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ARTICLE

Disease Ecology

Hantavirus in rodents in the United States: Temporal and spatial trends and report of new hosts

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Abstract

In North America, the rodent-borne hantavirus pulmonary syndrome is predominantly caused by the Sin Nombre virus, typically associated with the deer mouse *Peromyscus maniculatus*. Utilizing data from the National Ecological Observatory Network (NEON) hantavirus program, we assessed factors that may influence the spatial and temporal distribution of hantavirus in rodent populations across the United States. Between 2014 and 2019, the NEON hantavirus program conducted 104,379 small mammal captures and collected 14,004 blood samples from 49 species at 45 field sites. Our study identified 296 seropositive samples across 15 rodent species, including 8 *Peromyscus* species. We describe six new species with hantavirus seropositive samples not previously reported as hantavirus hosts. The highest number of seropositive samples was obtained from *Pe. maniculatus* ($n = 116$; 2.9% seroprevalence), followed by *Peromyscus leucopus* ($n = 96$; 2.8%) and *Microtus pennsylvanicus* ($n = 33$; 4.2%). Hantavirus seroprevalence showed an uneven spatial distribution, with the highest seroprevalence found in Virginia (7.8%, 99 seropositive samples), Colorado (5.7%, $n = 37$), and Texas (4.8%, $n = 19$). Hantavirus seropositive samples were obtained from 32 sites, 10 of which presented seropositive samples in species other than *Pe. maniculatus* or *Pe. leucopus*. Seroprevalence was inconsistent across years but showed intra-annual bimodal trends, and in *Pe. maniculatus* and *Pe. leucopus*, the number of captures correlated with seroprevalence in the following months. Seroprevalence was higher in adult males, with only one seropositive sample obtained from a juvenile *Peromyscus truei*.

Higher body mass, presence of scrotal testes, and nonpregnant status were associated with higher seropositivity. The NEON dataset, derived from a multiyear and structured surveillance system, revealed the extensive distribution of hantavirus across broad taxonomic and environmental ranges. Future research should consider winter season surveillance and continued analyses of stored samples for a comprehensive spatiotemporal study of hantavirus circulation in wildlife. Global changes are expected to affect the dynamics of rodent populations by affecting their availability of resources and demography and, consequently, may modify transmission rates of rodent-borne zoonotic pathogens such as hantavirus. This study can be considered a baseline to assess hantavirus patterns across host taxa, geographies, and seasons in the United States.

KEYWORDS
hantavirus, hosts, NEON, *Peromyscus*, rodent, trend

INTRODUCTION

Hantavirus infections, caused by viruses from the Hantaviridae family within the order Bunyavirales, have been documented in at least 140 animal species, including carnivores (e.g., red fox *Vulpes vulpes*) and bats (Guo et al., 2013; Milholland et al., 2018). Despite this broad host range, most hantavirus lineages are primarily associated with a few phylogenetically related rodent species (Milholland et al., 2018). Rodents usually present asymptomatic and persistent infections (Ermonval et al., 2016; Forbes et al., 2018).

In humans, hantavirus infections can lead to severe diseases, notably hantavirus pulmonary syndrome (HPS) (Laenen et al., 2019). The first report of HPS in the United States occurred in 1993 in the Four Corners region of New Mexico, and since then, numerous cases have been documented across the country, primarily related to the Sin Nombre virus (SNV) (Luis et al., 2015; Nichol et al., 1993; Van Hook, 2018). Human infections typically occur through the inhalation of aerosolized excreta, urine, or saliva from infected rodents (Bagamian, Towner, et al., 2012; Botten et al., 2002; Forbes et al., 2018).

New world hantaviruses are periodically identified in multiple hosts, usually with unknown zoonotic potential (Ermonval et al., 2016; Milholland et al., 2018; Mull et al., 2022). In North America, at least 17 hantaviruses are known to co-circulate, predominantly carried by New World rodents of the Family Cricetidae, subfamily Sigmodontinae (CDC, 2012; Milholland et al., 2018, 2019). The primary recognized rodent host of SNV is the deer mouse (*Peromyscus maniculatus*) (Childs et al., 1994; Ermonval et al., 2016; Luong et al., 2011). Nevertheless, SNV infections have also been identified in other rodent species, particularly cricetids such as *Peromyscus leucopus*, *Neotoma micropus*, and *Sigmodon hispidus*, among others (Childs et al., 1997; Milholland et al., 2018). The widespread distribution of *Pe. maniculatus* across the continental United States complicates the understanding of the roles other rodent species play in the distributional ecology of SNV (Chen et al., 2023; Forbes et al., 2018; Milholland et al., 2018).

Estimating the transmission risk of SNV to humans requires a comprehensive understanding of the complex hantavirus dynamics within multiple wildlife host populations that sustain viral circulation (Forbes et al., 2018; Goodfellow et al., 2021; Shubangi et al., 2022). Moreover, the biological, ecological, and environmental factors that maintain local hantavirus circulation within rodent communities remain insufficiently understood (Bagamian, Towner, et al., 2012; Carver et al., 2015; Heyman et al., 2012; Padula et al., 2004). This study aimed to enhance our understanding of the host species involved in hantavirus circulation in North America and to elucidate the spatial and temporal patterns of virus maintenance in the sylvatic cycle. To achieve this, we analyzed a comprehensive dataset from the Small Mammal Box Trapping and the Rodent Pathogen Status programs of the National Ecological Observatory Network (NEON) (NEON, 2023; Thibault, 2022), which provides open and interoperable data from multiple sites across the United States, employing sustained and standardized capturing and sampling protocols (Paull et al., 2023). This rodent-pathogen NEON dataset integrates trait data of rodents such as hantavirus seroprevalence, sex, reproduction status, life stage, and morphometrical measurements (NEON, 2023a; Thibault, 2022).

METHODS

NEON data

The NEON is a continent-scale observation facility designated to collect long-term, open-access ecological data, encompassing biological, chemical, and physical measurements and samples across the United States (NEON, 2023b). Between 2014 and 2019, the NEON Small Mammal Box Trapping program implemented the rodent hantavirus seroprevalence testing program (product DP1.10072.001) and generated the Small Mammal Box Trapping and Rodent Pathogen Status databases (product DP1.10064.001) (NEON, 2023b).

This database contains individual reports of small mammals captured and sampled at terrestrial field sites across 18 NEON domains (Figure 1). Due to regulatory changes and conservation concerns, blood samples were not collected in Hawaii, Puerto Rico, and Yellowstone National Park field sites (NEON, 2023a; Thibault, 2022). At each field site, captures are organized within multiple 90×90 meter grids, each containing 100 traps spaced 10 meters apart. Blood samples for hantavirus serology were collected in three designated "pathogen grids" at each NEON site. Captures and sampling typically occurred over one to three consecutive nights per sampling bout, with flexibility to accommodate logistical challenges posed by extreme or unexpected events such as flooding, fires, or severe weather conditions (Chesney et al., 2023; Paull et al., 2023). Sampling was scheduled to occur once every lunar cycle (approximately every 4 weeks) at 19 core sites, and every second lunar cycle at the 27 gradient sites (Springer et al., 2016). A maximum of 20 individuals was expected to be sampled per grid and collection date (Paull et al., 2023; Thibault, 2022). From 2017 onwards, only a subset of collected blood samples, up to 140 samples per site per year, was analyzed (Thibault, 2022).

Species sampled for hantavirus serology belonged to the families Cricetidae, Muridae, and Dipodidae. Each field site prioritized specific species, with *Peromyscus* species, particularly *Pe. maniculatus* and *Pe. leucopus*, identified by NEON as priority species for pathogen testing (Paull et al., 2023; Springer et al., 2016). Samples were collected only from individuals weighing more than 10 g and appearing to be in good physical condition (Thibault, 2022). NEON guidelines stipulate that individuals should not be resampled within an ongoing bout, though they may be resampled in subsequent bouts (Thibault, 2022). Serum samples were screened using IgG ELISA, following methodologies described by Klingström et al. (2002).

