HANTAVIRUS (BUNYAVIRIDAE) INFECTIONS IN RODENTS FROM ORANGE AND SAN DIEGO COUNTIES, CALIFORNIA

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Abstract. During a screening program to determine the extent of hantavirus activity in Orange and San Diego Counties, California, serum samples from 2,365 rodents representing nine genera and 15 species were tested for hantavirus antibodies. A reverse transcription–polymerase chain reaction on selected seropositive rodents was used to identify the specific hantavirus. Rodents positive for Sin Nombre virus (SNV) antibodies by Western blot included 86 (9.1%) of 948 deer mice (Peromyscus maniculatus), four (1.5%) of 275 California mice (Peromyscus californicus), one (0.5%) of 196 cactus mice (Peromyscus eremicus), 51 (12.2%) of 417 harvest mice (Reithrodontomys megalotis), and five (12.5%) of 40 California voles (Microtus californicus). All other specimens tested were negative for hantavirus antibodies. There was a correlation between age and sex of the reservoir host and prevalence of SNV antibody, especially among male deer mice and harvest mice. Few seasonal trends in antibody prevalence were observed and continued maintenance of SNV and El Moro Canyon virus was found at several foci over a 4–5-year period. Isla Vista virus was also found in voles and represents the first recorded in Orange County. Microhabitat selection on the part of these rodents based on plant density, plant height, and availability of food plants may explain, to some extent, all of the hantavirus-positive foci throughout the study area over a broad geographic range and the lack of antibody-positive rodents in dense chaparral, woodland, and riparian areas. The majority of rodents positive for SNV was identified from localities along coastal bluffs and the foothills of the Santa Ana Mountains, where trap success was high and P. maniculatus represented 43% of all rodents collected. Several residential, commercial, and industrial sites exist in these areas and the potential health risk should not be overlooked. This study represents an in-depth analysis of the prevalence, host distribution, and characteristics of rodent populations infected by three hantaviruses within a small, well-defined, geographic area.

Hantaviruses were first identified as etiologic agents of hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe.1-4 There are approximately 20 species of Hantavirus described; collectively, they comprise a genus of the family Bunyaviridae. They are distributed worldwide, most in close association with a single species or genus of rodent. Infection of the natural hosts (rodents) differs from human infection in that it is apparently asymptomatic and chronic. Humans contract most hantaviral infections by accidental inhalation of contaminated rodent urine, feces, saliva, or other body fluids.

At least four distinct species of Hantavirus cause HFRS.3 Early efforts to identify evidence of hantavirus infection in the New World were productive in rodent studies,1 but HFRS proved to be very uncommon in North America. A previously undescribed hantavirus (Prospect Hill virus [PHV]) was isolated from a vole (Microtus pennsylvanicus) in 1982.5 A wide variety of New World rodent species was found to have antibodies reactive with Hantaan virus. These included indigenous rodents (Muridae: Sigmodontinae and Arvicolinae) such as deer mice and white-footed mice (genus Peromyscus), wood rats (Neotoma), and voles (Microtus and Clethrionomys). In addition, the commensal rats Rattus norvegicus and R. rattus were found to harbor Seoul virus (SEOV) in port cities worldwide, including cities in the United States such as Baltimore and Houston.5-8

A few mild cases of HFRS have been identified in Baltimore that were most likely acquired from R. norvegicus.9 In 1993, a more common and serious form of hantavirus disease was discovered in the Americas and was associated with viruses harbored by indigenous rodents.10 Since an outbreak of hantavirus pulmonary syndrome (HPS)10-16 was recognized in the Four Corners region of the southwestern United States, more than 190 cases of this severe disease were reported in North America and at least 200 cases in South America. The mortality of HPS is approximately 50%. Subsequent cases have occurred in a largely sporadic manner over a wide geographic range. The epidemiology of recent cases has differed from earlier cases, with a much lower representation of Native American patients, a lower mortality, and an increased recognition of apparent occupational exposure (Rodriguez-Moran P, unpublished data).

