in paraffin, sectioned at 3  $\mu$ m, and stained with hematoxylin and eosin. Unfixed skin samples were submitted to the Athens Diagnostic Laboratory for examination for poxvirus particles. A homogenate of skin was clarified with deionized water. The resultant suspension was negative stained with 2% phosphotungstic acid and evaluated by direct transmission electron microscopy (Doan and Anderson, 1987). Sections of liver also were placed on slides coated with poly L-lysine (Sigma Chemical Company, St. Louis, Missouri, USA) for later immunohistochemical staining.

Histologically, the kidney had extensive, often angiocentric, necrotic foci that were heavily infiltrated by inflammatory cells. Macrophages predominated in these foci with fewer lymphocytes and plasma cells. The spleen was extensively necrotic. Non-suppurative inflammation was multifocal

to confluent between areas of necrosis in the liver. Numerous similar foci of mononuclear inflammation and necrosis were present in pulmonary interstitium, pancreas, and lamina propria of the intestinal tract, and were widely scattered in the myocardium. Within or peripheral to necrotic foci in the liver, kidney, and spleen were parenchymal cells or occasional macrophages enlarged by intracytoplasmic protozoal tachyzoites (Fig. 1). Individual tachyzoites were 3 to 4  $\mu$ m long and ovoid to elongate.

Poly-L-lysine-coated slides were stained with anti-*T. gondii* rabbit serum using an avidin-biotin complex procedure and reagents described by Lindsay and Dubey (1989). Numerous positively staining *T. gondii* tachyzoites were seen in lesions. No tissue cysts were found. Representative kidney slides containing the organisms have been deposited in the National Parasite Collection, Beltsville, Maryland (USA) (USNM HELM Coll No. 83990).

Mixed bacterial colonies were found in the multifocally ulcerated crop and within the kaolin layer of the proventriculus. Clusters of coccoid bacteria seen within skin sections of the head and neck were thought to be secondary invaders. Avian pox was suspected to be the inciting cause of the cutaneous lesions; however, no viral inclusion bodies were seen histologically in skin sections nor were viral particles seen by negative contrast electron microscopy.

The findings in this case prompted us to conduct a retrospective survey of wild turkey sera for antibodies to *T. gondii*. Sera from 130 apparently normal free-ranging wild turkeys collected between 1984 and 1989 from 21 locations in the southeastern United States (Table 1) were tested by the modified direct agglutination test (Dubey et al., 1993) at the U.S. Department of Agriculture Zoonotic Diseases Laboratory, Beltsville, Maryland. Five samples were selected from each of 20 mainland locations in Georgia, North Carolina, Kentucky, Missouri, and Louisiana. An additional 30 samples were from birds on Cum-

TABLE 1. Prevalence of *Toxoplasma gondii* antibody titers in 130 wild turkeys in the southeastern United States, 1984 to 1989.

Location	Sex <sup>a</sup>		Age <sup>b</sup>		Antibody titer		
	M	F	Ad	Juv	<1:25	1:25	1:100
<b>Georgia</b>							
Camden County	NA <sup>c</sup>	NA	NA	NA	30	0	0
Clarks Hill Wildlife Management Area	0	5	NA	NA	5	0	0
Floyd County	0	5	2	3	4	1	0
Glynn and McIntosh Counties	4	1	3	2	4	1	0
Lincoln County	1	4	4	1	5	0	0
Macon County	5	0	5	0	5	0	0
Rabun County	1	4	2	3	5	0	0
Randolph County	0	5	5	0	3	0	2
Wilkes County	0	5	5	0	5	0	0
<b>Kentucky</b>							
Anderson County	0	5	NA	NA	5	0	0
Lyon County	5	0	3	2	4	1	0
Owen County	5	0	5	0	4	1	0
<b>Louisiana</b>							
East Feliciana Parish	0	5	4	1	3	2	0
Livingston Parish	2	3	2	3	5	0	0
West Feliciana Parish	5	0	1	4	4	0	1
<b>Missouri</b>							
	0	5	1	4	5	0	0
<b>North Carolina</b>							
Bertie County	0	5	5	0	3	1	1
Caswell County	3	2	4	1	5	0	0
Cherokee County	0	5	0	5	3	2	0
Madison County	2	3	5	0	5	0	0
Onslow County	3	2	3	2	5	0	0

<sup>a</sup> M, male; F, female.

<sup>b</sup> Ad, adult; Juv, juvenile.

<sup>c</sup> NA, not recorded.

berland Island, Georgia. No wild or domestic feline definitive hosts of *T. gondii* existed on the island at the time samples were collected; bobcats were introduced to the island in 1988 (Diefenbach et al., 1993). Samples were tested at dilutions of 1:25, 1:100, 1:400, and 1:1,600.

Thirteen (10%) of 130 turkeys had antibody titers ( $\geq 1:25$ ) to *T. gondii* (Table 1). No birds had detectable antibodies at  $\geq 1:400$  dilution. Seropositive birds were found in all states from which samples were tested; no antibody positive birds came from Cumberland Island. Of the 13 seropositive birds, six were adult females, four were juvenile females, and three were juvenile males. These percentages of seropositive birds roughly correspond to the

age and sex composition of the sample in which 64% were female and 66% were adult. The 1:25 dilution was chosen as the cut-off titer for *T. gondii* infections in turkeys because it has been found successful in experimentally infected animals, including turkeys (J. P. Dubey, unpubl.). Experimentally validated cut-off points for interpretation of *Toxoplasma* antibody titers have not been established for any host species.

Earlier, Lindsay et al. (1994) found *T. gondii* antibodies in 12 (71%) of 17 wild turkeys from Alabama. *Toxoplasma gondii* was isolated from the heart in eight of 16 of these turkeys. Although a higher number of positive antibody titers was found in the Alabama birds, we found a

prevalence of 40% (two of five birds) at locations in Georgia, Louisiana, and North Carolina. Although the sample size is small, the higher prevalence at these three locations combined with the findings in Alabama is evidence that enzootic foci of toxoplasmosis may occur. Further serological testing may be required to answer these questions regarding the disparate antibody prevalences.

Although some populations of southeastern wild turkeys have circulating antibodies to *T. gondii*, this is only the second fatal infection reported. In the case reported by Howerth and Rodenroth (1985), the *T. gondii*-like tachyzoites appeared distorted because the bird had been frozen prior to necropsy. *Toxoplasma gondii* was not recovered from the turkey spleen when inoculated into mice, probably because freezing rendered *T. gondii* non-infective (Howerth and Rodenroth, 1985). In the present case, *T. gondii* was confirmed by immunohistochemistry. This bird may have been rendered susceptible to systemic toxoplasmosis by an earlier condition, possibly avian pox, as evidenced by the chronic skin lesions. Fatal infections with *T. gondii* are most commonly reported in immune-compromised individuals (Dubey and Beattie, 1988). The presence of circulating antibodies to *T. gondii* in several populations is evidence that wild turkeys often are infected; however, the diagnostic case accession data is evidence that turkeys rarely develop systemic clinical toxoplasmosis, possibly because of innate resistance.

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