Experimental Infection of *Peromyscus* Species Rodents with Sin Nombre Virus

Kaye Quizon, Kimberly Holloway, Mahmood Iranpour, Bryce M. Warner, Yvon Deschambault, Geoff Soule, Kevin Tierney, Darwyn Kobasa, Angela Sloan, David Safronetz

We demonstrate that 6 distinct *Peromyscus* rodent species are permissive to experimental infection with Sin Nombre orthohantavirus (SNV). Viral RNA and SNV antibodies were detected in members of all 6 species. *P. leucopus* mice demonstrated markedly higher viral and antibody titers than *P. maniculatus* mice, the established primary hosts for SNV.

Orthohantaviruses, a genus of enveloped, segmented, negative-sense, single-stranded RNA viruses, are the cause of hantavirus cardiopulmonary syndrome. Hantavirus species are primarily associated and coevolve with specific rodent host species (1,2). In North America, Sin Nombre virus (SNV) causes most confirmed cases of hantavirus cardiopulmonary syndrome (3) and is primarily maintained in *Peromyscus maniculatus* deer mice (4). *P. maniculatus* mice are widely distributed in North America (Figure) and are a complex of subspecies that diverge according to geographic location (2,7). Likewise, SNV and its related viruses are found to diverge in association with their rodent reservoirs (1). Although host switching is thought to be uncommon in SNV (1,2), other rodent species sharing a geographic site were recently found to carry the virus, potentially acting as additional reservoirs and sources of human infection (8). We evaluated the permissiveness of 6 colony-bred *Peromyscus* mouse species, whose founders originated from locations across North America, to infection by SNV originating from New Mexico.

The Study

We obtained geographically distinct peromyscine mice (of both sexes and ≥6 weeks of age) from breeding colonies maintained by the *Peromyscus* Genomic Stock Center, University of South Carolina (Columbia, SC, USA) (*P. maniculatus bairdii* prairie deer mouse, *P. maniculatus sonoriensis* Sonoran deer mouse, *P. polionotus subgriseus* Oldfield mouse, *P. leucopus* white-footed or wood mouse, *P. californicus insignis* California mouse), and the University of Manitoba (Winnipeg, Manitoba, Canada) (*P. maniculatus rufinus* deer mouse) (Figure) (9). We experimentally infected the mice with SNV strain no. 77734, which had been exclusively passaged in *P. maniculatus rufinus* mice (both the virus and the host used to propagate it originating from New Mexico) as previously described (9–11). At no point postinoculation did we note any deleterious effects of infection in rodents. We collected tissue samples (lung, heart, spleen, kidney) and either blood (days 7 and 14) or serum (days 21 and 42) samples from mice at 7, 14, 21, and 42 days postinfection (dpi) and evaluated them for the presence of viral (small-segment) RNA (10). We performed ELISA on serum samples, as previously described (12), to assess seroconversion.

We detected SNV RNA in all 6 species to varying degrees; peak median levels occurred at 21 dpi (Figure 2). The lowest levels of viral RNA (a surrogate metric for permissiveness and viral infection or replication) were in *P. maniculatus* mice, the established primary hosts for SNV.
trapping locations. Median viral RNA peaked at 21 dpi and were sustained through 42 dpi; however, only serum was positive at 42 dpi in *P. maniculatus rufinus* mice. *P. maniculatus bairdii* mice founders were trapped in southeastern Michigan (Figure), whereas *P. maniculatus rufinus* mice descended from founders from central and northwestern New Mexico (Figure). *P. leucopus* mice appear to be highly permissive to SNV based on detection of SNV RNA. We observed high levels of viral RNA in serum, heart, kidney, lung, and spleen samples at 21 dpi and 42 dpi. Further studies are required to confirm whether this finding translates to persistent infection, viral shedding, and possible transmission between animals.

**Figure.** Geographic distribution of *Peromyscus* mouse species represented in a serologic analysis of serum samples from experimentally infected peromyscine rodents. Pins indicate collection sites of colony founders: *P. californicus* (A), *P. leucopus* (B), *P. maniculatus bairdii* (C), *P. maniculatus rufinus* (D), *P. maniculatus sonoriensis* (E), and *P. polionotus* (F). Inset shows phylogenetic relationships with evolutionary distances estimated by Miller and Engstrom (5). Map adapted from Bedford and Hoekstra (6).
Although sex differences have been noted to have little effect on SNV infection in *P. mani culatus rufinus* mice (10), differences may exist in other rodent species. The numbers in our study are small; however, the data suggest sex-related differences might occur according to species. For example, male *P. mani culatus bairdii* mice have higher viral RNA titers at 42 dpi, whereas in *P. mani culatus sonoriensis* mice at 21 dpi, almost all positive animals are female. Meanwhile, *P. leucopus* mice showed indiscriminate viral RNA detection between sexes across all timepoints (Appendix Figure, https://wwwnc.cdc.gov/EID/article/28/9/22-0509-App1.pdf). Future studies are required to shed light on this aspect.

