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SIN NOMBRE VIRUS INFECTION OF DEER MICE IN MONTANA: CHARACTERISTICS OF NEWLY INFECTED MICE, INCIDENCE, AND TEMPORAL PATTERN OF INFECTION

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ABSTRACT: Sin Nombre virus (SNV), hosted by the deer mouse (*Peromyscus maniculatus*), is the principal cause of hantavirus pulmonary syndrome (HPS) in North America. To improve our understanding of factors that contribute to the occurrence of HPS, we conducted an extensive field study of the characteristics of newly infected (as determined by recent acquisition of antibody) deer mice and the temporal pattern of antibody acquisition (seroconversion) from 1994 through 2004 in Montana, USA. We sampled 6,584 individual deer mice, of which 2,747 were captured over multiple trapping periods. Among these 2,747 deer mice, we detected 171 instances of seroconversion. There was no relationship between seroconversion and the acquisition of scars. However, recently infected Montana deer mice were more likely to be older, more likely to be males, and more likely to be in breeding condition. In addition, recently infected male deer mice gained less weight over the 1-mo period following seroconversion than did those that did not acquire antibody, suggesting that SNV infection may have negatively impacted the health of infected rodents. Incidence was highly variable among years, and timing of infections was primarily associated with the breeding season (generally early spring through late fall).

Key words: Antibody, deer mice, epidemiology, hantavirus, Montana, *Peromyscus maniculatus*, seroconversion, Sin Nombre virus.

INTRODUCTION

Sin Nombre virus (SNV; family *Bunyaviridae*, genus *Hantavirus*) is the causative agent of hantavirus pulmonary syndrome (Childs et al., 1994) in the US and Canada. Other hantaviruses cause this severe respiratory disease elsewhere in the Western Hemisphere. After the discovery and identification of SNV in the southwestern US (Nichol et al., 1993), long-term studies of rodent reservoir populations were initiated in Arizona, New Mexico, Colorado, and Montana, USA (Mills et al., 1999). The goal was to obtain sufficient understanding of the ecological dynamics of rodent host–SNV relationship, such that we could develop measures to predict, control, and prevent human exposure to SNV.

Considerable information regarding the ecology of the principal rodent host of SNV, the deer mouse (*Peromyscus maniculatus*), and the prevalence of antibodies against SNV has been accumulated and reported from these studies (Douglass et al., 1996, 2001; Mills et al., 1997, 1998, 1999; Abbott et al., 1999; Calisher et al., 1999, 2001). Specifically, population sizes of rodent hosts of SNV are spatially and temporally variable, as is the prevalence of antibody to SNV. Also, deer mice with antibody to SNV tend to be older, sexually mature, and to have been involved in aggressive encounters, as evidenced by the presence of scars. The prevalence of antibody to SNV also is usually higher in males than in females (Calisher et al., 1999; Douglass et al., 2001). Finally, it appears that SNV is transmitted intraspecifically, during social encounters associated with breeding (Root et al., 2005).

Hantaviruses can cause persistent infections in their rodent hosts, and hantavirus RNA can be detected in tissues, even among rodents with high-titer antibody (Kuenzi et al., 2005). The established method for determining prevalence of

hantavirus infection in rodents is by detection of antibody. Although the presence of antibody indicates infection, it provides no information on when infections were acquired or whether the host is shedding infectious virus.

