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Source: Journal of Wildlife Diseases, 43(1) : 1-11

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-43.1.1

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Journal of Wildlife Diseases, 43(1), 2007, pp. 1–11
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DEMOGRAPHIC FACTORS ASSOCIATED WITH PREVALENCE OF ANTIBODY TO SIN NOMBRE VIRUS IN DEER MICE IN THE WESTERN UNITED STATES

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ABSTRACT: We used long-term data collected for up to 10 yr (1994–2004) at 23 trapping arrays (i.e., webs and grids) in Arizona, Colorado, Montana, and New Mexico to examine demographic factors known or suspected to be associated with risk of infection with Sin Nombre virus (SNV) in its natural host, the deer mouse (*Peromyscus maniculatus*). Gender, age (mass), wounds or scars, season, and local relative population densities were statistically associated with the period prevalence of antibody (used as a marker of infection) to SNV in host populations. Nevertheless, antibody prevalence and some of the risk factors associated with antibody prevalence, such as relative population density, gender bias, and prevalence of wounding, varied significantly among sites and even between nearby trapping arrays at a single site. This suggests that local microsite-specific differences play an important role in determining relative risk of infection by SNV in rodents and, consequently, in humans. Deer mouse relative population density varied among sites and was positively and statistically associated with infection prevalence, an association that researchers conducting shorter-term studies failed to demonstrate. Both wounding and antibody prevalence increased with mass class in both males and females; this increase was much more pronounced in males than in females and wounding was more frequent in adult males than in adult females. Prevalence of wounding was greatest among seropositive deer mice, regardless of mass class, but many deer mice without detectable wounds or scars eventually became infected. Many of these patterns, which will be useful in the development of predictive models of disease risk to humans, were only detected through the application of data collected over a long (10-yr) period and with abundant replication.

Key words: Antibody prevalence, Arizona, Colorado, deer mice, gender, hantaviruses, Montana, New Mexico, *Peromyscus maniculatus*, population density, risk factors, Sin Nombre virus, wounds.

INTRODUCTION

In 1993, an outbreak of severe respiratory distress in humans in the southwestern United States led to the recognition of hantavirus pulmonary syndrome (HPS). Although HPS has now been recognized in 32 states, from California to New York, and in Canada, the great majority of cases still occur in the western USA. Sin Nombre virus (SNV; family *Bunyaviridae*, genus *Hantavirus*), the principal cause of HPS in the USA, is hosted by the deer mouse (*Peromyscus maniculatus*). Although several other hantaviruses associated with other sigmodontine rodents have been identified in the USA, most HPS cases here are associated with SNV and the deer mouse.

Beginning in 1994, longitudinal studies of hantaviruses and their rodent hosts were initiated in Arizona, Colorado, Montana, and New Mexico (Douglass et al., 1996; Mills et al., 1999b). The purpose of these studies is to elucidate the natural history of hantavirus-host associations.

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More specifically, we attempt to track hantavirus infection in host rodents in ecologically and geographically diverse habitats to delineate the environmental and demographic bases of changes in infection prevalences and, most importantly epidemiologically, to devise measurements of increased risk factors for HPS to provide early warning to humans.

The epidemiology of HPS in North America is closely tied to the ecology and epizootiology of SNV infection in deer mice. Understanding the risk factors associated with SNV infection in deer mice is important because it leads to an understanding of the mechanisms of SNV transmission among its rodent hosts and the temporal and spatial variation in this transmission. This knowledge, in turn, may lead to improved prediction of relative risk of HPS for humans. Several factors have been associated with the presence of antibody to hantaviruses (as a marker of infection) in rodent hosts. An association between male gender and antibody prevalence has been reported for SNV (Childs et al., 1994; Mills et al., 1997; Calisher et al., 2005), as well as for numerous other hantaviruses (Glass et al., 1998; Levis et al., 1998; Abbott et al., 1999; Calderón et al., 1999; Cantoni et al., 2001; Yahnke et al., 2001; Escutenaire et al., 2002; Murua and Padula, 2004). An association between mass (as an indicator of age) and antibody prevalence has been demonstrated for several hantaviruses (Mills et al., 1997; Glass et al., 1998; Escutenaire et al., 2000; Douglass et al., 2001), implying that these viruses are transmitted horizontally among hosts. An association between the presence of wounds (or scars) and antibody prevalence has been demonstrated for Seoul virus (Glass et al., 1988) and several New World hantaviruses (Calisher et al., 1999; Douglass et al., 2001), implying transmission via aggressive encounters. Varying seasonal patterns in the prevalence of infection with hantaviruses in their host populations also have been reported (Niklasson et al., 1995; Escutenaire et al., 2000; Douglass et al., 2001; Calisher et al., 2005).