*P. mani culatus* mice were established early on as the primary reservoir for SNV (4). However, studies of this virus–host relationship rarely report the host tax on beyond the species level. Moreover, although levels of viral RNA tended to be negligible in the early stages of infection, an observation consistent with previous studies in susceptible animals (9,10,13), the detection of viral RNA at later timepoints probably means members of these species are nonetheless susceptible to SNV infection. That animals were positive at 42 dpi hints at the possibility of persistent infection, although longer-term studies would be needed to confirm.

Antibodies against the SNV nucleoprotein were detected in most serum samples collected from days 21 and 42 from the peromyscine rodents (Table). The lone exception was *P. californicus* mice, for which only a single animal had detectable SNV antibodies. Rates of seroconversion broadly mirrored the rates of detection by quantitative reverse transcription PCR. The development of antibodies reactive to the SNV nucleoprotein appeared to be delayed in male mice from 2 species (*P. mani culatus bairdii* and *P. mani culatus sonoriensis*), and only 1 of 6 of these rodents demonstrated positive serum samples at 21 dpi.

**Conclusions**

Overall, our data support recent observations from Goodfellow et al. (8) that rodents other than *P. mani culatus* mice are capable of carrying SNV without showing signs of disease. In that study, wild-caught members of *P. boylii* mice, *Mus muscu luminosus* mice, and *Tamias minimus* chipmunks trapped at 2 sites had detectable SNV RNA in their lung tissues. SNV sequences from these rodents were more similar to each other than to previously reported sequences, suggesting circulation of the virus within these rodent populations.

In our study, we experimentally infected 6 *Peromyscus* mouse species, including 3 that fall under the *P. mani culatus* species complex, and whose founders originate from locations across North America, with a strain of SNV originating from a single wild-caught animal. Although replicating SNV was detected in animals of all 6 species, permissiveness of each species to SNV infection varied. Susceptibility to SNV infection appears to be multifactorial and is not fully explained by characteristics relating to geographic proximity or genetic relatedness. Given the variability in the patterns of infection between subspecies within *P. mani culatus*, future studies should consider reporting the subspecies when studying this virus–host relationship. Furthermore, other *Peromyscus* mouse species may play an important role in the molecular evolution and transmission of SNV. Host-switching events are thought to give rise to SNV variants and even new hantaviral species (1,2,14). That SNV was capable of replicating to high levels in *P. leucopus* mice for sustained periods is notable. The geographic distributions of *P. mani culatus* and *P. leucopus* mice overlap greatly, presenting opportunities for these 2 populations and their respective viruses to come into contact. Together, these regions cover most of North America, including nearly the entirety of the contiguous United States.

These findings demonstrate the importance of broadening our understanding of the SNV–*P. mani culatus* virus–host relationship and highlight the benefit of identifying infected and infectious rodent reservoirs at the subspecies level to help elucidate epizootics and spillover events to humans. Furthermore,
although distinct eastern and western patterns of genetic evolution have been documented in *P. maniculatus* rodents and associated strains of SNV in North America (1,2), our study suggests that these patterns might not necessarily prevent western lineages of SNV from emerging into eastern populations of peromyscine rodents.

**Acknowledgments**

We thank Hippokratis Kiaris and Vimala Kaza for assistance with obtaining the mice from the *Peromyscus* Genetic Stock Center.

This work was funded by the Public Health Agency of Canada.

Animal studies were conducted in accordance with the Canadian Council of Animal Care guidelines and an animal use document approved by the Canadian Science Centre for Human and Animal Health’s institutional animal care and use committee. Work involving infectious SNV was performed in a Biosafety Level 4 laboratory of the Public Health Agency of Canada. When required, materials were inactivated according to approved procedures for subsequent analysis.

All authors declare no conflict of interest.

**About the Author**

Ms. Quizon is a laboratory biologist at the Public Health Agency of Canada in Winnipeg. Her primary research interests are zoonoses and the development of diagnostic methods for infectious diseases.