We standardized NEON data using the neonUtilities package, with all R codes to process NEON data products available (Lunch et al., 2018). The NEON Small Mammal Trapping database includes detailed individual data such as taxonomic identification, age (life stage), sex, reproductive condition (pregnancy status and testes, nipples, and vagina conditions), and standardized measurements of hind foot length and body mass. The protocol mainly considers the distances between the clitoris or genital papilla and anus to determine sex, testes presence and size for age, and vaginal opening to determine reproductive conditions (Paull et al., 2023). Additional measurements, such as ear length, tail length, and total length, are taken optionally to facilitate more accurate species identification (Paull et al., 2023).

Data analyses

Species of the genus *Peromyscus*, particularly *Pe. maniculatus* and *Pe. leucopus*, were designated as priority species for hantavirus testing in NEON protocols (Paull et al., 2023; Springer et al., 2016), as they are considered pivotal in the transmission dynamics of the SNV (Childs et al., 1994; Luong et al., 2011; Milholland et al., 2019). All our analyses were conducted on three datasets: (1) all small mammal species captured, (2) dataset filtered to only include *Pe. maniculatus*, and (3) dataset of *Pe. leucopus*. Seroprevalence was estimated as a proportion of the seropositive samples in relation to all blood samples. We examined associations between hantavirus seroprevalence and sex, reproductive status, and life stage using the prop.test() function in R (RStudiov2023.09; RStudio Team, 2023), which performs a parametric test for equality of proportions based on χ^2 statistics. Additionally, we assessed potential biases in the number of captures, blood samples, and seroprevalence metrics.

To explore temporal patterns, we constructed time-series models to assess the relationship between two time series: the number of captures and seroprevalence. We employed the sample cross-correlation function (CCF) in the "tseries" package in R (Trapletti et al., 2024) to identify lags of the x-series that might predict (Shrestha et al., 2024; Tenzin et al., 2011). The CCF calculates correlations between $+h$ and for $h = 0, \pm1, \pm2, \pm3$, examining whether the x-variable serves as a leading indicator for the y-variable, thereby determining the effect of capture numbers on future hantavirus seroprevalence. One or more $+h$ values with negative h is considered a correlation between x-variable at a time before and the y-variable at time and interpreted as the x-variable leading the y-variable (i.e., "x leads to y"). Conversely, positive h values suggest that the x-variable lagged behind the y-variable (i.e., "x lags to y"). The level of statistical significance for CCF analysis was set as $\alpha = 0.05$ (Shrestha et al., 2024).

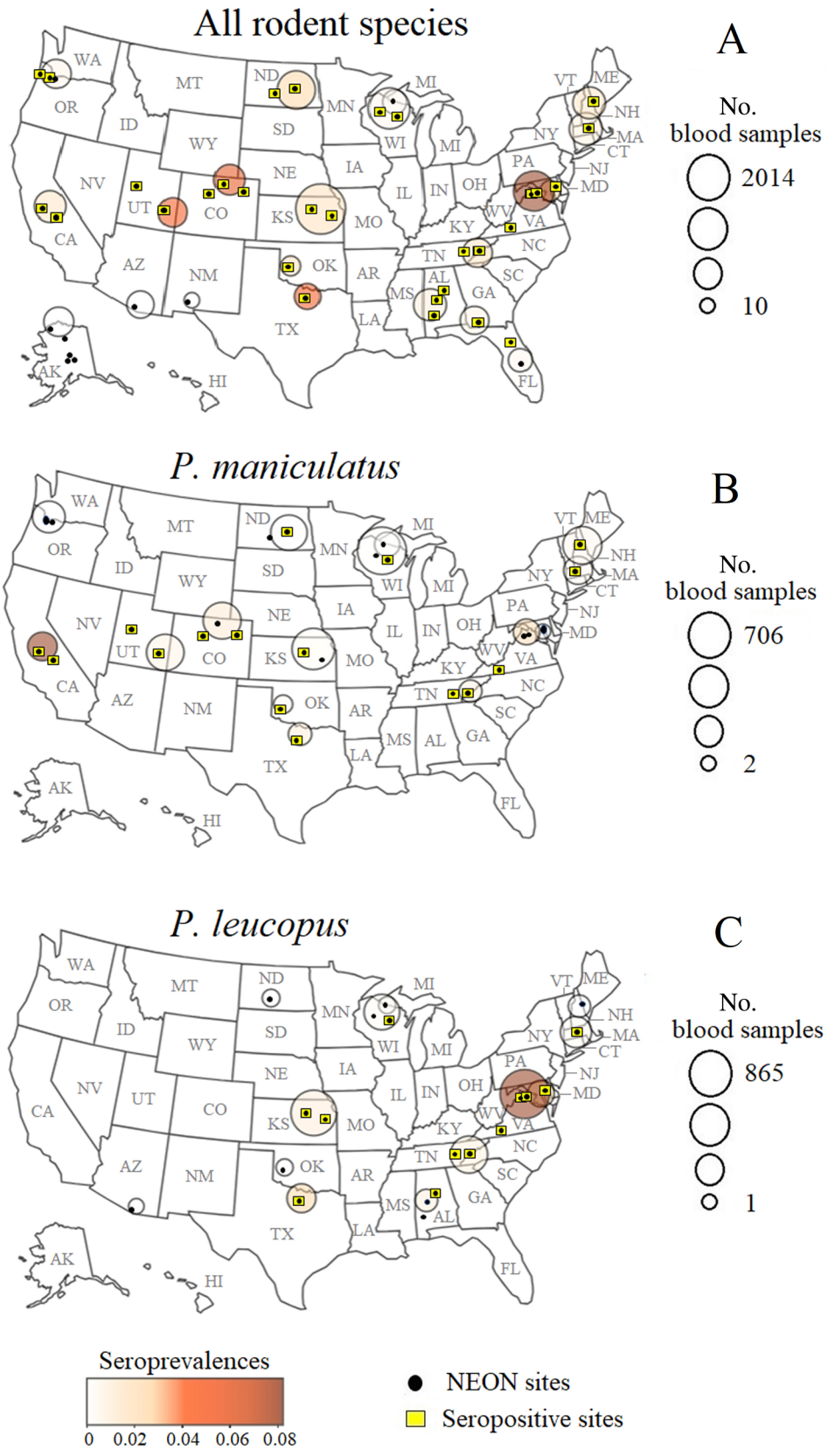

FIGURE 1 Distribution of National Ecological Observatory Network (NEON) field sites, blood samples, and seroprevalence. The figure illustrates the geographic distribution of NEON field sites across the United States (black dots), and sites with hantavirus seropositive samples (yellow squares). Data are aggregated by state to depict the number of blood samples (indicated by circle size) and the seroprevalence (represented by color scale). The number of blood samples are defined for three groups: (A) all rodent species, (B) *Peromyscus maniculatus*, and (C) *Peromyscus leucopus*. Sites with no seropositive samples (seroprevalence = 0) are shown as transparent circles.

In addition, we applied a multiplicative decomposition to the time series to describe trends and patterns of change across years and seasons (forecast Package) (Hyndman & Athanasopoulos, 2018). The time-series analysis included three components: *Trends*, indicating the direction and variability rate over years, allowing for the identification of stable or changing patterns; *Seasonality*, reflecting regular fluctuations at consistent intervals, such as seasons or years; and *Reminders*, representing residuals or noise, which capture irregular or random variations not explained by the other components and reflect data uncertainty. Finally, to assess the relationships between hantavirus seroprevalence and body measurements (i.e., body mass, hind foot length, total body length, ear length, and tail length), we used the wilcox_test() function from the rstatix package in R (Kassambara, 2023). This test is a nonparametric alternative to the *t* test, suitable for comparing two groups when data distributions are skewed.

