

LEPTOSPIRA ANTIBODIES DETECTED IN WILDLIFE IN THE USA AND THE US VIRGIN ISLANDS

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ABSTRACT: From 2011 to 2017, 4,534 serum samples from 13 wildlife species collected across the US and in one territory (US Virgin Islands) were tested for exposure to *Leptospira* serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona. Of 1,759 canids, 1,043 cervids, 23 small Indian mongooses (*Herpestes auropunctatus*), 1,704 raccoons (*Procyon lotor*), and five striped skunks (*Mephitis mephitis*), 27.0, 44.4, 30.4, 40.8, and 60%, respectively, were antibody positive for any of the six serovars. The most commonly detected serovars across all species were Bratislava and Grippotyphosa. Our results indicate that *Leptospira* titers are very common in a wide variety of wildlife species. These species may act as important reservoirs in the epidemiological cycle of the pathogen. Additional studies to determine the relationship between serologic evidence and shedding of the pathogen by wildlife are necessary to better understand the risk.

Key words: Canids, cervids, coyote, disease, *Leptospira*, leptospirosis, raccoon, white-tailed deer.

INTRODUCTION

As human populations continue to expand into more rural areas, the frequency of human-wildlife interactions increases along with the potential for transmission of various pathogens. This is true especially for diseases such as leptospirosis for which wildlife species are known reservoirs (Hartskeerl and Terpstra 1996). Leptospirosis is a bacterial disease with a worldwide distribution that can affect humans, livestock, pets, and wildlife (Sullivan 1974). Approximately 250 serovars of *Leptospira* exist (Evangelista and Coburn 2010), and certain leptospiral serotypes are known to be associated with specific hosts that serve as reservoirs of infection (Galton 1959). These maintenance hosts are often wildlife species that carry and shed the organism for long periods (Bolin 2003). Spirochetes from infected animals are secreted in the urine and

persist in the environment (Wobeser 2006). These leptospirae can contaminate water and moist soil (McKeever et al. 1958), which can lead to indirect transmission to incidental hosts (Levett 2001). The microagglutination test (MAT) is the most common serological test for antibody detection, but cross-reaction between serovars is a known limitation and can affect the interpretation of results (Bolin 1994). Antibodies against multiple serovars are often detected in infected animals, though the initially infecting serovar is assumed to be the one to which the animal develops the highest titer (Bolin 2003).

Transmission of *Leptospira* to livestock and humans often originates or is maintained by wildlife (Cirone et al. 1978). Infection in livestock can result in reproductive failure, causing significant financial impacts (Hartskeerl et al. 2011). Humans typically become infected either through occupational expo-

sure, via recreational activities, or from contact with urine of infected animals (either directly or by water or soil contaminated with urine; Adler and de la Peña Moctezuma 2010). Infection with *Leptospira* causes a wide spectrum of clinical disease in humans including mortality in extreme cases (Guerra 2009), but the disease is likely underdiagnosed since the symptoms are often nonspecific and similar to those of many other diseases (Meites et al. 2004).

Our objective was a national-level effort to estimate the antibody prevalence of *Leptospira* serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona in various wildlife species from across the US to better understand the potential risk to humans, pets, and livestock posed by exposure to wildlife.

METHODS

Sample collection

One of the objectives of the US Department of Agriculture's Wildlife Services is to mitigate conflicts between humans and wildlife. The National Wildlife Disease Program (a branch of Wildlife Services) opportunistically obtains blood samples from wildlife species that are removed as nuisance animals or trapped for active surveillance, and sera are archived for future testing. Wildlife species that are sampled as part of this program may include raccoons (*Procyon lotor*), skunks (family Mephitidae), small Indian mongooses (*Herpestes auropunctatus*), and various cervid and canid species.

All blood was collected postmortem via intracardiac puncture, and after clotting, the tubes were centrifuged ($125 \times G$), and sera were transferred to cryogenic vials. Sera were stored refrigerated (4 C) and shipped within 72 h on ice packs to National Wildlife Disease Program (Fort Collins, Colorado) and then stored at -80 C until testing. Collection site information (county, state, and GPS coordinates) were recorded for each sample.