Botten et al. (2000) detected SNV antigen in kidney within 7 days and in a variety of tissues within 14 days after experimental infection of deer mice. About half of the mice had detectable IgG antibody at 14 days after infection; all had detectable IgG antibody by 21 days after infection (B. Hjelle, pers. comm., 2002). In addition, Botten et al. (2002) detected SNV RNA in saliva samples from a subset of deer mice between 15 and 90 days after experimental infection, but virus was not detected uniformly. Botten et al. (2003) demonstrated that persistently infected deer mice had highly variable patterns of viral infection in regards to tissues involved and degree of viral replication. In a recent study (Safronetz et al., 2005), SNV RNA was amplified from tissues and excreta, including urine of some, but not all, naturally infected deer mice. These results, when taken together, suggest individual differences among naturally infected deer mice with respect to viral shedding. Laboratory studies of several hantaviruses, including Hantaan, Puumala, and Black Creek Canal viruses, have demonstrated that the quantity of virus shed and the risk of infection to other hosts is much higher during the first 2–6 wk after a host has become infected than later in the course of infection (Lee et al., 1981; Yanagihara et al., 1985; Gavrilovskaya et al., 1990; Hutchinson et al., 1998).

Over the past 10 yr we have collected enough data to begin examining characteristics of newly infected rodents (as determined by the recent acquisition of antibody). These are the rodents that likely are shedding the greatest quantities of virus and are most infectious for other hosts and for humans. It was our goal to investigate how gender, breeding condition, wounding, and breeding season relate to recent SNV infection in the deer mouse. It is possible that the associations between gender, breeding condition, breeding season, and antibody prevalence are not directly applicable to recent infection; this information is important for assessing human risk.

The first objective of this study was to use data regarding only deer mice that acquire antibody (seroconvert) between consecutive trapping sessions (a 4-wk interval) and to compare characteristics of those deer mice to a set of deer mice trapped during the same time periods that did not seroconvert. Specifically, we wanted to determine if recently seroconverted deer mice: 1) are more likely to be older, 2) are more likely to be males, 3) are more likely to be in breeding condition, 4) are more likely to have wounds or scars, or 5) have gained less weight than deer mice that have not seroconverted. A second objective was to determine the timing of antibody acquisition and to determine if 1) deer mice are more likely to seroconvert during the breeding season or during the nonbreeding season and 2) if there is interannual variation in seroconversion. The final objective was to determine the incidence of infection (seroconversion) with SNV.

MATERIALS AND METHODS

Study areas

Long-term reservoir studies were conducted in north central (Cascade, Cut Bank, C. M. Russell Wildlife Refuge) and western (Wisdom, Polson, Gold Creek) Montana, USA, in an array of habitats including scrublands, grasslands, and forests (Douglass et al., 1996, 2001; Mills et al., 1999). At each site we found high relative abundances of deer mice, and deer mice accounted for large proportions of the small mammal assemblages.

Trapping and processing

To track host populations and antibody prevalence, live trapping was conducted on 18 grids, from 1994 through 2004. Grids consisting of 100 Sherman live traps were

equally spaced in a square of 1 ha (Douglass et al., 1996). Two grids at each of six sites (12 grids) were monitored monthly for three consecutive nights and the animals tested for antibody to SNV. One grid at each site was a control (mice not bled) used to determine the effect of bleeding on rodents (Douglass et al., 2000). Two grids (at Cascade, Montana, USA) were monitored year-round for ~10 yr; the remaining 10 grids were operated only 6 mo per year because of snow cover.

Animal handling, blood collection, and safety procedures were performed following the guidelines of Mills et al. (1995). Whole blood samples from rodents were tested for IgG antibody to SNV using an enzyme-linked immunosorbent assay (ELISA) (Feldmann et al., 1993) at the Montana State University or the Montana Department of Health and Human Services.

Antibody acquisition and incidence

We define antibody acquisition (seroconversion) as a change from antibody-negative (no detectable antibody by ELISA) from one time point to antibody-positive (detectable antibody) at a later time point. If the period between the negative and positive result was ≥2 mo, these data were used only for interannual comparisons of incidence. We considered "recent" seroconversion to have occurred when an antibody-negative rodent captured during one trapping session was antibody-positive at the following trapping session (1 mo later). For monthly comparisons of seroconversion, we corrected for inconsistent trapping effort by using the number of seroconversions/10,000 trap nights of effort. To compare yearly seroconversion, data from all seroconverted animals were used to estimate the incidence of seroconversion by year based on estimated mouse-months of risk of exposure (Childs et al., 1987). Several animals, almost all juveniles, had antibody at one trapping session but did not have antibody when next captured. This was assumed to be related to the loss of maternal antibody, and once negative, these animals were considered at risk and included in the analyses.

