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The susceptibility of five species of wild animals to experimental infection with *Leptospira grippotyphosa*

JAMES R. REILLY

Abstract

Five species of wild animals including opossums, *Didelphis marsupialis,* striped skunks, *Mephitis mephitis,* red, *Vulpes vulpes,* and gray foxes, *Urocyon cinereoargenteus* and raccoons, *Procyon lotor,* were inoculated intraperitoneally with varying numbers of *Leptospira grippotyphosa,* organisms. Clinical signs were not detected, however, leptospiremia, leptospiruria and antibodies for the homologous organism, were demonstrated. Lesions attributed to leptospirosis were observed in liver and kidney tissue of infected animals. Infections were demonstrated in 5 of 9 opossums, 3 of 9 striped skunks, 3 of 9 red foxes, none of the gray foxes, and 9 of 9 raccoons. Therefore, it would appear that under conditions of this experiment that raccoons were most, opossums moderately, and skunks and red foxes least susceptible; gray foxes were not susceptible.

Introduction

The susceptibility of certain myomorph rodents has been investigated in the search for an experimental animal which might be used for the isolation of leptospires.[9,15] However, little attention has been focused upon the susceptibility of other wildlife species which may serve as nidi in the epizootiology of leptospirosis. It has been shown that the raccoon could not be experimentally infected either by feeding leptospiremic or leptospiruric mice carrying *Leptospira grippotyphosa*[12] or by forced ingestion of this organism in enteric coated capsules, designed to open in the duodenum.[11] Since opossums, striped skunks, and red foxes could be infected by the enteric route, the present investigation was undertaken to ascertain the relative susceptibility of opossums, striped skunks, red and gray foxes and raccoons to intraperitoneal inoculation with *L. grippotyphosa.*

Materials and Methods

The culture used to infect these animals was a 23 day subculture of *L. grippotyphosa* B699 recovered from passage in a Swiss strain of white mice. This strain was originally isolated from a cow which had aborted.[5] This culture was grown in BAP80-5FU medium, bovine albumin and polysorbate 80 medium[2] plus 5-fluorourcil (200 mg/ml) as a selective agent to inhibit growth of contaminating

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organisms.[6] The inoculum had a light transmittance of 36.5% at a wavelength of 600 mu (blue sensitive photo tube[1]) approximating 2.0 x 10^8 organisms per milliliter. Suitable dilutions were made in BAP80-5FU medium so that each milliliter would contain approximately 100, 1,000 or 10,000 organisms. Three each of opossums, striped skunks, red foxes and raccoons were given intraperitoneal injections of each dilution. Only one gray fox received each dilution due to the inavailability of this species.

Nine opossums were used as experimental animals, 5 adult females, 2 adult males and 2 immature males. All striped skunks, 5 females and 4 males, were adults. Seven of the red foxes were immature animals, 5 males and 2 females. In addition there was 1 adult red fox of each sex. All gray foxes were adults; 2 females and 1 male. With the exception of one immature male, all the raccoons used in the experiment were adults; 7 females and 1 male.

To determine if these animals which were taken from the wild had been previously infected, 2 blood samples were collected at intervals of not less than 3 weeks. Serums were prepared and tested in the microscopic agglutination test against 8 antigens, including *L. canicola, L. grippotyphosa, L. icterohaemorrhagiae, L. sejroe, L. pomona, L. ballum, L. hyos and L. autumnalis.* Urine collected from the animals by bladder tap[5] was cultured to minimize the possibility of including sero-negative carriers in the experiment.

An anesthetic solution containing phencyclidine HCL[2] (25 mg/ml) was used to facilitate handling of animals.[11] Anesthetic was not required for foxes because their temperament permitted them to be handled during sampling without danger to the animal or operator. Blood samples were collected and cultured, undiluted or diluted, in BAP80-5FU medium on postinoculation days (PID) 4, 5, 6 and 7. Serums collected from blood samples taken on PID 21, 28 and 42 were evaluated in the microscopic agglutination test against the homologous antigen. Urine and renal tissues were cultured when the animals were killed on PID 42. Kidney and liver tissues were fixed in 10% neutral formalin, and embedded in Paraplast[3]. Five micron sections were stained with Harris' hematoxylin and counter stained with Eosin y. Additional kidney tissue was impregnated with silver according to Levaditi's method.