The main etiologic agent of HPS is the Sin Nombre (Four Corners) virus (SNV), which is primarily associated with the deer mouse (Peromyscus maniculatus), and accounts for most of the cases nationwide (Hjelle B, unpublished data). Seventeen cases of HPS have been reported in California, with eight fatalities. Two other hantaviruses are known to occur in California. El Moro Canyon virus (ELMCV), which is closely related to SNV, has been associated with the harvest mouse (Reithrodontomys megalotis)17 and Isla Vista virus (ISLAV), which is a genetically distinct PHV-like virus in the California vole (Microtus californicus).18 Neither ELMCV nor ISLAV are currently known to cause human disease. Since 1988, the Orange County Vector Control District (OCVCD) has accumulated serum samples from rodents, of which 2,365 have been tested for hantavirus antibodies. Testing of rodent sera from Orange County began in 1993 shortly after the Four Corners HPS outbreak and the first antibody-positive P. maniculatus from the Los Angeles Basin (five of 34 collected in 1992) were confirmed.19

This paper summarizes an in-depth analysis of the prev-
alance, host distribution, and characteristics of rodent populations infected by three hantaviruses within a small, well-defined, geographic area.

MATERIALS AND METHODS

Collection sites and trapping methods. Rodents were collected from several sites throughout Orange County and northwestern San Diego County, along the Orange County border. Most of the locations where P. maniculatus and R. megalotis were collected were sage scrub habitats often associated with grasslands and disturbed, weedy (ruderal) vegetation or Sumac savannah grassland. Occasionally, both species of mice were collected in riparian habitats and on a few occasions from suburban residential sites approximately 0.2–0.4 km from natural habitats. Rodents were trapped in Sherman live traps (7.6 × 8.9 × 22.9 cm; H. B. Sherman Trap Co., Tallahassee, FL) baited with dry oats and placed during the early evening at sites considered to be suitable habitats. The traps were picked up early the next morning using TexaCal® snake tongs (TexaCal, Houston, TX). Traps were placed inside plastic biohazard material bags and transported to a staging and processing area.

Rodent processing. Handling and processing the rodents were done outdoors in the sunlight, which affords additional protection from active virus infection. Deactivation by UV light exposure has been demonstrated in related hantavirus-setting protection from active virus infection. Deactivation by UV light exposure has been demonstrated in related hantaviruses. The rodents were usually processed by a two-person team clad in polylaminated Tyvek®es.

The rodents were placed individually (while still in the trap) into an ice chest containing dry ice until they were killed. They were then transferred onto a dissection tray for identification. This information along with locality and other pertinent data were entered into a field catalogue with each entry designated with a unique field identification number. Each rodent was bled infrasternally by cardiac puncture with a 3/8 inch, 25- or 26-gauge needle and a 1-cm³ tuberculin syringe. The blood was ejected into a 25-ml plastic tube. Spent needles and syringes were disposed of in a Sharps icch containing dry ice until they were killed. Weights for 442 P. maniculatus were sub-

divided into four categories: < 10 g, 10–< 15 g, 15–20 g, and > 20 g and compared with seroprevalence. Weights for 259 R. megalotis were also subdivided into four categories: < 6 g, 6–< 8 g, 8–10 g, and > 10 g. Wound scoring was accomplished by examining 117 P. maniculatus and included all available antibody-positive specimens (51 males and 16 females) and subsamples of negative specimens (25 males and 25 females). The rodents were checked for the presence or absence of notches and scars on one or both ears and/or scars and areas of hair loss on the tail using a stereo-microscope.