RESULTS

From 2014 to 2019, the NEON small mammal trapping program recorded 104,379 captures, with *Pe. maniculatus* and *Pe. leucopus* comprising 21.9% and 17.1% of these captures, respectively (Table 1). A total of 127 species were captured, and 49 species were sampled, resulting in the collection of 14,004 blood samples over the 6-year period (Appendix S1: Table S1). Blood samples collected and analyzed represented 13.4% of all captures. For example, blood was collected for 17.1% of *Pe. maniculatus* captures (3919 blood samples) and for 19.2% of *Pe. leucopus* captures (3460 blood samples). From the pool of blood samples, *Peromyscus* species accounted for 64.7% of the total blood samples collected ($n = 9059$), principally *Pe. maniculatus* representing 28.1% and *Pe. leucopus* 25% of the sample pool (Figure 2, Table 1). We identified 135 blood sample records labeled with ambiguous scientific names, including 88 as *Peromyscus gossypinus*/*leucopus*, 47 as *Pe. leucopus*/*maniculatus*, and 71 blood sample records were labeled at the genus level, including *Peromyscus* sp. and *Myodes* sp.

Seropositivity was detected in 15 rodent species (30.6% of the species sampled) (Figure 2), with a total of 296 seropositive samples (2.1% seroprevalence). Most of the seropositive samples were collected from *Peromyscus* species (79.1%, 234 seropositive samples). The highest seroprevalence was obtained from *Peromyscus truei* (4.9%, 9 seropositive samples), followed by *Microtus pennsylvanicus* (4.3%, 33 seropositive samples) and *Pe. maniculatus* (3.0%, 116 seropositive samples). Filtering for species that contributed ≥1% ($n > 140$) of the blood samples from the total pool, we found no seropositives for *Myodes rutilus*, *Neotoma albigula*, *Zapus hudsonius*, and *Onychomys torridus* (Appendix S1: Table S1). We found seropositive samples in six species for which we did not find previous reports of hantavirus exposure or infection, including *Peromyscus keeni*, *Pe. gossypinus*, *Peromyscus polionotus*, *Myodes gapperi*, *Podomys floridanus*, and *Napaeozapus insignis*.

Spatial trends

Small mammals were captured across 46 terrestrial sites, and blood samples were collected from all sites except YELL, located in Yellowstone National Park. Captures and sampling activities in Puerto Rico (LAJA and GUAN sites) stopped in 2017. The site with the highest number of captures was SRER in Arizona, with 7285 captures, followed by ONAQ in Utah with 7157 captures, and HARV in Massachusetts with 7010 captures (Figure 1, Table 1; Appendix S1: Table S2). At the state level, Utah recorded the highest number of captures (9379 captures), followed by Kansas (8097), and North Dakota (7780). At the NEON site level, the KONZ site in Kansas yielded the

TABLE 1 Captures, sites, blood sampling, and hantavirus serology test results for all rodents, *Peromyscus maniculatus*, and *Peromyscus leucopus*.

Variable	All rodents	*Pe. maniculatus*	*Pe. leucopus*
No. captures	104,379	22,904 (21.9% of all captures)	17,999 (17.1% of all captures)
No. sites with captures	46	31 (67.4% of all sites)	23 (50% of all sites)
No. sites sampled	45 (97.8% of all sites)	30 (96.8% of sites with *Pe. maniculatus*)	21 (91.3% of sites with *Pe. leucopus*)
No. blood samples collected	14,004 (13.4% of all captures)	3919 (17.2% of *Pe. maniculatus* captures)	3460 (19.2% of *Pe. leucopus* captures)
No. seropositive samples	296 (2.1% of all blood samples)	116 (3.0% of *Pe. maniculatus* blood samples)	96 (2.8% of *Pe. leucopus* blood samples)
No. sites with seropositive samples	32 (71.1% of all sites)	17 (56.7% of sites with *Pe. maniculatus*)	12 (57.1% of sites with *Pe. leucopus*)

Note: Information on other species is detailed in Appendix S1: Table S1.

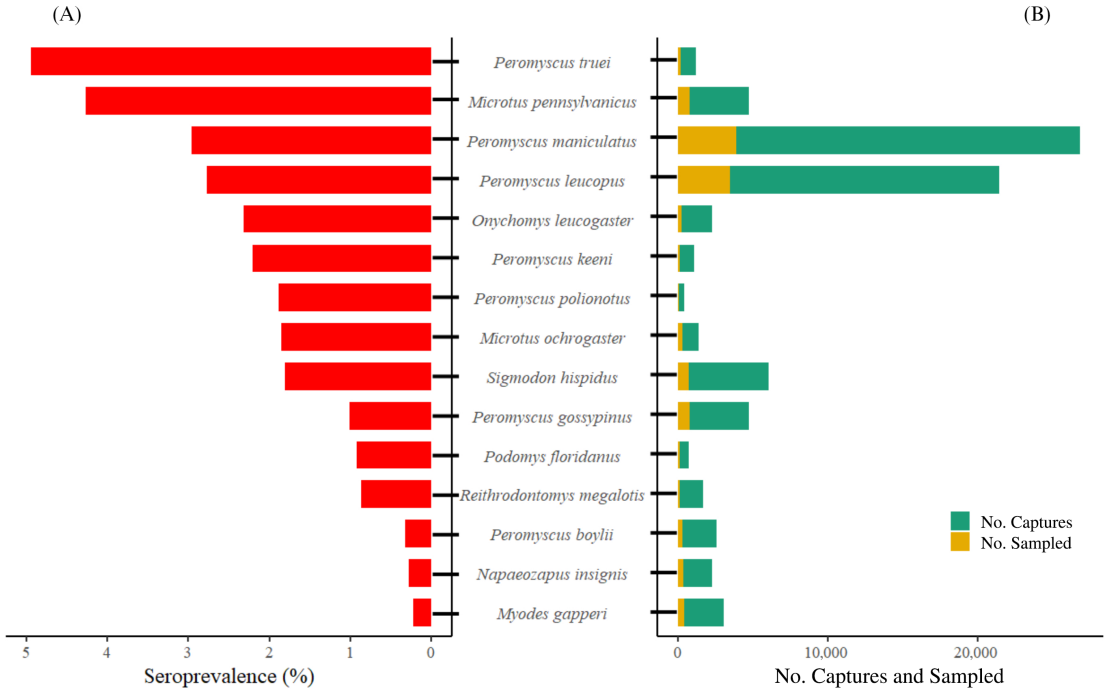

FIGURE 2 Captures, blood samples, and hantavirus seroprevalence by rodent species. (A) Hantavirus seroprevalence (seropositive/total blood samples per species) for each of the 15 seropositive rodent species. (B) Number of blood samples and the number of captures for each species. Notably, *Perosmyscus truei* and *Microtus pennsylvanicus* exhibit the highest seroprevalence, although both species have low numbers of blood samples and captures during the study period. In contrast, *Peromyscus maniculatus* and *Peromyscus leucopus* present high seroprevalence as well as substantial numbers of captures and blood samples.

highest number of blood samples, with 1109 samples collected, representing 23.6% of captures at that site. This was followed by the WOOD site in North Dakota, with 764 blood samples, accounting for 14.9% of site captures. The median number of captures per site was 415 and of blood samples per site was 77. At the state level, the largest numbers of blood samples were collected in Kansas (2014 blood samples), Virginia (1261), and North Dakota (1144). Seropositive samples were identified at 32 NEON sites, which constitute 71.1% of the sampled sites, distributed across 17 of the 23 surveyed states (73.9%) (Figure 1, Table 1; Appendix S1: Table S2).

At the site level, the highest seroprevalence was observed at CPER in Colorado, with 14.3% based on one seropositive sample from the seven blood samples collected. Blood samples were collected in 0.5% of the 1602 captures at the CPER site. Other NEON sites with notable seroprevalence included SCBI (11% seroprevalence, 65 seropositive samples) and MLBS (10.2% seroprevalence, 30 seropositive samples), both sites located in Virginia.

