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## TOXOPLASMA ANTIBODIES AMONG BOBCATS AND OTHER CARNIVORES OF NORTHERN CALIFORNIA<sup>1</sup>

H. P. RIEMANN, J. A. HOWARTH, R. RUPPANNER, C. E. FRANTI and D. E. BEHYMER

**Abstract:** The prevalence of antibodies to *Toxoplasma gondii* was investigated among five species of wild carnivores in Northern California. The highest prevalence was among bobcats (*Lynx rufus*), with 15 of 21 tested being serologically positive. Other results included serological evidence of toxoplasmosis in two of seven raccoons (*Procyon lotor*), one of three badgers (*Taxidea taxus*) and two of three coyotes (*Canis latrans*). Two gray foxes (*Urocyon cinereoargenteus*) were serologically negative.

One badger with an indirect hemagglutination antibody titer of 1:8192 was found to harbor *T. gondii* in its brain tissues.

### INTRODUCTION

The current widely accepted life cycle of *T. gondii* involves members of the cat family (Felidae) as the final host.<sup>2</sup> Cats shed oocysts in their feces for 1 to 2 weeks during the primary infection.<sup>1,3</sup> The sporulated oocysts can remain viable in the soil for periods up to or exceeding a year depending upon temperature and soil conditions.<sup>12,15</sup> *T. gondii* is transmitted through fecal contamination to a wide variety of intermediate host species including man. After infection, the protozoa can survive as cysts in the body tissues for the lifetime of the host.<sup>5</sup> Cysts in skeletal muscles facilitate transfer of *T. gondii* by ingestion. Humans may also acquire toxoplasmosis by eating raw or undercooked meat,<sup>6</sup> and possibly by handling raw meat.<sup>7,11</sup>

Attention has been focused on domestic cats as a primary source of infection because of their close association with humans. However, other members of the Felidae are equally capable of supporting the sexual life stage of *T. gondii* and shedding oocysts. In all probability enzootics of toxoplasmosis occur in nature with the role of domestic cats replaced by wild Felidae and the intermediate

hosts are other wildlife species. Wild Felidae may also be an important link in the transfer of *T. gondii* to livestock on open range.

Bobcats are a prominent species of wild Felidae in California and are found in nearly every life zone throughout the state. In the present investigation the intent was, therefore, to determine the prevalence of toxoplasma antibodies among bobcats and other carnivorous wildlife species that share the habitat and food sources of the bobcat.

### MATERIALS AND METHODS

The animals tested for antibodies to *T. gondii* included bobcats, raccoons, badgers, coyotes, and gray foxes. The animals were trapped for their pelts during the winter months of 1974 at two locations (Napa and Humboldt counties) in Northern California. Blood samples were collected from the heart using a 10 ml syringe and 16 ga needle. The head, including the brain, was collected after skinning and kept refrigerated until tested for isolation of the organism.

The serums were tested for specific antibodies by the microtiter system of

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the indirect hemagglutination (IHA) test.<sup>9</sup> Each serum was screened at a 1:64 dilution using commercial antigen.<sup>[2]</sup> Serums which gave a positive reaction with the sensitized cells but did not agglutinate the unsensitized cell control were titrated to the endpoint.

Brain tissues from nine of the seropositive animals were tested for the presence of *T. gondii* cysts. One gram of brain tissue was removed through the foramen magnum and a 20% suspension was made in 0.85% sterile saline using a mortar and pestle. One ml of the suspension was injected intraperitoneally into each of two toxoplasma-free mice. Cardiac blood samples were taken from the mice after 21 days and tested for specific antibodies. A positive serologic reaction was taken as evidence that the original brain tissues contained *T. gondii*.

#### RESULTS

Of 35 animals that were tested, 20 had serological evidence of exposure to *T. gondii* (Table 1). The highest antibody prevalence was in the bobcats, among which 15 of 21 were seropositive. Thirteen of the bobcats were collected from Napa County in central California and eight originated from Humboldt County in northern California. Seven of the bobcats from Napa County and all eight from Humboldt County were seropositive. The antibody titers of the bobcats ranged from 1:64 to 1:2048, but the majority had titers of 1:256 or 1:512.