Hantaviruses are hypothesized to be transmitted in host populations by aggressive encounters, or perhaps occasionally by environmental contamination with infectious excreta. Transmission under these circumstances would be expected to increase with host population density (density-dependent transmission [Begon et al., 2002; Keesing et al., 2006]). Thus, a positive association between antibody prevalence and population density should be expected (Mills et al., 1999a). Nevertheless, evidence for such an association in hantavirus host populations has been difficult to demonstrate.

In this study, we used longitudinal data collected over as many as 10 yr in Arizona, Colorado, Montana, and New Mexico and including more than 27,566 captures of deer mice to provide a detailed analysis of the risk factors associated with infection with SNV in these rodents. Within our study areas, we characterized the extent of variation in SNV infection in deer mice among locations and seasons and between genders. As well, we describe the association of SNV infection with deer mouse age, wounding, and population size.

MATERIALS AND METHODS

Permanent mark-recapture trapping arrays (grids in Montana, webs elsewhere) were established at ecologically diverse locations in Montana (1994), Colorado (1994), New Mexico (1994), and Arizona (1995). Descriptions of the sites and preliminary results of studies at these sites have been published (Douglass et al., 1996; Abbott et al., 1999; Calisher et al., 1999; Mills et al., 1999a; Parmenter et al., 1999). Small-mammal trapping was generally done for three nights each month (each 6 wk in Colorado) beginning in mid-1994, as weather and logistics permitted. Trapping was not conducted during the winter and early spring months (December–April) in Colorado; in Montana, winter trapping was conducted at only three trapping grids at a single site (Cascade). Collection of rodents and safety procedures at all sites followed recommended procedures published by the US Centers for Disease Control and Pre-

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vention (Mills et al., 1995a, b). The following data were recorded for each animal: date processed, species, weight and other standard measurements, the presence of wounds or scars, age (juvenile, subadult, adult), gender, reproductive status (for males whether testes were scrotal and for females whether the vaginal orifice was perforate, whether lactating, and whether mammary glands were enlarged), and capture status (new capture or recapture [captured in previous trapping sessions but not captured within the previous 2 days]). A sequentially numbered stainless steel tag was inserted into the cartilage of an ear of each animal, and an approximately 0.2-ml blood sample was taken from the retro-orbital capillary plexus. The rodent was then released on the trapping array from which it had been collected.

We used the presence of antibody to SNV as our marker of recent or previous infection. Blood samples were tested by a standard enzyme-linked immunosorbent assay (ELISA) for IgG antibody to SNV (Feldmann et al., 1993). Because of the broad cross-reactivity of the SNV nucleocapsid antigen, detected antibodies to SNV are not specific but can be used as an indication of infection with a hantavirus; the specific hantavirus can only be determined by detection and sequencing of viral RNA. Nevertheless, because hantavirus-host relationships are generally highly specific, we assumed that ELISA detection of IgG antibody in deer mice indicated infection with SNV.

