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## TOXOPLASMOSIS: A SEROLOGICAL SURVEY IN ONTARIO WILDLIFE\*

P. J. QUINN,<sup>[1]</sup> R. O. RAMSDEN<sup>[2]</sup> and D. H. JOHNSTON

**Abstract:** Sera from seven species of wild animals in Ontario were examined for antibody to *Toxoplasma gondii* using the Sabin-Feldman dye test. Of 158 sera tested, 53% of the red foxes (*Vulpes vulpes*), 56% of the striped skunks (*Mephitis mephitis*), 78% of the coyotes (*Canis latrans*), 33% of the black bears (*Ursus americanus*), 18% of the short tailed shrews (*Blarina brevicauda*) and none of the field voles (*Microtus pennsylvanicus*) had antibody.

Antibody to *T. gondii* was present in sera from wild animals captured throughout southern Ontario. A positive linear correlation between prevalence of toxoplasmosis and age of fox pups was calculated ( $p < 0.005$ ).

### INTRODUCTION

Toxoplasmosis has an extremely wide host and geographic distribution and may frequently infect and stimulate antibody production without clinical disease.<sup>4,11,13</sup> Information on the presence of toxoplasmosis in Ontario is scant. In a serological survey in the Toronto area Ffrench and Fish<sup>2</sup> found that 25.5% of 650 persons tested had detectable antibody to *Toxoplasma gondii* as determined by using the Sabin-Feldman dye test. Investigators at the Ontario Veterinary College have confirmed toxoplasmosis as the cause of death in dogs, cats, chinchillas and mink,<sup>5,10,14</sup> and as a cause of abortion in sheep.<sup>9</sup>

The three currently recognised modes of transmission are transplacental or congenital infection, fecal contamination of food from an infected cat and transmission through carnivorous eating habits.<sup>3,8</sup> In free-living mammalian species, carnivorous eating habits may be of particular importance in transmission, especially since the infection has been ob-

served in some of the prey rodent species.<sup>4,13</sup>

The present serological survey was undertaken because wild carnivore populations may reflect the prevalence of the disease in prey species (rodents and lagomorphs) and indicate foci of infection in Ontario.

### MATERIALS AND METHODS

Sera were collected from trapped wildlife from 14 counties in southern Ontario. Most specimens were from carnivores, particularly the red fox (*Vulpes vulpes*), with smaller numbers of coyote (*Canis latrans*), striped skunk (*Mephitis mephitis*), black bear (*Ursus americanus*), big short tailed shrew (*Blarina brevicauda*) and field vole (*Microtus pennsylvanicus*).

Trappers were paid per animal for fox pups, and this gave them incentive to capture all members of a litter. Fox pups were taken in April, May and June with the majority being captured early in this

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period. Soon after capture standard body measurements and weights were taken, familial relationships recorded and pups were ear tagged and bled.

Specimens for serology were either serum stored at  $-20^{\circ}\text{C}$ , or as whole blood absorbed on paper discs.<sup>9</sup> Paper discs were dried and stored at room temperature. The Sabin-Feldman dye<sup>12</sup> test was carried out on all specimens. Test sera and paper disc eluates were screened at a dilution of 1:16 and, if positive, were titrated in doubling dilutions to 1:1024. The minimum significant reaction was taken as 50% stained organisms at 1:16 serum dilution.

#### RESULTS

With the exception of the field vole, evidence of toxoplasmosis infection was found in all species examined (Table 1) and in all but one of 14 of the counties of Ontario from which samples were derived (Table 2, Figure 1).

Red fox pups constitute the largest single specimen group and estimates of ages in days were based on weight and body measurement as compared with captive pups. A study of the relationship of fox pup age to prevalence of infection was possible and results were tabulated in Table 3; for purposes of comparison mature captive foxes were included in the same table. When the data for fox pups with an estimated age of 20-79 days was examined (Table 3) using a student's T test, a positive linear correlation was apparent between the percentage of fox pups with serological evidence of toxoplasmosis and increasing fox pup age ( $p < 0.005$ ).

