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Authors: Dearing, M. Denise, Previtali, M. Andrea, Jones, Jeremy D., Ely, Patrick W., and Wood, Britta A.

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SEASONAL VARIATION IN SIN NOMBRE VIRUS INFECTIONS IN DEER MICE: PRELIMINARY RESULTS

M. Denise Dearing,[1,2] M. Andrea Previtali,[1] Jeremy D. Jones,[1] Patrick W. Ely,[1] and Britta A. Wood[1]

[1] Department of Biology, 257 South 1400 East, University of Utah, Salt Lake City, Utah, USA
[2] Corresponding author (email: denise.dearing@utah.edu)

ABSTRACT: The proportion of deer mice (*Peromyscus maniculatus*) with recently acquired Sin Nombre virus (SNV) infections is an indicator of epizootic intensity and may be key in predicting outbreaks of hantavirus cardio-pulmonary syndrome in humans. We investigated whether incidence of recent infections was related to season, sex, reproductive status, or habitat disturbance. In May and September, 2006, we sampled 912 deer mice at six sites in Utah. We determined SNV antibody prevalence and estimated the number of recent infections with an avidity enzyme-linked immunosorbent assay. Antibody prevalence in adults ($n=735$) was 22%, and putative maternal antibody prevalence in juveniles ($n=177$) was 7%. Sampling period explained a significant amount of the variance in the probability of recent infections, which were two times more common in May versus September. Additionally, prevalence of high-avidity maternal antibodies (i.e., from dams with older infections) in juveniles did not correspond to the antibody avidity patterns in adult females. In May, no juveniles had high-avidity antibodies compared to adult females (49%); in September, avidity could not be measured in juveniles because none were seropositive, despite large sample sizes ($n=84$) and an 11% seroprevalence in adult females. Based on the results, coupled with those from the literature, we speculate that the majority of new infections may occur predominantly in the spring and that SNV may impair reproductive output of females.

Key words: Avidity, deer mice, maternal antibody, *Peromyscus maniculatus*, Sin Nombre virus.

INTRODUCTION

Sin Nombre virus (*Bunyaviridae: Hantavirus*) is hosted by deer mice (*Peromyscus maniculatus*; Childs et al., 1994) and can cause human infections that can progress to hantavirus cardio-pulmonary syndrome (HCPS). Transmission between rodents presumably occurs through body-fluid transfer during aggressive interactions (Mills et al., 1997; Calisher et al., 1999; Safronetz et al., 2008). Infection in deer mice is chronic; however, it has been speculated that infectiousness may be greatest during early stages of infection when viremia is high (Botten et al., 2000; Botten et al., 2003). For hantaviruses in general, the number of recently infected animals is an indicator of the epizootic intensity (Bernshtein et al., 1999; Safronetz et al., 2006). With SNV, viral shedding in urine and saliva (critical sources for transmission to humans) may also be highest during early stages of infection (Safronetz et al., 2008). With another hantavirus, Puumala virus, the number of recent infections in the host (bank voles, *Clethrionomys glareolus*) is the best predictor of future human cases (Bernshtein et al., 1999). Therefore, information on the prevalence of recent infections in deer mice may be key in predicting the risk of HCPS.

Several factors may influence the prevalence of recent SNV infections in deer mice. Periods of increased contact rates between deer mice, such as during the onset of breeding activities in the spring, may be correlated with recent infections (Kuenzi et al., 2005). A greater proportion of recent SNV infections were recorded in spring versus fall for deer mice in Alberta, Canada (Safronetz et al., 2006). A separate study observed seroconversions nearly year round, with a greater number during the reproductive season, and individuals in breeding condition were more likely to seroconvert (Douglass et al., 2007). Sex

differences may also play a role in the timing of seroconversions (Calisher et al., 1999). Furthermore, differences in habitat quality may influence antibody prevalence and, potentially, the prevalence of recent infections. Populations of deer mice in habitats with all-terrain vehicle disturbance have lower SNV antibody prevalence and higher population turnover (Lehmer et al., 2008).

