

PREVALENCE OF ANTIBODIES TO *TOXOPLASMA GONDII* IN WILD MAMMALS OF MISSOURI AND EAST CENTRAL KANSAS: BIOLOGIC AND ECOLOGIC CONSIDERATIONS OF TRANSMISSION

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ABSTRACT: Sera from 273 wild mammals from Missouri and Kansas (USA), collected between December 1974 and December 1987, were tested for the presence of antibodies to *Toxoplasma gondii* using the Sabin-Feldman dye test. Sixty-five (24%) had antibodies at titers of $\geq 1:8$, including 38 (66%) of 58 carnivores, 14 (15%) of 94 omnivores, 13 (11%) of 117 herbivores, and none of four insectivores. The prevalence of antibodies in mice (*Mus musculus* and *Peromyscus* spp.) and rats (*Rattus norvegicus* and *Sigmodon hispidus*) was low (3%), while medium sized herbivores such as squirrels (*Sciurus* spp.), rabbits (*Sylvilagus floridanus*), and muskrats (*Ondatra zibethicus*) had prevalences of about 18%. Red foxes (*Vulpes fulva*) and mink (*Mustela vison*) had the highest prevalence of antibodies with frequencies of 90 and 66%, respectively. In 32 attempts to isolate *Toxoplasma gondii* from wild mammals with positive ($\geq 1:4$) titers, only six (19%) were successful: a gray squirrel (*Sciurus carolinensis*), a beaver (*Castor canadensis*), an opossum (*Didelphis marsupialis*), a red fox and two mink. These findings are consistent with the hypothesis that the probability of infection with *Toxoplasma gondii*, and therefore prevalence of antibodies in wildlife, is greatest in carnivores.

Key words: *Toxoplasma gondii*, prevalence of serum antibodies, wild mammals, dye test.

INTRODUCTION

There are a number of studies on the prevalence of serum antibodies to *Toxoplasma gondii* in wild mammals (Dubey and Beattie, 1988; Dreesen, 1990). These include studies from Iowa (USA) (McCulloch et al., 1967; Smith et al., 1992) and Illinois (USA) (Paine, 1969). No such study has been done in Missouri (USA), and only recently has one been done in Kansas (USA) (Brillhart et al., 1994). The prevalence of antibodies in domiciled adult cats from the Kansas City, Kansas, and Kansas City, Missouri, metropolitan area was 38%; it was 58% in stray cats from Iowa and northern Missouri (Dubey, 1973). Our objective was to determine the prevalence of *Toxoplasma gondii* antibodies among wild mammals in western Missouri and adjacent east central Kansas.

METHODS

From December 1974 to December 1987, serum samples were collected from the following wild mammals: one big brown bat (*Eptesicus*

fuscus), one red bat (*Lasiurus borealis*), two evening bats (*Nycticeius humeralis*), 38 opossums (*Didelphis marsupialis*), 12 eastern cottontail rabbits (*Sylvilagus floridanus*), one thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*), five gray squirrels (*Sciurus carolinensis*), five fox squirrels (*S. niger*), 14 beavers (*Castor canadensis*), two deer mice (*Peromyscus maniculatus*), 13 woodland white-footed mice (*P. leucopus*), one cotton rat (*Sigmodon hispidus*), 42 muskrats (*Ondatra zibethicus*), four Norway rats (*Rattus norvegicus*), 17 house mice (*Mus musculus*), one white-tailed deer (*Odocoileus virginianus*), 13 coyotes (*Canis latrans*), 10 red foxes (*Vulpes fulva*), four gray foxes (*Urocyon cinereoargenteus*), 52 raccoons (*Procyon lotor*), 29 mink (*Mustela vison*), four striped skunks (*Mephitis mephitis*), and two bobcats (*Felis rufus*).

