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Source: Journal of Wildlife Diseases, 16(2) : 189-194

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-16.2.189>

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## PREVALENCE OF ANTIBODIES TO *Toxoplasma gondii* IN STRIPED SKUNKS FROM SASKATCHEWAN AND ALBERTA

D. B. SCHOWALTER,<sup>1</sup> J. O. IVERSEN,<sup>2</sup> L. C. CORNER<sup>2</sup> and J. R. GUNSON<sup>1</sup>

**Abstract:** Fifteen percent (81 of 542) of striped skunks, *Mephitis mephitis*, collected in the prairie of Alberta and Saskatchewan during 1974 to 1978, were positive for antibodies against *Toxoplasma gondii*. The seropositive rates varied from 8% (6 of 78) for skunks less than six months of age to 47% (9 of 19) in animals three or more years old. Spring and summer transmission was indicated by a preponderance of high titres ( $\geq 1:1024$ ) in seropositive skunks collected April through September (22 of 40, 55%) compared to seropositives collected October through March (10 of 38, 26%) ( $P = < 0.05$ ). Prevalence was significantly greater among skunks collected in the relatively humid parkland (63 of 286, 22%) than in the arid prairie grassland biome (20 of 255, 8%) ( $P = < 0.01$ ). The results indicate that *T. gondii* is focally enzootic in Alberta and Saskatchewan.

### INTRODUCTION

Studies of human toxoplasmosis have indicated a generally higher prevalence of infection in warmer and humid equatorial regions than at higher latitudes or in very dry areas.<sup>25</sup> The dry climate and temperature extremes, characteristic of the Canadian prairies, could result in some geographic restriction of the transmission of *Toxoplasma gondii*.<sup>8,21,24,27</sup> In one serologic survey in Saskatchewan, a very low prevalence was found in domestic cats.<sup>11</sup> However, a subsequent Saskatchewan survey indicated a somewhat higher rate in cats and established the occurrence of antibodies in sheep and swine in the agricultural areas of the province.<sup>12</sup>

The collection of sera from a large number of striped skunks (*Mephitis mephitis*) in Alberta and adjacent Saskatchewan during the course of other investigations provided an opportunity to test for prevalence of *T. gondii* antibodies among a resident wildlife

species of the Canadian prairies. This paper describes the pattern of distribution of *T. gondii* near the northern limits of agriculture in North America.

### MATERIALS AND METHODS

Striped skunks were collected during a program of rabies monitoring in wildlife populations in Alberta and adjacent Saskatchewan. The methods of collection have been described elsewhere.<sup>10,11</sup> Animals were collected in the prairie grassland and parkland biomes. Land use varied from predominantly livestock grazing to predominantly cereal crops in other areas. Some areas of collection in southern Alberta were partially irrigated. Sera from 542 striped skunks were collected from late 1974 to early 1978. The sera were stored at -10 to -20 C until tested.

All sera were inactivated at 56 C for 30 min prior to testing. Each serum was tested for antibodies against *T. gondii*

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using commercial antigen <sup>□</sup> in the indirect hemagglutination (IHA) test by the microtitre system.<sup>15</sup> Serum samples with a 2+ reaction at a dilution of 1:64 or greater were considered positive for *T. gondii* hemagglutinating antibodies if there was no greater than a  $\pm$  reaction with unsensitized cells.<sup>6,14,15,18</sup> Quantification of titres by two-fold serial dilution was carried out on the positive sera. Various attack rates were compared statistically with the Critical Ratio (C.R.).<sup>20</sup>

## RESULTS

Of 542 skunk sera tested, 15% (81) were seropositive for *T. gondii* hemagglutinating antibodies. The seropositive rates for males and females were comparable.

Although seropositive skunks were collected throughout the prairie grassland and parkland biomes in both provinces, the seropositive rate was significantly higher (C.R. = <4.5; P = <0.01) in the parkland region (63 of 286, 22%) than in the more southerly prairie grasslands (20 of 255, 8%) (Figure 1).

For 230 skunks of known age from the parkland, the seropositive rates increased with the calculated age of the animals. Skunks less than 6 months old had a significantly lower rate (6 of 78, 8%) than older skunks (41 of 152, 27%) (C.R. = 3.4; P = <0.01). Prevalence by age group was 6 of 32 (19%) skunks 6 to 12 months old; 26 of 101 (26%) skunks 13 to 36 months old; and 9 of 19 (47%) skunks older than 36 months. The percentage of all seropositive skunks of known age with titres of 1:1024 or greater was significantly lower (C.R. = 2.2; P = <0.05) for skunks less than 6 months old (3 of 17, 18%) than older skunks (23 of 48, 48%).

The percentage of all seropositive skunks with titres of 1:1024 or greater was significantly higher (C.R. = 2.36; P = <0.05) for animals collected during the spring and summer months of April through September, 55% (22 of 40 seropositive skunks), than for animals collected during the fall and winter months, 26% (10 of 38 seropositive skunks) (Table 1). The geometric mean titre was 1:676 for seropositive skunks collected during April through September, whereas it was 1:338 for seropositives collected during October through March.

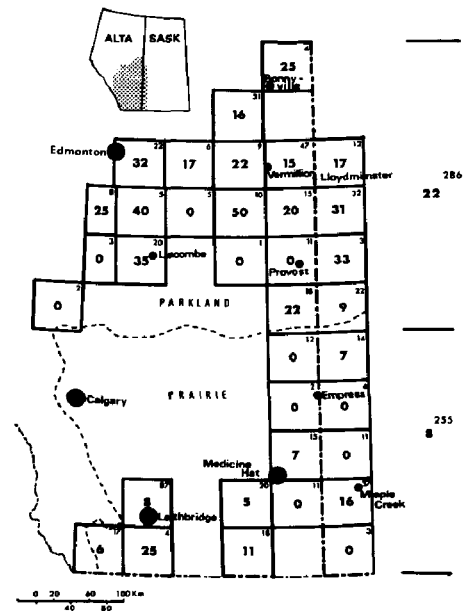


FIGURE 1. Map of Alberta and Saskatchewan showing percentage of *T. gondii* seropositive striped skunks (large bold-face numbers = % and smaller numbers = sample size per area). Numbers at the right of map represent summary for parkland (top) and prairie grassland (bottom) biomes. Broken line indicates boundary between biomes.