At the state level, Virginia exhibited the highest seroprevalence at 7.9% ($n = 99$ seropositive samples), followed by Colorado at 5.7% ($n = 37$) and Texas at 4.8% ($n = 19$). Notably, five of the 45 sites (11%) where hantavirus sampling was conducted did not yield any seropositive samples. These sites included locations in Michigan (826 blood samples), Alaska (570 blood samples), and Arizona (473 blood samples). Similarly, Kansas (2014 blood samples), Wisconsin (899 blood samples), and Massachusetts (745 blood samples) exhibited low seroprevalence (<1.2%) despite the high number of blood samples collected (Figure 1; Appendix S1: Table S2).

Peromyscus maniculatus were captured at 31 out of 46 sites, with the highest capture numbers recorded at ONAQ in Utah (2218 captures), followed by UNDE in Michigan (2057 captures) and HARV in Massachusetts (1997) (Table 1). Blood samples from *Pe. maniculatus* were collected at all sites where this species was captured, except in the YELL site, with seropositive samples detected at 56.6% of these sites (17 sites) (Figure 1, Table 1). The highest seroprevalence for *Pe. maniculatus* was observed at the STER site in Colorado, reaching up to 15.7% (27 seropositive samples). At the state level, North Dakota had the highest number of *Pe. maniculatus* captures (2937 captures), followed by Colorado (2610) and Utah (2606). The highest number of blood samples from *Pe. maniculatus* were collected in Kansas (505), Michigan (427), and Wisconsin (423). Notably, the highest seroprevalences for *Pe. maniculatus* were observed in Virginia (14.5%), Colorado (7.8%), and Texas (7.1%).

Peromyscus leucopus were captured at 23 sites across 14 states, with the highest capture numbers at SCBI in

Virginia (2768 captures), followed by ORNL in Tennessee (2327 captures) and HARV in Massachusetts (2239 captures) (Table 1). Blood samples from *Pe. leucopus* were collected at 21 of the 23 sites where this species was captured (91.3%), with seropositive samples identified at 12 (57.1%) of them (Figure 1, Table 1). The nine sites sampled for *Pe. leucopus* that did not yield seropositive samples presented a low number of blood samples (e.g., DELA with three samples, LENO with two, OAES with 21, SRER with one) (Figure 1; Appendix S1: Table S2). The SCBI site in Virginia had the highest number of blood samples from *Pe. leucopus* (457 blood samples), as well as the highest seroprevalence (12.9%, 59 seropositive samples). At the state level, Virginia also recorded the highest number of blood samples ($n = 865$) and the highest prevalence (8.2%) for *Pe. leucopus*. Following SCBI, the KONZ site in Kansas had the second highest number of blood samples from *Pe. leucopus* ($n = 411$). Hantavirus seropositive samples were identified in 10 sites where no seropositive samples were identified in *Pe. maniculatus* or *Pe. leucopus*. These sites were distributed across southeastern states (Alabama, Florida, Georgia), northern regions (North Dakota, Washington, Wisconsin), and Colorado (Figure 1).

Sex and life stage

A significantly higher number ($\chi^2 = 366.76$, df = 1, $p = 9.48 \times 10^{-82}$) of captured rodents were identified as males ($n = 52{,}433$) compared with females ($n = 46{,}441$). This pattern was consistent with the number of sampled individuals ($\chi^2 = 85.57$, df = 1, $p = 2.24 \times 10^{-20}$) and seroprevalence ($\chi^2 = 26.94$, df = 1, $p = 2.10 \times 10^{-7}$), with 7501 sampled males (2.6% seroprevalence) and 6409 sampled females (1.6% seroprevalence; Figure 3; Appendix S1: Table S3). A total of 5.3% of the captures ($n = 5535$) and 0.7% of the blood samples ($n = 94$) were recorded with undetermined sex or lacked sex identification. Seroprevalence was significantly higher ($\chi^2 = 13.55$, df = 1, $p = 0.\ 24 \times 10^{-3}$) in males with scrotal testes ($n = 122$, 3.6% seroprevalence) compared with non-scrotal males ($n = 70$, 1.6% seroprevalence), and pregnancy was negatively correlated with seroprevalence ($\chi^2 = 63.21$, df = 1, $p = 1.85 \times 10^{-15}$), with 1.7% seroprevalence in pregnant females (15 of 866 blood samples) and 1.9% in nonpregnant ones (128 of 6567 blood samples; Figure 3; Appendix S1: Table S3).

Regarding life stage, the majority of the captures ($\chi^2 = 124{,}997$, df = 1, $p = 2.2 \times 10^{-16}$) and blood samples ($\chi^2 = 16{,}857$, df = 1, $p = 2.2 \times 10^{-16}$) were from adults (captured males = 87,022; male blood samples = 11,889; Figure 3). Higher seroprevalence was found in adults (2.3%, 277 of 11,889 blood samples) compared with subadults (1.0%, 16 of 1580 blood samples) and juveniles (0.2%, one of 475 blood samples; $\chi^2 = 487.69$, df = 1, $p = 4.54 \times 10^{-108}$). The only seropositive sample from a juvenile was a *Pe. truei* rodent (Appendix S1: Table S3).

Time and seasonal trends

Captures were conducted annually from 2014 to 2019 at 13 sites, although blood samples were consistently collected each year only at the SCBI site in Virginia. The median number of captures per year per site was 415, while the median number of blood samples was 77. Captures tended to peak in May and September, corresponding to spring and summer months, exhibiting a moderate bimodal intra-annual pattern (Figure 4; Appendix S1: Figure S1). Conversely, captures decreased during winter months from December to February, a period when blood samples were infrequently collected. In January, only 43 blood samples were collected, all from 2018, with a single seropositive sample identified in a *Peromyscus boylii* rodent. In February, 73 blood samples were collected, with 51 samples from 2018 and 23 in 2019, yet no seropositive samples were found (Appendix S1: Figure S2).

Captures trends (Figure 4) displayed greater consistency across years compared with seroprevalence, as indicated by the more uniform residual structure (Appendix S1: Figure S2). Higher inconsistencies were noted for *Pe. maniculatus* compared with *Pe. leucopus* (Appendix S1: Figures S3 and S4). Hantavirus seroprevalence tended to peak during fall and spring, specifically in September (year 2014), between May and June (year 2015), between March and April, as well as in October–November (years 2016, 2017, 2018) (Figure 4; Appendix S1: Figure S3). Intra-annually, a bimodal seasonal trend was observed across all rodent species, yet no significant lagged correlations were found between the number of captures and seroprevalence within a 95% CI. Thus, the number of rodents captured did not explain or predict seroprevalence variation when considering all rodents collectively (Appendix S1: Figure S2).

For *Pe. maniculatus*, seroprevalence peaks typically occurred between September and November (years 2014, 2016, 2017, 2018) and between March and May (years 2018, 2019). In November 2018, a peak in *Pe. maniculatus* seroprevalence was observed when only two blood samples were collected, both testing positive, resulting in a seroprevalence of 100%. The CCF showed a positive correlation (CCF(r) = 0.2432; $p = 0.039$) at a −2-month lag in *Pe. maniculatus*, suggesting that an increase in the number of captures is associated with an increase in the average seroprevalence 2 months later (Figure 4; Appendix S1: Figures S2 and S4).

