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# EPIZOOTIOLOGY OF SIN NOMBRE AND EL MORO CANYON HANTAVIRUSES, SOUTHEASTERN COLORADO, 1995–2000

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ABSTRACT: Sin Nombre virus (SNV) is an etiologic agent of hantavirus pulmonary syndrome. To better understand the natural history of this virus we studied population dynamics and temporal pattern of infection of its rodent hosts in southeastern Colorado (USA) from 1995 to 2000. We present evidence for the presence of two hantaviruses, SNV in deer mice (*Peromyscus maniculatus*) and El Moro Canyon virus in western harvest mice (*Reithrodontomys megalotis*), at our study sites. Sin Nombre virus appeared only sporadically in deer mouse populations; overall prevalence of antibody to SNV was 2.6%. El Moro Canyon virus was enzootic: seroconversions occurred throughout the year; antibody prevalence (11.9% overall) showed a delayed–density-dependent pattern, peaking as relative abundance of mice was declining. Males of both host species were more frequently infected than were females. An apparently lower mean survivorship (persistence at the trapping site) for SNV antibody-positive deer mice could indicate a detrimental effect of SNV on its host, but might also be explained by the fact that antibody-positive mice were older when first captured.

*Key words:* Antibody, deer mice, hantaviruses, *Peromyscus maniculatus, Reithrodontomys megalotis,* rodents, western harvest mice.

## INTRODUCTION

The 1993 recognition of hantavirus pulmonary syndrome (HPS) as a disease entity in the American Southwest led to studies of both the principal etiologic agent of this disease, Sin Nombre virus (SNV), and its principal rodent host, the deer mouse, *Peromyscus maniculatus* (Childs et al., 1994; Boone et al., 1998; Abbott et al., 1999; Calisher et al., 1999; Kuenzi et al., 1999; Drebot et al., 2001). These studies were meant to elucidate the natural history of hantaviruses in North America, while at the same time researchers were attempting to understand the highly variable nature of hantavirus infections of their rodent hosts and the ecologic basis for those variations. The goal is to understand the relationships between the viruses and their hosts in order to predict changes in the risk of HPS to humans and, perhaps, to devise HPS prevention and control plans (Mills et al., 1999).

In addition to SNV, other hantaviruses have been isolated from rodents in the United States. El Moro Canyon virus (ELMCV) has been detected in western harvest mice (*Reithrodontomys megalotis*) essentially throughout its range; Black Creek Canal (BCCV) and Muleshoe viruses have been detected in hispid cotton rats (*Sigmodon hispidus*) in Florida and Texas, respectively; New York-1 virus (NYV) and Blue River virus have been detected in white-footed mice (*Peromyscus leucopus*) in New York and in the central United States, respectively; Limestone Canyon virus (LCV) has been detected in brush mice (*Peromyscus boylii*) in the southwestern USA; Bayou virus has been found in rice rats (*Oryzomys palustris*) in Louisiana; Bloodland Lake virus has been detected in prairie voles (*Microtus ochrogaster*) in Missouri; Isla Vista virus has been detected in California voles (*Microtus californicus*) in California; and Prospect Hill virus has been identified in meadow voles

(*Microtus pennsylvanicus*) in the northern USA and Canada. The molecular biology of these and other hantaviruses has been reviewed extensively (Morzunov et al., 1998; Monroe et al., 1999; Drebot et al., 2001). Similarities between the hantavirus and host phylogenies indicate a long-term parallel or co-evolutionary relationship between each virus-host pair.

To investigate SNV and its relationship to deer mice, in 1994 we established study sites in montane shrubland and semidesert shrubland ecosystems in western Colorado (Calisher et al., 1999). Because the dynamics of hantavirus infection in host populations may vary among ecosystems, we also established study sites in a geographically and ecologically distinct area in southeastern Colorado at the Pinyon Canyon Maneuver Site (PCMS; described in detail in US Department of the Army, 1980). The rodent assemblage at PCMS is more diverse than that in western Colorado. Certain species reach their range limits in or near PCMS, including southern and eastern species. Thus, the area is of considerable zoogeographic interest (Ribble and Samson, 1987). This study allowed us to explore the effects of such diversity on both rodent populations and virus prevalence.

