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# Serological Evidence for Zoonotic Hantaviruses in North Carolina Rodents

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ABSTRACT: In a survey of seven species of wild rodents ($n$ = 423) collected between October 1993 and March 1994 from the three principal ecological biomes of North Carolina (USA), we found hantavirus antibodies in seven (2%) of 301 *Peromyscus* spp. Hantavirus antibodies were detected in *P. leucopus* and *P. maniculatus* captured from mountain and coastal island biomes. Three mice were positive for Sin Nombre virus, while four others had antibodies to Seoul virus or a related agent. Two mice serologically positive for Sin Nombre virus were collected from inside a private mountain domicile. We conclude that the risk of human exposure to hantaviruses in North Carolina resembles that for most other areas of the continental United States.

*Key words:* Hantavirus, *Peromyscus* spp., Sin Nombre virus, Seoul virus, serological survey, zoonotic diseases.

Sin Nombre virus (SNV) is a member of a group of newly recognized zoonotic hantaviruses that are enzootic in several North American sigmodontine rodent species. The SNV was first identified following an epidemic of unexplained adult respiratory distress syndrome that occurred in residents of the Four Corners area (Utah, Colorado, Arizona, New Mexico) of the southwestern United States in May 1993 (Nichol et al., 1993). At this writing, over 98 confirmed cases of hantavirus-related unexplained adult respiratory distress syndrome, presently designated hantavirus pulmonary syndrome (HPS), have been documented from 21 states, resulting in over 51 deaths from the disease (Centers for Disease Control and Prevention, 1994c). The human case-fatality rate associated with these novel agents is higher than that for all other known hantaviruses. Hantaviruses typically persist asymptomatically in their natural rodent reservoirs with chronic periods of virus shedding, despite the presence of specific antibodies to the agent. Most rodent-to-human transmission of hantaviruses has occurred via the respiratory route from virus-laden rodent urine, feces, and salivary excretions (Tsai, 1987). Aspects of the biology, epidemiology, and preventive measures for these agents have been recently reviewed (Weigler, 1995).

The SNV and related hantaviruses exist predominantly in *Peromyscus maniculatus* (deer mouse) throughout its range over most of the United States, excluding the Southeast (Childs et al., 1994). However, additional cases of HPS have occurred in the USA outside this habitat range, including one associated with a closely related virus detected in cotton rats (*Sigmodon hispidus*) in Florida (Centers for Disease Control and Prevention, 1994a) and another newly recognized hantavirus detected in autopsy specimens from a HPS fatality in Louisiana (Morzunov et al., 1995). Recent investigations following a case of HPS that occurred on Long Island, New York (USA) in January 1994 (Centers for Disease Control and Prevention, 1994b) led to serological and virological (Song et al., 1994) evidence for a hantavirus closely related to SNV apparently enzootic in certain New York *P. leucopus* (white-footed mouse) populations as well. Thus, it appeared that both of these *Peromyscus* spp., with their combined habitats spanning most areas of the continental United States, together play important roles in hantavirus epizootiology. An additional case of HPS recently was reported from a mountainous area of Virginia in the mid-Atlantic region

of the continental USA ecologically contiguous with portions of North Carolina. Our objective was to determine whether serological evidence existed for hantaviruses in North Carolina wild rodents, including sympatric *P. maniculatus* and *P. leucopus* populations (Nowak, 1991).

Rodents in this survey were captured by use of live traps (H. B. Sherman Live-Trap Company, Tallahassee, Florida) and grain bait. Traps were placed approximately 1 hr prior to sundown and retrieved within 2 hr following sunrise the next morning. Trapping was done in counties representing the three principal ecological biomes (mountain, piedmont, and coastal island) of North Carolina. This included the counties of Buncombe (35°45′N, 82°30′W), Henderson (35°25′N, 82°33′W), Jackson (35°15′N, 83°5′W), and Yancey (35°50′N, 82°15′W) together for the mountain biome; Wake (35°45′N, 78°40′W) for the piedmont biome; and Currituck (36°15′N, 75°47′W) for the coastal island biome. All captures were made between October 1993 and March 1994. Animal trapping and handling was performed humanely under conditions compliant with the provisions of federally recommended standards (Shaw, 1988).

Captured rodents were anesthetized using methoxyflurane (Pittman-Moore, Mundelein, Illinois, USA) to effect via the open drop method (Barry, 1972), bled via cardiac puncture, euthanized by cervical dislocation, and then frozen at −70 C for future virological studies. Blood was heparinized to maximize the volume for testing, given the small amount of blood available for some species, frozen at −20 C, and transported to the laboratory for testing within 90 days following rodent capture. Hantavirus antibody testing was performed in laboratories of the Special Pathogens Branch of the National Center for Infectious Diseases, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia (USA). Heparinized whole blood specimens were tested for Immunoglobulin G (IgG) antibodies to SNV using a recombinant viral nucleocapsid protein enzyme linked immunosorbent assay (ELISA), in parallel with separate ELISAs employing the heterologous antigens of Seoul (SEOV) and Prospect Hill hantaviruses, as described by Feldmann et al. (1993). The ELISAs were performed in standardized 4-fold dilutions from 1:100 through 1:6400, where titers of ≥1:400 were considered positive.

