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Assessment of Ecologic and Biologic Factors Leading to Hantavirus Pulmonary Syndrome, Colorado, U.S.A.

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Aim. To understand the ecologic parameters of Sin Nombre virus (SNV; family *Bunyaviridae*, genus *Hantavirus*) infections in the deer mouse *(Peromyscus maniculatus),* environmental variables impacting the rodent populations, and the conditions under which SNV is amplified. This may help us understand the antecedents of human risk for developing hantavirus pulmonary syndrome (HPS) as a consequence of SNV infection.

Method. Each 6 weeks, we trapped, measured, tagged, bled, and released rodents at three widely spaced sites in Colorado, USA: Fort Lewis (1994-2001), Molina (1994-2001), and Pinyon Canyon Maneuver Site (1995-2001). The ELISA method was used to test rodent blood samples for IgG antibody to SNV antigen.

Results. Where rodent species richness was high, the prevalence of infection of deer mice (as determined by the presence of antibody) with SNV was low, and vice versa. There was a higher prevalence of antibody to SNV in male than in female rodents, and seasonal differences were observed in acquisition of SNV between male and female deer mice. Long-lived infected deer mice served as transseasonal, over-winter reservoirs for the virus, providing the mechanism for its survival.

Conclusion. Prevalence of rodent infection appears to be associated with fluctuations in deer mouse populations and, indirectly, with timing and amount of precipitation and the resulting biologic events (a "trophic cascade"). Together with information regarding transseasonal maintenance of SNV, seasonal differences in acquisition of SNV between sexes, group foraging, and various other factors may expand our understanding of the risk factors for acquiring HPS. Taken together and applied, we anticipate developing methods for preventing this disease as well as diseases caused by other rodent-borne viruses.

Key words: *antibodies, viral; ecology; epidemiology; hantavirus; hantavirus pulmonary syndrome; lung diseases; rodentia; USA*

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are found essentially worldwide and are major causes of morbidity and mortality in Asia and Europe (1). Infections with some of these viruses have been associated with illnesses causing significant mortality after acute, systemic disorders characterized by fever, hemorrhagic manifestations, including intravascular coagulopathy, and renal failure (1). These illnesses usually are given the clinical diagnosis of hemorrhagic fever with renal syndrome but also are known as Korean hemorrhagic fever, epidemic hemorrhagic fever, hemorrhagic nephrosonephritis, nephropathia epidemica, and many other, more local, names. The first recognized etiologic agent of hemorrhagic fever with renal syndrome, Hantaan virus, was isolated from a striped field mouse, *Apodemus agrarius*, the natural reservoir of this virus in Korea (1). Several other viruses cause hemorrhagic fever with renal syndrome-like diseases, among which are Seoul, Dobrava, and Puumala viruses. Antibody to Seoul-like viruses have been weakly associated with chronic renal disease in residents of U.S. cities with large populations of rats (2).

In mid-May 1993, an outbreak of fatalities in adults with acute cardiopulmonary distress was recognized in the southwestern U.S.A., first in New Mexico and, shortly thereafter, in Colorado, Arizona, and Utah. A virus belonging to the *Hantavirus* genus subsequently was shown to be associated with this syndrome, now called hantavirus pulmonary syndrome (3,4). Serologic and virologic investigations of the 1993 outbreak indicated that this virus, called Sin Nombre virus (SNV), had the deer mouse, *Peromyscus maniculatus*, as its principal vertebrate host. The deer mouse is the most common mammal in North America, but it does not occur along the Atlantic coast. Later studies showed that SNV could be detected wherever deer mice occurred and that human hantavirus pulmonary syndrome occurred in associa-

tion with these rodents essentially throughout the U.S.A. and in many parts of Canada. Elegant molecular epidemiologic studies of specimens from patients with hantavirus pulmonary syndrome and deer mice captured in or near case-patient residences showed that SNV was distinct from other known hantaviruses (5). Hantavirus pulmonary syndrome now has emerged as a significant public health problem throughout the Americas (6).

To date, 38 hantaviruses have been detected in rodents (order *Rodentia*, family *Muridae*) of the subfamily *Murinae* (6 viruses, of which 5 are known to cause human disease), subfamily *Arvicolinae* (7 viruses, of which 1 is known to cause human disease), and subfamily *Sigmodontinae* (25, of which 17 are known to cause human disease). A hantavirus has also been isolated from a shrew (order *Insectivora*, family *Soricidae*) but is not known to cause human disease (1,7-9). It is likely that many more hantaviruses are yet to be detected.