Serology

All samples were tested at Colorado State University using methods described previously (Pedersen et al. 2015). Briefly, the MAT was used to detect antibodies to six *Leptospira* serovars: Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona, with a titer of

$\geq 1:100$ considered positive. These serovars were selected for their propensity to cause disease in humans, livestock, and pets (Acha and Szyfres 2001). The MAT results were reported as the endpoint dilution of serum where 50% agglutination of cells was observed (1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:>6400). Titers $> 1:6400$ were not measured to their endpoint since titers $\geq 1:800$ were considered evidence of recent or current infection, as has been reported previously (Chatfield et al. 2013; USDHHS 2013). The serovar in which agglutination was detected at the highest dilution was considered the reactive serogroup, which for some samples included multiple serovars.

Data analysis

Antibody prevalence and 95% confidence intervals were calculated with Microsoft Excel (Microsoft Corp., Redmond, Washington, USA). Maps were created using ArcGIS version 10.5 (ESRI, Redlands, California, USA).

RESULTS

We tested 4,534 sera from 13 different wildlife species collected in 46 states and the US Virgin Islands from March 2011 through March 2017 (Tables 1, 2 and Fig. 1). Antibodies to *Leptospira* were detected in 36.7% (Table 1). The majority of the samples were collected from coyotes (*Canis latrans*; $n=1,247$), raccoons ($n=1,704$), and white-tailed deer (*Odocoileus virginianus*; $n=1,029$) collected from across the US with widespread detection of antibody positives (Fig. 1). Antibody prevalence varied widely between species, but all six serovars were detected in species when at least 50 samples were available for testing (Tables 1, 3). Evidence of recent or active infection at the time of sampling was detected in white-tailed deer, arctic foxes (*Vulpes lagopus*), coyotes, red and gray foxes (*Vulpes vulpes* and *Urocyon cinereoargenteus*, respectively), gray wolves (*Canis lupus*), small Indian mongooses, raccoons, and striped skunks (*Mephitis mephitis*; Table 3). However, there were no apparent associations between species and serovars with evidence of current or recent infections (Table 3).

Bratislava was the most common serovar detected in white-tailed deer. Bratislava and

TABLE 1. Antibody prevalence with 95% confidence intervals of sera collected from various wildlife species in the USA and Virgin Islands from 2011 to 2017 and tested by microagglutination test for exposure to *Leptospira* serovars Bratislava, Canicola, Hardjo, Icterohaemorrhagiae, and Pomona. A titer of ≥ 100 was considered positive.