Results

Infection was confirmed either by culture or serology in 5 of 9 opossums, 3 of 9 striped skunks (Table 1), 3 of 9 red foxes and 9 of 9 raccoons (Table 2). Three gray foxes were not susceptible to the challenge of 100, 1,000 or 10,000 *L. grippotyphosa* organisms. The age of the animals did not appear to be a factor in susceptibility.

Opossum: Leptospiremia was demonstrated on PID 6 in 1 opossum which had been inoculated with 100 organisms and on PID 4 and 5 in 2 others which had received 10,000 organisms. Homologous agglutinins were detected at titers of 1:100 in one animal of each group on PID 28, but they could not be demonstrated in the serum of the latter opossum which had a leptospiremia on PID 4. Renal shedding was demonstrated on PID 42 by culture of urine or kidney triturates from those animals which previously had positive hemocultures. Histopathologic hepatic changes consisted of numerous foci of acute inflammation in the region of portal triads and the central vein. Renal lesions consisted of a mild glomerulitis with an occasional focus of interstitial nephritis. Leptospires were not observed in kidney tissue.

Striped skunk: The organism was demonstrated, on PID 4, in 1 skunk which had been injected with 1,000 organisms and on PID 6 in 1 which had received 10,000 organisms. Renal shedding was demonstrated on PID 42 in 2

[1] Spectronic 20, Bausch and Lomb, Rochester, N.Y.
[2] Sernyl, Parke, Davis and Co., Detroit, Mich.
[3] Aloe Scientific, St. Louis, Mo.

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skunks which had been injected with 10,000 organisms; 1 of these animals had manifested a leptospiremia on PID 6. Hepatic changes consisted of congestion, marked degenerative change, extensive bile duct hyperplasia and bile stasis. The most prominent histopathologic change in skunk kidneys consisted of severe glomerulitis characterized by atropic glomeruli with complete loss of the capillary bed, thickening of Bowman's capsule and a chronic focal interstitial nephritis. Leptospires were not observed in kidney sections.

Fox: The organism was cultured from the blood of 1 red fox on PID 4, 5 and 6, which had been inoculated with 100 organisms. Leptospiremias were demonstrated by hemaculture on PID 6 in 1 and on PID 4 and 6 in 2 other red foxes which had been inoculated with 1,000 organisms. Agglutinins for the infecting organism were detected in sera of 2 red foxes from each of the 3 groups i.e. those receiving 100, 1,000 and 10,000 organisms. Leptospires were cultured on PID 42 from the red fox of the first group which had a positive blood culture

TABLE 1. *The susceptibility of Opossums and Striped Skunks to intraperitoneal inoculation with varying numbers of* L. grippotyphosa.

		Demonstration of Infection								
Species and		Culture Blood				Kidney	Serology Reciprocal of titer			
challenge	Age	PID*	4	5	6	7	42	21	28	42
Opossum										
100	A		—	—	+	—	+	—	1100**	100
	A		—	K†						
	A		—	—	—	—	—	—	—	—
1,000	A		—	—	—	—	+	—	—	—
	J		—	—	—	—	—	1100	—	—
	J		—	—	—	—	—	—	—	—
10,000	A		+	—	—	—	—	—	—	—
	A		—	—	—	—	+	100	100	—
	A		—	+	K					
Total			1	1	1		3	2	2	1
Skunk										
100	A		—	—	—	—	—	1100	—	—
	A		—	—	—	—	—	—	—	—
	A		—	—	—	—	—	—	—	—
1,000	A		—	—	—	—	—	—	—	—
	A		—	—	+	—	—	100	100	—
	A		—	—	—	—	—	—	—	—
10,000	A		—	—	—	—	—	—	—	—
	A		+	—	—	—	+	1100	1100	—
	A		—	—	—	—	+	—	—	—
Total			1		1		2	3	2	

* PID = Post infection day.
** Incomplete agglutination.
† Killed by anesthesia.

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on PID 4, 5 and 6. Pathologic changes in the liver consisted of patchy areas of fibrosis and chronic focal centrolobular hepatitis. Kidney changes included numerous foci of chronic interstitial nephritis. Leptospires were not observed in sections of the kidney.

Gray Fox: Infection could not be demonstrated in the 3 gray foxes which had received intraperitoneal inoculations of 100, 1,000 and 10,000 organisms respectively. In addition histopathologic changes consistent with leptospirosis were not observed in tissue section.