We investigated the roles of seasonality, reproductive status, sex, and disturbance on incidence of recent infections of SNV in deer mice. We predicted that recent infections would be higher in the spring, due to increased reproductive activity, compared to fall when reproduction is subsiding; and that deer mouse populations in disturbed habitats would have a greater proportion of recent infections due to their high population-turnover rate. Additionally, we compared patterns of avidity of maternal antibodies in juveniles to that of adult female deer mice. Maternal antibody prevalence often reflects prevalence in adult females (Boulinier and Staszewski, 2008). We extended this hypothesis to examine whether prevalence of high- and low-avidity antibodies were similar between juveniles and adult females.

MATERIALS AND METHODS

Study Sites and Sampling Periods

Deer mice were sampled from six sites in Juab County, Utah (Sites 3, 4, 16, 17 located at 39.5N, 112.9W and Sites 5, 10 located at 39.4N, 112.24W, see Lehmer et al., 2008 for details). All sites were in sagebrush steppe and classified as high or low disturbance, based on a composite index calculated from the percent cover of shrubs minus that of bare ground (Table 1).

Sites were sampled in May and September, 2006 around the new moon. Animals were live-trapped for three consecutive nights on a web of 148 traps over 3.1 ha (Mills et al., 1999). Animals were identified to species and sex, weighed, and marked with ear tags. Deer mice were classified as "reproductive" if they had any of the following: descended testes, protruding nipples, or perforate vagina. Ani-

TABLE 1. Antibody prevalence and percentage of recent infections of SNV in deer mice.

Site		May						September					
		Adult males		Adult females		Juveniles		Adult males		Adult females		Juveniles	
No.	DI[a]	% Pos. (*n*)[b]	% RI (*n*)[c]	% Pos. (*n*)	% RI (*n*)	% Pos. (*n*)	% RI (*n*)	% Pos. (*n*)	% RI (*n*)	% Pos. (*n*)	% RI (*n*)	% Pos. (*n*)	% RI (*n*)
4	44.27	27 (30)	50 (8)	10 (39)	50 (4)	6 (17)	100 (1)	10 (79)	13 (8)	8 (52)	25 (4)	0 (14)	0 (0)
3	42.04	45 (31)	43 (14)	27 (56)	47 (15)	5 (19)	100 (1)	19 (54)	11 (9)	13 (39)	33 (3)	0 (14)	0 (0)
10	37.17	45 (20)	67 (9)	20 (20)	75 (4)	13 (16)	100 (2)	23 (31)	29 (7)	5 (38)	50 (2)	0 (16)	0 (0)
16	−3.58	47 (17)	71 (7)	36 (14)	60 (5)	6 (17)	100 (1)	10 (30)	0 (2)	17 (30)	0 (4)	0 (18)	0 (0)
5	−6.20	10 (10)	100 (1)	19 (16)	67 (3)	17 (6)	100 (1)	37 (27)	30 (10)	14 (22)	33 (3)	0 (12)	0 (0)
17	−14.07	46 (28)	31 (13)	33 (18)	33 (6)	29 (17)	100 (5)	17 (18)	50 (2)	13 (16)	0 (2)	0 (10)	0 (0)
Total		39 (135)	50 (52)	24 (164)	51 (37)	13 (93)	100 (11)	18 (239)	21 (38)	11 (197)	22 (18)	0 (84)	0 (0)

[a] DI = disturbance index: sites with positive values are "low-disturbance sites" and those with negative values are "high-disturbance sites."

[b] % Pos. = percentage of individuals seropositive for antibodies against SNV from the total sample; *n* = total number of deer mice sampled.

[c] % RI = percentage of individuals with recent infections among seropositive individuals.

mals ≤14 g were classified as juveniles, whereas those >14 g were classified as adults (Mills et al., 1997; Borucki et al., 2000; Douglass et al., 2001). We collected blood from the retro-orbital sinus upon initial capture during each trapping season. Animals were released at location of capture.