Data were summarized and analyzed using Microsoft Access XP, SPSS, and SAS. Data were analyzed by trapping array (web or grid). In many cases patterns were similar among arrays at a given site and these arrays were combined for further analysis. When patterns were similar among sites, but sample sizes were not adequate to allow analysis by site, sites were combined by region (Arizona, western Colorado, southeastern Colorado, Montana, New Mexico). In some cases, where patterns were similar among regions, all data were combined into a single analysis. Antibody prevalence was expressed as "period prevalence," where the period was equal to the entire sampling period for each site. Thus, prevalence was the total number of antibody-positive individual rodents captured divided by the number of individual rodents tested during the sampling period. An individual mouse was counted as antibody-positive if it was found antibody-positive at one or more captures. To analyze the association between age and antibody prevalence, deer mouse body weight was used as a surrogate for age and animals were divided into six (one juvenile, one subadult, and four adult) mass classes at 4-g intervals (Douglass et al., 2001). If an individual mouse was captured in more than one mass class, it could appear multiple times in the analysis, but it appeared only once in each mass class. These same inclusion criteria applied to wounding. Because not all investigators kept accurate data concerning palpable pregnancies, we could not remove pregnant females from weight class calculations. Thus, pregnant females may be assigned to a higher mass class than their nonpregnant weight would justify. Because of differences in trapping effort between Montana (100 traps per grid) and all other sites (145 traps per web), we expressed relative deer mouse population abundance using trap success. Trap success was calculated as the number of individual mice captured, divided by the number of trap nights (number of traps multiplied by the number of nights) multiplied by 100.

Some investigators have recommended the use of a correction for an "experiment-wise" error rate (e.g., Bonferroni correction [Miller, 1991]) when multiple statistical comparisons are made with the same data set. However, the application of such a correction to our data would presuppose a universal null hypothesis with which we are not concerned and would increase the likelihood of type II errors. We agree with others who argue that these corrections should not be used when assessing evidence about specific hypotheses (Perneger, 1998; Nakagawa, 2004).

RESULTS

For purposes of our analyses we included only those sites where the deer mouse was a numerically dominant member of the rodent assemblage. Central and southern Arizona sites dominated by brush mice (*Peromyscus boylii* [Abbott et al., 1999; Kuenzi et al., 1999]) were eliminated, as were desert sites in New Mexico that were dominated by rodents in the family Heteromyidae (Parmenter et al., 1999). The final database included information about 27,566 captures of 11,458 individual deer mice trapped on 23 trapping arrays at the 11 sites. Most trapping sites were operated for 9–10 yr; a few were operated for shorter intervals (Table 1).

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TABLE 1. Period prevalence of IgG antibody to Sin Nombre hantavirus in male and female deer mice at sites in Arizona, Colorado, Montana, and New Mexico, 1994–2004. Prevalence estimates do not include 67 mice that were missing data for serological results, gender, or capture site.

Array (state)	Sampling dates	Prevalence in males: % (*n*)	Prevalence in females: % (*n*)	*P* value, Fisher's exact test
Fort Lewis A (Colorado)	June 1994–October 2004	22.9 (398)	8.9 (448)	<0.001
Fort Lewis B (Colorado)	June 1994–October 2004	30.0 (373)	17.9 (392)	<0.001
Molina A (Colorado)	October 1994–September 2004	9.2 (251)	5.9 (272)	0.183
Molina B (Colorado)	October 1994–September 2004	13.1 (191)	7.4 (202)	0.068
PCMS-1 (Colorado)	January 1995–August 2001	7.1 (282)	0.5 (191)	<0.001
PCMS-2 (Colorado)	January 1995–August 2001	2.2 (180)	0.8 (118)	0.652
Navajo-1 (New Mexico)	October 1997–August 2004	12.9 (232)	6.7 (210)	0.038
Navajo-2 (New Mexico)	January 1995–August 2004	4.0 (124)	6.7 (103)	0.386
Zuni-1 (New Mexico)	September 1994–August 2004	10.3 (155)	1.8 (110)	0.006
Zuni-2 (New Mexico)	October 1997–August 2004	20.7 (144)	4.9 (104)	<0.001
Cascade-11 (Montana)	June 1994–September 2004	8.4 (655)	6.7 (524)	0.321
Cascade-12 (Montana)	June 1994–September 2004	5.2 (385)	1.7 (359)	0.009
CMR-17 (Montana)	June 1994–September 2004	6.0 (251)	1.4 (216)	0.014
CMR-18 (Montana)	June 1994–September 2004	6.7 (165)	5.8 (120)	0.811
Cutbank-14 (Montana)	June 1994–September 2004	9.0 (210)	3.9 (155)	0.060
Cutbank-15 (Montana)	June 1994–September 2004	22.7 (216)	12.5 (176)	0.012
Gold Creek-8 (Montana)	June 1994–September 2004	14.2 (212)	4.7 (170)	0.002
Gold Creek-9 (Montana)	June 1994–September 2004	11.0 (155)	7.4 (148)	0.325
Polson-5 (Montana)	June 1994–September 2004	33.8 (724)	22.4 (715)	<0.001
Polson-6 (Montana)	June 1994–September 2004	16.3 (240)	13.3 (211)	0.427
Grand Canyon E (Arizona)	August 2002–October 2004	30.0 (263)	16.6 (217)	<0.001
Grand Canyon M (Arizona)	August 2002–October 2004	40.4 (109)	8.8 (91)	<0.001
Grand Canyon T (Arizona)	August 2002–October 2004	15.0 (133)	7.7 (91)	0.142
Overall		16.5 (6,048)	9.5 (5,343)	<0.001