Data organization and analysis

Analyses were conducted using a dataset that consisted of the 99 deer mice that acquired antibody over a one-trapping-session interval and a set of 812 deer mice that remained antibody-negative. The negative group was selected controlling for time (month) and location (grid). For example, if one or more deer mice seroconverted within the August-September interval of 1995 at the CMR collection site on grid number 17, all deer mice ($n=4$) that did not seroconvert over the same interval at CMR grid 17 were selected into the comparison group of seronegative rodents.

Our primary purpose was to assess the associations between a dichotomous outcome variable (seroconversion) and several dichotomous independent variables (age, gender, breeding condition, and wounding status). Seroconversion was classified as "yes," for deer mice that seroconverted from one consecutive trapping session to the next, or "no," for those that did not seroconvert. Ideally, a single multivariate analysis could be used to simultaneously investigate the associations and interactions among all variables. However, the number of variables was large in relation to the number of seroconverted rodents, and, as expected, the single multivariate approach yielded no statistically significant results. Therefore the comparisons of interest, 1) age×seroconversion status, 2) breeding condition×seroconversion status, and 3) wounding×seroconversion status, were each analyzed using 2×2 tables with gender included as a potential effect modifier or confounder. The Breslow-Day test was used to determine whether the associations between seroconversion status and age, breeding condition, and wound status were dependent on gender. If the Breslow-Day test was not statistically significant (evidence of no interaction effect), gender was assessed as a potential confounder by comparing the crude odds ratio with the Mantel-Haenszel common odds ratio. Ultimately, odds ratios were presented as measures of the degree of association between the independent variables and the outcome variable, seroconversion.

Statistical analysis of the difference in weight gain between seroconverted and nonseroconverted male deer mice, controlling for age (juvenile versus adult), was conducted using a 2×2 (seroconversion×age group) analysis of variance.

Male and female deer mice were classified as adult if they were ≥18 g at the time of first capture and juvenile if they were <18 grams at the time of first capture (Fairbairn, 1977). A male deer mouse was considered in breeding condition if (on one or both months of the consecutive trapping sessions) the testes were scrotal; a female deer mouse was considered in breeding condition if at least one of the following conditions was satisfied: 1) vagina perforated, 2) nipples enlarged, or 3) pubic symphysis open. A deer mouse was considered wounded if we detected scars or wounds on its

TABLE 1. Numbers (percentages) of recaptured male and female deer mice that seroconverted between consecutive trapping sessions by age category.

Gender/age	Recaptures[a]	Seroconversions[b]	Breslow-Day *P* value[c]	OR (95% CI)[d]
Males				
Juvenile	151	10 (6.6%)		3.03 (1.50, 6.12)[e]
Adult	322	57 (17.7%)		
			0.012	
Females				
Juvenile	156	13 (8.3%)		0.82 (0.39, 1.70)
Adult	275	19 (6.9%)		

[a] Number of consecutive-session recaptures.

[b] Number of consecutive-session seroconversions.

[c] Statistically significant Breslow-Day test indicates that association between age and seroconversion is dependent on gender.

[d] OR = odds ratio; CI = confidence interval.

[e] Statistically significant association between age and seroconversion.

body one or both months of the consecutive trapping sessions.

RESULTS

Results are based on 244,600 trap nights of effort on sampling grids in Montana. We captured 6,584 individual deer mice; 2,747 were captured during two or more trapping sessions; 2,610 were captured on two or more consecutive monthly trapping sessions; and 99 of these acquired antibody between consecutive trapping sessions.