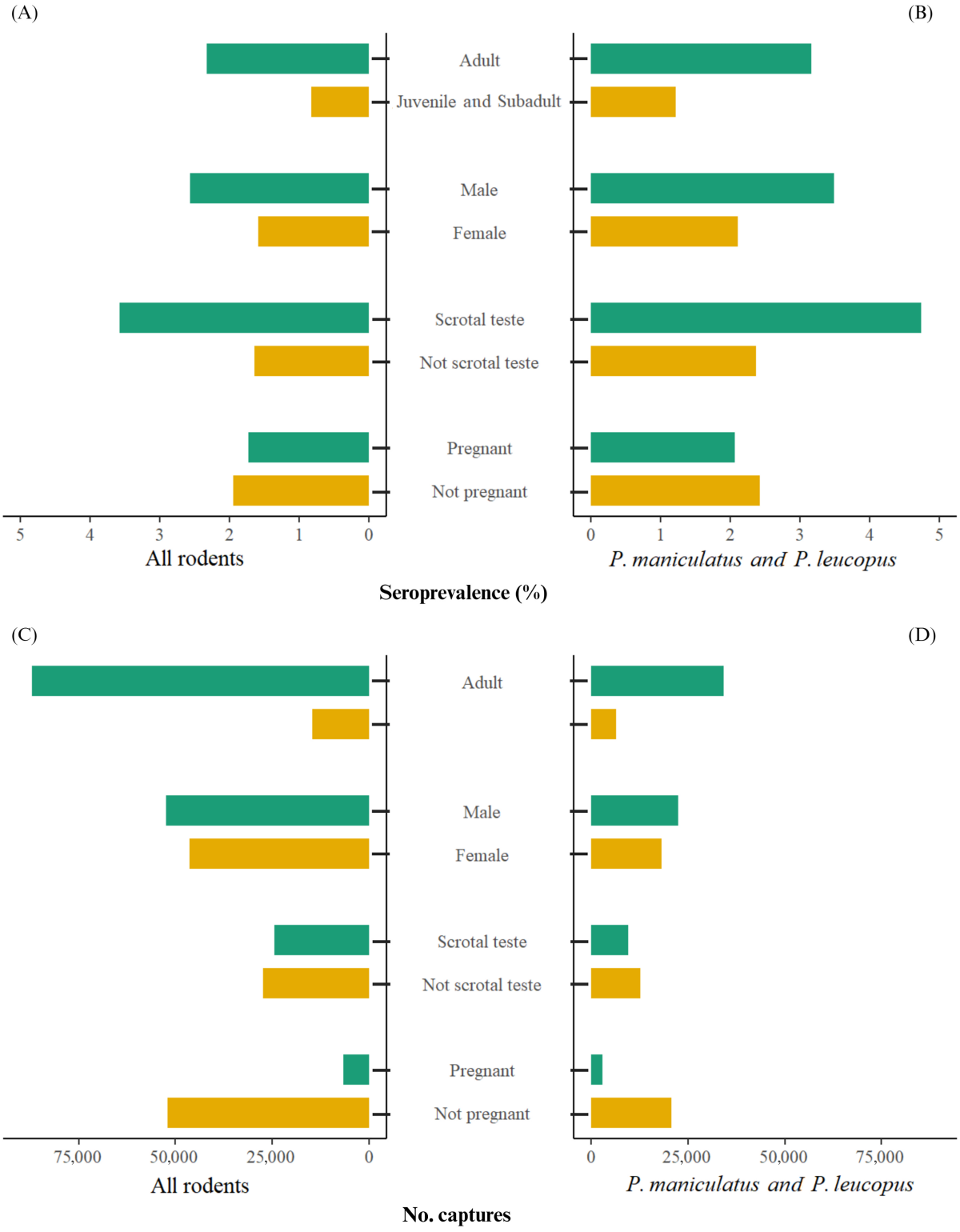

FIGURE 3 (A, B) Seroprevalence, (C, D) numbers of captures, and (E, F) numbers of blood samples according to sex, life stage, and reproductive status. These variables are shown separated for all rodents and for *Peromyscus maniculatus* and *Peromyscus leucopus* grouped. For life stage, juveniles and subadults were grouped. Significant differences in seroprevalence were identified for scrotal testes and pregnancy status. For better visualization, all variables are presented as binomial, with two possible outcome categories. More details about the results of these variables can be found in Appendix S1: Table S4.

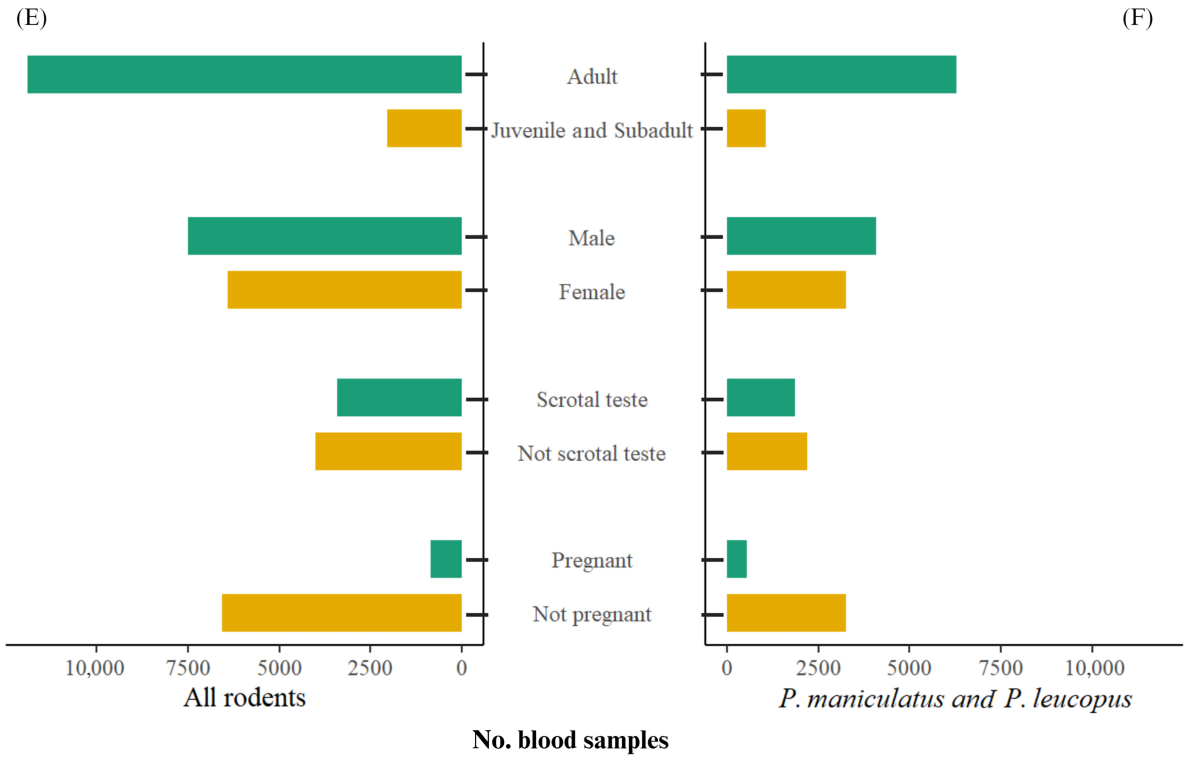

FIGURE 3 (Continued)

The seroprevalence of *Pe. leucopus* exhibited bimodal peaks in spring (March–April) and fall (September–October) (Figure 4; Appendix S1: Figure S1). The 2016 peak in *Pe. leucopus* seroprevalence observed in April (20%) was based on four seropositive samples out of 20 collected, while the 2018 peak observed in March resulted from two seropositive samples out of 13 collected. We identified that the average number of *Pe. leucopus* captures could predict future seroprevalence, showing both positive and negative cross-correlations. Significant positive correlations were observed at lags −9 (CCF(r) = 0.3297; $p = 0.005$), −10 (CCF(r) = 0.3082; $p = 0.008$), and −11-months (CCF(r) = 0.2455; $p = 0.037$), suggesting that an increase in *Pe. leucopus* captures is associated with increased seroprevalence 9–11 months later. Conversely, significant negative correlations were found at lags −4 (CCF(r) = −0.344; $p = 0.003$) and −5-months (CCF(r) = −0.3354; $p = 0.004$), sthat an increase in *Pe. leucopus* captures is associated with a decrease in seroprevalence 4–5 months later (Figure 4; Appendix S1: Figure S1).