Variation in SNV antibody prevalence among sites

The period prevalence of antibody to SNV was highly variable among sampling sites and trapping arrays, ranging from 2.2% to 40.4% in males and 0.5% to 22.4% in females (Table 1). Sites with the highest prevalence in male deer mice also had relatively higher prevalence in female mice. Although antibody prevalences sometimes were very similar among trapping arrays at the same site, there were sometimes large differences among arrays (e.g., Zuni, Polson, Grand Canyon; Table 1).

Association between antibody prevalence and gender

The period prevalence of SNV antibody in male deer mice was higher than that in female deer mice at 22 of 23 trapping arrays (Table 1). Overall, the male bias in antibody prevalence was statistically significant ($P \leq 0.05$; Fisher's exact test) at only 13 of the 23 trapping arrays.

Association between antibody prevalence and mass (age)

Combining data on deer mice from all sites (the patterns were similar for all sites except southeastern Colorado), there was a J-shaped curve relating SNV antibody prevalence to mass class (Fig. 1a). In the four adult mass classes (>14 g), there was a statistically higher antibody prevalence in male than in female deer mice ($P < 0.001$, Fisher's exact test; Fig. 1a). In the juvenile and subadult mass classes, there were no statistically significant differences in antibody prevalence between genders. When the juveniles were excluded, antibody prevalence appeared to increase as a linear function of mass class and appeared to increase more rapidly in males than in females (Fig. 1a).

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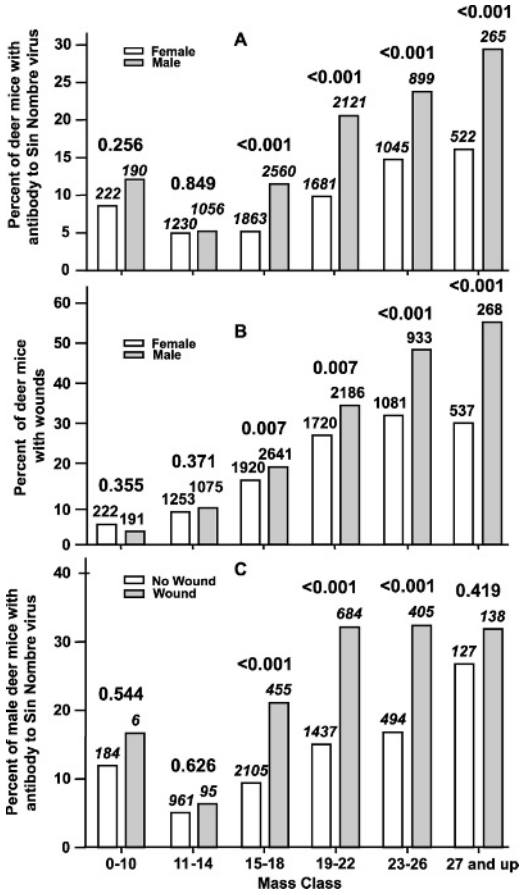


FIGURE 1. Percentage of male and female deer mice (a) with antibody to Sin Nombre virus, (b) with detectable wounds or scars, and (c) with antibody and with or without wounds, in each of six mass classes on 23 mark-recapture trapping arrays in Arizona, Colorado, Montana, and New Mexico, 1994–2004. Sample sizes (n=number of individual male deer mice tested) and probabilities (Fisher's exact test) are provided above bars.