To 30 November 2001, hantavirus pulmonary syndrome had been laboratory-diagnosed for 288 humans (109 case-fatalities, or 37.8%) in 31 states of the U.S.A.; 115 of the 288 were residents of the area adjoining New Mexico, Colorado, Arizona, and Utah, ie, the Four Corners area. The percentage of cases by race was: Caucasian (78%), Native American (19%), African-American (2%), and Asian (1%). A few hantavirus infections were also diagnosed in Canada and many in South America.

Ages of patients in the U.S.A. have ranged from 10 to 75 years (mean, 37 years) but a mild illness due to SNV was also detected in a 4-year old; 40% of patients with hantavirus pulmonary syndrome have been women. More than 60% of the first recognized case-patients died, but the case-fatality rate now is 38%, probably reflecting improved surveillance and detection of more mild cases. Thirteen fatalities among 26 cases (50% case-fatality rate) have occurred in Colorado, even with modern intervention measures. Most or all Colorado cases ostensibly have been caused by SNV (L. Eskew, U.S. Centers for Disease Control and Prevention, personal communication, 2001).

The clinical course of hantavirus pulmonary syndrome caused by SNV differs significantly from disease resulting from infection with Old World hantaviruses Hantaan, Seoul, Dobrava, and Puumala. Onset of hantavirus pulmonary syndrome caused by SNV is characterized by a prodrome including fever, myalgia, and various respiratory symptoms. Patients with hantavirus pulmonary syndrome have acute pulmonary edema and shock; pathogenesis of the disease appears to be related to the presence of viral antigens in pulmonary capillaries (10). Patients may have abrupt onset of acute respiratory distress associated with interstitial pulmonary edema and die a week or less after onset. Other symptoms reported in the early stages of hantavirus pulmonary syndrome include headache, abdominal pains, nausea, and vomiting. Other organ systems do not seem to be involved, although mild renal insufficiency has been observed in a few patients (just as pulmonary involvement has been observed in patients infected with hantaviruses known to cause hemorrhage, fever, and renal impairment).

The pathophysiology of hantavirus pulmonary syndrome may be similar to that of hemorrhagic fever with renal syndrome, except, of course, that the principal affected organs are the lungs, not the kidneys. Nonetheless, antigen of SNV has been detected in lung, kidney, heart, liver, and spleen tissues. The cause of death in patients with hantavirus pulmonary syndrome is not clear but the catastrophic failure of the lungs certainly is central to it: capillaries leak profusely, flooding air spaces with fluid, and attendant pH imbalance is the feature proximal to death. Severely ill patients showed evidence of shock. Clearly, it is of critical importance to prevent infections causing such life-threatening illnesses.

Before we can prevent hantavirus pulmonary syndrome, we must understand the dynamics of SNV in its rodent host and the environmental variables impacting the rodent populations. Only when these human hantavirus pulmonary syndrome antecedents are understood, will we be able to prevent or interrupt virus transmission. Therefore, in 1994 we began long-term studies of hantaviruses and their rodent hosts in Colorado. The results thus far have provided insights to the complex natural history of these viruses and to the dynamics of deer mouse populations. This report summarizes data regarding deer mouse population fluctuations, gender differences in prevalence of infection in deer mice, evidence for an inverse correlation between rodent species richness and prevalence of SNV infection in deer mice, navigational instincts of deer mice, transseasonal maintenance of SNV, seasonal differences in acquisition of SNV between sexes, group foraging by deer mice, evidence for a "trophic cascade", and various other factors that may make important contributions to the general risk for acquiring hantavirus pulmonary syndrome.

Methods

Description of Sites

Study areas in western Colorado, at Fort Lewis (La Plata County, southwest Colorado; N 37 13′ 30.9", W 108 10′ 51.1", elevation 2,438 m) and Molina (Mesa County, west central Colorado; N 39 09′ 45.8", W 108 03′ 18.4", elevation 1,951 m) were chosen because they were within a few kilometers of 1993 case-patient residences. We established trapping webs at both sites, beginning in June 1994 at Fort Lewis and in October 1994 at Molina (11). Studies in southeastern Colorado, at the Pinyon Canyon Maneuver Site, were begun in January 1995 and ended in August 2001. Four sites were established at Pinyon Canyon Maneuver Site, with trapping webs in a pinyon pine (*Pinus edulis*)-juniper (*Juniperus spp.*)/short grass prairie habitat (N 37 33.024′, W 103 59.560′, elevation 1,585 m), at the head (N 37 32.754′, W 103 49.343′, elevation 1,524 m) and within the canyon (N 37 32.193′, W 103 49.125′, elevation 1,341 m), and at a functioning windmill (N 37 31.327′, W 103 53.545′, elevation 1,585 m).

