

A SURVEY OF HANTAVIRUS ANTIBODY IN SMALL-MAMMAL POPULATIONS IN SELECTED UNITED STATES NATIONAL PARKS

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Abstract. Hantavirus activity in 39 National Parks in the eastern and central United States was surveyed by testing 1,815 small mammals of 38 species for antibody reactive to Sin Nombre virus. Antibody-positive rodents were found throughout the area sampled, and in most biotic communities. Antibody was detected in 7% of 647 deer mice (*Peromyscus maniculatus*), 2% of 590 white-footed mice (*P. leucopus*), 17% of 12 rice rats (*Oryzomys palustris*), 3% of 31 cotton rats (*Sigmodon hispidus*), and 33% of 18 western harvest mice (*Reithrodontomys megalotis*). Antibody was also found in three of six species of voles, and in one of 33 chipmunks (*Tamias minimus*). Prevalence among *Peromyscus* was highest in the northeast. Although few cases of hantavirus pulmonary syndrome have been identified from the eastern and central regions, widespread infection in reservoir populations indicates that potential exists for human infection throughout much of the United States.

Hantaviruses are lipid-enveloped, trisegmented, RNA viruses of the family Bunyaviridae. Several New-World hantaviruses have been implicated as etiologic agents of hantavirus pulmonary syndrome (HPS), a serious human disease with a mortality of approximately 50%.¹ The hantaviruses are rodent-borne zoonotic agents responsible for a wide spectrum of human disease in Asia, Europe, North America, and South America. A hallmark of the hantaviruses is their typical association with a single species of murid rodent host, in which they establish a chronic, apparently asymptomatic, infection that involves shedding of infectious virus in bodily secretions and excretions. The Old World hantaviruses are associated with the murid subfamilies Murinae (Old World rats and mice) and Arvicolinae (voles), while the New World viruses are associated with the subfamilies Arvicolinae and Sigmodontinae (New World rats and mice).

The 1993 outbreak of HPS in the southwestern United States led to the identification of Sin Nombre virus (SNV) as the causative agent and the implication of the deer mouse (*Peromyscus maniculatus*) as the principal reservoir.^{2,3} The deer mouse is one of the most widespread and common small mammals in North America, occurring throughout the continental United States, except for the Southeast and the Atlantic seaboard (Figure 1), and occupying nearly every dry-land habitat. Indeed, cases of HPS have occurred throughout most of the range of the deer mouse in the United States. However, a few cases have occurred outside the range of the species, illustrating that other North American rodents are host to hantaviruses with the potential for causing serious human disease. Two HPS cases in New York have been attributed to New York virus, a Sin-Nombre-like hantavirus associated with the white-footed mouse (*P. leucopus*).⁴ A single case in south Florida led to the isolation of Black Creek Canal virus from its putative host, the cotton rat (*Sigmodon hispidus*).^{5,6} Three HPS cases in Louisiana and east Texas have been associated with Bayou virus, whose host appears to be the rice rat (*Oryzomys palustris*).⁷

Rodent trapping and sampling associated with the investi-

gation of numerous HPS cases in the western United States quickly resulted in the accumulation of considerable data concerning the prevalence of hantavirus infection in reservoir populations in this region of the country (Ksiazek TG, unpublished data). Subsequently, additional hantaviruses and hantavirus reservoir species were identified in the western United States, including El Moro Canyon virus in the western harvest mouse (*Reithrodontomys megalotis*), which has not been associated with human disease.⁸

The relative rarity of cases in the eastern United States, and subsequent lack of sampling and testing of rodent populations, has resulted in a paucity of data concerning hantaviruses in rodent populations over this area. This study was undertaken to provide the following information concerning hantavirus infection in reservoir populations in the eastern United States: 1) the identification of hantavirus reservoir species, 2) the geographic range of infection in reservoir species, and 3) the variation in prevalence of infection among host species, geographic locations, and principal biotic communities.

MATERIALS AND METHODS

Thirty-nine natural areas under the administration of the U.S. National Park Service were selected for sampling (Figure 1). Most parks were in the eastern United States; nine were west of the Mississippi River/Great Lakes area; none were west of the Rocky Mountains. The 38 eastern and central parks (all except Yellowstone) were divided into five groups based on geographic region and assigned to one of five field teams for sampling. Each field team was led by a graduate student in mammalogy or professional biologists from a U.S. Navy Disease Vector Ecology and Control Center. All trapping was conducted between May and August 1994, except at Fort Pulaski National Monument (NM), Natchez Trace Parkway, and Horseshoe Bend National Military Park (NMP), which were sampled in December 1994, March 1995, and April 1995, respectively. Most parks were visited for three nights (range = 2–6). With assistance from