Fox pup litter size and prevalence of antibody to *T. gondii* were compared (Table 4). These data suggest the possibility of a linear relationship; however, because of insufficient data, this could not be confirmed statistically.

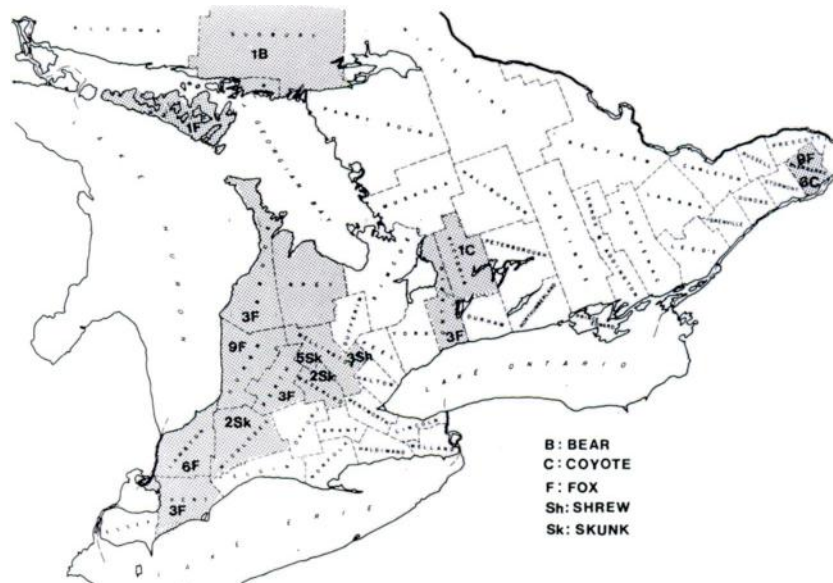


FIGURE 1. Locations in Southern Ontario where animals were captured for this study. Shaded areas indicate counties from which animals were obtained; individual species with antibody to *T. gondii* are plotted by county.

TABLE 1. *Toxoplasma* Antibody Titer in Various Species of Wildlife (Sabin-Feldman Dye Test).

	1/16	1/32	1/64	1/128	1/256	1/512	1/1024 and above	Total Tested	Total with Demonstrable Antibody	Percent with Demonstrable Antibody
Red Fox (pup)	9	4	3	1	2	1	17	70	37	53
Red Fox (mature)	—	—	—	4	—	2	2	13	8	62
Striped Skunk	7	—	—	—	3	—	4	25	14	56
Black Bear	1	—	—	—	—	—	—	3	1	33
Coyote (pup)	6	—	—	—	1	—	—	9	7	78
Short-Tailed Shrew	1	—	1	—	—	1	—	17	3	18
Field Vole	—	—	—	—	—	—	—	21	0	0

TABLE 2. Geographic Distribution of Specimens and Prevalence of Antibody to *T. gondii*.

	Red Fox	Striped Skunk	Coyote	Black Bear	Field Vole	Short-Tailed Shrew
Ontario Counties						
Kent	3/3*	—	—	—	—	—
Lambton	6/22	0/1	—	—	—	—
Middlesex	0/2	2/2	—	—	—	—
Huron	9/12	—	—	—	—	—
Perth	3/4	—	—	—	—	—
Waterloo	0/1	2/3	—	—	—	—
Wellington	—	5/9	—	—	0/21	3/17
Bruce	3/4	—	—	—	—	—
Grey	0/1	—	—	—	—	—
Ontario	3/4	—	—	—	—	—
Victoria	—	—	1/1	—	—	—
Glengarry	9/12	—	6/8	—	—	—
Manitoulin	1/5	—	—	—	—	—
Sudbury	—	—	—	1/3	—	—
Source unknown	8/13	5/9	—	—	—	—

\* numerator—number serologically positive for *T. gondii*  
denominator—total animals tested

TABLE 3. Red Fox: Relationship of Age to the Prevalence of Antibody to *T. gondii*.