IgG and avidity assays

We performed enzyme-linked immunosorbent assays (ELISA) for immunoglobulin G (IgG) in deer mouse blood against SNV nucleocapsid antigen as described in Feldmann et al. (1993). Samples were considered positive if the optical density was three times greater than negative sera. Positive samples (1:100 dilution) were retested against negative antigen to eliminate false positives.

All seropositive juveniles (i.e., deer mice ≤14 g) were considered to have maternal antibodies and not IgGs to their own infection. The 14-g cutoff for maternal antibodies was previously established by three independent studies (Mills et al., 1997; Borucki et al., 2000; Calisher et al., 2007). In addition to these studies, we estimated the minimum body size of a deer mouse for which antibodies to its own SNV infection could be detectable by using values from the literature combined with data from our sites. Using the growth curve for the appropriate subspecies, we used the age at which deer mice are weaned (30 days) to estimate the body size at which individuals become independent and are likely to encounter an infected individual (Dice and Bradley, 1942; Layne, 1968). The estimated body size at the time of weaning for this subspecies was 11 g. At our study sites, juveniles 11–14 g represented 78% of the sample, which supports the notion that juveniles are beginning to venture out of the nest at this size. From the 30-day time point, we extrapolated out 14 days, which is the minimum time for a detectable antibody response (Botten et al., 2000). Using the growth curve, we estimated that the body size of a 44-day-old deer mouse is 15 g. This result implied that 15 g is the minimum size for which antibodies to an infection acquired as a juvenile could be detected. This exercise lent further support to the notion that seropositive deer mice under 14 g are unlikely to have acquired their own infection, and that the detectable antibody is maternal.

We used an avidity ELISA to categorize infections as "recent" (≤30 days) or "older" (>30 days) based on methods of Safronetz et al. (2006). Samples with a relative avidity index (RAI) ≥0.5 were considered high avidity and classified as "older" infections, while those <0.5 were considered low avidity and classified as more "recent" infections (Safronetz et al., 2006). We recognize that we were attempting to measure a continuous variable (time since infection) as a discrete one (recent versus older) and that a difference between an avidity of 0.50 and 0.51 is arbitrary. However, Safronetz et al. (2006) reported that all animals experimentally infected within 30 days had avidities <0.5, whereas only two of 14 animals infected for more than 30 days had avidities <0.5, with the vast majority of avidity scores being above 0.6 RAI. Thus, we used 0.5 RAI as a means to place animals into two categories, recent and older infections, for the statistical analysis.

Preliminary analyses revealed that, despite the large number of juvenile captures (n=177), few were seropositive (n=12). Furthermore, none of the seropositive juveniles for which the avidity ELISA was run (n=11) had high-avidity maternal antibodies. To further probe this pattern, we augmented the dataset with 15 additional samples from seropositive juveniles collected from five neighboring sites at the same time and manner as the six main sites (Lehmer et al., 2008).

Statistics

We used generalized linear mixed models to determine which factors were related to the probability of adult deer mice having a recent infection. The dichotomous response variable of having a recent versus an older infection was modeled as a function of sex, reproductive status, sampling period, disturbance (fixed effects), and site (random effect). Four of the 159 adult deer mice that tested seropositive were trapped in both sampling periods; only the initial sample was included in the analysis for reasons of statistical independence.

RESULTS

We captured 912 individual deer mice at the six focal sites. Of these, 159 adults tested seropositive. Antibody prevalence varied with season and demographic category (Table 1). Adult males had nearly two times the antibody prevalence of females. Of the 912 deer mice trapped, 177 (19%) were juveniles. There was no male bias in antibody prevalence of juveniles (5% males and 8% females), and within the juveniles, antibody prevalence declined with increasing mass (5–