At Fort Lewis, the habitat is montane shrubland (12) superimposed on intrusive igneous rocks forming laccoliths (13). Vegetation comprises predominantly ponderosa pine (*Pinus ponderosa*), Gambel's oak (*Quercus gambelii*), a variety of grama grasses (*Bouteloua spp.*), and many other, more minor, floral components. At the Molina site, the habitat is semi-desert shrubland superimposed on Mancos shale (11,13). Vegetation includes juniper, pinyon pine, and various shrubs and grasses.

The Pinyon Canyon Maneuver Site is under the management of the Directorate of Environmental Compliance and Management, U.S. Department of the Army, Fort Carson, Colorado. Complete descriptions of this tract were published (14,15). In general terms, Pinyon Canyon Maneuver Site is a short grass prairie/pinyon-juniper community (16), with topography consisting of broad, moderately sloping uplands bordered by the Purgatoire River Canyon on the east, limestone hills on the west, and an extruded basalt hogback ridge on the south.

Sampling Methods

Sampling was done for 3 days each 6 weeks as weather permitted. Webs were established as follows: twelve 7.6x8.9x 22.9 cm non-collapsible traps (H.B. Sherman Traps Inc., Tallahassee, FL, USA) were placed on the ground at 5-m intervals for 20-m and then at 10-m intervals for 80-m, in each of 12 rows, with an additional trap placed at the central point. In all, there were 145 traps in each of two webs; the location of each trap was marked with a construction flag. Traps were baited with a mixture of cracked corn, oats, and peanut butter (4:2:1), and allowed to remain open overnight. When temperatures were expected to be $<5°C$, cosmetic balls of nonabsorbent material were placed in each trap, so that trapped animals would have insulating material available to retain heat until they were processed and released. Each morning, traps were examined and rodents were taken to a central processing area where they were identified, weighed, sexed, and bled by inserting a capillary tube into the retroorbital plexus, before their release at their site of capture. We followed standard methods for sampling rodents and for minimizing hazard from potentially infected animals (17). The handling and processing of rodents by these methods does not have a significant impact on the subsequent survival or probability of recapturing most species (18,19). Each trap in which a rodent was captured was washed thoroughly with a mild solution of detergent to destroy virus-contaminated material, then rinsed thoroughly and air-dried before replacing the trap in the field. This procedure had the added benefit of removing or diminishing scent cues deposited by former inhabitants, which could influence successive captures (20).

Antibody Determination

Blood samples were stored on wet or dry ice and transported to our Fort Collins laboratory, where they were stored in a mechanical freezer (-70C). These samples were thawed once for removal of a sample to be tested for antibody, refrozen in the mechanical freezer, and later shipped on dry ice to the U.S. Centers for Disease Control and Prevention, Atlanta, for confirmatory testing. Tests for IgG antibody to SNV antigen were done by enzyme-linked immunosorbent assays (ELISA), according to the method of Feldmann et al (21). Samples were tested at a screening dilution of 1:100 and end-point titers determined subsequently. Because rodents infected with hantaviruses are persistently infected (22), antibody to these viruses can be used as an indicator of current infection. In addition, the nucleocapsid SNV antigen used in these tests is broadly cross-reactive among hantavirus species, such that antibody to this antigen only indicates infection with any of a number of closely related hantaviruses, not necessarily with SNV (21). Nonetheless, because SNV is the only hantavirus we have detected in deer mice, we took the presence of antibody to this virus in deer mice as evidence of SNV infection.

Results

Rodent Populations and Prevalence of Antibody to SNV

Between 1994 (at the Fort Lewis and Molina sites) or January 1995 (at the Pinyon Canyon Maneuver Site) and late 2001, numbers of rodents varied from few to many (complete data not shown). During this period at the Fort Lewis site, deer mice were absent or numerous (mean, 31.2 per 3-day trapping period), ranging from 0 to 157 in October 1999 (Fig. 1). There was no direct association visible between antibody prevalence (mean, 13.1%; range 0-42.9%; excluding intervals during which only 1-3 mice were trapped) and numbers of deer mice (Fig. 1). Between April and October 1996, 0/69 had antibody to SNV and between August 2000 and May 2001, 1/77 had such antibody. At the Molina site, numbers of deer mice ranged from 6 to 62 (mean, 22.8), with the greatest number trapped in October 1997 (Fig. 2). Mean antibody prevalence was 8.9% (range 0-33%). Between August 1996 and August 1997, 1/60 deer mice had antibody and between June 2000 and July 2001, 1/95 had antibody.