	Estimated Age (days)						Mature 2 yrs. or more
	20-29	30-39	40-49	50-59	60-69	70-79	
Foxes with antibody to <i>T. gondii</i>	0	4	13	3	11	6	8
Total foxes tested	3	11	22	4	23	7	13
% of foxes with antibody to <i>T. gondii</i>	0	36	59	75	48	86	62

TABLE 4. Red Fox: Relationship of Family Size to the Prevalence of Antibody to *T. gondii*.

Number of pups in the litter	1	2	3	4	5	6	7	8	9
Number of litters of this size examined	7	5	5	2	3	1	—	—	1
Number of fox pups with antibody to <i>T. gondii</i>	2	4	8	3	9	4	—	—	7
Total number of fox pups tested	7	10	15	8	15	6	—	—	9
% of fox pups tested with antibody to <i>T. gondii</i>	29	40	53	38	60	67	—	—	78

TABLE 5. Relationship of Food Habits to Presence of Antibody to *T. gondii*.

Food Habits	Species	Animals with antibody to <i>T. gondii</i> (serologically positive/total tested)	Percentage positive
Carnivorous	Shrew	3/17	18
	Fox	37/70	53
	Coyote	7/9	78
Omnivorous	Skunk	14/25	56
	Bear	1/3	33
Herbivorous	Vole	0/21	0

#### DISCUSSION

In Table 5 prevalence data have been related to a rough classification of the diet of the seven species tested. Food habits are known to vary with prey abundance, season and local conditions<sup>15</sup> so this division can only be arbitrary. The results of this study, though limited, support the belief that wild carnivores in nature are more likely to become infected than wild herbivores living in the same geographic areas.

The frequency of positive serological evidence of *T. gondii* infections may not reflect the actual rate at which indivi-

duals become infected. Laboratory mice are highly susceptible and rarely survive infection, whereas other species are more resistant and so are more likely to show serological evidence of infection.<sup>3</sup> Production of antibody is influenced by several factors, including age of the host, strain of the infecting organism and persistence of infection. Some species have detectable antibody for only a few months after infection,<sup>3</sup> and others such as some birds, never develop antibody detectable by the dye test.<sup>3</sup> We are uncertain of the status of shrews and the field mouse with respect to these problems.

In studies of human infection with *T. gondii* a positive correlation has been found with age.<sup>11</sup> Our studies of the red fox pups indicated that the prevalence of infection rose from 0% at 20-29 days of age to 86% at 70-79 days of age. The presence of *T. gondii* specific antibody in very young pups due to consumption of vixen's colostrum was considered to be minimal, because serological evidence of infection appeared to coincide with increasing age and consumption of raw meat.

The acquisition of toxoplasmosis by wild carnivores can be attributed largely to their diet; however, the source of infection for their prey species is unknown. The role of the domestic cat (*Felis domesticus*) in the life cycle of *T. gondii* is now well established, but the part that feral cats play in the transmission of this parasite to other free-living mammals is

uncertain. *T. gondii* oocyst production has been observed in the bobcat (*Lynx rufus*), mountain lion (*Felis concolor*) and other felidae.<sup>3</sup> The extent to which this occurs under natural conditions has not yet been established. Oocyst transmission through feline feces may explain natural infections of herbivores since oocysts are able to survive for up to one year in moist soil.<sup>7</sup>

Our studies have demonstrated that toxoplasmosis infects several wild species and is widespread geographically throughout southern Ontario. How this infection affects wildlife and what relationship wildlife infections have to human or domestic animal infections is unknown. Because of the public health and economic importance of this disease we believe that further investigations of wildlife involvement are required.

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