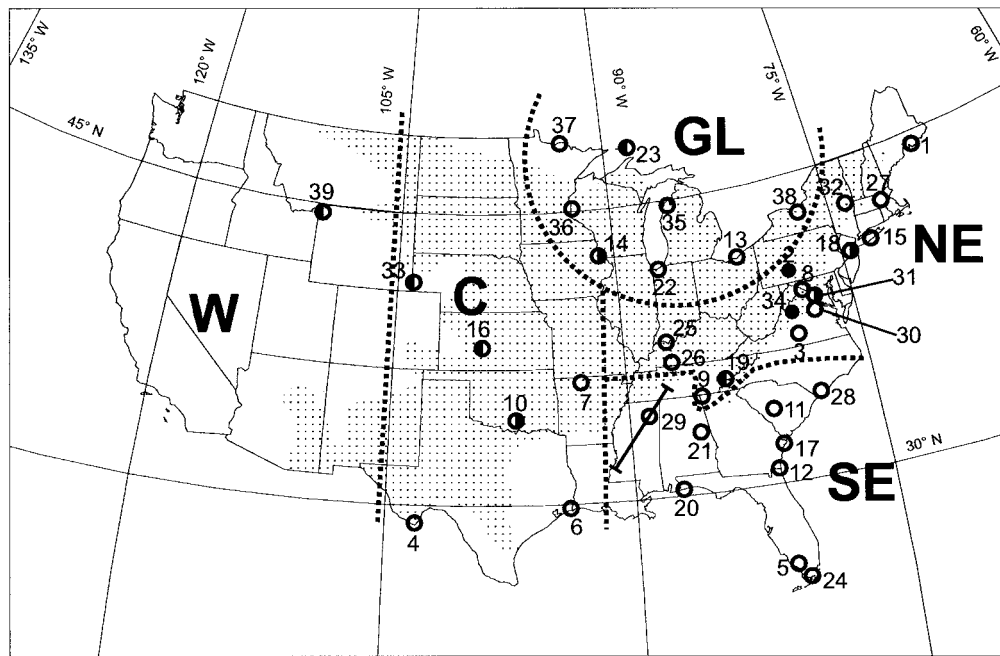


FIGURE 1. Locations of 39 National Parks included in a study of hantavirus antibody prevalence. The range of *Peromyscus maniculatus* (deer mouse) is indicated by vertical hatching. The range of *P. leucopus* (white-footed mouse) is indicated by horizontal hatching. The range where *P. maniculatus* and *P. leucopus* occur together is indicated by dots. Parks represented by circles shaded on the left half had antibody-positive deer mice; antibody-positive white-footed mice are represented by shading on the right half; parks with both species antibody-positive are represented by closed circles; and those with neither species antibody-positive, or none tested, by open circles. Numbers refer to parks named in Table 1. Dotted lines represent boundaries between northeastern (NE), southeastern (SE), Great Lakes (GL), central (C), and western (W) regions (geographic distributions of *Peromyscus* species are taken from Carleton²⁹). The bar associated with Natchez Trace Parkway indicates the approximate extent of the park through parts of Tennessee, Mississippi, and the northwestern corner of Alabama.

the park naturalist, resource manager, or other representative, trapping sites were chosen to represent all major habitat types within the park. Each night, 100–200 Sherman ($8 \times 9 \times 23$ cm; H.B. Sherman Trap Co., Tallahassee, FL) and approximately 20 Tomahawk live-capture traps ($14 \times 14 \times 40$ cm; Tomahawk Trap Co., Tomahawk, WI) were placed in traplines of 10–20 traps at 5-m intervals and baited with rolled oats, usually mixed with peanut butter. Animals were collected each morning and carried to a central field processing station where they were anesthetized and sampled according to standardized procedures.^{9–11} Specific identification, sex, weight, reproductive condition, and habitat of capture were recorded. Frozen whole blood samples were sent to the Centers for Disease Control and Prevention (CDC) for serologic analysis. Fluid-preserved voucher specimens (carcasses)¹² were checked by museum staff for proper identification and, along with frozen tissues, were deposited in the Museum of Southwestern Biology (MSB), University of New Mexico. The investigation at Yellowstone National Park (NP), Wyoming, was conducted similarly, except that sampling was carried out by park staff and was of longer duration (April–June) (M. Johnson, Yellowstone NP, unpublished data).