Pinyon Canyon Maneuver Site trapping sites and rodent population dynamics are described in a separate article (Calisher et al., 2005). Herein we describe the epizootiology of SNV and ELMCV in their rodent hosts at PCMS.

## MATERIALS AND METHODS

### Description of study sites

Calisher et al. (2005) provided details about the location and characteristics of the sites and procedures related to capturing, processing, and testing rodents. In brief, PCMS, Las Animas County, southeastern Colorado (USA), is under the auspices of the Directorate of Environmental Compliance and Management, Fort Carson, Colorado. The area has a dry continental climate, with elevations ranging from 1,300 to 1,700 m (US Department of the Army, 1980; Shaw et al., 1989).

Two primary sites were chosen for mark-release-recapture serologic studies: Mouth of Red Rock Canyon (MRC; 37°32.759′N, 103°49.352′W), a meadow with grasses and forbs, containing a permanent water source at the head of a shallow canyon; and Red Rock Canyon (RRC; 37°32.169′N, 103°49.105′W), a site within that canyon. At each site, trapping was conducted at approximately 6-wk intervals from January 1995 through November 2000. Detailed descriptions of these sites have been published, as have descriptions of the trapping methods used (Calisher et al., 2005).

We conducted 26,480 trap nights, of which 17,255 were at MRC and 9,225 were at RRC. An approximate 0.2-ml blood sample was taken from the retroorbital plexus of each rodent (except for sciurids) upon its first capture at each trapping session.

### Antibody testing

Blood samples were stored on wet ice or dry ice and transported to the Arthropod-borne and Infectious Diseases Laboratory (Fort Collins, Colorado, USA), where they were stored in a mechanical freezer (−75 C). These samples were thawed once for removal of an aliquot, which was tested for antibody, refrozen in the mechanical freezer, and later shipped on dry ice to the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) for confirmatory testing. Tests for immunoglobulin G antibody to SNV antigen were done by enzyme-linked immunosorbent assay (ELISA), using the method of Feldmann et al. (1993). Samples were tested at a screening dilution of 1:100. The ELISA detects antibodies to all known sigmodontine hantaviruses, including SNV, ELMCV, LCV, BCCV, and NYV.

### Data analysis

Data were entered and Chi-square analyses conducted using EPI-5 (Dean et al., 1990). All other statistical analyses were conducted using SPSS (Norusis, 1993) and SAS (SAS Institute Inc., 2000) statistical software.

Quarterly rodent abundance was expressed as trap success, calculated as the number of individual rodents captured per 100 trap nights. Seasons were defined as winter (January–March), spring (April–June), summer (July–September), and autumn (October–December). Antibody prevalence was calculated as the number of mice with antibody to SNV, divided by the number tested during the given period.

Minimum longevities (maximum known time of residence on the trapping sites) of all deer mice and western harvest mice were determined from recapture data (Calisher et al., 2005). Mean minimum longevities of infected

individuals (i.e., those with antibody) were determined using two methods: 1) by calculating the mean number of weeks from the time an individual was first captured until it was last captured and 2) by calculating the mean number of weeks from the time an individual was first captured with antibody until it was last captured. Mice that were only captured once (or only captured once with antibody under method 2) were not included in calculations. Differences in mean minimum longevity were tested for statistical significance using the Monte Carlo estimates of the exact *P* values (PROC NPAR1WAY; SAS Institute Inc., 2000).

## RESULTS

### Verification of virus identity

In 1996, one of us (J.E.R.) identified SNV-like S and M segment RNA partial sequences from a seropositive deer mouse captured at a site 20 km west of MRC. These sequences fell within the clade formed by published sequences for SNV in deer mice from New Mexico. In addition, a seropositive western harvest mouse captured at MRC had ELMCV-like S and M segment RNA partial sequences. Further evidence that antibody to SNV in deer mice was due to infection with SNV and that antibody to SNV in western harvest mice was due to ELMCV came from comparative titrations by ELISA (see below).