Most house mice, Norway rats, and a few of the woodland white-footed mice were taken dead in mouse snap-traps during the course of pest eradication either in or around residences. Most other small mammals were taken either alive or dead by members of field biology classes from Avila College, Kansas City, Missouri. All bats were obtained as donations from the public to the Animal Care Unit at the University of Kansas Medical Center. Bats were bled from the retro-orbital sinus while under ether anesthesia,

TABLE 1. Prevalence of antibody titers determined by the dye test from sera of wild mammals from Missouri and East Central Kansas.

Mammal	Number tested	Positive titer at <1:8	Positive titer at ≥1:8	Prevalence at ≥1:8 (%)
Insectivores				
Bats	4	0	0	0
Totals	4	0	0	0
Herbivores				
Mice and rats ^a	37	1	1 ^b	3
Squirrels ^c	11	0	2 ^d	18
Cottontail rabbit	12	1	2	17
Muskrat	42	1	7	17
Beaver	14	0	1	7
White-tailed deer	1	0	0	0
Totals	117	3	13	11
Omnivores				
Opossum	38	1	5	13
Raccoon	52	7	7	13
Striped skunk	4	0	2	50
Totals	94	8	14	15
Carnivores				
Coyote	13	0	8	62
Red fox	10	0	9	90
Gray fox	4	0	1	25
Bobcat	2	0	1	50
Mink	29	2	19	66
Totals	58	2	38	66

^a Number includes 17 house mice, 15 *Peromyscus* spp., four Norway rats, and one cotton rat.

^b One woodland white-footed mouse (*Peromyscus leucopus*) had a titer of 1:8.

^c Number includes five gray squirrels, five fox squirrels, and one thirteen-lined ground squirrel.

^d Two gray squirrels had a titer of 1:16 and 1:2,000, respectively.

and were subsequently released back to the wild. Most serum samples from larger mammals, either killed during the legal trapping and hunting seasons in the state of Missouri or salvaged from roadways after being killed by automobiles, were taken from clotted ventricular heart blood.

Most mammals bled for this study came from either Jackson (39°07'N, 94°15'W) or Johnson (38°47'N, 94°03'W) Counties, Missouri. All bats came from Wyandotte County, Kansas (39°03'N, 94°37'W). Some of the rodents also came from Camden (38°09'N, 92°59'W) and Miller (38°08'N, 92°35'W) Counties, Missouri, and Johnson (39°02'N, 94°38'W) and Miami (38°25'N, 94°47'W) Counties, Kansas. Two-hundred-sev-

enty-three sera were tested for antibodies against *Toxoplasma gondii* using the Sabin-Feldman dye test as modified by Frenkel and Jacobs (1958). The degree of hemolysis of blood varied considerably, as did the volume retrieved from each mammal, but the highest minimal dilution accepted for testing was 1:16, and most sera were tested with minimal dilutions of 1:4 or 1:2. All titers testing greater than 1:2 are reported, although the meaning of a low titer in respect to chronic infection is undetermined.

In 32 cases where a positive titer was detected, brain tissue that had been kept refrigerated was ground with alundum and injected subcutaneously into small groups of mice. After 3 wk these mice were bled from the retro-orbital sinus while under ether anesthesia, and the sera were run in the dye test. In one instance tissues from an opossum were fed to a cat in an attempt to recover oocysts of *Toxoplasma gondii*.