Whole blood was tested for antibody reactive with SNV recombinant nucleocapsid protein antigen by ELISA according to a standardized protocol.^{13, 14} Briefly, blood specimens were initially diluted 1:25 in 5% skim milk in 0.01 M phosphate-buffered saline with 0.5% Tween 20 and subsequently diluted from 1:100 through 1:6400 in four-fold dilutions in microtiter plates. Samples were tested against the recombi-

nant nucleocapsid antigen and a recombinant control antigen.¹³ A conjugate mixture of anti-*Rattus norvegicus* and anti-*P. leucopus* (heavy and light) IgG (Kirkegaard and Perry, Gaithersburg, MD) was used to detect bound immunoglobulin for rodent species. Non-rodent species were tested with conjugates made to similar species. The conjugates were selected based on a search of a number of commercially available products and measuring their binding to target species in checkerboard titrations. Adjusted optical densities (ODs) for each dilution were calculated by subtracting the OD₄₁₀ of the control antigen from the OD₄₁₀ of the SNV antigen. Titers were assigned based on an adjusted OD value exceeding 0.20 for each dilution. A second measure consisting of the sum of the adjusted OD for all four dilutions was also calculated. Sera were considered positive if their titer was 1:400 or their sum adjusted OD was 0.95. The cut-off values were determined by assessment of rodents found positive by several serologic tests during the initial investigation of the Four Corners outbreak,³ and have been reassessed periodically among large populations of rodents collected in North and South America. Antibodies to other North American hantaviruses are cross-reactive with SNV antigen. This assay would detect, but would not distinguish among, infections by New York virus (from the white-footed mouse),⁴ Prospect Hill-like viruses (from arvicoline rodents),¹⁵ El Moro Canyon virus (from the western harvest mouse),⁸ Black Creek Canal virus (from the cotton rat),⁶ and Bayou virus (from the rice rat).⁷

Data were analyzed using SPSS software.¹⁶ Trap success was computed as the number of captures per 100 trap nights,

and trap nights were equal to the number of traps set multiplied by the number of nights. Specific trap success for *Peromyscus* species was calculated similarly, except that the number of trap nights was corrected for nonavailability of traps by subtracting half the number of captures of all other species combined. Mass classes were calculated by dividing the range of the masses for each species at the 33rd and 66th percentiles. Mass classes for deer mice were I (4.0–17.7 g), II (17.8–20.9 g), and III (21.0–37.0 g). Those for white-footed mice were I (6.0–16.9 g), II (17.0–20.0 g), and III (20.1–37.0 g). For regional analyses, parks were divided into Northeast, Southeast, Great Lakes, Central, and Western regions (Figure 1).

RESULTS

Trapping success. A total of 23,833 trap nights yielded 1,911 small mammals of 41 species from 39 National Parks (Tables 1 and 2). The most commonly captured animals were two species of *Peromyscus*: the deer mouse *P. maniculatus* (34% of captures) and the white-footed mouse *P. leucopus* (32%). The cotton mouse *P. gossypinus* and the red-backed vole *Clethrionomys gapperi* each accounted for about 5% of captured animals. These four common species accounted for 78% of small mammals captured. A total of 472 of the 1,911 captures were from Yellowstone NP. The intensive effort in Yellowstone accounted for a large proportion of some species, including 367 (56%) of the 659 deer mice; all of the mountain voles (*Microtus montanus*, $n = 33$), least chipmunks (*Tamias minimus*, $n = 35$), western jumping mice (*Zapus princeps*, $n = 14$), and Uinta ground squirrels (*Spermophilus armatus*, $n = 2$), and the only long-tail vole (*Microtus longicaudus*). Excluding Yellowstone, the average number of captures per park was 38 (range = 1–116).

Trapping effort in the 38 eastern and central parks varied from a low of 180 to a high of 881 trap nights. The effort at Yellowstone NP was 3,561 trap nights. Trap success varied from < 1% to 34%; the overall average was 8.0% (Table 1).

Relative density of reservoir species populations, as indicated by specific trap success, varied greatly among parks for deer mice and white-footed mice (Table 1). Deer mice were captured in 11 parks. Specific trap success was highest (13%) in Isle Royal NP, Michigan, followed by Yellowstone NP and Scott's Bluff NM, Nebraska (near 10%). Six of the remaining parks had deer mouse trap success rates of 5–7%, and two (Indiana Dunes National Lakeshore [NL], Indiana and Acadia NP, Maine) had much lower trap success rates (0.2% and 1.5%, respectively). White-footed mice were captured in 25 parks. Specific trap success varied from < 1% at Big Bend NP, Texas, and Rock Creek Park (Washington, DC) to 17% at Effigy Mounds NM, Iowa, and St. Croix National Scenic River (NSR; Table 1), Wisconsin.