Association between antibody prevalence and frequency of wounds or scars

As did the prevalence of antibody, the prevalence of wounds in deer mice increased with age for both males and females (Fig. 1b). Thus, scars and antibody should be positively correlated because of the cumulative association of both variables with age. We minimized the confounding effect by comparing wounding frequencies between deer mice with and without antibody within each mass class (Fig. 1c). The patterns of association of antibody prevalence and wound or scar prevalence by mass class were very similar at all sites except those in southeastern Colorado, which did not show any clear pattern. When all sites were combined, deer mice with wounds or scars had higher antibody prevalences than those without wounds or scars in all six mass classes. However, the antibody prevalence differences between wounded and unwounded mice were not statistically significant in the oldest adults and in the juvenile and subadult mass classes (Fig. 1c).

Association between antibody prevalence and season

Seasonal differences in antibody prevalence varied among sampling regions (Arizona, western Colorado, southeastern Colorado, Montana, and New Mexico) for both male and female deer mice (Table 2). In western Colorado (2,041 m to 2,389 m elevation) and Montana (834 m to 1,990 m), antibody prevalences were highest in spring and summer. Although the pattern in northern Arizona (2,040 m) was less clear, the highest antibody prevalence in male deer mice was in the summer. The highest prevalence in New Mexico (2,040 m to 2,161 m) also was in the summer, although there were no statistically significant differences for male deer mice. The highest prevalence for male deer mice in lower-altitude southeastern Colorado (1,493 m) was in the autumn.

Association between antibody prevalence and deer mouse relative population density

Using period antibody prevalence data and mouse relative population density data for all 23 trapping arrays, we found a positive association between overall array-specific antibody prevalence and relative deer mouse population density (trap success) presented as individual deer mice captured per 100 trap nights at each array (F=11.56, P=0.002, r^2=0.355; Fig. 2). To avoid bias, trap success was calculated using only those months (May–October) when trapping occurred at all

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TABLE 2. IgG antibody to Sin Nombre virus in deer mice at Arizona, Colorado, Montana, and New Mexico sites, by gender and season, 1994–2004.

Site and state	Season[a]	Males: No. positive/no. tested (% prevalence)	Females: No. positive/no. tested (% prevalence)
Western Colorado[b]	SP	67/253 (26.5) (AU)[d]	41/256 (16.0) (SU,AU)
	SU	111/523 (21.2)	62/588 (10.5) (SP)
	AU	134/730 (18.4) (SP)	64/754 (8.5) (SP)
		$P=0.023$[e]	$P=0.004$
Southeastern Colorado	WI	3/98 (3.1)	1/78 (1.3)
	SP	5/222 (2.3) (AU)	0/153 (0.0)
	SU	5/123 (4.1)	0/73 (0.0)
	AU	15/214 (7.0) (SP)	1/132 (0.8)
		$P=0.104$	$P=0.421$
New Mexico	WI	30/208 (14.4)	5/138 (3.6) (SU)
	SP	25/181 (13.8)	11/180 (6.1)
	SU	33/198 (16.7)	17/171 (9.9) (AU,WI)
	AU	27/190 (14.2)	4/138 (2.9) (SU)
		$P=0.862$	$P=0.044$
Montana	WI[c]	16/320 (5.0) (SP,SU,AU)	4/231 (1.7) (SP,SU,AU)
	SP[c]	156/806 (19.4) (AU,WI)	87/632 (13.8) (AU,WI)
	SU	318/1703 (18.7) (AU,WI)	177/1579 (11.2) (AU,WI)
	AU	155/1521 (10.2) (WI,SP,SU)	106/1367 (7.8) (WI,SP,SU)
		$P<0.001$	$P<0.001$
Arizona	WI	32/162 (19.8) (SU)	10/113 (8.8)
	SP	45/199 (22.6) (SU)[f]	17/173 (9.8)
	SU	82/266 (30.8) (WI,SP)	23/216 (10.6)
	AU	86/337 (25.5)	20/268 (7.5)
		$P=0.056$	$P=0.643$

[a] WI = December, January, February; SP = March, April, May; SU = June, July, August; AU = September, October, November. Although start dates varied among arrays (see Table 1), period of analysis is 1994 through 2004 for all regions except Arizona (2002–2004).