<sup>□</sup> International Biological Laboratory, Inc., Rockville, Maryland 20852, USA.

TABLE 1. Distribution of antibody titres against *Toxoplasma gondii* among seropositive striped skunks by month and by season; Alberta and Saskatchewan; 1974-1978.

Month Collected	Reciprocal of titre							Percentage of seropositives with titres of $\geq 1:1024$
	64	128	256	512	1024	2048	$\geq 4096$	
<b>SPRING AND SUMMER</b>								
April			1	1	4			
May	2	2	1	1	3	2	1	22/40 (55%)
June					1		1	
July		1		2	2		1	
August			1	3	3	1	2	
September		2	1		1			
<b>FALL AND WINTER</b>								
October				2			1	
November		2	2	1	1			10/38 (26%)
December	5	2	2	4	3	2		
January					1			
February			3	1				
March	2		1	1	2			

## DISCUSSION

The results presented here and in other serologic surveys have demonstrated that *T. gondii* infection occurs in widely separated populations of striped skunks.<sup>3,16,18,26</sup> In extensive serologic surveys for *T. gondii* in California, striped skunks were among the more frequently seropositive species.<sup>1,5,18,22</sup> In Ontario, the infection was enzootic in certain skunk populations.<sup>16</sup> Therefore, the striped skunk, a relatively abundant and easily obtained animal throughout its range, may be a potential indicator species for the level of enzooticity of *T. gondii* in a given geographic area. The result of this study supports the concept

that the agent is enzootic in Alberta<sup>21</sup> and Saskatchewan.<sup>12</sup>

Domestic and feral cats are the definitive hosts in the life cycle of *T. gondii*<sup>2,4,7,13</sup> and no other animals have been found to shed oocysts. Feral house cats are important hosts for *T. gondii* and appear to be responsible for reinforcing the infection in wildlife areas.<sup>18</sup> In prairie grassland and parkland biomes of the study area, feral cats were considered a major nuisance by skunk collectors and frequently were captured in traps, preventing the capture of skunks. More significant were regular observations of joint denning of cats and striped skunks and cats were occasionally gassed with

skunks in the latter's communal dens (pers. commun., Per Andersen, Dept. of Biology, University of Calgary, Alberta). This information indicates a broad similarity of habitat use by both animals. The prevalence of toxoplasmosis among rural-dwelling and feral cats in Saskatchewan (viz., 28%)<sup>12</sup> provides further circumstantial evidence of skunk infection via soilborne oocysts from the cats or from eating small vertebrates (e.g., mice) that had been infected by cats.

In Alberta and Saskatchewan, a pronounced geographic difference in the relative abundance of *T. gondii* in skunks was evident between the prairie grassland and parkland biomes. The oocysts shed in cat feces may remain viable for long periods but are inactivated by dryness.<sup>8</sup> The prairie grassland portion of the study area is characterized by potential annual evapotranspiration which considerably exceeds average annual rainfall.<sup>9,17</sup> The dryness caused by the "Chinook" winds of the prairies may be particularly critical in the winter. The parkland biome, the region characterized by higher seropositive rates, is characterized by greater rain and snowfall. The more humid conditions of the parkland favor the characteristic stands of aspen that distinguish the parkland from the prairie grassland biome. The higher humidity of the parklands would enhance survival of oocysts of *T. gondii* and increase the opportunity of transmission.<sup>8,27</sup>

Rolling topography<sup>9,17</sup> also may be involved in the enhancement of transmission. In such areas, culverts are

often placed under roads to facilitate drainage. The culverts may remain moist and cool throughout the summer while surrounding open fields may have dried. In addition to serving as "environmental sheaths", culverts represent convenient passageways for movement of skunks and cats. The highest prevalence of antibodies in skunks in the southern aspect of the study area occurred near Maple Creek and the adjacent Cypress Hills, an unglaciated relic area where elevation and topography promote greater precipitation effectiveness.<sup>17</sup>

For the Canadian prairies, we postulate that transmission of *T. gondii* to skunks reaches a peak during the summer season. The hypothesis is based on: 1) the greater percentage of higher titres during the months of April through September (viz., the prairie spring and summer); and 2) the annual cycle of the skunk. Although the temporal significance of the titres of the IHA test for *T. gondii* in skunks is not known, interpretations from studies in other species have indicated a high titre ( $\geq 1:1024$ ) represents an acute infection.<sup>19</sup> Skunks remain relatively sedentary and inactive throughout the winter during the denning period.<sup>10,23</sup> Striped skunks apparently breed in February or March throughout most of their range.<sup>23</sup> On the Canadian prairies, skunks become increasingly active and mobile during April. The majority of young are born during May and the young of the year represent an influx of non-immunes into the population. Increased movement, scavenging for food, and the recruitment of non-immunes would enhance *T. gondii* transmission to skunks during the summer.

### Acknowledgements

Special thanks are extended to Dr. H.N. Vance, Director of Animal Health Division, Government of Alberta, for financial support.

The laboratory assistance of Roberta Saunders, Audrey Robertson, and Morreen Iversen and the field assistance of Bill Wynnyk and William Johnson is gratefully acknowledged.

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*Received for publication 16 May 1979*

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