Raccoon: This species was the most susceptible to intraperitoneal inoculation with *L. grippotyphosa*. Infections were produced in all 9 individuals regardless of the number of organisms injected. Leptospiremia was demonstrated in 9 raccoons at least once between PID 4 and 7. Renal shedding was demonstrated in 5 animals by culture of urine or kidney triturates, on PID 42, but only in 1 raccoon which had been infected with 10,000 organisms. Serologic response to the homologous organism was detected on PID 21 in 6 animals and a maximum

TABLE 2. *The susceptibility of Red Foxes and Raccoons to intraperitoneal inoculation with varying numbers of* L. grippotyphosa.

Species and challenge	Age	Demonstration of Infection								
		Culture Blood				Kidney	Serology Reciprocal of titer		
		PID*	4	5	6	7	42	21	28	42
Red fox										
100	J		—	—	—	—	—	100	100	—
	J		+	+	+	—	+	100	1000	100
	A		—	—	—	—	—	—	—	—
1,000	A		—	—	—	—	—	—	—	—
	J		—	—	+	—	—	100	1100**	—
	J		+	—	+	—	—	100	1100	—
10,000	J		—	—	—	—	—	100	1100	—
	J		—	—	—	—	—	—	—	—
	J		—	—	—	—	--	100	1100	—
Total			2	1	3		1	6	6	1
Raccoon										
100	A		—	—	+	—	+	100	100	100
	A		—	+	—	+	+	—	100	—
	A		—	—	—	+		100	100K†	
1,000	A		—	—	+	+	+	100	100	100
	A		—	+	+	+	+	1100	100	100
	A		+	+	+	K				
10,000	J		+	+	+	+	+	100	100	100
	A		+	+	+	+	—	100	1000	11000
	A		+	—	+	+	—	—	—	—
Total			4	5	7	7	5	6	7	5

* PID = Post infection day.
** Incomplete agglutination.
† Killed by anesthesia.

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titer of 1:1,000 was attained on PID 28 in 1 animal which had received a challenge of 10,000 organisms. Hepatic changes consisted of marked congestion, mild lymphocytic infiltration, bile stasis and bile duct hyperplasia. Focal to extensive diffuse chronic interstitial nephritis and a mild glomerulitis characterized by a thickening of Bowman's capsule were the renal changes. Forms suggestive of leptospires were observed in kidney sections from one animal, an immature male which had received 10,000 organisms.

Discussion

Although some individuals of all species, with the exception of gray foxes, responded to the challenge, apparent clinical disease was not produced. In this respect it may be that an insufficient number of organisms were inoculated to produce an overwhelming infection or pathogenicity had been reduced by cultural passage. However, a descending order of susceptibility was established, i.e. raccoon, opossum, striped skunk and red fox.

The ready susceptibility of the raccoon may indicate that it should be considered a reservoir or maintaining host which provides a particular condition of biologic empathy for *L. grippotyphosa;* the opossum, striped skunk and red fox would be transient or incidental hosts.[1] Roth et al.[13] reported that a maintaining host is easily infected and becomes an asymptomatic carrier with a high leptospiral infection balancing a high serologic reactor rate. This was true of raccoons in this experiment, since the organism was recovered 9 times and there were 7 serologic reactors; a host type index of 9-7. On the other hand the red fox may be considered an incidental or transitory host, one producing a high incidence of serologic reactors and a somewhat lesser leptospiral infection rate; a host type index of 3-6.

The opossum and the striped skunk should probably be included in this category although there is insufficient experimental evidence for placing the opossum here. However, the infection produced a severe glomerulitis in the skunk which was characterized by atropic glomeruli, complete loss of capillary tufts and thickening of Bowman's capsule. The host type indices were 5-3 and 3-3, respectively.

It has been reported that sciuromorph and myomorph rodents are maintaining hosts for *L. grippotyphosa*,[7,10,14] and that these rodents can be the source of infection in food chain transmission to opossums, striped skunks and red foxes. However, previous investigations[11,12] have shown that raccoons could not be infected with *L. grippotyphosa* B699 either by feeding carrier mice or enteric coated capsules containing the organism. This may indicate that the interspecific predator-prey structure may not be involved in the transmission of this organism from rodents to the raccoon although the latter is highly susceptible. Thus it is possible that transmission of *L. grippotyphosa* among raccoons may be intraspecific and may be accomplished by genital contact[1,10] or by contact with urine-contaminated wet environments.

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