Common name	Species	n	% Positive (95% confidence interval)							
			Bratislava	Canicola	Grippityphosa	Hardjo	Ictero	Pomona	All serovars	
Arctic fox	<i>Vulpes lagopus</i>	233	5.2 (3.0–8.8)	9.0 (6.0–13.4)	2.2 (0.9–4.9)	6.0 (3.6–9.8)	3.4 (1.8–6.6)	2.6 (1.2–5.5)	18.0 (13.6–23.5)	
Axis deer	<i>Axis axis</i>	1	0 (0–79.4)	0 (0–79.4)	0 (0–79.4)	0 (0–79.4)	0 (0–79.4)	0 (0–79.4)	0 (0–79.4)	
Black-tailed deer	<i>Odocoileus hemionus columbianus</i>	6	0 (0–39.0)	0 (0–39.0)	33.3 (9.7–70.0)	0 (0–39.0)	16.7 (3.0–56.4)	0 (0–39.0)	33.3 (9.7–70.0)	
Coyote	<i>Canis latrans</i>	1,247	11.2 (9.5–13.0)	3.6 (2.7–4.8)	17.9 (15.9–20.1)	5.7 (4.5–7.1)	6.4 (5.2–7.9)	7.5 (6.1–9.1)	29.4 (26.9–31.9)	
Gray fox	<i>Urocyon cinereoargenteus</i>	88	8.0 (3.9–15.5)	5.7 (2.5–12.6)	23.9 (16.2–33.7)	5.7 (2.5–12.6)	3.4 (1.2–9.6)	5.7 (2.5–12.6)	34.1 (25.0–44.5)	
Gray wolf	<i>Canis lupus</i>	71	4.2 (1.5–11.7)	2.8 (0.8–9.7)	9.9 (4.9–19.0)	4.2 (1.5–11.7)	1.4 (0.3–7.6)	4.2 (1.5–11.7)	19.7 (12.1–30.4)	
Moose	<i>Alces alces</i>	3	0 (0–56.2)	0 (0–56.2)	0 (56.2)	0 (0–56.2)	0 (0–56.2)	0 (0–56.2)	0 (0–56.2)	
Mule deer	<i>Odocoileus hemionus</i>	4	0 (0–49.0)	0 (0–49.0)	0 (0–49.0)	0 (0–49.0)	0 (0–49.0)	0 (0–49.0)	0 (0–49.0)	
Raccoon	<i>Procyon lotor</i>	1,704	18.5 (16.7–20.4)	6.9 (5.8–8.2)	25.8 (23.8–28.0)	18.9 (17.1–20.8)	15.7 (14.1–17.5)	6.9 (5.8–8.2)	40.8 (38.5–43.1)	
Red fox	<i>Vulpes vulpes</i>	120	9.2 (5.2–15.7)	4.2 (1.8–9.4)	20.0 (13.8–28.0)	5.0 (2.3–10.5)	6.7 (3.4–12.6)	7.5 (4.0–13.6)	35.0 (27.1–43.9)	
Small Indian mongoose	<i>Herpestes auroreopunctatus</i>	23	13.0 (4.5–32.1)	8.7 (2.4–26.8)	8.7 (2.4–26.8)	21.7 (9.7–41.9)	0 (0–14.3)	0 (0–14.3)	30.4 (15.6–50.9)	
Striped skunk	<i>Mephitis mephitis</i>	5	0 (0–43.5)	0 (0–43.5)	60.0 (23.1–88.2)	0 (0–43.5)	0 (0–43.5)	0 (0–43.5)	60.0 (23.1–88.2)	
White-tailed deer	<i>Odocoileus virginianus</i>	1,029	37.1 (34.2–40.1)	2.9 (2.1–4.1)	6.1 (4.8–7.8)	3.4 (2.5–4.7)	2.4 (1.7–3.6)	8.5 (6.9–10.3)	44.8 (41.8–47.9)	

TABLE 2. Number of samples collected by wildlife species and state from 2011 to 2017 and tested for exposure to *Leptospira* serovars Bratislava, Canicola, Grippotyphosa, Hardjo, Icterohaemorrhagiae, and Pomona.

Common name	Species	<i>n</i>	State (<i>n</i>)
Arctic fox	<i>Vulpes lagopus</i>	233	Alaska (233)
Axis deer	<i>Axis axis</i>	1	Hawaii (1)
Black-tailed deer	<i>Odocoileus hemionus columbianus</i>	6	Washington (6)
Gray fox	<i>Urocyon cinereoargenteus</i>	88	Alabama (6), Arkansas (1), California (3), Georgia (10), Maine (20), Minnesota (1), New York (3), Pennsylvania (31), Texas (13)
Moose	<i>Alces alces</i>	3	Alaska (3)
Mule deer	<i>Odocoileus hemionus</i>	4	California (2), Wyoming (2)
Red fox	<i>Vulpes vulpes</i>	120	Alabama (1), Alaska (9), California (7), Connecticut (4), Illinois (5), Indiana (1), Maine (11), Massachusetts (9), Michigan (6), Minnesota (8), New Hampshire (3), New Jersey (19), New York (4), Pennsylvania (28), Tennessee (1), Wyoming (4)
Small Indian mongoose	<i>Herpestes auropunctatus</i>	23	Hawaii (23)
Striped skunk	<i>Mephitis mephitis</i>	5	Vermont (5)

Grippotyphosa were the most common serovars detected in canids, and Grippotyphosa was the most commonly detected serovar in raccoons and striped skunks. Hardjo was the most common serovar detected in small Indian mongooses (Table 1). No significant differences were observed within species between each year of the 6 yr sampling period.