### Prevalence of antibody to SNV

Of a total 1,293 blood samples from deer mice, 494 from pinyon mice (*Peromyscus truei*) and 623 from western harvest mice, 34 (2.6%), two (0.4%), and 68 (10.9%), respectively, had antibody. Some mice were captured more than once and had more than one sample taken. In terms of individuals, 19 of 754 (2.5%) tested deer mice, two of 277 (0.7%) pinyon mice, and 42 of 451 (4.2%) western harvest mice had antibody. No other rodents (see Calisher et al., 2005) had antibody reactive with SNV. End-point titers ranged from 100 to ≥51,200 for deer mice and from 100 to 12,800 for western harvest mice. The geometric mean titer of 24 antibody-positive deer mouse bloods was 5,381, and of 36 western harvest mouse bloods, the mean titer was 1,088. These differences in titers might be due to heterologous antibody (ELMCV antibody reacting with SNV antigen) or to lower reactivity of the anti-species conjugate used in the ELISA test (a mixture of anti-*Peromyscus* and anti-*Rattus* conjugate was also used for testing western harvest mice), or they may be attributable to a combination of these factors.

Relative abundance (as indicated by trap success) varied seasonally and among years (Calisher et al., 2005). Relative abundance of deer mice generally peaked in autumn or winter, decreased in spring to a summer low, and increased again in autumn (Fig. 1). Exceptions to this pattern occurred in the autumn of 1997 and the winter of 2000, when populations were very low, perhaps as a result of unfavorable weather conditions (Calisher et al., 2005). The prevalence of antibody to SNV in deer mice decreased to zero soon after the beginning of our study in 1995. Antibody in deer mice was detected again in late 1998, and prevalence of antibody peaked at 17.4% in November 1999. Antibody prevalence then abruptly declined, reaching zero by the spring of 2000 (Fig. 1).

Relative abundance of western harvest mice followed a generally similar pattern (Fig. 2), except that populations were relatively high in summer of 1997, and the western harvest mouse population seemed to take longer to recover from the 1997–98 population crash. Western harvest mice appeared to be absent from our trapping sites during the summer and much of the autumn of 1998. Antibody prevalence generally increased as relative abundances were declining, and, except for summer 1995 and autumn 1998—when samples sizes were very small, antibody prevalence was generally highest when populations were lowest (Fig. 2). There was a significant negative correlation between trap success and antibody prevalence (Spearman's correlation coefficient=−0.547, $P$=0.01).

