

# Sin Nombre virus prevalence from 2014–2017 in wild deer mice, *Peromyscus maniculatus*, on five of the California Channel Islands

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## Abstract

Sin Nombre virus (SNV) is a zoonotic virus that is highly pathogenic to humans. The deer mouse, *Peromyscus maniculatus*, is the primary host of SNV, and SNV prevalence in *P. maniculatus* is an important indicator of human disease risk. Because the California Channel Islands contain permanent human settlements, receive hundreds of thousands of visitors each year, and can have extremely high densities of *P. maniculatus*, surveillance for SNV in island *P. maniculatus* is important for understanding the human risk of zoonotic disease. Despite the importance of surveillance on these heavily utilized islands, SNV prevalence (i.e. the proportion of *P. maniculatus* that test positive to antibodies to SNV) has not been examined in the last 13–27 years. We present data on 1,610 mice sampled for four consecutive years (2014–2017) on five of the California Channel Islands: East Anacapa, Santa Barbara, Santa Catalina, San Nicolas, and San Clemente. Despite historical data indicating SNV-positive mice on San Clemente and Santa Catalina, we detected no SNV-positive mice on these islands, suggesting very low prevalence or possible loss of SNV. Islands historically free of SNV (East Anacapa, Santa Barbara, and San Nicolas) remained free of SNV, suggesting that rates of pathogen introduction from other islands and/or the mainland are low. Although continued surveillance is warranted to determine whether SNV establishes on these islands, our work helps inform current human disease risk in these locations and suggests that SNV prevalence on these islands is currently very low.

## KEYWORDS

hantavirus, *Peromyscus maniculatus*, Rodentia, Sin Nombre virus

## 1 | INTRODUCTION

Sin Nombre Virus (SNV) is the zoonotic pathogen that causes the majority of hantavirus pulmonary syndrome (HPS) in humans in North America (Yates et al., 2002). SNV represents a significant health threat to infected individuals with fatality rates that can exceed 35% (Maurice et al., 2017). The prevalence of SNV in

its primary rodent host (deer mice, *Peromyscus maniculatus*) is an important component of estimating the risk of human exposure (Calisher et al., 2011). Individual *P. maniculatus* remain persistently infected, but SNV prevalence in mouse populations can be highly variable in time and space (Luis et al., 2018; Yates et al., 2002). As such, quantifying changes in SNV prevalence in *P. maniculatus* through time provides important information on ecological factors

controlling disease dynamics, allows us to detect potential disease hotspots, and can help guide public health recommendations.

The California Channel Islands contain a large number of residents and receive a large number of visitors (Schoenherr et al., 1999); two of the islands (San Nicolas and San Clemente) contain U.S. Navy installations, Santa Catalina Island contains two towns (Avalon and Two Harbours) and the remaining five islands constitute Channel Islands National Park, which receives over 300,000 visitors each year. SNV prevalence in deer mouse populations can vary widely among islands. Reports of SNV-positive mice in 1994 were as high as 71% on Santa Cruz Island (Jay et al., 1997), but mice on other islands exhibit lower SNV prevalence (e.g. 14.3% on Santa Catalina and 2.9% on San Clemente) and some islands (Anacapa, San Nicolas, and Santa Barbara) have no historical evidence of SNV-positive mice (Jay et al., 1997; Orrock & Allan, 2008). Despite significant human use of these islands, SNV surveillance studies are now 13–27 years old. Moreover, the original surveillance data from some islands with long-term human settlements are limited. For example, our current knowledge of SNV prevalence in mice on Santa Catalina and San Clemente islands is derived from only seven and 34 mice, respectively, that were sampled from undescribed locations and collected decades ago; of these 41 mice, one from each island was positive for SNV (Jay et al., 1997). Because SNV prevalence can exhibit temporal variation on the islands (Graham & Chomel, 1997; Orrock & Allan, 2008), contemporary studies are urgently needed. Given the frequency of human habitation and visitation on these islands, the goal of this study is to use multiple years of sampling across five islands to inform current human disease risk as well as to enable a comparison of current disease risk to previous decades.