DISCUSSION

Although human cases of leptospirosis in the US are most often reported in Hawaii (Levett 2001), our results suggest that leptospirosis is widespread across the country in a variety of wildlife hosts. In Hawaii, the small Indian mongoose, in addition to preying on native species, may be an important contributor to the epidemiological cycle of leptospirosis. The antibody prevalence was reported as 22% ($n=241$) and 23% ($n=180$) in two studies conducted from 1969 to 1973 (Higa and Fujinaka 1976; Tomich 1979), which is similar to the 30% ($n=23$) we detected, but we examined different serovars and used a different serological test. We identified Bratislava, Grippotyphosa, and Hardjo (Table 1) and are unaware of these serovars being documented previously in small Indian mongooses. All mongooses were collected in Honolulu County, which not only is an important tourist destination but is also where 70% of the state's population resides. Additional studies to determine whether small Indian mongooses are shedding the bacteria are recommended to fully assess the risk they pose to people.

Since the majority of the cervid samples we tested were collected from white-tailed deer, we limited our conclusions to this species. Although we identified antibodies to all six serovars in white-tailed deer, Bratislava was most commonly identified. Although Bratislava has been reported previously in this species (Goyal et al. 1992), serovars Pomona and Grippotyphosa have been reported more often (Shotts and Hayes 1970; Ingebrigtsen et al. 1986). The overall antibody prevalence