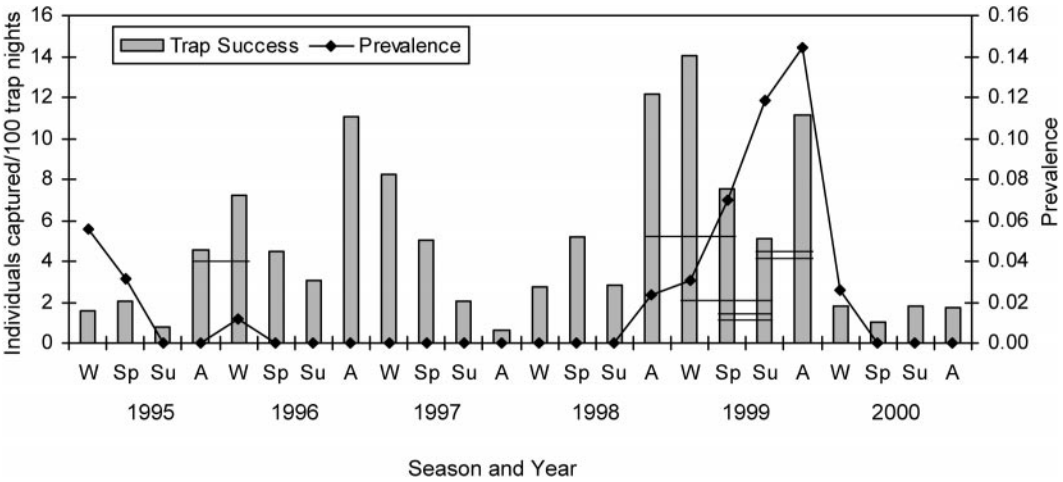


FIGURE 1. Relation between relative abundance (trap success expressed as numbers of individuals captured per 100 trap nights) and prevalence of immunoglobulin G (IgG) antibody to Sin Nombre virus in deer mice (*Peromyscus maniculatus*), Pinyon Canyon Maneuver Site, southeastern Colorado, 1995–2000. Horizontal lines represent individuals seroconverting between collection periods. W=winter, Sp=spring, Su=summer, and A=autumn.

Males represented 60% (451/754) of the tested individual deer mice but 95% (18/19) of antibody-positive individuals ($P=0.001$, Fisher's exact test). Males represented 45% (185/409) of the total tested individual western harvest mice and a disproportionate 76% (32/42) of the antibody-positive harvest mice ($P<0.001$, Fisher's exact test).

#### Seroconversions

Seroconversions (SNV antibody negative to SNV antibody positive, low titer to at least a fourfold higher titer) were not

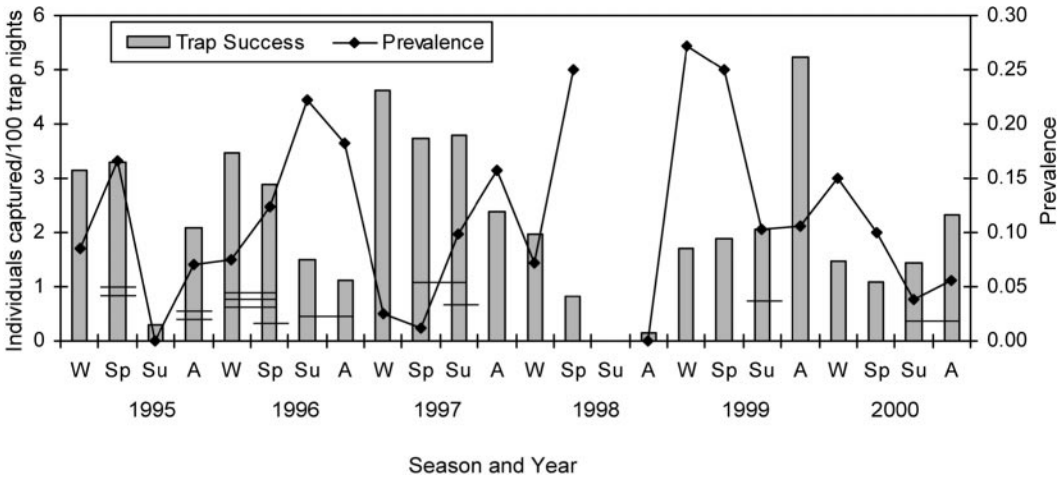


FIGURE 2. Relation between relative abundance (trap success expressed as numbers of individuals captured per 100 trap nights) and prevalence of immunoglobulin G (IgG) antibody to Sin Nombre virus in western harvest mice (*Reithrodontomys megalotis*), Pinyon Canyon Maneuver Site, southeastern Colorado, 1995–2000. Horizontal lines represent individuals seroconverting between collection periods. W=winter, Sp=spring, Su=summer, and A=autumn.

TABLE 1. Incidence of immunoglobulin G antibody reactive with Sin Nombre virus in deer mice (*Peromyscus maniculatus* [*P. man*]) and western harvest mice (*Reithrodontomys megalotis* [*R. meg*])[a] recaptured and sampled at least twice at Pinyon Canyon Maneuver Site, southeastern Colorado, January 1995–November 2000.