RESULTS

Antibodies to *Toxoplasma gondii* were found in 65 (24%) of 273 mammals tested (Table 1). Antibodies were most prevalent in carnivores (66%) and successively less common in omnivores (15%), herbivores (11%) and insectivores (0%). From 32 serologically positive wild mammals, *Toxoplasma gondii* was isolated from only six: one of two gray squirrels with a titer of 1:2,000 (other titer, 1:16), from a beaver with a titer of 1:4,000, from one of three opossums with a titer of 1:128 (range of titers, 1:8 to 1:128), from one of four red foxes with a titer of 1:256 (range of titers, 1:64 to 1:512), and from two of eight mink both with titers of 1:1,000. The range of titers was 1:8 to 1:2,000. For the opossum, tissues were fed to a cat and oocysts of *Toxoplasma* were subsequently recovered. In addition, no isolations resulted from subinoculation of brain tissue from two house mice (titers, 1:6, 1:8), one rabbit (titer of 1:16), five muskrats (range of titers, 1:8 to 1:16), three raccoons (range of titers, 1:4 to 1:16), and three coyotes (range of titers, 1:32 to 1:64). Successful isolation was associated with high titers ≥ 1:128, but high titers did not assure isolation from brain tissue, as subinoculations from six mammals with titers ≥ 1:128 did not result in antibody production of recipient mice.

DISCUSSION

We review here the prevalence of antibody to *Toxoplasma* in wild mammals of the United States (Table 2). All of the studies necessarily represent a small sampling of the total populations, with ages of the surveyed animals unknown and, in some cases, with different serological tests and different interpretations of threshold titers. However, we wish to focus on the results of these studies, to look for patterns, and to formulate working hypotheses that could be tested by further studies. For this reason we analyzed the data on the basis of biological and ecological considerations.

We first regrouped the published data according to a similar interpretation of food categories as arranged in this paper; these were based upon food habits as presented by Schwartz and Schwartz (1981). For example, in the paper by Marchiondo et al. (1976), observations were reported on 63 herbivores, 103 omnivores, and 279 carnivores. For Table 2, 87 coyotes and 10 foxes, originally classified by Marchiondo et al. (1976) as omnivores, were moved to the category of carnivores. This was done because, based on the stomach analyses of 770 coyotes, 1,006 red fox, and 305 gray fox, diets consisted primarily of flesh of mammals or fowl (96, 95, and 89%, respectively), with rabbits and rodents forming the bulk of the food items in all three species (Schwartz and Schwartz, 1981). The 233 dogs in that paper (Marchiondo et al., 1976) were omitted as we did not consider them to be wildlife and, as they don't shed oocysts, it is doubtful that they significantly affect the life cycle of *Toxoplasma gondii*. If animals in other reports were similarly disparate from the categorization in this study, they also were re-categorized.

The prevalences found in our study were most similar to what was found in Georgia (USA). Surprisingly, data in Kansas, Iowa, and Illinois (USA) were quite different from our data in the adjacent state of Missouri. Most specimens from Missouri were

collected from oak-hickory (*Quercus* spp. and *Carya* spp.) woodlands; similarly, Georgia lies within the deciduous woodland biome. Both areas are characterized by forest canopies resulting in shade and relatively high humidity. In contrast, Kansas, Iowa, and Illinois contain extensive elements of the temperate grassland biotic community, characterized by less shade, less rain, higher rates of evaporation, and therefore more desiccation. Thus we propose that similar biotypes or soil types might result in similar prevalences among the various consumer categories of wildlife.

We speculate, because of cyst formation in nervous and muscle tissue, that carnivores and omnivores, over the course of time, are more likely to be exposed to *Toxoplasma gondii* than herbivores. It is probably a far less common event for an herbivore to ingest an oocyst along with soil or water than for an omnivore or carnivore to ingest cyst-infected flesh from prey or carrion. Based on the average prevalence of antibodies (Table 2), about one of every ten meals (0 to 21%, mean = 9%) consisting of raw herbivore flesh (less commonly if only small rodents are eaten [Table 1]) will probably contain infectious tissue cysts of *Toxoplasma gondii*.