Morphometry and hantavirus seroprevalence

Body mass data were available for 87,570 (83.9%) captures and 12,863 (91.9%) blood samples, while hind foot length was recorded for 83,239 (79.7%) captures and 12,356 (88.2%) blood samples (Appendix S1: Figure S5). Total length measurements were missing in 91.5% of captures ($n = 95{,}719$) and 89.6% of blood samples ($n = 12{,}549$). Ear length was not recorded in 80.2% of captures ($n = 83{,}737$) and in 72.3% of blood sample records ($n = 10{,}124$), while tail length was absent in 73.0% ($n = 76{,}239$) of captures and 63.9% of blood samples ($n = 8950$). Specifically, for *Peromyscus* species, body mass was recorded in 44,499 (88.3%) captures and in 8454 (93.32%) blood samples, while hind foot length was available for 42,445 (84.2%) of the captures and in 8253 (91.1%) of the blood sample records.

In *Pe. maniculatus*, seropositive individuals were found to have larger body masses (20.8 g) when compared with seronegative ones (18.6 g; $W = -6.67$, df = 104.8, $p = 1.22 \times 10^{-9}$), and ear lengths tended to be smaller in seropositive rodents (16.7 g) when compared with seronegatives (17.0 g; $W = 3.72$, df = 33.4, $p = 7.26 \times 10^{-4}$). Similarly, for *Pe. leucopus*, seropositive rodents tended to have a larger body mass (23.5 g) when compared with seronegative individuals (22.7 g; $W = -2.14$, df = 91.2, $p = 0.035$) (Figure 5; Appendix S1: Table S4 shows detailed values for all body measurements).

DISCUSSION

This study assessed hantavirus seroprevalence across 49 species in the United States using data from the

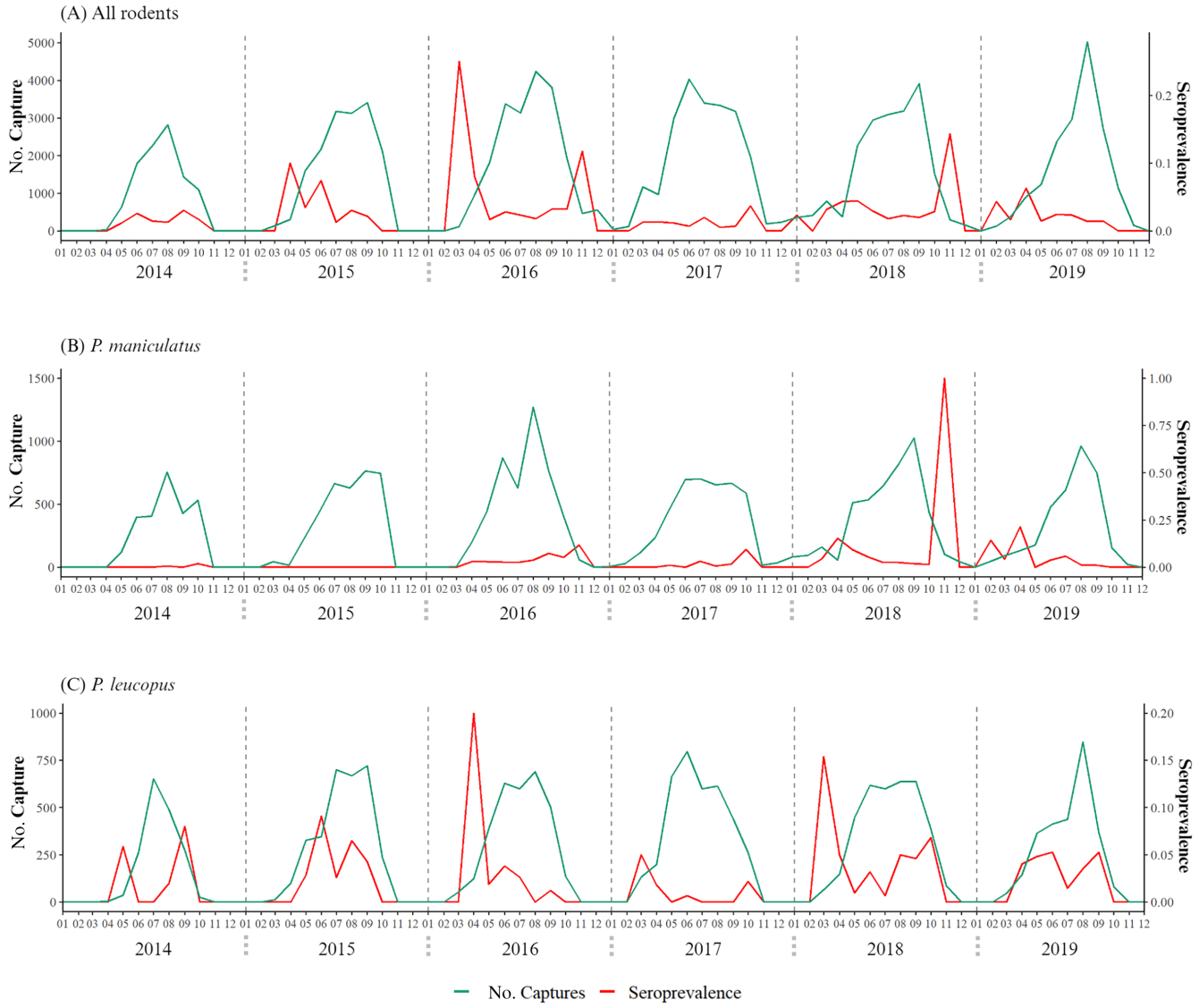

FIGURE 4 Numbers of captures and seroprevalence for (A) all rodents, (B) *Peromyscus maniculatus*, and (C) *Peromyscus leucopus* across the 2014–2019 period. Peaks in seroprevalence (e.g., February 2016 in all rodents and in *Pe. leucopus*, December 2018 in *Pe. maniculatus*) tend to coincide with a lower number of captures. The number of blood samples and seropositive rodents is shown in Appendix S1: Figure S3.

NEON. We described the distribution of hantavirus exposure across species, seasons, and geographical regions, contributing to the understanding of hantavirus circulation among known and newly identified host species. Our findings indicate that *Peromyscus* species generally exhibit higher seroprevalence levels, in agreement with scientific literature identifying *Pe. maniculatus* as the primary host of SNV (Childs et al., 1994). Nevertheless, other rodent species also play significant roles in supporting hantavirus circulation, particularly in regions where *Pe. maniculatus* and *Pe. leucopus* show lower seroprevalence and multiple hantaviruses coexist (Ermonval et al., 2016; Rowe et al., 1995).

Since hantaviruses were described only three decades ago, new hosts and hantaviruses continue to be identified, aided by enhanced screening efforts and advancements in molecular tools (Bellomo et al., 2021; Milholland et al., 2018; Perry et al., 1997). Globally, researchers have identified at least 140 small mammal species hosting nearly 30 different recognized hantaviruses (ICTV, 2024), with at least 42 species hosting hantaviruses with zoonotic potential (Chen et al., 2023; Forbes et al., 2018; Guo et al., 2013; Guzmán et al., 2015; ICTV, 2024; Milholland et al., 2018). Although hantaviruses usually circulate within a single or a few rodent species, these viruses can circulate among host