| Species/location[b] | Sex | Number at risk[c] | Number of new infections | Cumulative % with antibody | Mouse mo of observation[d] | Incidence[e] |
|---|---|---|---|---|---|---|
| *P. man*/MRC | Total | 115 | 2 | 1.7 | 491 | 0.41 |
| | M | 71 | 1 | 1.4 | 316 | 0.32 |
| | F | 44 | 1 | 2.3 | 175 | 0.57 |
| *P. man*/RRC | Total | 142 | 4 | 2.8 | 553 | 0.72 |
| | M | 78 | 4 | 5.1 | 298 | 1.34 |
| | F | 64 | 0 | 0 | 255 | 0 |
| *R. meg*/MRC | Total | 77 | 10 | 13.0 | 241 | 4.15 |
| | M | 36 | 7 | 19.4 | 106.5 | 6.57 |
| | F | 41 | 3 | 7.3 | 134.5 | 2.23 |
| *R. meg*/RRC | Total | 17 | 2 | 11.8 | 34.5 | 5.80 |
| | M | 11 | 2 | 18.2 | 21.5 | 9.30 |
| | F | 6 | 0 | 0 | 13 | 0 |

[a] No pinyon mice (*Peromyscus truei*) seroconverted during the study period.
[b] MRC = Mouth of Red Rock Canyon; RRC = Red Rock Canyon.
[c] Number of mice without antibody when first captured.
[d] Total time intervals between successive captures when mice were antibody-negative, plus half the interval between the time when mice changed from antibody-negative to antibody-positive status.
[e] New infections per 100 mice per month.

detected in pinyon mice or in juvenile deer mice. Six seroconversions were detected in adult (>18 g) male deer mice, one each in the periods October 1998–May 1999, March–September 1999, May–August 1999, and May–September 1999, and two between September and November 1999 (Fig. 1). One adult female deer mouse seroconverted between November 1995 and January 1996 (Fig. 1).

Ten adult male (>8 g) and three adult female (>8 g) western harvest mice seroconverted during this study (Fig. 2). Among the males, seroconversions were detected in the periods April–May 1995, April–June 1995, October–November 1995 (two), March–April 1996, March–May 1996, April–June 1996, September–November 1996, June–July 1997, and September–November 2000. The three females that seroconverted did so in the periods March–April 1996, July–August 1997, and August–September 1999.

### Incidence

Incidence, calculated as the number of new SNV infections (i.e., seroconversions) per 100 mouse months of observation, was determined for deer mice and western harvest mice at RRC and MRC (Table 1). Although the incidence for male deer mice at RRC (1.34) was more than four times the incidence at MRC (0.32), the number of male deer mice at RRC (78) was only slightly greater than at MRC (71). The incidence for male western harvest mice at RRC (9.30) was 40% greater than at MRC (6.57), but the number of male harvest mice at RRC (11) was less than a third of the number at MRC (36).

### Minimum longevity

When all captures (including preseroconversion captures) were used to calculate mean minimum longevity for SNV antibody-positive individuals, one female deer mouse with SNV antibody persisted to 9.1 wk; mean minimum longevity (persistence on the trapping site) of 107 antibody-negative female deer mice that were recaptured at least once was 17.2 wk (range 4–60 wk). Mean longevity of 14 male deer mice with SNV antibody was

18.5 wk (range 6–42 wk), as compared with a mean of 17.7 wk (range 4–81 wk) for 143 recaptured male deer mice without SNV antibody. This difference was not statistically significant ($P$=0.708, Monte Carlo estimate for the exact test).

When preseroconversion captures were discarded, no female deer mice with SNV antibody were recaptured; mean postinfection longevity of six recaptured male deer mice with antibody was 9 wk (range 6–18 wk), compared with the mean of 17.7 wk for 143 antibody-negative male mice. This difference in mean minimum longevity was marginally statistically significant ($P$=0.047, Monte Carlo estimate for the exact test).