[b] Pooled data from Colorado sites Fort Lewis and Molina.

[c] WI and SP for Montana represent only a single trapping site (Cascade).

[d] Prevalences with superscripts are statistically different from prevalences in those seasons indicated by the superscript; Fisher's exact test, $P<0.05$, except as noted.

[e] Probability of overall Fisher's exact testing differences in antibody prevalence among seasons.

[f] $P=0.058$

sites. A single point (at the upper right in Fig. 2) exercises a large amount of influence on the data. Even when we delete that point, the resulting model provides a statistically significant positive relationship between prevalence and population density ($F=7.97$, $P=0.01$, $r^2=0.281$).

DISCUSSION

The collection of long-term data allows the analysis of characteristics of host-virus systems that are subject to high variation in the short term because of extrinsic factors. Although highly variable and unpredictable in the short term, these characteristics (antibody prevalences, population densities, gender bias, associations with season, etc.) may have measurable values that distinguish sites, habitats, and populations, and these values may manifest as long-term averages.

Our results, based on large sample sizes and over periods up to 10 yr, have confirmed and refined conclusions of several short-term studies demonstrating

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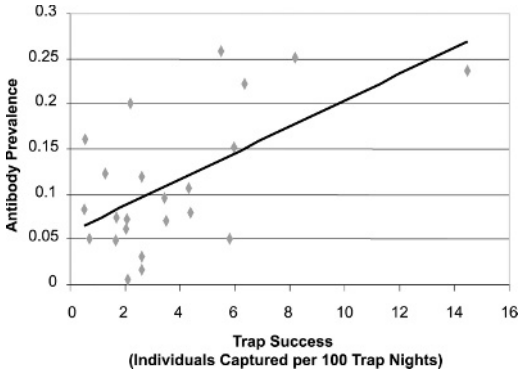


FIGURE 2. Regression of prevalence of antibody to Sin Nombre virus in deer mouse populations on the relative population density (% trap success expressed as number of captures per 100 trap nights) at 23 trapping arrays in Arizona, Colorado, Montana, and New Mexico, 1994–2004. Trap success values were restricted to the months of May through October, when all arrays were in operation.

the association of SNV infection with gender, age, and wounding. We also provide new information concerning spatial and temporal variation in prevalence of infection with SNV in populations of deer mice. We demonstrate the important relationship of relative population size with antibody prevalence, a relationship that has eluded many previous researchers, including ourselves. Furthermore, differences in population size and resulting patterns in infection prevalence appear to be associated with long-term, site-specific differences that are manifest on a local scale and are only discernable through the examination of long-term data.

The overall prevalence of antibody to SNV was highly variable among sites and even between replicate trapping arrays at the same site. Prevalence of infection in male deer mice varied from 2% to 30% at arrays in southeastern and western Colorado, respectively. Although similar differences in prevalence among sites have been observed (Mills et al., 1997), previous studies were conducted over a short time period and were suspected of being related to local temporal variation in antibody prevalence. The prevalences reported here are long-term averages and thus likely represent site-specific differences and not short-term temporal variation at a given site.

The positive relationship between long-term relative population density and antibody prevalence suggests that deer mouse population density may be an important factor in determining frequency of virus transmission. Thus, SNV in deer mouse populations appears to follow density-dependent transmission (Begon et al., 2002; Keesing et al., 2006) as predicted (Mills et al., 1999a). Several previous studies (Mills et al., 1997; Boone et al., 1998; Douglass et al., 2001) failed to show a positive relationship between deer mouse abundance and antibody prevalence. We believe this is because previous studies sought to demonstrate simultaneous relationships in populations changing over time, without taking into account the seasonal time-lag relationship between population density and prevalence (Niklasson et al., 1995; Mills et al., 1999a). The use of long-term, site-specific average densities and prevalence reduces the effect of temporal variation so that inherent site-specific differences emerge. The relatively low r^2 value for the regression of antibody prevalence on population density could indicate a poor fit to the linear model, or simply a high variability in the data. The linear model exhibited a better fit than any of several simple nonlinear models we tested. We believe the low r^2 is simply a reflection of the high variability in the data, and that this variability results from the numerous extrinsic factors affecting transmission rates other than host population density. Based on a very limited sample size, the data suggest that there may be an upper limit to antibody prevalence at around 25%. Although short-term studies have shown that prevalences can, at least temporarily, reach much higher levels in localized areas (e.g., Childs et al., 1994), there is likely an upper limit to the prevalence that can be sustained over the

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long term. At its maximum, prevalence may be balanced by the recruitment of uninfected young (Mills et al., 1999a).