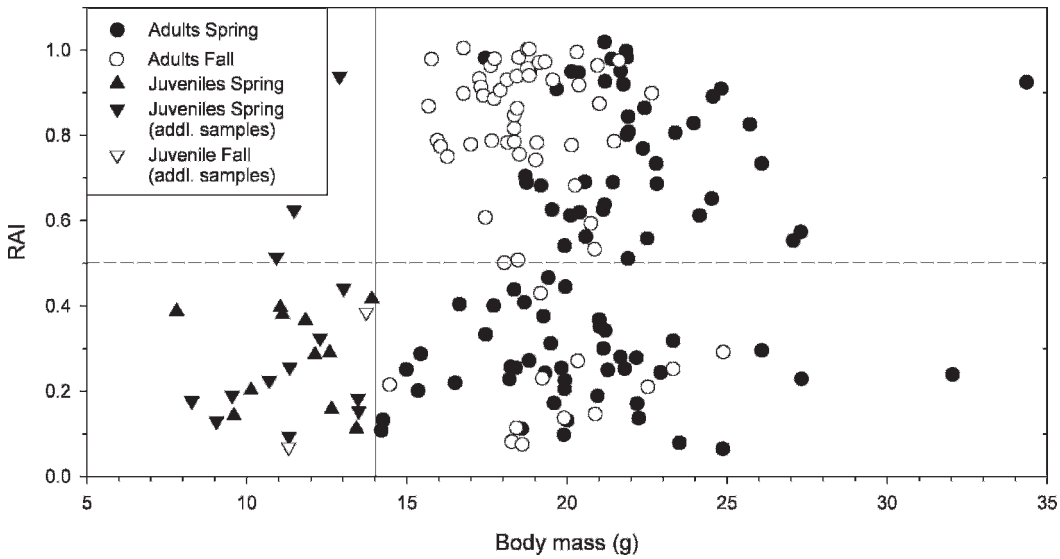

FIGURE 1. Relative avidity index (RAI) and body size of SNV-positive deer mice captured in May (solid symbols) and September (open symbols) 2006. Solid line represents body mass cutoff (14 g) for juveniles versus adults, and dashed line indicates the threshold of RAI=0.5 for discriminating between low-avidity (recent infections) and high-avidity (old infections) antibodies.

10.9 g, 12.5%; 11–12.9 g, 9%; 13–14 g, 3%). Three of the 12 antibody-positive juveniles were recaptured at a later date. All three had reverted to seronegative. None of the juveniles captured in the fall were seropositive (Table 1).

Sampling period was the only fixed factor that explained a significant amount of variance in the probability of recent infection (n=149 adult deer mice, $P<0.001$, coefficient estimate: -1.40 ± 0.40 for September, with May as the reference category). The probability of having a recent infection in May was two-and-a-half times greater than in September (least square means May: 0.52 ± 0.06 vs. Sep: 0.21 ± 0.06). The percentage of seropositive adults with recent infections was 50% in May versus 21% in September. Site also explained a small amount of the variation (variance components=0.09 ± 0.20). There was no significant effect of disturbance, sex, or reproductive status.

The avidity patterns of maternal antibodies in juveniles did not approximate that of adult females, particularly for old infections (Table 1 and Fig. 1). None of the seropositive juveniles from the six core sites had maternal antibodies that originated from a dam with an old infection (Table 1). The additional testing of 15 seropositive juveniles from five other sites revealed a few juveniles with maternal antibodies from mothers with older infections (n=3, with one of them being borderline with an RAI of 0.51). Thus, for all seropositive juveniles combined (n=26), 23 had avidities indicative of recent infections in the mother, whereas only three had avidities indicative of an older infection in the mother. In September, when the percentage of seropositive adult females with older infections was the highest (78%), no juveniles were seropositive despite a large sample size of juveniles (n=84; Table 1).

DISCUSSION

In this study, we examined four factors thought to influence the timing of SNV infection. Only one, sampling period, influenced the probability of a recent infection; in May, deer mice were approx-

imately three times more likely to have a recent infection than in September. None of the three other factors predicted the probability of recent infection.