Blood processing. The whole blood samples were stored in a refrigerator (4 °C) for no more than 1 hr before they were centrifuged, and sera were transferred into 0.5-ml disposable, microcentrifuge tubes and stored in a freezer at −20°C prior to shipment. These tubes were packed in dry ice and shipped in the early stage of the study to the Centers for Disease Control and Prevention (Atlanta, GA) and the Viral and Rickettsial Disease Laboratory (VRDL), California Department of Health Services (CDHS) (Berkeley, CA). They were subsequently sent to the University of New Mexico School of Medicine (UNMSM, Albuquerque, NM) where serologic analysis was supervised by one of the authors (BH). Testing of rodent sera prior to September 1993 did not include SNV antigen, but used detergent lysates of Vero E6 cells that were chronically infected with hantaviruses other than SNV.

Reverse transcriptase–polymerase chain reaction studies. Representative specimens of antibody-positive P. maniculatus, R. megalotis, and M. californicus were sent on dry ice to the UNMSM where RT-PCR and sequencing analyses were performed on necropsied tissues. These analyses permitted differentiation of hantaviruses from infected rodents that cannot be distinguished by serology using a single antigen. Since the detection of ELMCV and ISLAV antibodies depends upon cross-reactivity with SNV-N and PHV antigens, respectively, lung tissue samples from antibody-positive mice were used for the RT-PCR to genetically distinguish between the viruses. The viral cDNA was amplified with primers specific for SNV, ELMCV, and ISLAV to identify the viral species present in each sample.

Antibody testing. A recombinant-expressed nucleocapsid antigen of SNV was available for serologic testing at the UNM by August 1993. A Western blot format was used for routine screening essentially as described, except that narrow membrane strips with SNV-N alone were used instead of membranes containing multiple hantavirus antigens. An affinity-purified fusion protein linking the intact SNV-N protein to a T7 phage gene 10 leader protein (apparent molecular weight ~ 55 kD) was used as a target. Only SNV antigen was used in these studies. There was no systematic attempt to detect antibodies to SEOV.

Serum samples were incubated at 4°C with the Western blot membrane containing bound SNV-N at a dilution of 1:400 overnight with rocking. After the membrane was washed as previously described, a secondary alkaline phosphatase–conjugated antibody was applied at a 1:1,000 dilution (Kirkegaard and Perry, Gaithersburg, MD). Since a limited number of such conjugates are commercially available, the conjugate species was chosen to be the one that is most likely to react with antibodies from the rodent specimen under in-
investigation. For serum samples from indigenous sigmodontine rodents (*Peromyscus*, *Reithrodontomys*, *Neotoma*), a goat anti-*Peromyscus leucopus* conjugate was used. For *Mus musculus* and *Rattus rattus*, anti-mouse and anti-rat conjugates were used, respectively. Since conjugates were not available for Heteromyidae (*Chaetodipus*) and Sciuridae (*Spermophilus*) rodents, an anti-mouse conjugate was used at 1:500 dilution. After incubation for 4 hr at room temperature with the conjugate, the membranes were washed again three times with wash buffer (10 mM sodium phosphate, pH 7.4, 0.1 M NaCl, 0.5% Triton X-100, 0.5% deoxycholic acid) and the bound alkaline phosphatase was detected with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrate. Positive reactivity consisted of staining of the SNV-N recombinant fusion protein at 55 kD.

**RESULTS**

**Reverse transcriptase–polymerase chain reaction.** Virus-specific primer pairs were used to amplify SNV, ELMCV and ISLAV genomic cDNA from *P. maniculatus* (deer mouse), *R. megalotis* (harvest mouse), and *M. californicus* (vole) tissue RNA templates,14,17,18 Of six deer mouse tissues, eight harvest mouse, and two vole tissues examined, all but one deer mouse RNA produced the expected PCR product, indicating the presence of SNV in antibody-positive deer mice, ELMCV in antibody-positive harvest mice, and ISLAV in antibody-positive voles. The one exception was a deer mouse that was negative for either viral RNA by RT-PCR analysis.