Male bias in antibody prevalence was evident at most but not all arrays, despite the analysis of relatively large sample sizes of male and female deer mice (e.g., there was a nonstatistically significant female bias at Navajo-2, and prevalences were similar between genders at Cascade 11 and at CMR-18, Table 1). The lack of male bias at these sites was not a broad regional phenomenon because nearby trapping sites did reveal significant male bias in antibody prevalence. Differences in degree of male bias in prevalence of antibody to hantaviruses have been hypothesized to be because of intersite or interspecies differences in social behavior (degree of aggression, venereal transmission, communal nesting [Mills et al., 1999a]). Our finding of large differences among local populations (among trapping arrays) of the same species at the same site demonstrate that differences in male bias in antibody prevalence may not require interspecific differences in social interactions or even differences in behavior because of different environmental conditions (e.g., winter huddling in colder climates).

We did not observe a significant male bias in juvenile (<15 g) mice. Equal prevalence of infection in male and female juvenile deer mice was also detected by Mills et al. (1997) during a short-term study in the southwestern United States and Glass et al. (1998) demonstrated a similar pattern in a short-term collection of cotton rats (*Sigmodon hispidus*) infected with Black Creek Canal hantavirus in southern Florida. Thus, our results support the hypothesis (Mills et al., 1999a) that the antibody detected in members of the smallest (youngest) mass classes was maternal antibody passed equally to each gender. Maternal antibody then wanes and leaves older animals susceptible to infection via an undefined gender-biased mechanism. When our data are compared to those from previously published studies regarding hantavirus infections, the degree of male bias in antibody prevalence among adult rodent hosts appears to be greater in some other host species (e.g., cotton rats and Black Creek Canal virus [Glass et al., 1998], brush mice, *Peromyscus boylii*, and Limestone Canyon virus [Abbott et al., 1999]) than we found in deer mice. Careful comparative analyses will be required to determine whether these differences are because of site differences, or because of differences in social structure and behavior among hosts (Mills et al., 1999a).

We demonstrated a clear increase in antibody prevalence with mass class (used as a surrogate for age) for female as well as for male deer mice (Fig. 1a). The increase in antibody prevalence across ages in females was not clear in data presented previously (Mills et al., 1997). In addition, antibody prevalence increased with age more rapidly in male deer mice than in female deer mice, suggesting a higher rate of SNV transmission among male than among female deer mice, especially adults. This pattern of more rapid increase in transmission with age in male hosts was also observed for cotton rats (Glass et al., 1998).

The higher antibody prevalence in male mice with wounds or scars in all mass classes except the juvenile classes suggests that wounding is somehow related to infection, as has been suggested previously (Glass et al., 1988; Calisher et al., 1999; Douglass et al., 2001). The failure to find statistically significant results for the heaviest mass class (Fig. 1c) may simply relate to the relatively smaller sample of mice in that category. Associations between wounds and antibody presence have been taken as evidence that hantaviruses are frequently transmitted by aggressive (social) encounters (Glass et al., 1988; Calisher et al., 1999; Douglass et al., 2001). Under this hypothesis, one would predict a pattern of more rapid acquisition of wounds by adult males.

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Such a pattern of more rapid acquisition of wounds by adult males is observed in Figure 1b. Indeed, as demonstrated by a comparison of Figures 1a and 1b, the patterns of acquisition of wounds and acquisition of antibody are very similar.