Using all captures, the mean longevity of three female western harvest mice that seroconverted was 29.3 wk (range 6–42 wk); mean minimum longevity for 45 recaptured female western harvest mice without SNV antibody was 13.5 wk (range 4–42 wk; $P$=0.530, Monte Carlo estimate for the exact test). Mean minimum longevity for 17 male western harvest mice with SNV antibody was 12.0 wk (range 6–31 wk), compared to 12.5 wk (range 5–39 wk) for 38 male western harvest mice that remained SNV antibody-negative ($P$= 0.531, Monte Carlo estimate for the exact test). When preseroconversion captures were discarded, no female western harvest mice with antibody were recaptured. Mean longevity of six recaptured male western harvest mice with antibody was 8.5 wk (range 6–17 wk), compared with the mean of 12.5 wk for 38 male mice without antibody ($P$=0.30, Monte Carlo estimate for the exact test).

Finally, deer mice and western harvest mice that were antibody-positive on first capture were not less likely to be recaptured than those that were SNV antibody-negative on first capture. For deer mice, eight of 17 antibody-positive mice were recaptured and 264 of 689 antibody-negative mice were recaptured ($P$=0.15, Fisher's exact test); for western harvest mice, eight of 41 antibody-positive and 101 of 386 antibody-negative mice were recaptured ($P$=0.45, Fisher's exact test).

### Wounds and antibody

No scars were observed on SNV antibody-positive adult female western harvest mice (one capture) or antibody-positive adult female deer mice (four captures). For male mice, antibody-positive mice had a higher (though not statistically significant) frequency of scars: six of 29 (21%) antibody-positive adult male deer mice had scars, compared to 103 of 628 (16%) antibody-negative adult males ($P$=0.61, Fisher's exact test); six of 47 (13%) adult male western harvest mice had scars compared to 22 of 259 (8%) antibody-negative adult males ($P$=0.41, Fisher's exact test).

## DISCUSSION

Our genetic sequencing analyses demonstrated the presence of SNV in deer mice and of ELMCV in western harvest mice at PCMS. Thus, at least two hantaviruses occur at PCMS. We assume that antibody to SNV in deer mice was due to infection with SNV and that antibody to SNV in western harvest mice was due to infection with ELMCV. Given the very low prevalence of antibody in pinyon mice (1% overall), it is unlikely that this species supported its own co-evolved hantavirus. We suspect that the antibody detected in pinyon mice represented spillover of SNV or ELMCV from deer mice or western harvest mice. Spillover of SNV into pinyon mouse populations has been demonstrated previously (Childs et al., 1994). The fact that rodents of all other species are seronegative indicates a lack of spillover of either SNV or ELMCV into other species.

Antibody to SNV in deer mice was uncommon. At least one SNV antibody-positive deer mouse was captured during only 10 of 41 (24%) trapping periods; virus was apparently locally extinct for most of 1996, 1997, and 1998 and again after January 2000. Of 1,293 samples from these mice, only 34 had antibody to SNV, and 30 of

those 34 (88%) were found in consecutive trapping periods between October 1998 and January 2000. The 2.6% overall SNV antibody prevalence in deer mice at PCMS is markedly lower than prevalences previously documented in western Colorado (9.5%, Calisher et al., 1999), Southern California (9%, Bennett et al., 1999), the southwestern USA (11%, Mills et al., 1997), or at 39 National Parks across the USA (7%, Mills et al., 1998). Reasons for the sporadic occurrence of SNV infection in deer mice are unclear. Deer mouse populations may have to remain above a certain threshold density in order to support virus infection. However, although comparisons are inexact because of differences in effort, rodent populations at PCMS were as high as or higher than those observed in western Colorado during approximately the same period (Calisher et al., 1999). Nevertheless, the overall prevalences in western Colorado (9–10%) were much higher. Deer mouse populations at PCMS were as high in 1996–97 as they were in 1999, when prevalence of infection reached 17.4%. Yet the virus was apparently absent during this period. This indicates that an element of chance is involved; density-dependent processes are irrelevant if virus is not present in the population. Temporary disappearance of hantaviruses from host populations does not seem to be a rare phenomenon. Absence of evidence of infection in host populations has been observed for periods of up to 46 mo in brush mouse populations in southern Arizona (Kuenzi et al., 1999; unpubl. data) and of up to 23 mo in deer mouse populations in northwestern New Mexico (Yates et al., unpubl.). These disappearances often coincide with periods of low host population density or follow population crashes.