Omnivorism should result in an intermediate prevalence rate, between herbivores and carnivores, because a larger portion of their diet would consist of plant material, uncontaminated with tissue cysts implicated in the carnivore cycle of *Toxoplasma gondii*. Our study supports this hypothesis with the prevalence of antibody in carnivores > omnivores > herbivores, as do studies of antibody prevalence in wild mammals from California, Iowa, Maryland, Georgia, and Florida (USA) (Table 2). Mean prevalences for carnivore, omnivores, and herbivores in all studies were 52, 21, and 9%, respectively. Obvious exceptions to this trend were the omnivores studied in Illinois with an extremely low prevalence of antibody (0%), omnivores studied in Kansas having a high prev-

TABLE 2. Prevalence of antibodies to *Toxoplasma* reported in domestic cats and wild mammals of the United States, showing type of test used to determine them, and the lowest reciprocal titer considered to be positive (threshold titer). Number of samples is listed in parentheses.

	State										
	California ^a	New Mexico ^b	Kansas ^c	Missouri/ Kansas ^d	Iowa ^e	Iowa ^f	Illinois ^g	Maryland ^h	Georgia ⁱ	Florida ^j	Mean %
Domestic cats	38% (47)	8% (91)	nd ^k	16% (510) ^l	58% (157) ^l	42% (74)	13% (8)	14% (650) ^m	14% (7) ⁿ	24% (67) ^o	25%
Herbivores	2% (935)	21% (63)	13% (464)	11% (117)	0% (23)	0.3% (618)	11% (9)	19% (119)	11% (44)	3% (754)	9%
Omnivores	34% (61)	33% (6)	52% (52)	15% (94)	6% (17)	13% (55)	0% (38)	23% (77)	20% (148)	15% (879)	21%
Carnivores	49% (171)	29% (143)	50% (2)	66% (58)	50% (6)	nd	nd	nd	72% (64)	46% (13)	51%
Type of test	IHA ^p	DT ^q	MAT ^r	DT	DT	MAT	IHA	DT, IHA ^m	DT	IHA	IHA
Threshold titer	64	32	25	8, ^d 2 ⁱ	16, ^c 2 ⁱ	32	64	16, ^b 32 ^m	16, ⁱ 32 ⁿ	64	64

^a California, Franti et al. (1976).
^b New Mexico, Marchiondo et al. (1976).
^c Kansas, Brillhart et al. (1994).
^d Missouri and Kansas, This study.
^e Iowa, McCulloch et al. (1967).
^f Iowa, Smith et al. (1992).
^g Illinois, Paine (1969).
^h Maryland, Jacobs and Stanley (1962).
ⁱ Georgia, Walton and Walls (1964).
^j Florida, Burrige et al. (1979).
^k nd = not done.
^l Iowa, Dubey (1973).
^m Maryland, Childs and Seegar (1986).
ⁿ Georgia, Dubey et al. (1981).
^o Florida, Burrige and Hennemann (1980).
^p IHA = indirect hemagglutination test.
^q DT = dye test.
^r MAT = modified agglutination test.

alence of antibody (52%), and the carnivores studied in New Mexico having the lowest prevalence of antibody (29%) observed in that group across the United States. Domestic cats from that state also had an unusually low prevalence of antibody (8%). This was especially surprising since the prevalences observed in herbivores (21%) and omnivores (33%) from New Mexico were as high or higher for these respective groups than observed anywhere else, except for omnivores in Kansas. Since survival of oocysts would not likely be high in the relatively arid southwestern U.S., possibly cannibalism or increased carnivorousness by omnivores and herbivores may have been responsible for the apparently high prevalence in these two groups. Such dietary modifications could be associated with the increased rigors of the arid environment. Vertical congenital transmission is possible, but its frequency in wild mammals is unknown, and a mechanism whereby it might be increased in the arid southwestern U.S. is unknown. The possibility of lateral transmission by blood sucking micropredators has not been established.