Although the prevalence of SNV antibody increased with age in both males and females, prevalence seemed to reach a peak much earlier in deer mice with visible wounds than in those for which no wounds were detected (Fig. 1c). It is possible that this pattern indicates that aggression is not the only mechanism of transmission of SNV among deer mice and those not involved in fighting can be infected by other mechanisms (e.g., naso-nasal and naso-anal contact, mutual grooming, communal huddling, aerosol, or venereal transmission) and longevity in and near the trapping site may be an important factor. Failure to observe a wound or a scar does not necessarily indicate that mice have not received bite wounds. Our inspections were brief, and many wounds were likely either healed or hidden under the fur and therefore not detected.

Clear seasonal changes in antibody prevalence were seen only in Montana: prevalences were highest in spring and summer and declined in fall and winter. We caution, however, that winter prevalence in Montana was estimated on the basis of three webs at a single location where trapping was conducted year-round for a 10-yr period. The prevalences in western Colorado were lowest in winter and fall and highest in spring. However, because the sampling sites were generally inaccessible during March and April, spring in western Colorado is represented only by the May sampling data. The seasonal pattern at the Grand Canyon, Arizona, may not yet be considered as clear because it is based only on approximately 2 yr of data and the lack of a seasonal pattern in southeastern Colorado may be because of the fact that SNV was absent from deer mouse populations during most of the 6-yr study there (Calisher et al., 2005). In New Mexico, sampling was conducted consistently each month, although sample sizes of antibody-positive deer mice were relatively low. It is possible that seasonal patterns at these high desert sites in New Mexico were obscured by the relatively large interannual variation driven by El Niño cycles (Yates et al., 2002).

In conclusion, we used long-term data with abundant replication to examine several factors that are known or suspected to be associated with the prevalence of infection by SNV in populations of deer mice. Overall period prevalences were quite variable across arrays for both male and female deer mice. Period prevalences of antibody to SNV also were related to average population size, gender, and age (as indicated by mass class). The long-term continuity of these studies, the accumulation of large numbers of samples, and averaging across many years allowed the demonstration of patterns (including the association between population size and antibody prevalence and the existence of consistent microsite-specific differences in risk factors associated with infection) that were not discernable from shorter-term studies. In spite of the replication and relatively long sampling period of our study, patterns were variable and statistical analyses were sometimes limited by inadequate sample sizes, particularly when samples were subdivided by site, array, or mass class, and when analyses depend on the sample size of deer mice with antibody. This suggests that SNV and host dynamics can only be described by using very large sample sizes and a very large number of replicates in space and time. Even 10 yr is a short period for monitoring biologic systems in a changing environment. The long-term continuity of these studies is critical to our goal of understanding fluctuations in the transmission of SNV in deer mice and its relationship to human disease.

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ACKNOWLEDGMENTS

This has been a truly collaborative effort on the parts of the authors and many others who have assisted in large and small ways. K. Hughes, T. Wilson, and W. Semmens assisted with field work done by the group at Montana Tech and S. Zanto, Department of Health, Helena, tested samples collected in Montana. R. McIntosh at Yavapai College; R. Stahn, J. McCury and others at Tusayan, Kaibab National Forest; J. Dunnum, C. Parmenter, C. Hice at the University of New Mexico, Albuquerque; and numerous graduate students and other volunteers of the Arthropod-borne and Infectious Diseases Laboratory, Colorado State University, assisted us in many of our efforts. R. Carns, Mesa County Health Department and T. Schountz, Mesa State College, Grand Junction, Colorado, set traps and saw to many needs. Mr. and Mrs. T. Elkins allowed us to trap on their property, and D. Schafer and D. Zalesky, San Juan Basin Research Center, Fort Lewis, Colorado, provided logistical support. Many employees of the US Army, at both Fort Carson, Colorado Springs, and at the Piñon Canyon Maneuver Site, made housing and other arrangements and sometimes went out of their way to see to it that we had access and approval to conduct trapping. Serological analyses at the US Centers for Disease Control and Prevention were conducted by P. Stockton, L. Pezzanite, K. Thurman, K. Slaughter, D. Cannon, T. Stevens, J. Betts, and A. Comer. Herminia Alva, US Centers for Disease Control and Prevention, assisted with preparation of the manuscript and figures. This study was funded in part by the US Centers for Disease and Prevention. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the funding agency.

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Received for publication 26 February 2006.

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