Prevalence of hantavirus antibody. Of 1,911 captured and identified small mammals, 96 were not tested because blood samples were either not taken or were taken in quantities insufficient for testing, leaving 1,815 small mammals from 38 species tested for antibody reactive with SNV. Antibody was detected in five of 12 sigmodontine, three of six arvicoline, and one of seven sciurid rodent species (Table 2). The SNV antibody prevalence was approximately 3% for

two arvicoline species (three of 86 red-backed voles, and one of 33 mountain voles); however, one of three tested prairie voles (*Microtus ochrogaster*) had antibody. The five sigmodontine rodents with antibody included two of five species of *Peromyscus* (45 of 647 deer mice and 10 of 590 white-footed mice), two of 12 rice rats, one of 31 cotton rats, and six of 18 western harvest mice. Antibody was also detected in one of 33 least chipmunks. The prevalence of antibody in deer mice in Yellowstone (25 of 364 = 6.9%) was very similar to that in the more eastern parks (20 of 283 = 7.1%).

Antibody-positive deer mice were found in seven of the 11 parks in which the species was captured, and seven of the eight parks where > 16 deer mice were captured (Table 1). Overall prevalence of infection in deer mice was 7%, and in those parks where > 16 deer mice were captured, it varied from 0% (0 of 35) in Sleeping Bear Dunes National Lakeshore (NL), Michigan to 17% (4 of 24) at Allegheny Portage Railroad National Historic Site (NHS), Pennsylvania (Table 1).

Antibody-positive white-footed mice were found in only 6 of the 26 parks where they were captured, and overall prevalence of antibody was < 2% (Table 1). The highest prevalence of infection occurred at sites where relatively few animals were captured: one (33%) of three at Rock Creek Park, two (29%) of seven at Allegheny Portage Railroad NHS, and two (14%) of 14 at Gateway National Recreation Area (NRA), New York.

Western harvest mice (which only occur west of the Mississippi River) were captured at two parks and had a high prevalence of antibody in both: four (36%) of 11 at Fort Larned NHS, Kansas, and two (29%) of seven at Scott's Bluff NM, Nebraska.

Twelve rice rats were captured from five parks in the Southeast: three from Big Cypress National Preserve (N Pres), Florida, one from Congaree Swamp NM, South Carolina, two from Fort Pulaski NM, Georgia, two from Horseshoe Bend NMP, Alabama, and four from Natchez Trace Parkway (Tennessee, Alabama, and Mississippi). Both antibody-positive animals came from Fort Pulaski. Cotton rats were captured from nine parks in the Southeast. The only antibody-positive individual was one of nine captured from Fort Larned NHS, Kansas.

All of the antibody-positive arvicoline rodents were captured from parks west of the Mississippi River. The antibody-positive red-backed voles were captured in Voyageurs NP, Minnesota (one of 30) and Yellowstone (two of 19). The single infected mountain vole was captured in Yellowstone, and the prairie vole was from Scott's Bluff, NM.

Antibody prevalence for major reservoir species by region and biotic community. Antibody prevalence in both species of *Peromyscus* was highest in the northeastern region, followed by the central region, while prevalences were lowest in the southeastern and Great Lakes regions. Among deer mice, antibody prevalence was 11% in the Northeast (11 of 96), 8% in the Central (eight of 95), 7% in the West (25 of 364), and 1% (one of 92) in the Great Lakes region. No deer mice were captured in the Southeast. Antibody prevalence for white-footed mice was 3% in the Northeast (eight of 289), 2% in the Central (one of 54), and < 1% in the Great Lakes region (one of 180). There were no anti-

TABLE 1

Results of small-mammal trapping at 39 United States National Parks from May through August 1994, including total captures, trap nights, and trap success (%); and trap success for *Peromyscus maniculatus* and *P. leucopus*, as well as numbers Sin Nombre virus antibody-positive for both species*