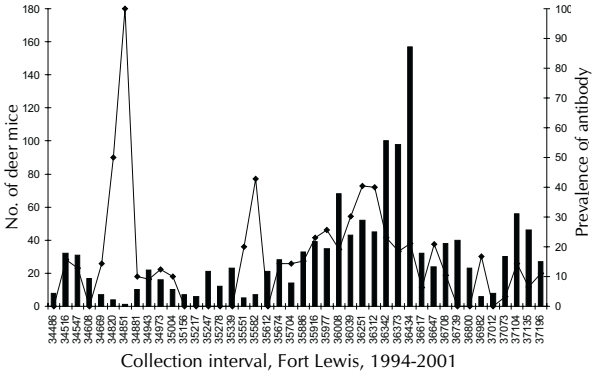

Figure 1. Number (bars) of deer mice (*Peromyscus maniculatus*) and prevalence of IgG antibody to Sin Nombre virus (line) at Fort Lewis, Colorado, USA, 1994-2001.

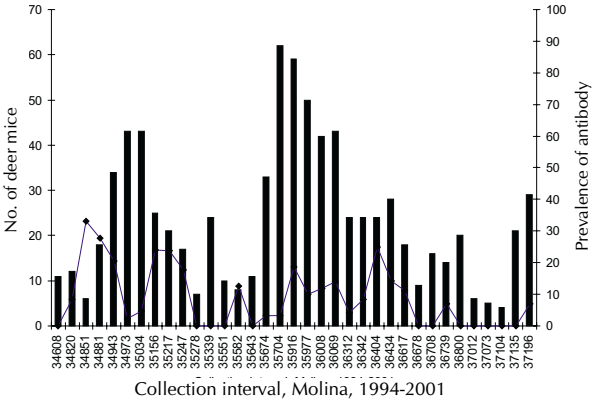

Figure 2. Number (bars) of deer mice (*Peromyscus maniculatus*) and prevalence of IgG antibody to Sin Nombre virus (line) at Molina, Colorado, USA, 1994-2001.

In contrast to these southwestern and west-central Colorado sites, numbers of deer mice and prevalence of antibody to SNV at the Pinyon Canyon Maneuver Site in southeastern Colorado usually were higher and lower, respectively (Fig. 3). Numbers of deer mice per trapping period ranged from 3 to 89 (mean, 30.4), with the greatest numbers usually collected in late fall to late winter. Mean antibody prevalence in deer mice was 1.9% (range, 0-17.4%) but from July 1995 to September 1998 only 3/919 deer mice were found to have antibody to SNV. In the 14-month period beginning October 1998, antibody prevalences in the 7 trapping intervals were 2.7%, 3.4%, 8.0%, 12.5%, 14.0%, 17.4%, and 9.1%. Thereafter (March 2000 – June 2001), none of 84 deer mice were shown to have antibody.

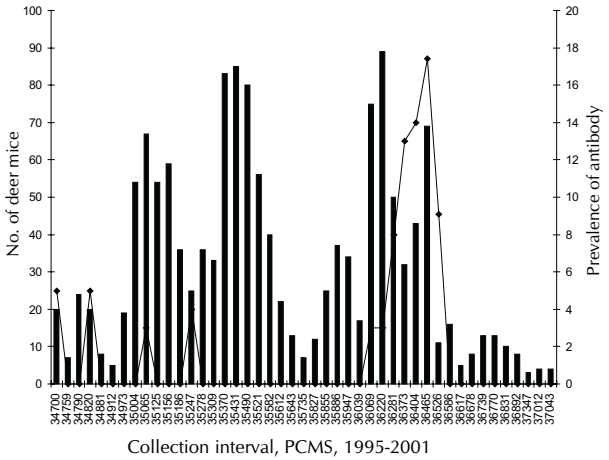

Figure 3. Number (bars) of deer mice (*Peromyscus maniculatus*) and prevalence of IgG antibody to Sin Nombre virus (line) at Pinyon Canyon Maneuver Site, Colorado, USA, 1995-2001.