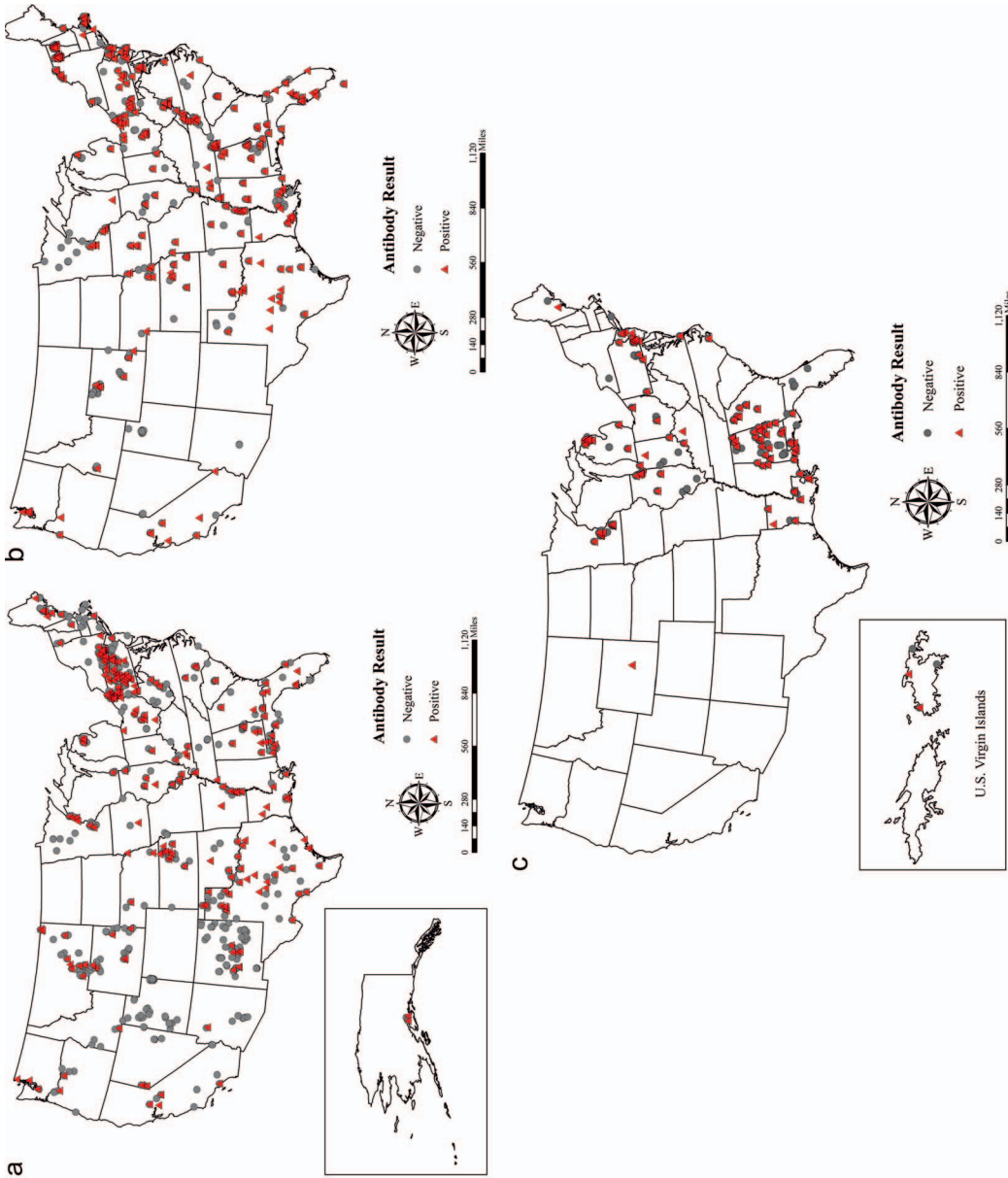


FIGURE 1. Collection site of serum collected from (a) coyotes (*Canis latrans*), (b) raccoons (*Procyon lotor*), and (c) white-tailed deer (*Odocoileus virginianus*) from 2011 through 2017 in the USA and US Virgin Islands and tested by microagglutination test for exposure to *Leptospira* serovars Bratislava, Canicola, Grippityphosa, Hardjo, Icterohaemorrhagiae, and Pomona.

we detected (44.8%) was much higher than the 3–27% antibody prevalence reported in studies conducted in various states (Shotts and Hayes 1970; Fournier et al. 1986; Ingebrigtsen et al. 1986; New et al. 1993), except for a Minnesota study in which 43% were antibody positive (Goyal et al. 1992). Approximately 40% of white-tailed deer in our study demonstrated titers to the serovars of *Leptospira* for which we tested. Though we detected evidence of recent infection in only 3% (Table 3), because of the widespread geographic distribution of white-tailed deer, they could be important contributors to the epidemiological cycle of this pathogen. Additional studies are recommended to determine with what frequency white-tailed deer shed *Leptospira*, especially since this is a very popular and abundant game species with a population of more than 30 million in the US (Vercauteren et al. 2011), meaning that hunters could be at an elevated risk of exposure. In addition to the risk to humans, white-tailed deer often utilize the same pastures as livestock for grazing, which could lead to transmission of *Leptospira* to livestock (Davidson et al. 1985), resulting in reproductive losses and subsequent economic impacts (Grooms 2006).

Dogs are considered maintenance hosts for serovar Canicola. Until recently, vaccinations provided protection only against Canicola and Icterhaemorrhagiae, but Pomona, Grippotyphosa, and Bratislava have also been detected in dogs (Moore et al. 2006) and wild canids (Clark et al. 1960; Amundson and Yuill 1981; Åkerstedt et al. 2010). *Leptospira* infections in free-ranging canids have frequently been associated with serovar Icterohaemorrhagiae (Åkerstedt et al. 2010), which is assumed to occur via exposure to contaminated urine or by feeding on infected rodents (Reilly et al. 1970). In the wild canids that we tested, exposure to all serovars was common, but Grippotyphosa was the only serovar where titers $\geq 1:800$ (i.e., actively infected) were identified in all five of the canid species examined (Table 3). This pattern was consistent with another study conducted on coyotes in Kansas (Marler et al.