Two of three coyotes and two of seven raccoons were also seropositive for *T. gondii*. One of the coyotes, an old toothless male, had a titer of 1:512. The other coyote, an 18 kg male had a titer of 1:1024. All three of the coyotes were trapped in Napa County. One of the seropositive raccoons, a 9 kg male from Napa County, had a titer of 1:512. The other seropositive raccoon, a female from Humboldt County, had a titer of 1:128.

One of two male badgers from Napa County was seropositive with a titer of 1:8192, the highest antibody titer of the entire group of animals tested.

Only two gray foxes, males from Napa County, were tested and both were seronegative for *T. gondii*.

Of the total 35 animals, 11 of 24 from Napa County were seropositive compared to nine of 11 from one location in Humboldt County. However, due to the small sample sizes and differences in numbers of the various species trapped in each area, the difference was not significant. Similarly, although sex was not reported for four animals, nine of 20 male animals were seropositive compared to eight of 11 female animals. The difference by sex was not statistically significant.

Isolations of *T. gondii* from brain tissues were attempted on nine seropositive animals from Napa County. The single brain found to be infected was from the badger with a titer of 1:8192. The mice that were inoculated responded with titers of 1:64.

#### DISCUSSION

Although limited numbers of animals were tested, it is evident that a high percentage of wild carnivores become infected with *T. gondii* at some time during their lifetime. Most likely the bobcat (or other wild Felidae) assumes, in nature, the role of the domestic cat and reinforces the spread of toxoplasmosis by seeding its habitat with oocysts. Since the sporulated oocysts remain viable in the soil for long periods of time, a single infected bobcat has the potential to expose a large number of foraging and grazing species, such as rodents, birds, deer or livestock, to *T. gondii*.<sup>8,10,14</sup> After infection, the host often remains chronically infected with the cysts of *T. gondii* and thereby forms a source of infection for bobcats as well as other carnivorous species preying on the infected animals.

[2] Industrial Biological Laboratories, Inc., 481 Stonestreet Ave., Brockville, Md. 20850.

TABLE 1. Prevalence of Antibodies to *Toxoplasma gondii* Among Carnivores.\*

Species	Tested No.	Positive No.	Negative <64	Reciprocals of dilutions						
				64	128	256	512	1024	2048	≥4096
Bobcat ( <i>Lynx rufus</i> )	21	15	6	2	2	4	5	1	1	
Raccoon ( <i>Procyon lotor</i> )	7	2	5		1		1			
Badger ( <i>Taxidea taxus</i> )	2	1	1							1
Coyote ( <i>Canis latrans</i> )	3	2	1				1	1		
Fox ( <i>Urocyon cinereoargenteus</i> )	2	0	2							
TOTAL	35	20	15	2	3	4	7	2	1	1

\* Animals trapped in Napa and Humboldt counties, California.

The low isolation rate from seropositive carnivores may have been due in part to using only brain tissues for the inoculation. Other investigators have reported finding the brain negative, but heart and lung tissues positive in experimentally infected bobcats.<sup>8</sup> High antibody titers have been shown to increase the chances of isolation. One investigator testing sheep noted that 83% of those with titers of 1:256 or higher were chronically infected.<sup>4</sup> If this principle applies to species other than sheep, an estimated 13 of the 20 seropositive carnivores tested here may have been harboring *T. gondii* in their tissues.

Bobcats may maintain an essential function in the life cycle of *T. gondii* in areas remote from human populations and provide oocysts for the infection of livestock on open range. Chronically infected sheep and cattle have the potential of spreading the parasite in a meat supply to human. Surveys of livestock in Northern California recently indicated *T. gondii* antibody prevalences of 32% in range cattle and 28% in sheep

which were ranged in remote mountain areas during the summer months.<sup>19</sup> However, the degree of exposure to *T. gondii* attributable to bobcats in comparison to domestic cats under these circumstances has yet to be determined.

A word of caution should be given to wildlife biologists, hunters, trappers and others who come into contact with bobcats or other wildlife species. In addition to the possibility of infection from fecal contamination from infected bobcats, there may be a hazard from skinning, butchering, biopsy procedures, necropsies, and the like. Although the hazard to persons having contact with wildlife is probably no greater than that among farmers, veterinarians and others having contact with domestic animals, common sanitary precautions such as proper disposal of the feces from caged bobcats, washing of hands before eating and not allowing pets (especially cats) to eat raw meat or viscera from game animals should help prevent the spread of *T. gondii* from wildlife to pets and humans.

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