TABLE 1. Distribution by sex and species of North Carolina wild rodent captures for hantavirus testing, October 1993 to March 1994.

| Species | Male | Female | Total (%)[a] |
|---|---|---|---|
| *Mus musculus* | 15 | 16 | 31 (7%) |
| *Microtus pennsylvanicus* | 7 | 7 | 14 (3%) |
| *Microtus pinetorum* | 29 | 18 | 47 (11%) |
| *Oryzomys palustris* | 5 | 5 | 10 (2%) |
| *Peromyscus* spp[b] | 136 | 120 | 256 (61%) |
| *Peromyscus leucopus* | 25 | 20 | 45 (11%) |
| *Sigmodon hispidus* | 8 | 12 | 20 (5%) |
| Totals | 225 | 198 | 423 (100%) |

[a] Total captured (percent of total rodent captures).
[b] Either *P. leucopus* or *P. maniculatus* in mountain regions where both species co-exist and are phenotypically similar.

Sera were obtained from 423 rodents (seven species) captured from the three surveyed biomes (Table 1) during the study period. In the mountain biome, *P. maniculatus* and *P. leucopus* were reported as *Peromyscus* sp. because of their overlapping distributions and close phenotypic similarities in that region. *Peromyscus maniculatus* does not occur in other areas of North Carolina, in which case specific identity was possible. Antibodies to hantaviruses were detected in seven *Peromyscus* spp. captured on six different trapnights, including three SNV-positive and four SEOV-positive mice (Fig. 1). Two of these positive specimens (one SNV, one SEOV) originated from a coastal island biome where *P. leucopus* is the only resident species of *Peromyscus*. The remaining five positive specimens (two SNV, three SEOV) were *Peromyscus* spp. captured in three of four counties sampled from the mountain biome. No positive rodents were detected from the piedmont biome, but

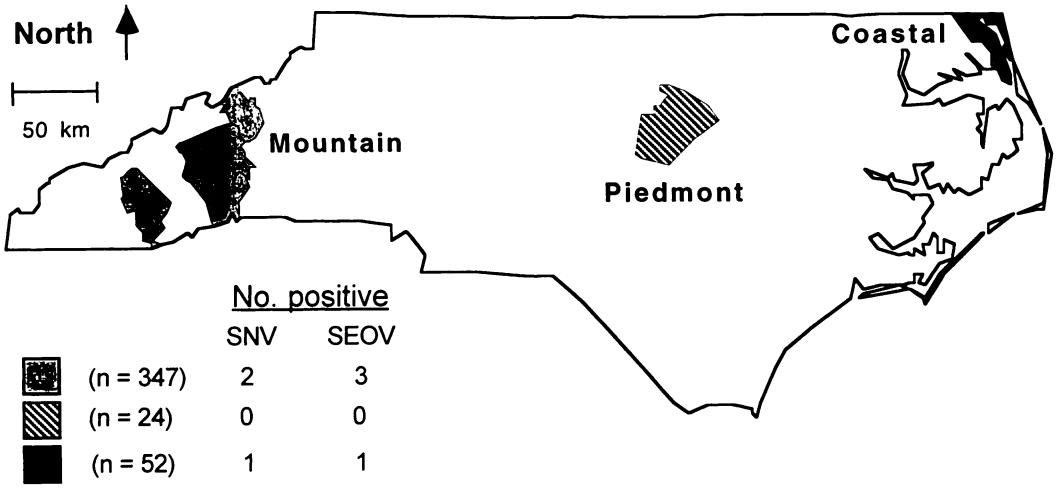


FIGURE 1. Hantavirus test results from wild-captured rodents ($n$ = 423) by ecological biome of North Carolina, USA, October 1993 to March 1994. Abbreviations: SNV, Sin Nombre virus; SEOV, Seoul virus.

the small sample size limited our power to detect infected rodent populations, if they occurred there.

Unlike published results from other states following the 1993 epidemic of HPS (Childs et al., 1994), the prevalence of SNV antibodies in *Peromyscus* spp. in the present survey was extremely low, with only three (1%) of 301 *Peromyscus* spp. seropositive. However, two SNV-positive mice in the mountain biome originated from inside a private mountain residence; thus HPS could occur in North Carolina. Both hantavirus-positive coastal *P. leucopus* captures originated within 100 m of private households. We have observed that *Peromyscus* spp. occasionally move into structures surrounding human habitat in North Carolina. The finding of SEOV-positive *Peromyscus* spp. was not anticipated since it has been previously known only from *Rattus* spp. (Tsai et al., 1985). This result could be evidence for a newly-recognized host for SEOV, or perhaps antigenic cross-reactivity with as yet undescribed hantaviruses pre-existing in the region. Yamada et al. (1995) have noted occasional serological cross-reactivities to the nucleocapsid protein of SEOV in *P. maniculatus* experimentally infected with SNV and in wild captured specimens. Previous efforts to sequence genomic hantavirus RNA recovered from tissues of seropositive rodents have been successful (Elliott et al., 1994) and may help to clarify these ambiguities. On the basis of available nucleic acid sequence data, the zoonotic SNV-related agents described to date appear to be more closely related to Prospect Hill virus and Puumula virus than to SEOV or to prototypic Hantaan virus of the Asian continent (Spiropoulo et al., 1994).

In summary, we observed serologic evidence for SNV infection among *P. maniculatus* and *P. leucopus* in mountain and coastal island biomes of North Carolina. This supports the hypothesis that both rodent species can act as reservoirs for zoonotic hantaviruses, at least in eastern regions of the United States (Song et al., 1994). Advances in molecular technology, along with heightened scientific interest in the novel agents, will undoubtedly help to recognize and characterize additional hantavirus variants in other rodent species across the North American continent. Improved awareness that many of these novel agents can present significant zoonotic health hazards should strengthen the importance of personal protective measures for individuals exposed to *Peromyscus* spp.

and their excreta (Centers for Disease Control and Prevention, 1993), thereby helping to prevent further hantavirus related morbidity and mortality.

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