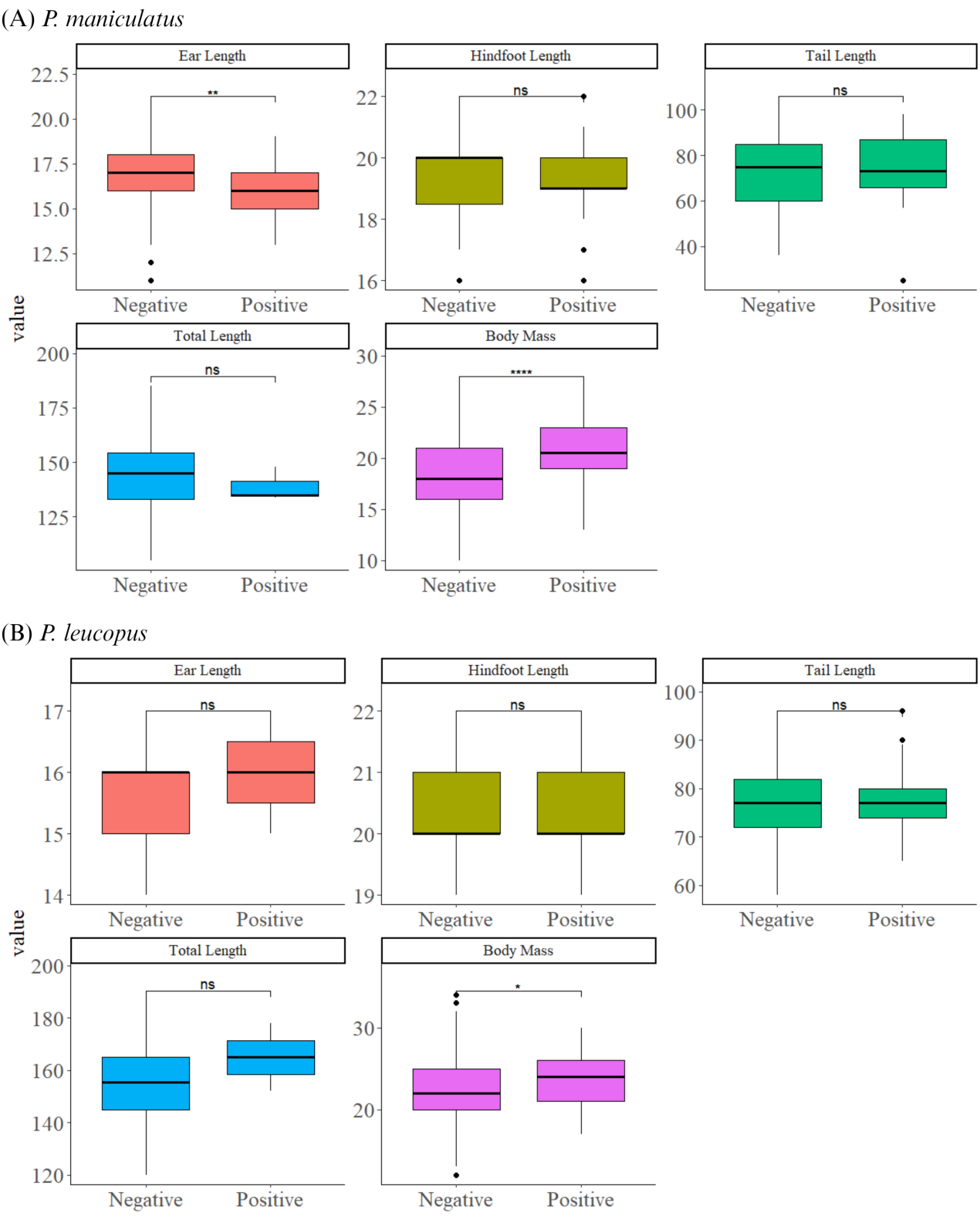

FIGURE 5 Differences of biological measurements in (A) *Peromyscus maniculatus* and in (B) *Peromyscus leucopus* between seropositive and seronegative individuals. Body mass was found to be significantly different between seropositive and seronegative individuals in both *Pe. maniculatus* and *Pe. leucopus*, and ear length was significantly different only for *Pe. maniculatus*. Body mass, which has the larger number of records, tends to have a consistent distribution in both species. In contrast, ear length and hindfoot length presented higher variability. The midline within each box represents the median value. The box limits indicate the interquartile range (IQR), spanning from the 25th to the 75th percentile. The whiskers extend to the minimum and maximum values within 1.5 times the IQR. Data points beyond the whiskers represent outliers (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, and ns=non-significant).

assemblages, and multiple hantaviruses can infect a single host species (Guzmán et al., 2015; Milholland et al., 2018).

The serological test employed by NEON does not distinguish hantavirus lineages (Klingström et al., 2002; Thibault, 2022). Even though our study identified seropositive samples in species previously recognized as SNV hosts (Figure 2; Chen et al., 2023; Ermonval et al., 2016; Milholland et al., 2018), from these findings, we cannot rule out that the immune response detected in blood samples reflects an exposure to other hantaviruses different from SNV or coinfections with different hantaviruses. For instance, *Pe. truei* has been described as a host for both SNV and Prospect Hill virus (*Orthohantavirus prospectense*) (ICTV, 2024; Milholland et al., 2018; Neill et al., 1996; Rollin et al., 1995), and *S. hispidus* has been found infected by SNV and Black Creek Canal viruses (*Orthohantavirus nigrorivense*) (Lewis, 2005; Milholland et al., 2018). Since not all hantaviruses are recognized to be pathogenic to humans (MacNeil et al., 2011; Mills, 1998; Springer et al., 2016), understanding the cocirculation of pathogenic and nonpathogenic hantaviruses is critical to assess human health risks (Chen et al., 2023; Luong et al., 2011).

In our study, we identified six seropositive species that, to our knowledge, represent newly described hosts for hantavirus, including *Pe. keeni*, *Pe. gossypinus*, *Pe. polionotus*, *My. gapperi*, *Po. floridanus*, and *N. insignis*. Nevertheless, the taxonomic identification of these potential novel hosts might have been misclassified, or they may have been recorded under invalid or synonymous scientific names in earlier reports. For instance, while we found no prior reports of hantavirus infection in *Pe. keeni*, the Integrated Taxonomic Information System (ITIS; www.itis.gov) recognizes 16 synonyms for this species and also accepts it as a subspecies of *Pe. maniculatus*. Consequently, past records of hantavirus infection in *Pe. keeni* might have been documented under a different species name, including *Pe. maniculatus*. Similarly, we found no report of hantavirus in *My. gapperi*, a species recently prioritized for hantavirus surveillance (Mull et al., 2022). Nonetheless, SNV was reported in *Clethrionomys gapperi* (Kuenzi et al., 2001), an accepted synonym of *My. gapperi* (Kryštufek et al., 2020). We identified one seropositive sample of *My. gapperi* in Massachusetts (HARV site), which is distant from the previous report of *C. gapperi* in Montana (Kuenzi et al., 2001). Thus, if the previous report truly corresponds to *My. gapperi*, the specimen identified in this study would represent the first record of the species in the Northeastern United States.

The seropositive samples found in *My. gapperi* were collected in sites where seropositive samples were also collected from *Pe. maniculatus* or *Pe. leucopus* rodents. Similar to previous findings of Kuenzi et al. (2001), the seroprevalence in *My. gapperi* was low, with results also based on an antibody test that does not distinguish among hantavirus lineages. Therefore, the epidemiological role of *My. gapperi* may not be crucial, as there are other well-known reservoirs in the area, and there is no evidence that it may carry another hantavirus for which it may have more relevance.

On the other hand, *Pe. gossypinus*, another plausible novel host for hantavirus, can be misclassified in the field as *Pe. maniculatus* (Perry et al., 1997). Seropositive samples of *Pe. gossypinus*, however, were collected in all the sites from Alabama and southern Georgia in which the species was captured. These sites fall outside the accepted distributional range of *Pe. maniculatus* by the International Union for Conservation of Nature and Natural Resources (IUCN, 2024b). Besides *Pe. gossypinus*, two other new hosts were found in these southern areas, including *Pe. polionotus* and *Po. floridanus*. In the Southern United States, the circulation of hantavirus could be related to Black Creek Canal or Bayou hantaviruses, both known to cause human pulmonary syndrome in humans (Morzunov et al., 1995) and associated with *S. hispidus* and *Oryzomys palustris*, respectively (Chen et al., 2023; Holsomback et al., 2009; McIntyre et al., 2005). Considering that these areas are beyond *Pe. maniculatus* and *Pe. leucopus* distributions, and the local presence of at least two zoonotic hantaviruses, the description of these new hosts is a valuable contribution in the assessment of human health risk.