The results suggest that SNV infections may be seasonally dependent. It is possible that deer mice that have survived the winter are in poor physical condition and thus are more likely to acquire infections (Nelson 2002). In addition, over-wintering deer mice with SNV infections may be more infectious in early spring if recrudescent virus is released from adipose stores during the winter (Botten et al., 2003). Thus, it is possible that stressful conditions in winter may drive the differences in the proportion of recent infections in May versus September. Additionally, reproductive activity generally begins to increase at the end of winter, peaks during early summer, and declines towards the fall (Douglass et al., 2001). In our study, 79% of adults appeared reproductive in May, compared to only 6% in September. Reproduction may enhance transmission through elevated contact rates (Glass et al., 1988). In addition, reproductive steroid hormones can depress the immune system (Grossman, 1985), making animals more susceptible to infection. Furthermore, reproduction is a costly metabolic process that competes for resources with the immune system (Martin et al., 2007).

That this study was conducted in a single year limits our ability to firmly establish the role of season on SNV infections. However, the pattern reported herein mirrors that reported by Safronetz et al. (2006), i.e., a greater proportion of recently infected deer mice occurred in May versus September. The fact that they conducted their study in a different year (2005) and in a vastly different habitat (prairie) suggests that the pattern of higher transmission rates in the spring may be a general one. This spring pattern of recent infections in Safronetz's study (2006), and in ours, may seem to conflict with the result of Douglass et al. (2007), as they reported seroconversions in deer mice across nearly all months of the year, with the greatest number of seroconversions in September. However, in the Douglass et al. (2007) study, they did not report the per capita seroconversion rate, i.e., force of infection, but rather seroconversions per trapping effort. Thus, it is unknown what fraction of the population seroconverts over time. Furthermore, as there was not a statistical comparison of seroconversion values, it is not possible to ascertain from their results whether the September seroconversions were significantly greater than any other months of the year.

One notable difference between our study and the Safronetz et al. (2006) work is in the magnitude of recent infections. In our study, the proportion of recently infected adults was approximately four times higher in both sampling periods. These results suggest that the force of infection may be greater in Utah, USA compared to Alberta, Canada. It is possible that a difference in the force of infection may explain the greater number of HCPS cases in the southwestern USA than those in Canada (Safronetz et al., 2008), although other differences in deer mouse ecology and human behavior could also play a role (Douglass et al., 2005).

The paucity of juveniles with maternal antibodies from females with older infections was surprising. Because IgGs are transferred during gestation and lactation (Becker et al., 2007), we expected avidity patterns in adult females to roughly approximate that of juveniles. The stark discordance suggests that juveniles produced by infected dams, particularly those with older infections, are missing from our sample. This result is perplexing. Exclusive trapping of juveniles with low-avidity antibodies is a doubtful explanation for this pattern. It is also unlikely that the pattern results from selective transfer of low-avidity antibodies, because high-avidity antibodies tend to transfer more efficiently (Avanzini et al., 1998). Howev-

er, it is possible that females with old infections have low titers and, thus, transfer fewer antibodies (Boulinier and Staszewski, 2008). This explanation has interesting implications for offspring immunity for young produced during recent versus older infections. Lastly, it is possible that the reproductive success of infected females with older infections is impaired. This effect is most likely to ensue near or following parturition, given that the body masses of some females with old infections were indicative of an advanced pregnancy (Fig. 1, all deer mice >25 g). Reduced survival of seropositive juveniles was reported by Douglass et al. (2001), although we cannot establish from this paper whether seropositive animals in this study were seropositive in subsequent captures, thereby eliminating any possibility of deer mice with maternal antibodies. The possibility of reduced survival of juveniles with maternal antibodies is intriguing, given that in other host–hantavirus systems, the presence of maternal antibodies enhanced maturation of juveniles (Kallio et al., 2006).

The pattern we observed in the avidity of juveniles is similar to that observed by Safronetz et al. (2006) in that few juveniles had high-avidity antibodies in May and none had them in September, despite the majority of the adult samples having high-avidity antibodies. Together, these studies suggest the possibility of a potential cost of SNV infection for females in the form of reduction in reproductive output. Such a cost could markedly alter the population dynamics of both host and pathogen. The difference in avidity between juveniles and females warrants a systematic study to examine the effect of SNV on female reproductive output.

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