No.	State	Park	Biome	Elevation (m)	Total			<i>P. maniculatus</i>			<i>P. leucopus</i>		
					Captures	Trap nights	Success (%)	Positive	Tested	Success (%)	Positive	Tested	Success (%)
1	ME	Acadia NP	Coniferous forest	SL-466	45	420	10.7	0	6	1.5	0	33	8.2
2	PA	Allegheny Portage Railroad NHS	Deciduous forest	850	33	350	9.4	4	24	6.9	2	7	2.1
3	VA	Appomattox Court House NHP	Deciduous forest	228	20	453	4.4				0	14	3.1
4	TX	Big Bend NP	Desert scrub	1,143	38	833	4.6				0	4	0.5
5	FL	Big Cypress N Pres	Coastal wetland	SL	14	394	3.6						
6	TX	Big Thicket N Pres	Mixed forest	SL-90	9	701	1.3						
7	AR	Buffalo NR	Deciduous forest	183-610	22	881	2.5				0	11	1.3
8	MD	Chesapeake and Ohio Canal NHP	Deciduous forest	3-190	22	491	4.5				0	19	4.3
9	GA	Chickamauga and Chattanooga	Deciduous forest	225	17	431	3.9						
10	OK	Chickasaw NRA	P/F ecotone	244-366	73	814	9.0				1	20	2.7
11	SC	Congaree Swamp NM	Deciduous forest	35	20	443	4.5						
12	GA	Cumberland Island NS	Coastal wetland	SL	8	414	1.9						
13	OH	Cuyahoga Valley NRA	Deciduous forest	300	37	723	5.1				0	22	3.1
14	IA	Effigy Mounds NM	Deciduous forest	182-304	102	489	20.9				1	80	16.9
15	NY	Fire Island NS	Coastal wetland	4	21	560	3.8				0	17	3
16	KS	Fort Larned NHS	Prairie	620	84	834	10.1	6	37	5	0	19	2.5
17	GA	Fort Pulaski NM	Coastal wetland	SL	3	452	0.7						
18	NY	Gateway NRA	Coastal wetland	4	14	480	2.9				2	14	2.9
19	TN	Great Smoky Mountains NP	Deciduous forest	488-1,607	45	362	12.4	2	27	7.6	0	6	1.8
20	FL	Gulf Islands NS	Coastal wetland	SL-15	15	585	2.6						
21	AL	Horseshoe Bend NMP	Deciduous forest	300	21	399	5.3				0	9	3
22	IN	Indiana Dunes NL	P/F ecotone	178	37	622	5.9	0	1	0.2	0	27	5
23	MI	Isle Royale NP	Coniferous forest	183-241	50	304	16.4	1	40	13.4			
24	FL	Biscayne NP	Coastal wetland	-18	4	518	0.8						
25	IN	Lincoln Boyhood Home N Mem	Deciduous forest	150	39	721	5.4				0	18	2.5
26	KY	Mammoth Cave NP	Deciduous forest	128-274	48	880	5.5				0	44	5
27	MA	Minuteman NHP	Deciduous forest	SL	19	420	4.5				0	14	3.4
28	NC	Moore's Creek NB	Mixed forest	12	5	478	1.0						
29	MS	Natchez Trace Parkway	Deciduous forest	91-823	92	781	11.8				0	40	6.1
30	VA	Prince William Forest Park	Mixed forest	39-76	26	472	5.5				0	26	5.5
31	DC	Rock Creek Park	Deciduous forest	110	8	560	1.4				1	3	0.5
32	NY	Saratoga NHP	Deciduous forest	90	21	420	5.0				0	20	4.8
33	NE	Scotts Bluff NM	Prairie	1,182-1,400	72	627	11.5	2	58	9.4			
34	VA	Shenandoah NP	Deciduous forest	335-975	116	683	17.0	5	39	6.1	3	72	11.2
35	MI	Sleeping Bear Dunes NL	Mixed forest	128-274	81	555	14.6	0	35	7.3	0	20	4.4
36	WI	St. Croix NSR	Mixed forest	323-345	68	198	34.3				0	31	17.3
37	MN	Voyageurs NP	Coniferous forest	335	89	344	25.9	0	16	5.5			
38	NY	Women's Rights NHP	Deciduous forest	140	1	180	0.6						
39	WY	Yellowstone NP	Coniferous forest	1,900-2,400	472	3,561	13.3	25	364	10.5			
Overall					1,911	23,833	8.0	45	647	2.8	10	590	2.6

* Numbers refer to parks mapped in Figure 1. Although some parks occur in multiple states, only the primary state (where most trapping was done) is listed. Trap nights include Sherman and Tomahawk traps. NP = national park; SL = sea level; NHS = National Historic Site; NHP = National Historic Park; N Pres = National Preserve; NR = National River; NRA = National Recreation Area; P/F = prairie/forest; NM = National Monument; NS = National Seashore; NRA = National Recreation Area; NMP = National Military Park; NL = National Lakeshore; N Mem = National Memorial; NB = National Battlefield; NSR = National Scenic River.

TABLE 2
Small-mammal species captured and tested for antibody reactive to Sin Nombre virus*