Factors associated with seroconversion

The proportions of male and female deer mice that seroconverted between successive trapping sessions by age class are shown in Table 1. The association between age and seroconversion was dependent on gender (X^2=6.36, df=1, P=0.012; Breslow-Day test). Specifically, adult male deer mice were more likely to seroconvert between consecutive trapping sessions than juvenile male deer mice (OR=3.03; 95% CI=1.5–6.12), whereas there was no association between age and seroconversion status for female deer mice (OR=0.82; 95% CI=0.39–1.70).

The association between breeding condition and seroconversion status (Table 2) was dependent on gender (X^2=5.48, df=1, P=0.019; Breslow-Day test). Specifically, male deer mice in breeding condition were much more likely to seroconvert between consecutive trapping sessions than male deer mice that were not in breeding condition over the 1-mo interval (OR=3.26, 95% CI=1.90–5.59). There was no association between breeding condition and seroconversion for female deer mice (OR=1.13, 95% CI=0.55–2.32).

The association between wounding status and seroconversion status (Table 3) was not dependent on the gender of the deer mice (X^2=0.07, df=1, P=0.799; Breslow-Day test). Gender was not a confounding variable, with no discernable difference between the crude odds ratio and the Mantel-Haenszel adjusted odds ratio (OR=1.22, 95% CI=0.76–1.97). Based on the adjusted odds ratio, there was no association between seroconversion and the presence of wounds at either the first or second capture. We also tested for a relationship between seroconversion and the simultaneous (during the same month) acquisition of scars: there was no significant relationship between the incidence of wounds and the incidence of seroconversion for either male (P=0.30) or female (P=0.25) deer mice (Fisher's exact test).

TABLE 2. Numbers (percentages) of male and female deer mice that seroconverted between consecutive trapping sessions by breeding status.

Gender/[a] breeding condition	Recaptures[b]	Seroconversions[c]	Breslow-Day *P* value	OR (95% CI)[d]
Males				
No	187	43 (8.4%)		3.26 (1.90, 5.59)[e]
Yes	286	24 (23.0%)		
			0.019	
Females				
No	244	17 (7.0%)		1.13 (0.55, 2.32)
Yes	193	15 (7.8%)		

[a] Yes = in breeding condition at one or both consecutive captures; No = not in breeding condition at either capture.

[b] Number of consecutive-session captures.

[c] Number of consecutive-session seroconversions.

[d] OR = odds ratio; CI = confidence interval.

[e] Statistically significant association between age and seroconversion.

Change in weight and seroconversion

There were no differences in the initial (first capture) weights between seroconverting and nonseroconverting mice ($F_{1,469}=1.78$, $P=0.61$). For both juveniles and adults, nonseroconverting deer mice gained, on average, approximately twice as much weight as their seroconverting counterparts (Table 4). The interaction effect associated with the 2×2 (age×seroconversion status) analysis of variance (ANOVA) was not statistically significant ($F_{1,466}=1.579$, $P=0.210$), suggesting that the magnitude of mean weight gain between seroconverting and nonseroconverting male deer mice was not dependent on the age group. Therefore we collapsed on age and compared the mean weight gain by seroconverting male deer mice (0.64 g) to nonseroconverting male deer mice (1.39 g; not shown in Table 4). Although the seroconversion main effect was not statistically significant at the 0.05 level ($F_{1,466}=3.5$, $P=0.062$), the low *P* value suggests that male deer mice recently infected with SNV may, on average, gain less weight than the deer mice that did not become infected with SNV. We did not compare weight change between females that acquired antibody and those that did not because of potential weight gain associated with pregnancy.

TABLE 3. The numbers (percentages) of male and female deer mice that seroconverted between consecutive trapping sessions by wound status.