As at the western Colorado sites, antibody prevalence usually increased after increases in rodent population sizes. Increases and decreases in prevalence of SNV infections in deer mice, not only in Colorado but in nearby New Mexico and Arizona (23), appear to be associated with commensurate increases and decreases in deer mouse populations and to be related indirectly to timing and amount of precipitation (data not shown). The "trophic cascade" resulting from increased precipitation leads to increased availability of food and resulting increases in breeding.

Under natural conditions, it simply is not possible to determine all the possible ecologic variables that may impact availability of rodent food. Nonetheless, we made rudimentary attempts to quantify a few of these variables. For example, we measured the production of acorns of Gambel's oak at the Fort Lewis site in each September 1994-2001. Acorns were collected from the ground surface in a 1 square meter area. Few acorns were observed in 1994 and none in 1995, 1997, 1999, or 2001. The same trees that produced about 350 g/m^2 of acorns in 1998 produced about 467 g/m^2 of acorns in 2000.

Whenever we trapped at Fort Lewis, Molina, and Pinyon Canyon Maneuver Site in 2000, we collected insects in pitfall traps containing propylene glycol as a preservative. Commonly observed insects were identified as belonging to the orders *Arachnida* (class *Araneida*), *Insecta* (classes *Coleoptera*, *Diptera*, *Hemiptera*, *Hymenoptera*, *Lepidoptera*, and *Orthoptera*).

Total insect biomass at Fort Lewis increased slightly from June to July, then decreased until October. At Molina, insect biomass was lower than at Fort Lewis and decreased from the June peak until October. In contrast, insect biomass at Pinyon Canyon Maneuver Site was higher than that at Fort Lewis or Molina and insects continued to be caught until November, albeit with progressively lower biomass. These collections are being continued to determine potential availability of insects as food sources for rodents.

Gender Differences in Prevalence of Infection in Deer Mice

If SNV is transmitted between deer mice by intraspecific agonistic behaviors, such as fighting, it would follow that the prevalence of antibody to SNV would be higher in males than in females. We found that the proportion of antibody-positive male deer mice to antibody-positive female deer mice decreased as the overall prevalence increased. Depending on the interval, we have found disparities ranging from 80:20 to 50:50. Glass et al (24) found that a much greater proportion of males had antibody to Seoul virus in rats (*Rattus norvegicus*) in the U.S.A., and Calisher et al (25) reported a similar association for SNV and deer mice in Colorado. At Fort Lewis, 73/497 (14.7%) female deer mice and 116/471 (24.6%) male deer mice had antibody to SNV. Therefore, males represented 61.4% of the seropositives at the Fort Lewis site but 48.7% of the population. The difference was significant (chi-square = 7.62; $p < 0.005$). At Molina, 27/374 (7.8%) female deer mice and 48/304 (15.8%) male deer mice had antibody to SNV. Males therefore represented 64% of the seropositives at the Molina site but 44.8% of the population. Again, the difference was significant (chi-square = 8.05; $p < 0.005$). We found similar associations with other rodents (data not shown), including 95% of seropositive western harvest mice (ostensibly infected with another hantavirus, El Moro Canyon virus) at Pinyon Canyon Maneuver Site being males, whereas males represented approximately 50% of the population of western harvest mice.

Scarring can be used as a surrogate for past fighting experiences in mice, and fighting appears to be a principal means by which SNV is transmitted from infected to uninfected mice (24,25). At the Fort Lewis site, between 1994 and 2001, 25/57 (43.9%) male deer mice had scars or wounds (missing toes, torn ears, other wounds on the ears or elsewhere on the body) and antibody to SNV, whereas 27/209 (12.9%) had scars and no antibody (Yates corrected chi-square = 25.33, $p < 0.001$). At the same place and during the same time, 7/12 (58.3%) female deer mice had scars and antibody to SNV, whereas 33/308 (10.7%) had scars and no antibody (Yates corrected chi-square = 9.08, $p = 0.003$). When data for males and females were pooled, 32/69 (46.4%) had scars and antibody to SNV, whereas 60/517 (11.6%) had scars and no antibody (Yates corrected chi-square = 44.73, $p < 0.001$).