Although no western harvest mice were captured at our trapping sites from July to October 1998, seropositive western harvest mice were found in 30 of 40 (75%) of those periods in which they did occur. The overall SNV antibody prevalence (10.9%; range 0% to 37.5%) was much higher than that in deer mice (2.6%; range 0% to 17.4%). By comparison, Bennett et al. (1999) reported an SNV antibody prevalence of 12% in 417 western harvest mice sampled in Southern California, and Mills and Childs (2001) reported an overall prevalence of near 20% in a sample of about 100 western harvest mice collected during several studies in the western USA.

It has been hypothesized that prevalence of antibody to hantaviruses in host rodent populations will follow a delayed–density-dependent temporal pattern (Niklasson et al., 1995; Mills et al., 1999; Yates et al., 2002). Increasing and peaking host populations will consist predominantly of uninfected juveniles that are likely not to have antibody (i.e., that are not infected). Sin Nombre virus antibody prevalence will rise most quickly in declining or low-density populations in which reproduction has stopped and the juvenile dilution effect has halted. Further, since the rate of encounters between hosts and, therefore, virus transmission events is likely to be positively correlated with population density, the prevalence of antibody in those declining or low-density populations will be proportional to the peak population density prior to the decline. This model requires a stable enzootic presence of the virus in the host population. Thus, it cannot be applied to the data illustrated in Figure 1, since SNV was present only sporadically in deer mice at our study sites at PCMS. Our data support the delayed–density-dependent model for western harvest mice in that they demonstrate relatively lower SNV antibody prevalence at peak relative abundances and highest prevalence after populations have begun to decline from peak levels. There was a significant negative relationship between population density and prevalence of infection (Spearman's correlation coefficient $=-0.46$, $P=0.035$). However, as was seen during a similar study in Montana (Douglass et al., 2001), there was no clear evidence that peak prevalence was proportional to pre-

vious peaks in population density. This may be due, in part, to the lack of a consistent seasonal pattern of host reproduction and population dynamics at PCMS.

On a spatial scale, there was a general correlation between local relative abundance and SNV antibody prevalence (or incidence) for deer mice and western harvest mice. Relative abundance (trap success) of male deer mice at RRC (4.8 captures per 100 trap nights) was much higher than at MRC (1.9). Accordingly, both overall SNV antibody prevalence and incidence for male deer mice were higher at RRC (3.4% and 1.3%, respectively) than at MRC (0.9% and 0.3%). Although differences in relative abundance and SNV antibody prevalence were not as great, the same pattern was observed for western harvest mice. Relative abundance of males of both species at RRC (0.8 captures per 100 trap nights) was lower than at MRC (1.4), and both antibody prevalence and incidence were higher at RRC (12.7% and 9.3%) than at MRC (9.0% and 6.6%).

Seroconversion rates, certain evidence of recent infections, paralleled those we have documented in western Colorado (Calisher et al., 1999). That is, male deer mice usually acquired their infections in the summer and fall. We attributed this phenomenon in western Colorado to closer contact with other infected deer mice because of greater numbers of mice. At PCMS, all but one of these SNV seroconversions occurred during a single period, as SNV antibody prevalence was increasing. Western harvest mice were rare at our western Colorado sites, so that no comparisons can be made. Nonetheless, most SNV seroconversions in western harvest mice at PCMS occurred during periods of relatively high populations (Fig. 2) and occurred most of the year except during the winter, indicating a more enzootic than epizootic situation.