## 2 | MATERIALS AND METHODS

We live-trapped mice on all five islands between July–September in 2014, 2015, 2016, and 2017. Individual mice were only sampled once, that is, individuals were not sampled again if they were recaptured at a later date. Two of the islands (East Anacapa Island and Santa Barbara Island) are part of the Channel Islands National Park, two islands contain U.S. Navy installations (San Nicolas Island and San Clemente Island), and one island contains several permanent human settlements (Santa Catalina Island). Because our sampling effort included several ongoing projects as well as sampling specifically performed for this study, the number of sites sampled differed on each island (Figure 1, Table S1) as did the area of each island sampled (see Figure 1 for locations of each sampling site used in this study) and the precise layout of a trapping site. A trapping site is defined as an area where live traps were deployed in one or more transects, a single grid, or around a focal sampling point (e.g. traps placed outside of an old barn structure) within 50 m or less of each other, with traps in a single site typically spanning a distance of up to approximately 100 m. Sites differed in the number of traps present (range = 8–100 traps), with a mean of 27 traps per site (see

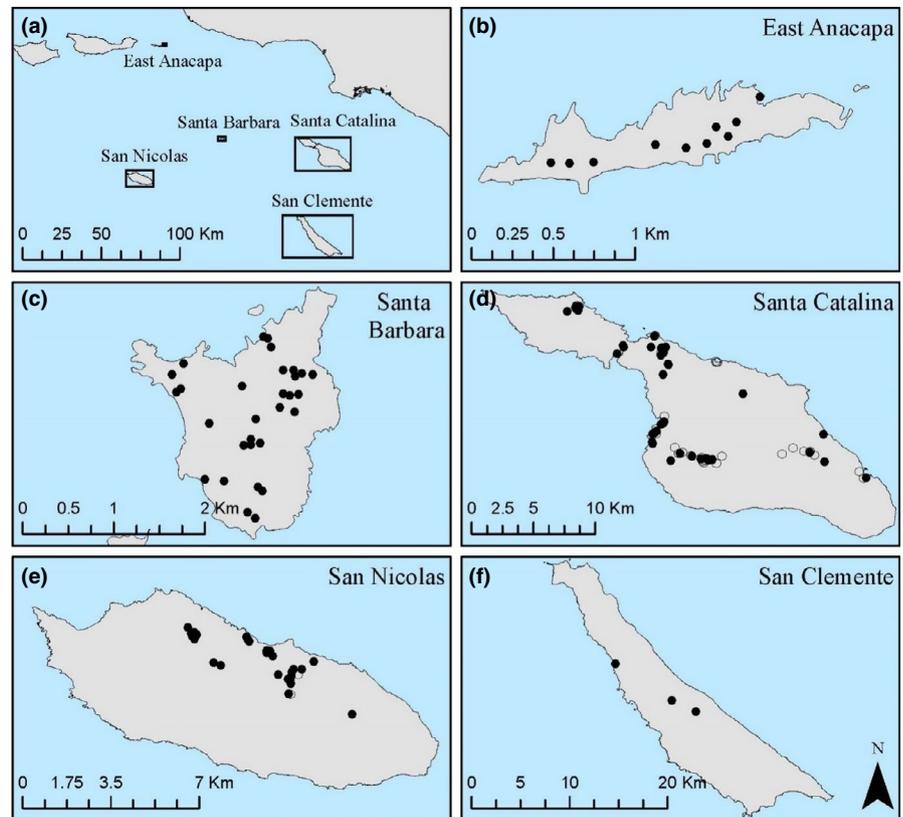
### Impacts

- We performed extensive multi-year sampling on five Channel Islands to document the current prevalence of antibodies to Sin Nombre virus (SNV) in its primary reservoir host *Peromyscus maniculatus*.
- Of 1,610 individual samples, no *P. maniculatus* tested positive for SNV, suggesting this pathogen is extinct or extremely rare on the two islands where it was historically found and that this pathogen has not colonized the three islands where it was not found in the past.
- The lack of *P. maniculatus* with SNV antibodies suggests that the risk of human contact with SNV is low on these five islands and that migration events resulting in pathogen establishment on these islands are rare.