Order/family	Subfamily	Species	No. positive	No. tested	% positive	NT	Total	
Rodentia								
Dipodidae	Zapodinae	<i>Zapus hudsonius</i>	0	20	0.0	0	20	
		<i>Zapus princeps</i>	0	14	0.0	0	14	
		<i>Napaeozapus insignis</i>	0	3	0.0	0	3	
Heteromyidae		<i>Chaetodipus hispidus</i>	0	2	0.0	0	2	
		<i>Chaetodipus penicillatus</i>	0	2	0.0	0	2	
		<i>Dipodomys merriami</i>	0	4	0.0	0	4	
Muridae	Arvicolinae	<i>Clethrionomys gapperi</i>	3	86	3.5	21	107	
		<i>Microtus longicaudus</i>	0	1	0.0	0	1	
		<i>Microtus montanus</i>	1	33	3.0	0	33	
		<i>Microtus ochrogaster</i>	1	3	33.3	0	3	
		<i>Microtus pennsylvanicus</i>	0	26	0.0	3	29	
		<i>Synaptomys cooperi</i>	0	1	0.0	0	1	
		Murinae	<i>Mus musculus</i>	0	16	0.0	1	17
			<i>Rattus norvegicus</i>	0	20	0.0	0	20
			<i>Rattus rattus</i>	0	9	0.0	0	9
			Sigmodontinae	<i>Neotoma albigula</i>	0	1	0.0	0
	<i>Neotoma floridana</i>	0		13	0.0	2	15	
	<i>Neotoma micropus</i>	0		1	0.0	0	1	
	<i>Ochrotomys nuttalli</i>	0		7	0.0	1	8	
	<i>Oryzomys palustris</i>	2		12	16.7	0	12	
	<i>Peromyscus attwateri</i>	0		46	0.0	0	46	
	<i>Peromyscus eremicus</i>	0		22	0.0	1	23	
	<i>Peromyscus gossypinus</i>	0		101	0.0	2	103	
	Sciuridae	<i>Peromyscus leucopus</i>	10	590	1.7	24	614	
		<i>Peromyscus maniculatus</i>	45	647	7.0	12	659	
		<i>Reithrodontomys megalotis</i>	6	18	33.3	2	20	
		<i>Sigmodon hispidus</i>	1	31	3.2	1	32	
		<i>Glaucomys volans</i>	0	1	0.0	1	2	
<i>Sciurus carolinensis</i>		0	1	0.0	0	1		
<i>Spermophilus armatus</i>		0	2	0.0	0	2		
<i>Spermophilus</i> sp.		0	1	0.0	0	1		
<i>Tamias minimus</i>		1	33	3.0	2	35		
<i>Tamias striatus</i>		0	32	0.0	0	32		
<i>Tamiasciurus hudsonicus</i>		0	8	0.0	10	18		
Lagomorpha								
Leporidae		<i>Sylvilagus floridanus</i>	0	1	0.0	1	2	
Insectivora								
Soricidae		<i>Blarina brevicauda</i>	0	5	0.0	8	13	
		<i>Sorex cinereus</i>		0		2	2	
		<i>Sorex fumeus</i>		0		1	1	
Carnivora								
Mustelidae		<i>Mephitis mephitis</i>	0	1	0.0	0	1	
		<i>Mustela erminea</i>		0		1	1	
		<i>Mustela frenata</i>	0	1	0.0	0	1	
		Grand total	70	1,815	3.9	96	1,911	

* NT = not tested. Orders are given in bold. Nomenclature follows that of Wilson and Reeder.³⁰

body-positive specimens among 67 white-footed mice tested from parks in the Southeast and no white-footed mice were captured in the West.

Antibody-positive *Peromyscus* were found in all major biotic communities except desert scrub (represented only at Big Bend, where only four *Peromyscus* were captured) and mixed (pine and hardwood) forest, from which there were no positive animals among 112 *Peromyscus* captured in five parks. Among the other major biomes, antibody prevalence among *Peromyscus* species was highest in prairie (eight of 114 = 7%; two parks), followed by coastal/wetland (two of 31 = 6%; two parks), coniferous forest (26 of 459 = 6%; four parks), deciduous forest (18 of 469 = 4%; 15 parks), and prairie/forest ecotone (one of 48 = 2%; two parks).

Characteristics of infected populations. For two species,

antibody prevalence was higher among males than females. Among male deer mice, antibody prevalence was 9% (33 of 365) compared with 4% (12 of 279) for females (χ^2 with Yates' correction = 4.76, $P = 0.03$). For white-footed mice, antibody prevalence was approximately equal among males (six of 337 = 1.8%) and females (four of 248 = 1.6%). All six antibody-positive western harvest mice were males (50% prevalence); no antibody was detected in the six females tested ($P = 0.05$, by Fisher's exact test). The five antibody-positive arvicolines had a near-equal sex distribution: the mountain vole was female, the prairie vole male; two of three red-backed voles were female.

For deer mice, there was a trend ($\chi^2 = 2.66$, $P = 0.10$) toward increasing antibody prevalence with increasing mass class (a surrogate for age). Four percent of the smallest mass

class were infected (nine of 205) compared with 7% of the intermediate mass class (15 of 206) and 9% of the heaviest mass class (20 of 226). For white-footed mice, the SNV antibody prevalences were 1% (two of 171), 2% (four of 212), and 2% (four of 195) for the small, medium, and large mass classes, respectively. Although the sample size of western harvest mice ($n = 18$) was too small to allow meaningful grouping into mass classes, there were no positive individuals among four animals weighing 6–10 g; five of seven animals weighing 11 g were positive, and one of seven animals weighing 12–14 g was positive.