Gender/wounding	Recaptures[a]	Seroconversions[b]	Breslow-Day *P* value	OR (95% CI)[c]
Males				
No wound	234	28 (12.0%)		
Wound[d]	168	23 (13.7%)		
			0.799	1.22 (0.76, 1.97)
Females				
No wound	247	15 (6.1%)		
Wound	139	11 (7.9%)		

[a] Number of consecutive-session captures.

[b] Number of consecutive-session seroconversions.

[c] OR = odds ratio; CI = confidence interval.

[d] At either of the two consecutive captures.

TABLE 4. Mean mass (standard deviation) at two consecutive captures (time 1, time 2) and mean weight gain (standard deviation) between consecutive trapping sessions for seroconverting mice and a set of nonseroconverting mice matched for month.

Gender/seroconversion[a]	Number of mice	Mean mass at time 1	Mean mass at time 2	Mean weight gain
Juveniles				
No	139	14.60 (2.10)	16.85 (2.24)	2.25 (2.12)
Yes	10	15.10 (2.24)	16.10 (2.96)	1.00 (2.00)
Adults				
No	264	21.58 (2.81)	22.11 (3.14)	0.53 (2.27)
Yes	57	21.56 (2.77)	21.84 (3.06)	0.28 (2.35)

[a] Yes = seroconverted; No = did not seroconvert.

Incidence of appearance of antibody to SNV in deer mice

The intra-annual distribution of incidence is best demonstrated by the monthly number of seroconversions/10,000 trap nights (Fig. 1a). Seroconversion occurred between all trapping periods except for October-November and November-December. Incidence increased steadily in the spring through early summer and then remained relatively constant through October.

Peaks in yearly incidence for deer mice occurred in 1995, 1997, and 2004 (Fig. 1b). From 1994 through 2004, annual incidence varied from less than one seroconversion per 100 mouse-months of exposure to three seroconversions per 100 mouse-months.

A large proportion (99 of 171=57.9%) of antibody acquisitions was detected between consecutive trapping periods (Fig. 2). However, some antibody negative deer mice were absent from, or at least were not recaptured on, grids or webs for as long as 12 mo before being recaptured with antibody.

DISCUSSION

Researchers studying hantavirus infection in host populations have demonstrated that recently infected mice shed large amounts of infectious virus and are most likely responsible for transmission of infection to other hosts or to humans (Lee et al., 1981; Yanagihara et al., 1985; Gavrilovskaya et al., 1990; Hutchinson et al., 1998). Thus, studies of recently infected deer mice are likely to contribute significantly to our understanding of the timing and mechanisms of transmission of hantaviruses among deer mice and from deer mice to humans. Under typical trapping conditions at our grids, documentation of SNV infection and seroconversion in deer mice was rare. It took 10 yr of trapping and data on 6,584 individual deer mice to accumulate data on 99 deer mice that seroconverted between successive trapping sessions. This sample size was barely large enough to investigate the characteristics of deer mice that had recently seroconverted. The analyses and conclusions presented here could not have been achieved in a shorter-term study; the use of long-term studies with ample replication is necessary for studying the transmission of SNV to other hosts and to humans.

Characteristics of deer mice that recently seroconverted were generally similar to characteristics of antibody-positive deer mice demonstrated in previous studies (Mills et al., 1997; Douglass et al., 2001). With respect to age, gender, and breeding condition, recently infected deer mice in Montana tended to be older, were more likely to be male, and were more likely to be in breeding condition than were a cohort of deer mice that did not acquire antibody over successive trapping sessions.