Figure 1 and Table 1, as well as Supporting Information for additional details). Sampling sites often included natural habitats (e.g. grasslands, chaparral, and wooded habitats where *P. maniculatus* is common) and also included human settlements on four of the five islands we sampled. For example, several sampling sites were located near buildings regularly used by the National Park Service on East Anacapa and Santa Barbara islands, in unused buildings on San Nicolas Island, and placed near housing and barn structures in Avalon and Middle Ranch on Santa Catalina Island. Sampling on San Clemente Island was conducted on permanent trapping grids in natural habitat as part of ongoing ecological field studies. Upon capture, a small blood sample was taken from the tail of each individual (Abatan et al., 2008) and the individual was released at the site of capture. Blood samples were collected and stored on absorbent filter paper (Nobuto strip; Advantec Type I Blood Sampling Paper, Toyo Roshi Taishu, Ltd., Tokyo, Japan) and kept at  $-20^{\circ}\text{C}$  until assayed for antibodies to SNV. This work was approved by the Research Animal Review Committee of the University of Wisconsin-Madison (Protocol L005041).

Testing of *Peromyscus* blood for antibodies to SNV was performed by IDEXX Laboratories using a multiplex fluorescent immunoassay (MFI). The MFI was developed using dilutions of samples from SNV positive and negative *P. maniculatus* (kindly provided by A. Kuenzi, Montana Technological University), confirmed by western blot of the reference standards and was used to plot a receiver operating characteristic (ROC) curve for the MFI to determine optimal values for sample classification (Crowther, 2008). Fluorescence values corresponding to  $>99\%$  sensitivity and  $>99\%$  specificity were used as threshold values to discriminate negative or positive test results in all samples tested. Additional details of this assay are provided in the Appendix S1. To assess the performance of the MFI assay and facilitate comparison with previous studies that used Enzyme-linked Immunosorbent assay (ELISA) approaches (Graham & Chomel, 1997; Jay et al., 1997; Orrock & Allan, 2008) developed by the Centers for Disease Control and Prevention (CDC; Feldmann

**FIGURE 1** Islands (a) and sites (b–f) where deer mice, *Peromyscus maniculatus*, were sampled for SNV on five of the California Channel Islands in 2014, 2015, 2016, and 2017. Hollow circles represent sites where trapping was conducted, but no *P. maniculatus* were captured; see text for additional information about how sites were designated. The total number of sites sampled on each island was 10 on East Anacapa (B), 30 on Santa Barbara (C), 77 on Santa Catalina (D), 29 on San Nicolas (E), and three on San Clemente (F). Some sites were sampled more than once during the 4-year study; see supporting information figures that show sites sampled in each sampling year (Figures S1–S5)



**TABLE 1** Summary of the number of individual *Peromyscus maniculatus* sampled for antibodies to Sin Nombre virus on five of the California Channel Islands

| Island        | Year |      |      |      | Total | Effort (TN) | Samples per TN |
|---------------|------|------|------|------|-------|-------------|----------------|
|               | 2014 | 2015 | 2016 | 2017 |       |             |                |
| East Anacapa  | 108  | 104  | 99   | 94   | 405   | 752         | 0.54           |
| Sta. Barbara  | 100  | 126  | 101  | 115  | 442   | 1,034       | 0.43           |
| Sta. Catalina | 31   | 38   | 44   | 98   | 211   | 4,355       | 0.05           |
| San Nicolas   | 72   | 82   | 55   | 99   | 308   | 740         | 0.42           |
| San Clemente  | 49   | 51   | 94   | 50   | 244   | 3,737       | 0.07           |

Note: None of the individuals sampled tested positive for antibodies to Sin Nombre virus. Effort describes the number of trap-nights (TN) used on each island; see Table S2 for sex-specific data.