**Seroprevalence.** A total of 2,365 rodents representing nine genera and 15 species was tested and included specimens collected from 1988 through June 1997. Of this total, 86 (9.1%) of 948 *P. maniculatus*, four (1.5%) of 275 *R. megalotis*, one (0.5%) of 196 *P. eremicus*, 51 (12.2%) of 417 *R. megalotis*, and five (12.5%) of 40 California voles (*M. californicus*) were positive for SNV antibodies. Four of the positive *M. californicus* were from a residential backyard and two of them were confirmed as ISLAV by RT-PCR on lung tissue. These ISLAV-positive *Microtus* represent the first recordings in Orange County. All other species of rodents were seronegative for SNV antibodies and included 28 *Chaetodipus californicus*, four *Chaetodipus fallax*, 137 *Mus musculus*, 107 *Neotoma fuscipes*, 91 *Neotoma lepida*, 41 *Peromyscus boylii*, two *Rattus norvegicus*, 59 *Rattus rattus*, 19 *Spermophilus beecheyi*, and one *Thomomys bottae*.

Species of *Peromyscus* positive for SNV antibodies were collected in every month of the year and there appeared to be a gradual increase in antibody prevalence in *P. maniculatus* from December to June. Positive *R. megalotis* were also collected in all months, but there was no apparent seasonal trend in antibody prevalence. However, there appears to be a correlation between age and sex of the reservoir host and prevalence of hantavirus antibodies, especially among males. Of the 556 male *P. maniculatus* tested, 61 (11%) were antibody positive for SNV while 25 (6.7%) of 370 females were positive. Of 257 male *R. megalotis*, 48 (18.7%) were antibody positive, while only three (1.9%) of 158 females were positive. When only the positive mice are considered, all of the *P. maniculatus* were adults with a weight range of 16–28 g (mean = 21.1) and males represented 73% of the total. Among the 51 positive *R. megalotis*, males represented 94% of the total and all but one female were adults with a weight range of 5–12 g (mean = 9.6). Figure 1 compares prevalence of antibody to SNV with the different weight classes of 442 *P. maniculatus* and shows that heavier (older) deer mice had a higher antibody prevalence than smaller subadults and that older males had a higher prevalence than older females. To test the possible correlations between age, sex, and antibody prevalence, chi-square values for each weight category of males and females were calculated. They indicated that significantly more mice in the > 20 g category had a higher prevalence (*P* < 0.001 for females and *P* < 0.001 for males). Antibody prevalence among all other weight categories of both sexes were not significantly different (*P* > 0.01). Similar results were ob-
tained for 259 *R. megalotis* tested for SNV antibodies (Figure 2). The highest antibody prevalence occurred in males heavier than 10 g. Chi-square values for each weight category of male *R. megalotis* indicated significantly more mice in the >10 g category had antibody (*P* < 0.01), while no significant differences were found between other male and female weight categories and antibody prevalence (*P* > 0.05).

Examination of 67 adult SNV antibody-positive *P. maniculatus* (51 males and 16 females) and 50 negative animals (25 males and 25 females) for evidence of intraspecific aggression (scarring on exposed parts of the ears and tail) indicated that more males had notches on the ears and/or scars, eschars, and areas of hair loss on the tail than females (87% and 51%, respectively). Fifty (98%) of 51 positive males had some type of scarring while 64% of antibody-negative males, 37.5% of antibody-positive females, and 60% of antibody-negative females had scarring. Chi-square analysis of antibody-negative males and females and antibody-positive females based on a 1:1 expected distribution ratio indicated no significant correlation with scarring (*P* > 0.05). However, there was a significant correlation between antibody-positive males and scarring (*P* < 0.0001).

Rodent trapping records in Orange County from 1984 through 1992 indicated that *P. maniculatus* and *R. megalotis* were collected more frequently in disturbed (ruderal) habitats consisting of introduced weeds and ornamental shrubs, grassland and sage scrub habitats, or grassland/sage scrub/chaparral ecotones, and were less common in chaparral, woodland, and riparian habitats. Selection of trapping locations from 1993 through 1995 was based on these early observations to ensure greater sample of the reservoir hosts. Therefore, most were represented by elements of grassland and sage scrub (i.e., grassland-ruderal, flood plain sage scrub, mulefat scrub, buckwheat sage scrub, etc.).