Because antibody-positive individuals are more likely to have wounds than are

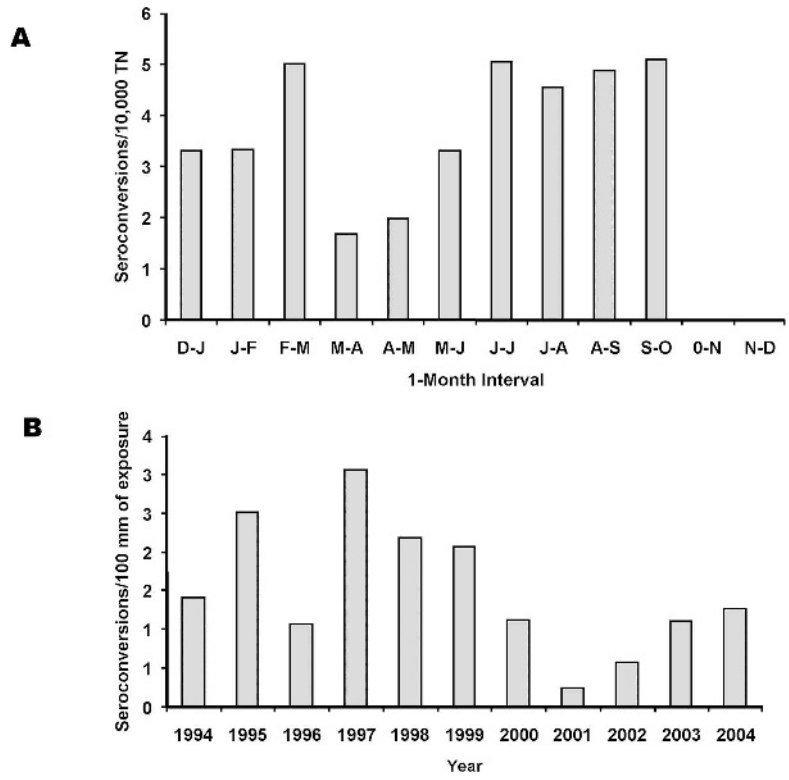

FIGURE 1. Temporal distribution of acquisition of antibody (seroconversion) to Sin Nombre virus in deer mice in Montana, 1994–2004. (A) Seroconversions per 10,000 trap-nights (TN) by monthly period. (B) Seroconversions per 100 mouse-months (mm) of exposure by year.

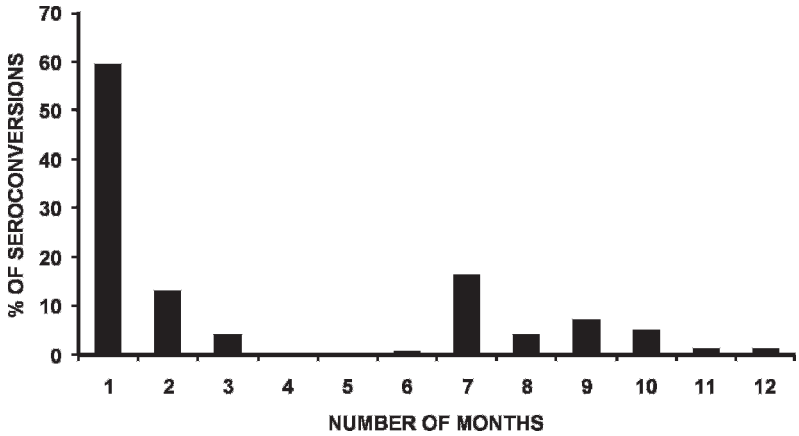

FIGURE 2. Temporal distribution of acquisition of seroconversions to Sin Nombre virus in Montana, 1994–2004, expressed as number of months between last antibody-negative capture and first antibody-positive capture.

antibody-negative individuals (Bennett et al., 1999; Calisher et al., 1999; Douglass et al., 2001), we expected to find an association between seroconversion and the acquisition of scars. The failure to find an association might be the result of the small sample size of deer mice that seroconverted from one trapping session to the next. Alternatively, it is possible that the frequently shown association between scars and antibody prevalence may be, at least in part, the result of a postinfection phenomenon. A mechanism for such a response was suggested for rats infected with Seoul hantavirus (Glass et al., 1988; Hinson et al., 2004; Klein et al., 2004). Specifically, rats infected with Seoul virus were more aggressive and spent more time fighting with intruders than did uninfected mice. These authors proposed that infection with a hantavirus altered the behavior of infected rats, causing them to be more aggressive after antibody acquisition. Further studies will be necessary to determine the validity of this hypothesis for SNV. Finally, it is possible that both wounding and seroconversion might be related to a third variable, such as population density, that might result in increased aggression *and* increased transmission, but through a mechanism (e.g., environmental contamination) that might be independent of aggression.