Rodent Species Richness and Prevalence of SNV Infection in Deer Mice

As noted above, the mean prevalence of deer mouse antibody to SNV at Fort Lewis was 13.1% (range, 0-42.9%), at Molina the mean was 8.9% (range, 0-33%), and at Pinyon Canyon Maneuver Site it was 1.9% (range, 0-17.4%). The richness of rodent species at these sites varied inversely with these prevalences. At Fort Lewis, where (except for the odd capture of a species) essentially two species (deer

mice and least chipmunks, *Tamias minimus*) were captured, the mean antibody prevalence was the highest of the three sites. At Molina, where essentially four species (deer mice, least chipmunks, pinyon mice [*P. truei*], and Hopi chipmunks [*T. rufus*]) were captured, the mean antibody prevalence was intermediate. At Pinyon Canyon Maneuver Site, where rodents belonging to 19 species were captured, the mean antibody prevalence was the lowest (Fig. 4).

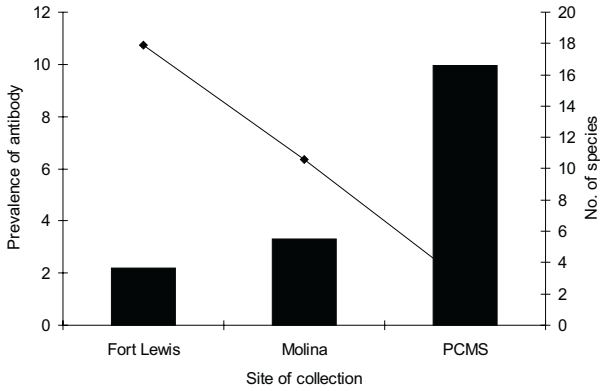

Figure 4. Prevalence of IgG antibody to Sin Nombre virus (line) and mean number (bars) of rodent species at Fort Lewis, Molina, and Pinyon Canyon Maneuver Site (PCMS), Colorado, USA, 1994-2001.

Other Observations

Gender differences. We observed seasonal gender differences in the acquisition of SNV infection by deer mice. Incidence of antibody acquisition at each trapping site was about the same for each gender but most seroconversions (recent infections) in deer mice at Fort Lewis and at Molina occurred during late summer to late fall for males and mid-winter to early spring for females.

Transseasonality. Given our failure to detect SNV and El Moro Canyon virus in our study sites at one time or another, an obvious question is "How are these viruses maintained transseasonally, ie, year-to-year?" Most individual deer mice did not persist on the trapping webs much beyond a month after they were first captured. A small number persisted for more than a year, which suggests that longevity of even a few infected deer mice, serving as transseasonal reservoirs, could provide a mechanism for over-winter virus maintenance. Furthermore, the rates of seroconversion in deer mice at the Fort Lewis and Molina sites were higher than the seroprevalence, suggesting that the longer deer mice live, the greater the probability they eventually become infected with SNV; one deer mouse had antibody to SNV detected for the first time 14 months after it had been initially captured.

Multiple captures. As a complement to surveillance for hantaviral infection in rodents at these communities, we examined demographic and ecological characteristics of these populations to evaluate factors that might influence transmission of virus. Occasionally during these studies, we captured two rodents in a single trap, presenting an opportunity to compare our dual capture results with those previously reported by others. To summarize our multiple capture data, dual captures of rodents are unusual but not rare and tend to occur among individuals of certain species (26). Because most often the pairs were comprised of rodents of the same species, that males more often were captured as pairs than were females, and that pairs of rodents of the same species could be recaptured as pairs, these data suggest that such captures are non-random, group foraging encounters which have implications for transmission of hantaviruses.

Navigational instincts. As do house mice (*Mus musculus*), deer mice invade homes, particularly in rural areas. The Pinyon Canyon Maneuver Site is a former cattle ranch, now returning to its natural condition as short-grass prairie. We often stay in an old bunkhouse, used by many research groups at irregular intervals. The house is well maintained but has openings through which mice can pass to and from the outside. For safety and cleanliness, we removed mice we found inside the house, but between April 1996 and April 1998, we live-trapped and released them rather than trapping and killing them (27). Nineteen deer mice and a pinyon mouse (which did not return) were examined and tagged. At first, we simply released these animals approximately 50 m from the house, but when we realized that they were returning, we released them at increasing distances (50-1,500 m) from the house; the distances were measured by pace counts by at least two investigators.