Table 1 shows these habitats from which rodents were tested for Hantavirus antibodies. *Peromyscus maniculatus* was the dominant species (35–60% of the total samples) in all categories except chaparral-woodland-riparian (24% of the total) where *Neotoma fuscipes* and *P. californicus* were more prevalent. Countywide, only three (4.1%) of the 74 *P. maniculatus* from chaparral, woodland, and riparian habitats were antibody positive for SNV. The majority of these samples were from the Santa Ana Mountains and north Orange County at elevations ranging from 300 to 1,000 m (1,000–3,300 feet), and no antibody-positive *P. maniculatus* were recovered from those regions of the county. The antibody prevalence in *P. maniculatus* from all other habitat categories (except wetlands) ranged from 9.5% to 11.6%. Unlike previous data published for the state of California, all positive *P. maniculatus* from Orange County were trapped at elevations ranging from 30 to 300 m and antibody prevalence decreased with altitude. *Reithrodontomys megalotis* comprised only 5% of the total rodents caught in chaparral-woodland-riparian areas, but ranged from 14% to 26% of the total in all other categories Antibody prevalence for *R. megalotis* ranged from 6.7% in agricultural areas to 19.5% in grassland-sage scrub habitats.

The majority of antibody-positive *P. maniculatus, R. megalotis, and M. californicus* was identified from localities along coastal bluffs and the foothills where several residential, commercial, and industrial sites exist or where construction is currently underway. These include residential backyards, three retirement communities, office trailers at an engineering firm, a sand and gravel company, two preschools, canyons adjacent to a government research facility, a microwave relay building, flood control channels in urban areas, agricultural land, and open fields and ravines near residential sites and shopping malls. Other more rural sites include two state parks, seven county regional parks, private ranch land, a military reservation, two county landfills, proposed housing and condominium developments, and proposed transportation corridors. These collections represent SNV antibody-positive rodents and, although no human cases of any hantavirus infections have been reported from Orange County, the potential risk should not be overlooked.
The map in Figure 3 shows the proximity of all sites where antibody-positive rodents were collected within the county. Note that all four positive P. californicus and the single positive P. eremicus were collected in areas where positive P. manicutatus were also collected. At Newport Coast, two antibody-positive P. californicus (of a total of 70) were trapped along the same 300-m transect line as 11 P. manicutatus (total = 150) between March 1995 and May 1997. This is not surprising since P. manicutatus and P. californicus represented 61% and 25%, respectively, of the total number of rodents trapped along the transect. In one instance a male P. californicus was caught in the same trap as a male P. manicutatus, an indication that these species may come into close contact while foraging, possibly under high population density situations. Antibody-positive R. megalotis (probably to ELMCV) were collected concurrently with SNV-positive P. manicutatus from Newport Coast, Laguna Beach, Rancho Mission Viejo, San Clemente, and San Juan Capistrano in Orange County and along San Mateo Creek in San Diego County. In June 1997, antibody-positive P. manicutatus, R. megalotis, and M. californicus (probably to ISLAV) were found at a single site in Laguna Beach along the same trap line during two nights of trapping. The presence of multiple hantaviruses at a single focus seems to be a common occurrence in Orange County. In addition, temporal maintenance data for a few selected foci in the county over several years have shown that the antibody prevalence to one virus may increase while the other decreases in the same year. For example, in San Juan Capistrano, the prevalence of SNV fluctuated from 31.8% (seven of 22) in 1994 to 9.4% (three of 32) in 1995, to 0% (0 of 11) in 1996, to 23.5% (four of 17) in 1997. Concurrently, the prevalence of ELMCV was 5.3% (one of 19), 29.7% (11 of 37), 0% (zero of one), and 14.3% (one of seven) for each year, respectively. Similar results were obtained from sites in San Clemente between 1992 and 1997, Irvine from 1994 to 1997, and Newport Coast from 1995 to 1997. In most cases, rodents were trapped from other areas adjacent to or within a few kilometers of the previously mentioned positive sites on the same date or within 1–3 weeks and no positive mice were recovered. Figures 4 and 5 show all collecting sites and the number of antibody-positive animals of the total number taken for P. manicutatus and R. megalotis. Several positive foci separated by negative foci are shown in both of these figures. This phenomenon is also quite common in the Orange County area and was also observed in northeastern California and Nevada21 and the Four Corners region.21