1979). Although 70% of the canid species that we tested were coyotes, the antibody prevalence was similar for all five species (Table 1).

Raccoons are considered good biological indicators of whether various diseases, such as leptospirosis, are circulating in the environment (Bigler et al. 1975). They are also abundant and widely distributed across the US, providing additional support to their role as indicators of human and ecological health (Burger and Gochfeld 2001). Additionally, they do not appear to display clinical signs of infection, making them favorable reservoir hosts for leptospirosis (Hamir et al. 2001). In addition to the human health aspect, raccoons are often observed in barns where cattle or swine are housed (Diesch et al. 1970). This increases the potential for transmission of *Leptospira* to livestock, which can also have a financial impact since infection can result in mastitis, abortion, and premature birth (Waitkins 1986). Since the raccoons that we sampled were collected from 42 states, and at least one positive was detected in all but three states (Fig. 1b), it is likely that the antibody prevalence we detected is representative of that circulating across the country, even if there are some slight regional variations in prevalence. The highest antibody prevalence in raccoons was detected for Grippotyphosa (Table 1), but evidence of recent infection of all six serovars was identified (Table 3), suggesting that raccoons are exposed to a wide range of serovars and thus have the potential to transmit them to various host species. Raccoons are also a concern because they may transmit *Leptospira* to dogs and then subsequently to the pet's owners (Davis et al. 2008) or veterinarians (Whitney et al. 2009). However, as with other species, additional studies to determine shedding in raccoons are recommended since the association between serology and shedding is unclear.

Though very few striped skunks were tested, the antibody prevalence we detected (60%) was consistent with other studies that have reported prevalence values ranging from 13% to 46.8% (Ferguson and Heidt 1981;

TABLE 3. Number of wildlife serum samples that tested antibody positive for exposure to *Leptospira* serovars Bratislava, Canicola, Grippotyphosa (Grippe), Hardjo, Icterohaemorrhagiae (Ictero), and Pomona by microagglutination test titer. Sera were collected from various wildlife species in the USA and the US Virgin Islands from 2011 to 2017. CI = confidence interval.