**References**

1. Dredge MA, Gavrillovskaya I, Mackow ER, Chen Z, Lindsay R, Sanchez AJ, et al. Genetic and serotypic characterization of Sin Nombre–like viruses in Canadian *Peromyscus maniculatus* mice. Virus Res. 2001;75:75–86. https://doi.org/10.1016/S0168-1702(01)00227-1

2. Dragoo JW, Lackey JA, Moore KE, Lessa EP, Cook JA, Yates TL. Phylogeography of the deer mouse (*Peromyscus maniculatus*) provides a predictive framework for research on hantaviruses. J Gen Virol. 2006;87:1997–2003. https://doi.org/10.1099/vir.0.81576-0

3. Warner BM, Dowhanik S, Audet J, Grolla A, Dick D, Strong JE, et al. Hantavirus cardiopulmonary syndrome in Canada. Emerg Infect Dis. 2020;26:3020–4. https://doi.org/10.3201/eid2612.202808

4. Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis. 1994;169:1271–80. https://doi.org/10.1093/infdis/169.6.1271

5. Miller JR, Engstrom MD. The relationships of major lineages within peromyscine rodents: a molecular phylogenetic hypothesis and systematic reappraisal. J Mammal. 2008;89:1279–95. https://doi.org/10.1644/07-MAMM-A-195.1

6. Bedford NL, Hoekstra HE. *Peromyscus* mice as a model for studying natural variation. eLife. 2015;4:e06813. https://doi.org/10.7554/eLife.06813

7. Kalkvik HM, Stout IJ, Doonan TJ, Parkinson CL. Investigating niche and lineage diversification in widely distributed taxa: phylogeography and ecological niche modeling of the *Peromyscus maniculatus* species group. Ecography. 2012;35:54–64. https://doi.org/10.1111/j.1600-0587.2011.06994.x

8. Goodfellow SM, Nofchissey RA, Schwalm KC, Cook JA, Dunnum JL, Guo Y, et al. Tracing transmission of Sin Nombre virus and discovery of infection in multiple rodent species. J Virol. 2021;95:e01342-21. https://doi.org/10.1128/JVI.01342-21

9. Botten J, Mirowsky K, Kusewitt D, Bharadwaj M, Yee J, Ricci R, et al. Experimental infection model for Sin Nombre hantavirus in the deer mouse (*Peromyscus maniculatus*). Proc Natl Acad Sci U S A. 2000;97:10578–83. https://doi.org/10.1073/pnas.180197197

10. Warner BM, Stein DR, Griffin BD, Tierney K, Leung A, Sloan A, et al. Development and characterization of a Sin Nombre virus transmission model in *Peromyscus maniculatus*. Viruses. 2019;11:7183. https://doi.org/10.3390/v11020183

11. Safronetz D, Prescott J, Feldmann F, Haddock E, Rosenke R, Okumura A, et al. Pathophysiology of hantavirus pulmonary syndrome in rhesus macaques. Proc Natl Acad Sci U S A. 2014;111:7114–9. https://doi.org/10.1073/pnas.1401998111

12. Warner BM, Sloan A, Deschambault Y, Dowhanik S, Tierney K, Audet J, et al. Differential pathogenesis between Andes virus strains CHI-7913 and Chile-9717869 in Syrian Hamsters. J Virol. 2021;95:108–29. https://doi.org/10.1128/JVI.00108-21

13. Botten J, Mirowsky K, Kusewitt D, Ye C, Gottlieb K, Prescott J, et al. Persistent Sin Nombre virus infection in the deer mouse (*Peromyscus maniculatus*) model: sites of replication and strand-specific expression. J Virol. 2003;77:1540–50. https://doi.org/10.1128/JVI.77.2.1540-1550.2002

14. Black WC IV, Doty JB, Hughes MT, Beaty BJ, Calisher CH. Temporal and geographic evidence for evolution of Sin Nombre virus using molecular analyses of viral RNA from Colorado, New Mexico and Montana. Virol J. 2009;6:102. https://doi.org/10.1186/1743-422X-6-102

Address for correspondence: David Safronetz, Special Pathogens Program, National Microbiology Laboratory Branch, Public Health Agency of Canada, 1015 Arlington St, Winnipeg, MB R3E 3R2, Canada; email: david.safronetz@phac-aspc.gc.ca
Experimental Infection of *Peromyscus* Species Rodents with Sin Nombre Virus

Appendix

**Appendix Figure.** Detection of Sin Nombre orthohantavirus S segment RNA by quantitative reverse transcription PCR at 7, 14, 21, and 42 days postinfection (dpi) in various tissues. Horizontal bars denote median values. Serum samples were collected instead of blood on 21 dpi and 42 dpi for serologic analysis. Colors indicate sex: red for female, blue for male.