**DISCUSSION**

Until now, most field studies of hantaviruses and their rodent hosts in the United States have been conducted in large geographic areas (one or more states) encompassing many different climates and habitats over a short period of time (one year or less). Our report represents one of the first long-term studies of the prevalence of hantaviruses in rodents within a small geographic area of California (782 square miles) and the probable influence of rodent densities, rodent behavior, and microhabitats on virus distribution. Preliminary information on the horizontal transmission of SNV...
via intraspecific aggression among male deer mice is presented, as well as information on the temporal maintenance of hantaviruses at individual collecting sites over several years. In addition, our data show the focality of hantavirus infections in rodent populations, the correlations of age, weight, and sex of reservoir hosts with antibody prevalence, and the distinct absence of SNV in urban peridomestic rodents, reinforcing the findings of other researchers around the country.21,24–26

Many newly developed areas in Orange County have encroached into sage scrub plant communities and peridomestic rodent species such as Mus musculus and Rattus rattus have frequently been trapped at the same sites where antibody-positive deer mice occur, leaving open the possibility of virus spillover. Twenty-six such sites were trapped during the course of this study, but none of the 137 house mice and 59 roof rats had antibodies for any hantavirus. House mice and roof rats are well established in older, developed, urban areas of the county but little or no overlap with P. maniculatus occurs except along flood control channels or agricultural sites such as strawberry fields and orange groves. Previous investigations27 have revealed the presence of Hantavirus antigens (Puumala virus) in the lungs of 14% of captured Mus musculus from Serbia, and antigen (probably from SEOV) was detected in 63% of captured Rattus norvegicus and 48% of captured Mus musculus from Yugoslavia. In addition, hantavirus was isolated from four pools of Mus musculus lung tissues from Yugoslavia, although these data have not been replicated.27

The obvious association of hantaviruses with adult male deer mice and harvest mice was shown to be statistically significant in this study. It has also been demonstrated in P. maniculatus from northeastern California and Nevada,25 Arizona, New Mexico, and Colorado,16,21 and in R. megalotis.26 The overall sex ratio for the total number collected in Orange County was approximately 60/40; 556 males/370 fe-
males (22 undetermined) and 257 males/158 females (two undetermined) for _P. maniculatus_ and _R. megalotis_, respectively. Sex ratios were calculated for _R. megalotis_ trapped in central California, and a preponderance of males was observed in the population and in field-trapped specimens of _P. maniculatus_ from several sites in California that ranged from 54% to 70% males. The explanation given most often by the majority of studies for this preponderance is that males have larger home ranges than females and thus have greater trap exposure and probability of capture. However, MacMillen found that the size of home ranges for males and females was not significantly different, but home ranges of males broadly overlapped with each other while those of females rarely overlapped. It seems possible that with a greater number of male mice present with broadly overlapping home ranges, animals infected with hantaviruses would come into contact with uninfected males more often during territorial disputes and transmit the virus through bit-
ing or other interactions, thus accounting for the higher prevalence among males. The preliminary analysis of wounds and scarring of 117 *P. maniculatus* indicated that more male than female deer mice had some degree of scarring on the ears and tail, and scarring was much more extensive in adult males. Ninety-eight percent of the antibody-positive males were scarred and they had the most extensive scarring, supporting the idea of virus transmission through aggressive behavior. Additional evidence supporting an association between intraspecific aggressive interactions and hantavirus infection in urban rats (*Rattus norvegicus*) has been demonstrated in Baltimore, Maryland. Marked and released animals showed direct correlations between seroconversions and wounds acquired between captures more often than expected by chance, and unwounded animals seroconverted less than expected. In addition, infection was highly asso-