DISCUSSION

Evidence of hantavirus infection was detected in reservoir populations throughout most of the geographic area encompassed by parks surveyed during this study: from Virginia to western Wyoming, and from southern Georgia to Isle Royale, on the Canadian border. Antibody prevalence was highly variable, and the distribution of infection among parks was patchy. No novel rodent hosts were identified by these serologic studies, and all antibody-positive animals belonged to taxonomic groups previously identified as being infected. Although evidence of antibody to hantavirus in rodent populations was found in only 11 of 39 parks, this result does not imply that the remaining parks are free of infection. It is more likely that our sampling was not sufficiently intensive to detect infection present at a low prevalence, and antibody was our only marker of infection. From other studies, we could expect 7–55% of antibody-negative animals to have detectable RNA by reverse transcription–polymerase chain reaction (RT-PCR).^{3,17} It is also possible that locally, the virus is subject to periodic extinctions and re-introductions from adjacent populations such that infection is always present regionally, but may or may not be found within a given local population at a given time. For the above reasons, any conclusions about differences in hantavirus activity among individual parks in the study area are discouraged. The finding of widespread infection by SNV and related viruses in the eastern and central United States supports the hypothesis that these viruses have been associated with sigmodontine and arvicoline reservoirs in North America on an evolutionary time scale and underscores the potential for endemic HPS throughout the contiguous United States. Most of the land area of the United States lies within the range of at least one sigmodontine rodent that is known to carry a hantavirus that is a recognized cause of HPS.

The 7% overall antibody prevalence in deer mice is close to that reported in studies from Montana¹⁸ and Kansas.¹⁹ It is somewhat lower than that seen during an extensive study in the southwestern United States in 1994 (11%),¹⁴ and much lower than that seen in the wake of the outbreak of HPS in the American Southwest in 1993 (30%).³ Most cases of HPS in the United States are due to SNV hosted by the deer mouse. It is tempting to explain the large difference in the numbers of human cases between the eastern and western United States in terms of differences in the reservoir population density or prevalence of infection. However, a comparison with epizootic conditions in the Four Corners area in 1993 is misleading because trapping efforts focused around case households and nearby controls, and compari-

sons with the more recent studies, cited above, do not reveal large differences in the prevalence of antibody. Furthermore, antibody prevalences in the only western park sampled (Yellowstone NP) and the more eastern parks were nearly identical.

Overall rodent density (as measured by trap success) was higher in Yellowstone NP than in most eastern parks. However, several eastern parks, including Effigy Mounds NM, Isle Royale NP, Shenandoah NP, Virginia, Sleeping Bear Dunes NL, St. Croix NSR, and Voyageurs NP, had comparable or slightly higher trap success. Specific trap success for deer mice was higher in Yellowstone NP than in any other park except Isle Royale NP. Nevertheless, higher trap success at a single park does not allow extrapolation to a regional scale. Most eastern parks were trapped over short periods and according to strict schedules; adverse weather, phase of the moon, and month of trapping would have confounded direct comparisons. The question of higher densities of deer mice in the western United States can be answered only by a more comprehensive study that measures true population densities and controls for both temporal and spatial variation.

Most parks where white-footed mice were captured had no antibody-positive individuals, in spite of relatively large sample sizes of animals tested. The overall antibody prevalence in white-footed mice in this study (2%) is much lower than that seen in a retrospective study in white-footed mice in New York State (12%).²⁰ However, in those parks where antibody-positive white-footed mice were found, prevalences were sometimes high (14%, 29%, and 33%; Table 1). Although small sample sizes were a contributing factor, the high variance in antibody prevalence indicates the distribution of infection in populations of white-footed mice is patchy. This pattern was also observed by White and others,²⁰ who found antibody prevalences as high as 48% at sites on Long Island, but < 1% in other areas of New York State.

Given the high degree of variation in antibody prevalence among individual parks within regions, perceived differences in hantavirus activity among regions should be interpreted cautiously. In addition, based on a single cross-sectional survey, any variation in time would be missed, and only spatial variation observed. Nevertheless, parks in the Great Lakes area that had populations of deer mice showed no (Sleeping Bear Dunes NL, Indiana Dunes NL, Voyageurs NP) or very low (Isle Royale NP) prevalence of antibody. The highest regional antibody prevalence for both deer mice and white-footed mice was seen in the Northeast. On a finer scale, antibody prevalence was strikingly high in the parks in the Appalachian Mountains. Sixteen of the 21 SNV antibody-positive deer mice and white-footed mice found east of the Mississippi River (Northeast, Southeast, and Great Lakes regions) were in the three parks in the Appalachian Mountains, Allegheny Portage Railroad NHS (six of 31 = 19%), Shenandoah NP (eight of 111 = 7%), and Great Smoky Mountains NP, Tennessee and North Carolina (two of 33 = 6%). Finally, SNV antibody-positive deer mice or white-footed mice were captured in several areas where no cases of HPS have been reported (Pennsylvania, Tennessee, Michigan, Iowa, Oklahoma, Nebraska, and Washington DC), in-

dicating the likelihood that HPS will eventually be recognized in these states.