Many reports have indicated that hantavirus infections do not have a significant detrimental effect on the host (Lee et al., 1981; Childs et al., 1989; O'Connor et al., 1997). In our study, one comparison ($n=6$) indicated decreased minimum longevity (persistence on the trapping site), for SNV antibody-positive male deer mice. This may indicate that infection with a hantavirus has a detrimental effect on the host. Douglass et al. (2001), while studying deer mouse populations in Montana, found that the average monthly survival of SNV antibody-positive juveniles and subadults (but not adults) was significantly lower than that of uninfected deer mice. An alternative explanation for the pattern we observed is that SNV antibody-positive rodents are, on average, older when they are first captured. This is demonstrated clearly by the consistent finding of a strong positive association between age and antibody acquisition (Mills et al., 1997; Abbott et al., 1999; Douglass et al., 2001). Thus, these older SNV antibody-positive animals would be expected to have a shorter remaining life span than the average SNV antibody-negative mouse. Similarly, in the single comparison that was marginally statistically significant, data regarding all captures of antibody-positive animals before they seroconverted were discarded, effectively shortening the calculated longevity of those individuals. Nevertheless, we presented this comparison because inclusion of time on the trapping arrays prior to seroconversion might decrease our ability to detect any postseroconversion effect on longevity. When the discarded captures were incorporated into the analysis, the difference in longevity disappeared. Finally, as is clear from Figure 1, a large proportion of the SNV antibody-positive deer mice were captured during a short period that preceded a major population decline during the winter of 1999–2000. Sin Nombre virus antibody-negative mice, captured throughout the study period, would thus be expected to have a higher average survivorship. Nevertheless, longevity comparisons were very similar when we restricted the comparison to autumn 1998 through winter 2000 (data not shown). We emphasize that our comparisons at PCMS are

based on small sample sizes. Larger field studies, as well as laboratory experiments, will be needed to definitively describe the effect (if any) of SNV infection on its natural host.

Based on previous studies by Glass et al. (1988) on Norway rats (*Rattus rattus*), studies by Douglass et al. (2001) involving deer mice, and our own observations of deer mice in western Colorado (Calisher et al., 1999), we anticipated finding a relationship between wounds or scars and prevalence of antibody to SNV in male deer mice and western harvest mice. This correlation between wounds and antibody has been interpreted as an indication of transmission of hantaviruses by intraspecific aggressive encounters among male mice. Although higher percentages of adult male deer mice and western harvest mice had scars than did their uninfected counterparts, sample sizes of SNV antibody-positive animals were very low, and the results of statistical comparisons were not significant.

Male deer mice and western harvest mice at PCMS were much more likely to be antibody positive than were females. This indicates that the mechanisms of transmission of SNV in deer mice and ELMCV in western harvest mice at PCMS are similar to the mechanisms of transmission of SNV in western Colorado and other areas of the USA where this pattern has been reported (Mills and Childs, 1998; Calisher et al., 1999).

We conclude that at PCMS, amplification of ELMCV in western harvest mice was more enzootic (characterized by fairly uniform and constant increases, never involving the majority of individuals of the particular species, with infections occurring uniformly throughout the year) than epizootic (characterized by regular or irregular increases involving large numbers of individuals of the particular species and seasonal acquisitions of infections). Visual inspection of the data indicates a time-lag association between prevalence of SNV antibody and relative abundance of harvest mice. The incidence of SNV infection in deer mice may have been barely sufficient to maintain the natural cycle of this virus in its rodent host. It may also be that, for reasons that are unclear, SNV is not enzootic in deer mouse populations at our study sites at PCMS, but, when introduced from nearby enzootic areas, the virus survives briefly, only to become locally extinct after a few infection cycles. The circumstances of transmission of SNV and ELMCV at PCMS may be quite different from those at other, ecologically less-diverse sites. For example, the 7-yr average diversity (Simpson's Diversity Index) at PCMS was 0.74, compared to 0.62 and 0.30 at two trapping sites in western Colorado (Mills et al., unpubl. data). Preliminary evidence accumulated in western Colorado (Calisher et al., 1999) and at other longitudinal study sites in the southwestern USA (Mills et al., unpubl. data) indicates that the less diverse the rodent assemblage, the higher the prevalence of SNV infection in deer mice, and that lack of diversity may be central to epizootic events. Ostfeld and Keesing (2000), considering vector-borne zoonotic diseases, and Mackelprang et al. (2001), considering SNV, came to similar conclusions.

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