**Figure 5.** Collection records of *Reithrodontomys megalotis* in Orange and northwest San Diego Counties, California, 1988–1997. Solid circles represent sites where antibody positive animals were collected; gray circles represent sites of animals with no detectable antibodies; numbers represent antibody prevalence at each site; the dashed line represents the northwest-southeast bisection of the county.
associated with the onset of sexual maturity and aggressive behavior.31

The structure, diversity, and abundance of available food plants at any given locality can also influence the distribution and abundance of rodents and thus their associated viruses. M’Closkey32 studied community structures of rodents in 1969 at a site in Orange County (now Newport Coast) and found that rodent diversity was positively correlated with shrub volume diversity and that *P. maniculatus* and *R. megalotis* favored disturbed, early succession habitats or open, patchy habitats with shrub heights less than 1 m. When niche separation was compared using discriminant analysis, significant differences were found between *P. maniculatus* and all other rodent species, including *R. megalotis*. In Los Angeles County, the highest density of *P. maniculatus* was found in a sparsely vegetated plot of boulder deposits that proved to be suboptimal for other species.30 Meverse31 examined the food preferences of rodents at Newport Coast and found that 56% of the diet of *P. maniculatus* consisted of lemonadeberry (*Rhus scoparia*), buckwheat (*Eriogonum fasciculatum*), California sagebrush (*Artemesia californica*), true sages (*Salvia sp.*), and grasses. The other 44% consisted primarily of small annual forbs and insects. *Reithrodontomys megalotis* fed primarily on grasses, *R. integrifolia*, *L. scoparius*, *A. californica*, and insects. All of these plants are common components of coastal sage scrub, but do not occur evenly throughout the range of the rodents. Optimal microhabitats with an abundance of suitable food plants occur at specific foci in Irvine, Laguna Beach, Mission Viejo, Newport Coast, San Clemente, and San Juan Capistrano. Consequently these areas have dense, localized populations of *P. maniculatus* and *R. megalotis* with antibody prevalences that range from 11% to 33%.

Table 1 shows that *P. maniculatus* was not very common in chaparral, woodland, and riparian plant communities. The apparent scarcity of deer mice in these habitats in Orange County may limit the prevalence and distribution of SNV among populations (as well as other species of *Peromyscus*) in these habitats. No obvious relationship could be discerned for *R. megalotis* and the distribution of ELMCV. Striking differences in the abundance of *P. maniculatus* and the prevalence of SNV antibodies can be seen in Orange County if it is bisected in a northwest-southeast direction (Figure 4). Nearly all of the 2,200 trap nights in the northern and eastern parts of the county were in chaparral, woodland, or riparian habitats. A few sites in northern Orange County were sage scrub and grassland. Overall rodent trapping success in this region was relatively low at 26% (8% for *P. maniculatus*), yet deer mice comprised 30% of all rodents trapped, an indication of a low population density. A total of 151 *P. maniculatus* and 66 (10%) were antibody positive. *Reithrodontomys megalotis* (Figure 5) displayed similar patterns of trap success (3% for northern and eastern Orange County and 7% for coastal areas) and comprised 12% and 18%, respectively, of the total rodents trapped. However, when antibody prevalence in both regions was compared, there was little difference and prevalence remained between 11% and 12% throughout the county regardless of habitat or rodent density. These data, in addition to showing the geographic distribution of antibody-positive rodents and differences in temporal changes in antibody prevalence, substantiate the conclusion that SNV and ELMCV have evolved independently in different reservoir rodents with no discernible cross-influence on each other. Factors such as temperature variation, relative humidity, rainfall, changes in abundance of food plants, and predator abundance, to name a few, must play a role in regulating rodent density and the prevalence of hantaviruses in southern California. Future research should focus on these factors in conjunction with long-term capture-recapture population studies of reservoir hosts, antibody prevalence, and plant community structure to provide more convincing evidence.

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