The recent description of a single clonal lineage for mouse-virulent *Toxoplasma gondii* (Sibley and Boothroyd, 1992) could be explained by a virtually asexual transmission pattern, as could happen by exclusive cannibalism, or by vertical transmission in nature, or by prolonged mouse to mouse passage in the laboratory. Although the latter explanation is more plausible, as suggested by the phraseology of: "testing as soon as possible after original isolation" (Sibley and Boothroyd, 1992), an exclusively cannibalistic transmission, first suggested by Weinman and Chandler (1954) and demonstrated vertical transmission within certain hosts (Beverley, 1959) should be seriously reconsidered. However, we disagree with the interpretation of data by Webster (1994) that antibody titers from rats, not exposed to cats, are evidence for vertical transmission. The antibody titers of the rats maintained with-

out contact from cats were low when compared with antibody titers regularly observed in infected laboratory rats, and present in his rats in contact with cats. While we do not know the causes of low titers, they often were non-specific based on negative results as found in wild animals examined by subinoculation, and could indicate *Hammondia hammondi* infection, which serologically cross-reacts with *T. gondii* (Frenkel and Dubey, 1975). In all of the studies reviewed, infections from *T. gondii* and *H. hammondi* could not be differentiated serologically. To do so, one would have to biotest all the tissues. *Toxoplasma* sp. can be subinoculated from one intermediate host to another, whereas *Hammondia* sp. would only infect cats, the definitive host. There is need for comparative serologic studies, using antigens of both *T. gondii* and *H. hammondi*.

It is interesting to note that in all cases except Iowa (McCulloch et al., 1967), where data are available, domestic cats had a lower prevalence of antibodies than did wild carnivores. There are at least two possible contributing factors. First, in most of the studies pertaining to cats, at least some, if not all, were domiciled, obtaining a large portion of their food either cooked or commercially prepared, and thereby inactivating any cysts of *Toxoplasma gondii* that might have contaminated the meat. Secondly, in these cat studies, some sera were obtained from sampling litters of young kittens, which were less likely to have eaten an infectious meal than an older cat. In the case of the Kansas City study (Dubey, 1973), if only cats greater than six months of age were considered, the prevalence increased from 16 to 38% ($n = 128$). In that same study, the "Iowa" cats with a prevalence of 58% were characterized as stray cats from Iowa and northern Missouri, and probably included a fairly large number of cats obtained from rural areas. Smith et al. (1992) also recorded a high prevalence in Iowa cats (42%) and a higher prevalence in adult cats (49%). Therefore, in Iowa, the behavioral differences between the typical

“barn cat” and their feral or truly wild counterparts probably were less distinct; that is, rodents and ground feeding birds probably formed a substantial part of their diets. Thus, the high prevalences of antibodies of 58% ($n = 157$) and 42% ($n = 74$), respectively, were similar to many values observed in the carnivore category. As yet we know of no large sampling of truly feral cats, but prevalences in bobcats averaged 62% (range, 44 to 73%) (Walton and Walls, 1964; Franti et al., 1976; Marchiondo et al., 1976), and the prevalence in feral cats from any particular area would probably approximate or exceed those found in the carnivore category. Also, we have insufficient data on prevalences in ground-feeding birds, because most of the surveys employed serologic tests which are not reliable indicators of infection in birds (Frenkel, 1981).

It is also interesting to note in this study the prevalences of antibodies in rabbits (two of 12), squirrels (two of 11), and muskrats (seven of 42) were similar (Table 1), as these three species are all medium sized primary consumers, forming a basal level of the predation pyramid. Their habitat preferences are roughly correlated with grassland, woodland, and marshland communities, respectively. We speculate that predators using these relatively larger prey species, and especially felids, are subject to similar frequencies of infectivity regardless of the habitat the predators utilize. Alternatively, felids may use each of these habitats in such a way that dispersion of infectious oocysts is similar in all three. Also noteworthy, in this study, is the very high prevalence in the red fox (nine of 10), and high prevalences in both of the mustelids—the striped skunk (two of four) and the mink (19 of 29). These animals, along with the coyote (eight of 13), the bobcat (one of two), and the gray fox (one of four), undoubtedly illustrate the cumulative efficacy of the predator-prey cycle of *Toxoplasma gondii* experienced by the secondary and tertiary consumers forming the apex of the predation pyramid.

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