et al., 1993), we compared the results of MFI to the protein-A/G horseradish peroxidase enzyme-linked immunosorbent assay (PAGEIA) protocol by Schountz et al. (2007). We used the PAGEIA because it is more rapid than the CDC ELISA (Feldmann et al., 1993; Schountz et al., 2007), it generates results highly consistent with the CDC ELISA (Schountz et al., 2007), and, in the rare instances when PAGEIA does not agree with CDC ELISA, the PAGEIA is more likely to classify samples as positive (Schountz et al., 2007). We felt that this latter property made the PAGEIA to CDC ELISA because it would err towards producing false-positive results, which seems preferable when dealing with a highly pathogenic virus. We used 40 samples (20 classified as SNV positive and 20 samples classed as SNV negative) from the three other nearby Channel Islands where SNV prevalence is known to be high (Graham & Chomel, 1997; Jay et al., 1997; Orrock & Allan, 2008) to evaluate the consistency

between MFI and PAGEIA. We used these samples (in addition to the rigorous assays developed with known positive and negative samples described above) because mouse populations on our focal islands historically had zero or very low prevalence (Jay et al., 1997; Orrock & Allan, 2008), and we felt it was imperative to test the performance of our MFI to accurately classify SNV-positive samples from the islands in order to detect the possible colonization of SNV on islands where it had never been found. We compared the MFI results with PAGEIA results from San Miguel, Santa Cruz, and Santa Rosa islands because these islands have historically had much higher SNV prevalence (Graham & Chomel, 1997; Jay et al., 1997; Orrock & Allan, 2008). These samples were collected using methods identical to the methods used to collect samples used in this study; full details on this assay are presented in the Supporting Information.

### 3 | RESULTS

We sampled 1,610 individual *P. maniculatus* from five islands over 4 years (Table 1) with a total of 10,618 trap nights of effort. None of the individuals sampled was positive for SNV antibodies. A total of 149 sites were sampled: 10 on East Anacapa, 30 on Santa Barbara, 77 on Santa Catalina, 29 on San Nicolas, and three on San Clemente and the mean trapping effort ranged from 25.5 to 1,245.7 trap nights per site (Table S1). The number of individuals sampled at a site ranged from 0 to 138, with a mean of  $11.37 \pm 1.69$  (SE) *P. maniculatus* sampled at each site. Trapping success (the number of individuals sampled per trap night of effort) ranged from 0.05 (Santa Catalina Island) to 0.54 (East Anacapa Island; Table 1). Given historic SNV prevalence on San Clemente Island and Santa Catalina Island (Jay et al., 1997) and our 244 and 211 samples from these two islands, respectively, the probability of failing to detect at least one SNV-positive mouse is extremely low (binomial probability of obtaining at least one positive result given the historical prevalence and the current sample size is 0.000191 and  $<0.000001$ , respectively). Results obtained via MFI were highly consistent with results from PAGEIA, with 39 of 40 samples classified as the same SNV status by the two methods. A single sample was classified as positive by the MFI but was classified as negative by PAGEIA.

### 4 | DISCUSSION

Our results provide important evidence suggesting that SNV may no longer be present on the two Channel Islands (Santa Catalina and San Clemente) where it was historically found (Jay et al., 1997), as we found no individuals seropositive for SNV over four consecutive years of sampling on the five islands in our study. Importantly, we note that earlier records of SNV on Santa Catalina and San Clemente were based on only two SNV-positive individuals in a total sample of 41 mice (Jay et al., 1997). Additionally, our work suggests that SNV has not become established on three of the islands where SNV was historically absent (East Anacapa, Santa Barbara, and San Nicolas) in the 7–20 years since these islands were last sampled (Jay et al., 1997; Orrock & Allan, 2008).

Despite sampling a much larger number of individuals compared to previous studies (we sampled over 30 times more individual mice on Santa Catalina and over seven times more individual mice on San Clemente), as well as sampling over four consecutive years, we did not find any mice on Santa Catalina or San Clemente that tested positive for antibodies to SNV (Table 1). While our findings contrast with the only previously published data from these islands (Jay et al., 1997), which did find SNV-positive individuals, it is important to note that Jay et al. (1997) found low prevalence on both of these islands and also sampled a limited number of individuals from undescribed locations, with one of seven individuals (14.3%) positive on Santa Catalina Island and one of 34 individuals (2.9%) positive on San Clemente Island. Taken together, these data indicate that (a) SNV prevalence may be extremely low on these two islands, (b) that SNV-positive mice may exist in spatially distinct areas that were not sampled, or