Laboratory infections of deer mice with SNV have been shown to have little (Netski et al., 1999) or no (Botten et al., 2000) physical effects on the rodent. However, Douglass et al. (2001), found a significantly lower rate of survival (residence time on the study site) of field-collected juvenile and subadult deer mice with antibody, suggesting that SNV may have some negative effect on naturally infected deer mice. In our investigation we found that male deer mice that seroconverted gained less weight than a cohort of deer mice that did not seroconvert. Although this difference in growth rate approached only statistical significance, these results are consistent with a hypothesis that SNV infection negatively affects the overall health of deer mice. It would take only a slight metabolic deficit to reduce the foraging effectiveness of infected mice, resulting in reduced weight gain, and, potentially, reduced survival.

Based on reduced winter sampling (two grids sampled in the winter), the lowest seroconversion rates in Montana occurred during late fall to early winter (November–January). The time of low rates of seroconversion in Montana coincided with the time that breeding activity was at its lowest, with fewer than 10% of deer mice showing signs of reproductive activity (Douglass et al., 2001).

We have data for only two grids during mid- to late winters (January–March). On those two grids, during late winter both the percentage of deer mice in breeding condition (Douglass et al., 2001) and the seroconversion rate began to increase in late winter (Fig. 1). On all grids, both the percentage in breeding condition and the rate of seroconversion increased through spring, with the seroconversion rate remaining fairly constant through summer, even after the proportion in breeding condition declined. The apparent decline in breeding is related to dilution of the population by young of the year, which may not reach maturity during that year. However, there may be a sufficient number of older, SNV-infected deer mice in the population to sustain transmission and, therefore, to sustain the rate of antibody acquisition (Calisher et al., 2001).

Of all the deer mice that seroconverted, the largest proportion seroconverted between successive trapping periods. However, most deer mice were captured (were residents captured during more than one trapping period) at the trapping sites for only a short period. The recapture rate of mice that seroconverted decreased rapidly after their first antibody-positive capture (Fig. 2). The increase of recaptured antibody-positive deer mice, after they had not been captured for 7 mo, likely is an artifact of the reinitiation of spring trap-

ping. Alternatively, the finding that initially antibody-negative deer mice were not trapped for long periods of time and then were captured as antibody-positive may suggest that deer mice may move long distances or have enlarged winter home ranges. Such movement may be important in reintroducing and maintaining viral infections in local populations, if they are wide-ranging and shedding SNV, as has been suggested (Root et al., 1999; Calisher et al., 2001).

In summary, this is the first field study to address the ecology and epidemiology of a hantavirus-host system in the subgroup of hosts that is likely to be most infectious (recently infected individuals) and therefore most epidemiologically important. Because acquisition of antibody is a rare event and deer mice generally are resident only briefly at sampling sites, long-term studies with ample replication are critical. Although statistical significance of results varied, we demonstrated that seroconverting deer mice are more likely to be males, and that these males are generally sexually mature adults. Seroconversion also occurred most frequently during the breeding season. We were surprised to find no statistically significant relationship between seroconversion and acquisition of scars, suggesting the possibility that the frequently shown association between scars and antibody prevalence may be due in part to scars that are acquired after antibody acquisition by rodents whose behaviors are influenced by viral infection. Deer mice that acquired antibody gained less weight than did a control group that did not acquire antibody, providing some additional evidence that SNV infection of deer mice may have a detrimental effect on their health. Timing of antibody acquisition coincided with the breeding season.

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