Common name	Species	n	Serovar	Titer							% Positive (95% CI)
				100	200	400	800	1600	3200	≥6400	
Arctic fox	<i>Vulpes lagopus</i>	233	Bratislava	5	4	3	0	0	0	0	5.2 (3.0–8.8)
			Canicola	17	4	0	0	0	0	0	9.0 (6.0–13.4)
			Grippe	1	1	2	1	0	0	0	2.2 (0.9–4.9)
			Hardjo	9	4	0	1	0	0	0	6.0 (3.6–9.8)
			Ictero	7	1	0	0	0	0	0	3.4 (1.8–6.6)
Coyote	<i>Canis latrans</i>	1,247	Pomona	2	2	0	1	1	0	0	2.6 (1.2–5.5)
			Bratislava	80	34	14	2	6	2	1	11.1 (9.5–13.0)
			Canicola	32	8	1	0	0	3	1	3.6 (2.7–4.8)
			Grippe	95	53	31	15	11	10	8	17.9 (15.9–20.1)
			Hardjo	43	19	6	1	2	0	0	5.7 (4.5–7.1)
Gray fox	<i>Urocyon cinereoargenteus</i>	88	Ictero	44	24	4	3	2	1	2	6.4 (5.2–7.9)
			Pomona	35	18	18	6	8	3	5	7.5 (6.1–9.1)
			Bratislava	4	2	1	0	0	0	0	8.0 (3.9–15.5)
			Canicola	3	1	1	0	0	0	0	5.7 (2.5–12.6)
			Grippe	7	8	3	0	0	0	3	23.9 (16.2–33.7)
Gray wolf	<i>Canis lupus</i>	71	Hardjo	4	1	0	0	0	0	0	5.7 (2.5–12.6)
			Ictero	3	0	0	0	0	0	0	3.4 (1.2–9.6)
			Pomona	2	1	1	0	0	0	1	5.7 (2.5–12.6)
			Bratislava	3	0	0	0	0	0	0	4.2 (1.5–11.7)
			Canicola	1	1	0	0	0	0	0	2.8 (0.8–9.7)
Raccoon	<i>Procyon lotor</i>	1,704	Grippe	3	1	1	0	0	1	1	9.9 (4.9–19.0)
			Hardjo	1	2	0	0	0	0	0	4.2 (1.5–11.7)
			Ictero	1	0	0	0	0	0	0	1.4 (0.3–7.6)
			Pomona	1	0	2	0	0	0	0	4.2 (1.5–11.7)
			Bratislava	132	69	51	24	19	13	7	18.5 (16.7–20.4)
Red fox	<i>Vulpes vulpes</i>	120	Canicola	63	34	13	3	0	2	3	6.9 (5.8–8.2)
			Grippe	144	123	60	37	28	16	32	25.8 (23.8–28.0)
			Hardjo	97	81	67	34	22	13	8	18.9 (17.1–20.8)
			Ictero	91	72	47	20	13	11	14	15.7 (14.1–17.5)
			Pomona	42	28	14	13	7	6	7	6.9 (5.8–8.2)
Small Indian mongoose ^a	<i>Herpestes auropunctatus</i>	23	Bratislava	2	6	3	0	0	0	0	9.2 (5.2–15.7)
			Canicola	5	0	0	0	0	0	0	4.2 (1.8–9.4)
			Grippe	9	6	2	1	3	0	3	20.0 (13.8–28.0)
			Hardjo	6	0	0	0	0	0	0	5.0 (2.3–10.5)
			Ictero	5	2	0	0	1	0	0	6.7 (3.4–12.6)
Striped skunk ^b	<i>Procyon lotor</i>	5	Pomona	5	4	0	0	0	0	0	7.5 (4.0–13.6)
			Bratislava	2	1	0	0	0	0	0	13.0 (4.5–32.1)
			Canicola	1	0	1	0	0	0	0	8.7 (2.4–26.8)
			Grippe	0	1	1	0	0	0	8.7 (2.4–26.8)	
			Hardjo	0	0	1	1	1	2	21.7 (9.7–41.9)	
			Grippe	1	0	0	0	2	0	60.0 (23.1–88.2)	

TABLE 3. Continued.

Common name	Species	n	Serovar	Titer							% Positive (95% CI)
				100	200	400	800	1600	3200	≥6400	
White-tailed deer	<i>Odocoileus virginianus</i>	1,029	Bratislava	203	115	50	9	2	1	2	37.1 (34.2–40.1)
			Canicola	21	9	0	0	0	0	0	2.9 (2.1–4.1)
			Grippio	38	16	4	2	2	1	0	6.1 (4.8–7.8)
			Hardjo	19	8	5	0	2	1	0	3.4 (2.5–4.7)
			Ictero	16	6	3	0	0	0	0	2.4 (1.7–3.6)
			Pomona	37	25	11	8	4	1	1	8.5 (6.9–10.3)

^a No positives for Ictero or Pomona.

^b No positives for Bratislava, Canicola, Hardjo, Ictero, or Pomona.

Richardson and Gauthier 2003). The striped skunk has been identified as an important maintenance host for Pomona and Canicola (Carpio et al. 1977), although Grippotyphosa was the only serovar we detected. However, the skunks we sampled were all collected in Vermont, and Grippotyphosa has been documented in skunks previously in the Northeast (Richardson and Gauthier 2003). Additional samples from across the country would be needed to more accurately characterize the antibody prevalence and serotypes circulating in skunks.

Though we only tested for exposure to six of the more than 250 existing leptospiral serovars, we detected more than 30% antibody prevalence across the range of species we examined. Since even wildlife species with small home ranges are typically very mobile compared to domestic animals, abundant opportunities for exposure exist, meaning this relatively high prevalence is not surprising. Further studies to examine the frequency with which wildlife shed *Leptospira* and to examine additional serovars are recommended to better understand the potential disease risk wildlife pose for humans, livestock, and pets.

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