(c) that SNV may have gone locally extinct on one or both of these islands during the 27-year intervening period since Santa Catalina and San Clemente were last sampled. Of these possibilities, the broad spatial area we sampled on Santa Catalina (Figure 1) and the extensive sampling effort (Table 1) suggest that there were not large undetected areas of SNV prevalence on Santa Catalina. Our sampling on Santa Catalina also included areas frequented by humans, including 23 trap nights in and around human structures in the town of Avalon, sampling in and around human structures at middle Ranch, and sampling near the town of two harbours. On San Clemente, our sampling had lower spatial coverage (i.e., three sampling sites; Figure 1), and future studies that span larger portions of the island will be critical for resolving spatial variation in SNV prevalence.

A final possibility explaining the paucity of SNV-positive mice on San Clemente and Santa Catalina is related to the SNV assays. First, it is possible that the original SNV-positive individuals tested with ELISA (one mouse each on Santa Catalina and San Clemente) represent false-positive testing errors. However, ELISA tends to be very reliable for detecting SNV-positive individuals (Schountz et al., 2007). Second, it is possible that the MFI assay we used failed to detect individuals that were truly SNV positive. This possibility seems highly unlikely based on three lines of evidence. (a) The sensitivity of our MFI assay was  $>99\%$  using samples of known SNV status (described in Appendix S1). (b) This sensitivity is further supported by our comparison of the PAGEIA assay outcomes to our MFI assay outcomes, which demonstrates that the MFI not only detected SNV-positive individuals, but that it was slightly more sensitive at detecting SNV seropositive mice than the PAGEIA assay. (c) Over the same time period examined here (2014–2017), the MFI detected a large number of SNV-positive individuals (135; Orrock, unpublished data) on three adjacent islands where SNV has historically been present (San Miguel, Santa Cruz, and Santa Rosa islands; Graham & Chomel, 1997; Jay et al., 1997; Orrock & Allan, 2008), suggesting that the MFI was highly effective at detecting SNV-positive individuals from populations where SNV is known to occur. As a result, we consider it likely that the lack of SNV we detected is indicative of a lack of seropositive individuals on the islands we studied.

The continued absence of SNV in mice on East Anacapa, Santa Barbara, and San Nicolas suggests that SNV may have never colonized these islands or that it did, but went extinct prior to sampling in 1993, 2007, and the sampling reported here. Mean SNV prevalence in the counties in southern California that are the likely source of potential *P. maniculatus* colonists (Ventura, Santa Barbara, Los Angeles, Orange, and San Diego Counties) was 0.08 (28 of 344 individuals positive; Jay et al., 1997), suggesting that immigration events from the mainland would have to be relatively large, frequent, or both to yield a successful introduction. For East Anacapa Island, colonization of SNV-infected individuals from nearby Santa Cruz Island (approximately 14 km away, compared to 21 km for the mainland), where prevalence has been found to be as high as 0.71 (Jay et al., 1997), could also serve as a means for SNV to reach the island. The lack of SNV colonization documented with our data (compared to 1993 and 2007 sampling) provides evidence that immigration rates sufficient to result in SNV introduction have not occurred. The low rates of immigration we infer

from a lack of SNV colonization are also consistent with molecular data on *P. maniculatus* (Durst, 2014) as well as significant variation in island SNV compared to mainland SNV (Hjelle et al., 1994) that all suggest that rates of immigration are currently very low or zero.

While our results suggest the absence or very low prevalence of SNV on the islands, future efforts will be essential for informing the ecology of SNV. For example, the absence of SNV on these islands underscores the importance of ongoing biosecurity efforts to prevent introductions of *P. maniculatus* from nearby islands or the mainland, as well as indicates the need for continued surveillance to detect any future colonization events that might occur. Our multi-year study included natural and human-related habitats; future efforts that continue to sample these areas as well as areas we did not sample (Figure 1), areas where introductions may be most likely (e.g. harbours) and areas where human exposure is most likely (e.g. within human settlements and military barracks), will be important for informing human disease risk.

## 5 | DECLARATION

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U. S. Government.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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