Three deer mice had been captured multiple times in our test grid (as far as 250 m from the house) before they were first captured in the house. Once captured in the house, however, they were not captured in traps of the grid (ie, outside the house). The mean distance traversed by the five deer mice that returned to the house was at least 394 m; one mouse returned after being released 500 m and 1,000 m, then 750 m, and 1,200 m from the house at consecutive daily trapping sessions of 3 days. Sometime within the subsequent 6 weeks, this mouse returned to the house from the 1,000-m release point and then from 750 m and 1,200 m away on consecutive days within the trapping period. Each of the mice returning to the house did so within 24 h of release, and two within as few as 6 h after release from 500 m and 750 m away. Nine mice were captured once; 6/8 mice captured twice were captured at least once more; one was captured 10 times, one 7 times, one 6 times, one 4 times, and two 3 times. Equal numbers of male and female, adult and juvenile mice were captured in the house, but only adult mice (5/5) returned to the house. Returning deer mice maintained or gained weight between captures and grew in length at approximately the same rate as deer mice captured in the test grid. None of the mice we captured had antibody to SNV.

Discussion

It could be expected that territorial defensiveness of the deer mouse would be more likely as their population density increased, and that behaviors associ-

ated with such defensiveness, including intraspecific agonistic behaviors, would lead to transmission of virus from an infected to an uninfected deer mouse. Indeed, we observed such an association (25) but it is far from clear that this was a general phenomenon. It may be informative that deer mouse populations at Pinyon Canyon Maneuver Site were relatively high at certain times, yet for the vast majority of the study period, few or no infected deer mice were captured. Except for the 14-month period beginning October 1998, during which the prevalence of SNV at Pinyon Canyon Maneuver Site increased to a peak of 17.4%, presence of SNV was, for the most part, nominal. This may be related to rodent species richness (see below). Interestingly, at Pinyon Canyon Maneuver Site, there appeared to be a lag between numbers of deer mice and increasing antibody to SNV, although this also was not clear-cut.

To sort out which of the perceived thousands of climatological, biological, and geological characteristics of an area impact the ecosystem, which are quantitative and which are qualitative, and what the effect of timing of rain or snow events has on habitat can be answered only with longitudinal studies, such as this one. In regards to timing of meteorological occurrences, if the annual mean precipitation for a given habitat is "X" mm and 20% of that usually occurs in each month from May to September, what is the effect of "X" mm occurring, instead, in October? In the red rocks country of the American southwest, the soil would not absorb all or even most of this "X" mm, it would simply run off into a low-lying area and be useless insofar as vegetation is concerned. Rodents dependent on the vegetation that would have flourished under normal conditions, would have to rely on other, perhaps less nourishing, food sources. The long-term effects of such events are unknown but is one of the many variables that must be investigated if we are to make long-term assessments of rodent-borne virus disease risk. Alternatively, when conditions are optimal, the resulting "trophic cascade" could lead to increased availability of rodent food, resulting in increases in breeding, and potentially increased prevalence of SNV. According to our data, there was no direct and immediate association between the years 1998 and 2000 of acorn production and the numbers of deer mice captured at the Fort Lewis site. Nonetheless, in combination with knowledge of other phenomena, the importance of primary production of vegetation might be clearer, as might knowledge of available insect biomass, which likely will be shown to be the result of climatic conditions. Obviously, we did not present complete details of some of the many studies summarized in this paper , e.g., insect biomass, because of the relatively preliminary nature of these studies. Nevertheless, it will be of obvious value to continue studies of acorn production and available insect biomass, and to estimate normalized difference vegetation indices (28) by use of satellite imaging. Normalized difference vegetation index provides a crude estimate of the amount of vegetation and is a means of monitoring vegetation changes that might prove to be predictive of rodent population density.

Differences in rates of acquisition, overall prevalence, and seasonality of acquisition of SNV infection between male and female deer mice indicate clear-cut biological distinctions, as well as gender differences. Male deer mice have a higher prevalence of infection, as determined by antibody status. Scarring or wounding, which correlated with the presence of antibody, might be indicative of close contact of uninfected with infected deer mice. However, scarring (evidence of fighting) does not by itself indicate much more than aging, so that an association of scarring and mass class (weight, ie, age) must also be shown. It is anticipated that use of these and similar indicators will provide insight into the life history of the rodent hosts of hantaviruses.