The rice rat and the cotton rat are reservoirs for hantaviruses (Bayou virus and Black Creek Canal virus, respectively) that are known human pathogens. The range of the rice rat is restricted to the southeastern United States, and the species rarely reaches high densities in any part of its range.²¹ The 12 rice rats captured during our study came from only five of the 16 parks within its range, and it was usually rare (< 10% of the captures for a given park). Nevertheless, three of only 14 animals captured in Big Cypress N Pres were rice rats (all antibody-negative) and two of only three animals captured at Fort Pulaski NM were rice rats (both were antibody-positive). Thus, although rice rats and Bayou virus are unlikely to be epidemiologically important on a broad scale, the potential exists for high rates of infection in local populations, and sporadic transmission of Bayou virus to humans.

The range of the cotton rat includes the southeastern and southcentral United States, most of Mexico and Central America, and parts of northern South America, and may be expanding to the north and west.²² The species has an affinity for natural and anthropogenic, grassy, nonforest habitats, and densities may be locally high (50–70/hectare),²² especially in autumn. Populations may reach plague proportions in agricultural areas.²³ During our study, cotton rats were captured in nine of the 16 parks within their range, and they often constituted a numerically important part of the rodent assemblage (29% of 14 captures at Big Cypress N Pres, 23% of 22 at Buffalo National River, Arkansas, 33% of three at Fort Pulaski NM, 53% of 15 at Gulf Islands NS, Florida and Mississippi). Prevalence of antibody in the species was low (one of 32 total; one of nine captured at Fort Larned NHS). Nevertheless, its local abundance and affinity for disturbed grassy habitats^{22, 24} would bring cotton rats into frequent contact with humans and suggests a high potential for sporadic cases of HPS within the range of the species.

The finding of antibody to hantavirus in North American arviculines is common. The only autochthonous New-World hantavirus recognized prior to 1993 was Prospect Hill virus, associated with the meadow vole (*Microtus pennsylvanicus*).¹⁵ Recently, Bloodland Lake virus was described from the prairie vole,²⁵ and Isla Vista virus from the California vole (*M. californicus*),⁴ and a genetically distinct hantavirus has been described from *M. montanus*.²⁶ No distinct hantavirus has been described from the red-backed vole. Antibody detected in this species may indicate that it is host to distinct hantaviruses or may represent interspecific spill-over of virus from known sympatric hosts. Viral isolation or sequencing of products from RT-PCR will be necessary to distinguish among these alternatives. Although none of the arvicoline viruses in North America have been associated with any human disease, Puumala virus, which is associated with the bank vole (*Clethrionomys glareolus*), is responsible for a relatively mild form of hemorrhagic fever with renal syndrome in Europe.

Although the known American hantaviruses are restricted to the sigmodontine and arvicoline rodents, the finding of antibody-positive chipmunks may not be unusual. During follow-up investigation of the original outbreak of HPS in the southwestern United States, 4% of 166 *Tamias dorsalis*,

5% of 19 *T. minimus*, and 13% of 30 *T. quadrivittatus* had antibody reactive with Prospect Hill virus.³ Sequences derived from RT-PCR products from these animals were identical or nearly identical to SNV from deer mice captured at the same sites. The high prevalence of infection among non-reservoir species during this earlier investigation was probably because of the unusually high rodent densities and epizootic conditions within the deer mouse population. Nevertheless, one might expect that sporadic infection may be identified in any species which is sympatric with a hantavirus reservoir species if enough individuals are sampled.

Increased frequency of infection in male hosts and in heavier mass classes of deer mice is in agreement with (but less pronounced than) that observed in other studies of hantavirus reservoir species,^{14, 19, 27, 28} suggesting that hantaviruses are transmitted horizontally within reservoir populations and that transmission may frequently result from bites inflicted during aggressive encounters among males.¹⁴

This study was designed to be extensive in scope, but was not intensive within specific parks or regions. It has provided us with a broad perspective of the geographic extent of infection within hantavirus reservoir populations, and how infection rates may vary among regions. Additional regional and site-specific studies are needed to provide an understanding of the dynamics of infection within local reservoir populations. These studies should address how transmission and infection in reservoir populations vary over time, among sites, and among specific habitats, and how these variables are influenced by human activities and relate to human exposure.

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