The negative association between rodent species richness and prevalence of SNV infection in deer mice may have far-reaching consequences. Decreased biodiversity of vertebrate host communities has been linked, at least in theory, to increasing transmission of certain vector-borne diseases (29). Mills et al (30) proposed a mechanism for a link between biodiversity and directly transmitted zoonotic diseases. Using data from long-term studies of rodent-borne hantaviruses in the southwestern United States, they demonstrated a negative correlation between richness of rodent communities and both the prevalence of antibody to hantaviruses in rodent reservoir host populations and hantavirus disease in nearby human populations. They further suggested that species evenness (the relative abundance of species in a community) was a better predictor of virus transmission rates than species richness (the total number of species in a community). If decreased biodiversity brings about increased prevalence of SNV infection in deer mice, then severe habitat changes, such as can be caused by extractive mining, outdoor recreational sporting activities, and expanded housing for humans, might be a cause for concern.

Long-term persistence of SNV is central to our understanding of transseasonal maintenance of this virus. If the virus disappears from an area, the only way it can reoccur is if infected and persistently shedding vertebrate hosts invade the area. If deer mice that are infected but not shedding virus persist in an area, the mechanism becomes much more complex. The data presented indicate that at least one mechanism for transseasonal persistence is the presence of long-lived, infected deer mice, some proportion of which shed virus (transseasonal reservoirs). The seroconversion rate further suggests that the longer uninfected deer mice live, the greater the probability they eventually will become infected with SNV.

Data regarding deer mice captured together suggest that such captures are non-random, group foraging encounters that have implications for transmission of hantaviruses. We expect that results of genomic studies of these mice will be instructive insofar as determining whether and how SNV trafficking occurs and whether infected deer mice move from "refugia", where they and their viruses have survived adverse environmental conditions.

With respect to deer mouse movement, we have shown that these rodents not only travel great distances but also appear to have navigational instincts, honed by experience (27).

Homing ability, site fidelity, and navigational proficiency of rodents are well documented (31,32). Teferi and Millar (33) studied the homing ability of deer mice in Alberta, Canada; 50% of deer mice in that study returned to their home sites (a short-grass prairie habitat). The mice traveled 650 m to 1,980 m (mean 1,500 m) and had to cross a river and pass optimal habitat patches to reach their home sites. Deer mice with previous homing experience were more successful and faster in returning home (100%) than inexperienced mice (60%). Teferi and Millar (33) suggested that these deer mice were able to navigate in a direct route to their home sites. We released mice in locations where they had no direct route to the house; they had to follow a winding road, climb over rocky outcroppings nearly 17 m high, or otherwise surmount obstacles and dangers, such as predators. Infected deer mice released and then returning to a house or uninfected deer mice released, infected, and then returning to a house would increase the likelihood of human contact with an SNV-infected mouse. The risk would be the same for other hantaviruses infecting other peridomestic rodents. Against current recommendations that rodents in homes be trapped and killed, some homeowners live-trap and release them outside their homes. Our data support killing mice in homes (so long as one prevents other mice from taking their places) and provide evidence that released wild mice return and may place the residents at risk. Accordingly, there are immediate practical aspects to this work.

Conclusion

There is a higher prevalence of antibody to SNV in male than in female rodents. The proportion of male to female rodents with antibody decreases as the overall prevalence increases. There is a positive correlation between observed wounds and presence of antibody; males acquire antibody to (infection with) SNV mostly during the late summer-late fall period. Females acquire antibody to SNV during the winter-early spring period.

Simultaneous multiple captures of rodents of the same species suggest group foraging, which may relate to trafficking of SNV. Long-lived infected individual deer mice may serve as transseasonal reservoirs of SNV. Habitat diversity may be inversely correlated with prevalence of SNV.

Most people infected with SNV acquired it through their vocations, although simply living in a rural area might be considered a risk factor. Working in dusty areas, such as barns and attics, renovating mobile homes in rural areas, cleaning (sweeping or wiping dust from) long-unoccupied vacation homes, having an air conditioner with a mouse nest inside, working at farming or gardening during which rodent nests or excreta may be exposed, camping, and other activities that would activate resting dust particles or otherwise expose one to them, all can be risk factors.

Future studies will include those of the rates of reproductive preparedness, which may be predictive of potential rodent population densities, and genomic analyses of deer mice to determine the potential for SNV trafficking. It is already clear that there is an extremely complex correlation between precipitation and other climatological events, and rodent population densities as well as infection with SNV. When more elements of this "trophic cascade" and the relative importance of each are understood, we will have a powerful tool with which to devise predictors of future events. Finally, if there is a correlation between the number of deer mice with antibody to SNV and human risk of acquiring infection with this virus, we will have met at least a portion of our goals. Application of these protocols and findings may have relevance to predicting disease risk caused by hantaviruses and other rodent-borne viruses worldwide.

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