Detecting hantavirus exposure in rare species or in those with low prevalence requires a large sampling size and extended field efforts. The comprehensive NEON hantavirus program explored a wide range of individuals, species, geographies, seasons, and ecosystems. The sampling effort of NEON partly explains the detection of hantavirus exposure in new areas and in multiple and novel host species, revealing hidden aspects of hantavirus dynamics. The identification of rare events would probably remain undetected with a lower sample size and in studies limited in time and space. For instance, we found only a few previous reports for species such as *Reithrodontomys megalotis* (Abbott et al., 1999; Bi et al., 2008), and only one report for *Onychomys leucogaster* (Banther-McConnell et al., 2024). Studies with modest sampling effort may lead to over- or underestimation of hantavirus prevalence in wildlife (Carver et al., 2010). For example, we found peaks up to 100% seroprevalence in periods with low sample sizes (e.g., November 2018 in *Pe. maniculatus*) and high seroprevalence in some species with low sample sizes such as *Pe. truei* (Figure 2; Appendix S1: Table S1).

Despite the large number of species and blood samples, and the great geographical extension of the NEON program, we did not find seropositive samples in 34 of the

species sampled, some of them previously described as hantavirus hosts. This absence of seropositive samples underscores the relevance of large-scale, continuous surveillance programs, particularly for pathogens like hantaviruses, which typically exhibit low prevalence and can infect rare species (Milazzo et al., 2012; Milholland et al., 2019; Mull et al., 2022). For instance, Mull (2023) utilized taxonomical and ecological characteristics to identify over 100 species in the Americas that should be targeted as potential hantavirus hosts. Furthermore, Milholland et al. (2018) found that out of nearly 37 endemic *Peromyscus* species in Mexico, 28 remain untested for hantaviruses, indicating a significant taxonomic gap in host identification. On the other hand, the NEON program, like other studies, relies on serological testing that does not differentiate between hantavirus lineages or assess their pathogenic potential (Bellomo et al., 2021). To deepen our understanding of hantavirus–host dynamics, it is crucial to identify hosts, explore their distributions and interactions, and assess the zoonotic potential of the hantaviruses they carry (Milholland et al., 2018).

Spatial distribution

The primary recognized reservoir of the SNV, *Pe. maniculatus*, was not captured at 15 sites, 14 of which were located beyond its known distribution range, reflecting its consistent occurrence within its stablished range (Dragoo et al., 2006). There were no seropositive samples at nearly half of the sites where *Pe. maniculatus* was sampled (13 of 30) (Figure 1). Most of these sites were near other NEON sites that presented seropositive *Pe. maniculatus*, indicating exposure to hantavirus in surrounding areas, as represented at the state level (Figure 1). For instance, although no seropositive samples were collected at NIWO in Colorado, which is approximately 35 km from the RMNP site where *Pe. maniculatus* seropositive samples were collected. This suggests that fine-scale ecological factors may be influencing hantavirus circulation, which should be explored as a means to better identify landscape-level drivers of hantavirus transmission risk. Interestingly, some of the sampled sites without seropositive *Pe. maniculatus* were located at the edges of the species' distributional range (IUCN, 2024b), which may represent areas with lower population densities or with a different rodent species richness structure. Seronegative *Pe. maniculatus* sites grouped in two clusters, one in the southwestern United States (New Mexico and Arizona) and another in the eastern regions (Maryland and Virginia). Notably, the eastern sites, especially those in Virginia, showed high seroprevalence in *Pe. leucopus* (Figure 1).

Among the 21 sites where *Pe. leucopus* rodents were sampled, in nine of them no seropositive samples were collected. Similar to the trend observed for *Pe. maniculatus*, these seronegative sites were typically located at the edge of the species' distributional range, such as SRER (Arizona), DELA and LENO (Alabama), BART (New Hampshire), and UNDE (Michigan). The only exception was OAES in Oklahoma, potentially explained by the limited number of blood samples collected (21 blood samples) (Appendix S1: Table S1). Thus, hantavirus exposure in *Pe. maniculatus* and *Pe. leucopus* appears widespread but not continuous across their range. On the other hand, the highest seroprevalence for *Pe. leucopus* was found in Virginia and Maryland, specifically where no seropositive *Pe. maniculatus* were collected. In fact, at two of these sites (BLAN and SERC) *Pe. leucopus* was the only seropositive species. Therefore, in the eastern areas, at the borders of *Pe. maniculatus* distribution, *Pe. leucopus* may play a crucial role in sustaining hantavirus circulation.

Our study identified 10 sites where seropositive samples did not correspond to the well-documented hantavirus hosts *Pe. leucopus* or *Pe. maniculatus*, despite being sampled in four and in six of those sites, correspondingly. Interestingly, in six of these sites, the seropositive samples were obtained from species identified in this study as potential novel host species. For instance, *Po. floridanus* was the sole seropositive species in Florida (OSBS), and along with *Pe. gossypinus*, the only seropositive species in Georgia (JERC). Additionally, a blood sample obtained from *N. insignis* was the only seropositive sample at STEI, in Wisconsin. In Washington, the two available NEON sites (ABBY and WREF) yielded three seropositive samples, all from *Pe. keeni*, another potential novel host for hantaviruses. These two sites from Washington, however, are located outside the distribution range described for *Pe. keeni* (IUCN, 2024a), although the sites tend to overlap with occurrences documented in the Global Biodiversity Information Facility (GBIF, 2024). The seropositive records collected from *Pe. keeni* would represent the first documentation of hantavirus in this species but also provide additional evidence of the species presence beyond the recognized boundaries currently stablished by the IUCN.

In the remaining five sites with seropositive samples unrelated to *Pe. maniculatus* or *Pe. leucopus*, the rodents involved have been previously described as hantavirus hosts. For example, in Colorado (STER), seropositive samples were collected from *On. leucogaster*, a species recently identified as a hantavirus host, with two positive cases reported in New Mexico (Banther-McConnell et al., 2024). At the NOGP site in North Dakota, the seropositive samples were collected exclusively from *Mi. pennsylvanicus*,

previously associated with Prospect Hill virus (*Or prospectense*), with pathogenic potential for humans (Lee et al., 1982). Although blood samples from *Mi. pennsylvanicus* were collected across seven states, seropositive samples were only obtained in Virginia and North Dakota. The low sample size in the other states (three blood samples in five states) limits our ability to rule out hantavirus exposure in *Mi. pennsylvanicus* across its extensive distribution in the United States (IUCN, 2024b). Nevertheless, our findings suggest that *Mi. pennsylvanicus* plays a role in areas where seropositive samples were collected, possibly supporting the cocirculation of Prospect Hill virus and SNV.

Finally, seropositive samples were collected from *R. megalotis* at CPER, Colorado, where no seropositive samples were identified for *Pe. maniculatus* or *Pe. leucopus*. Reports of hantavirus infection in *R. megalotis* are rare in the literature and have been associated with SNV, El Moro Canyon, and Huitzilac hantaviruses, with unknown pathogenic potential for humans (Lewis, 2005; Milholland et al., 2018; OPS, 1999). In summary, *R. megalotis* may have a role in hantavirus circulation, particularly in central and southwestern areas of the United States, where hantavirus exposure has been described in humans (New Mexico, California, Colorado) (Hjelle et al., 1994; Lewis, 2005; Milholland et al., 2019) and where *Peromyscus* species may play a minor epidemiological role.

Temporal trends and seasonality

Agreeing with previous studies, our analyses revealed variable rodent abundance and hantavirus seroprevalence across different seasons and years (Bagamian, Douglass, et al., 2012; Carver et al., 2010; Luis et al., 2010; Semmens et al., 2001). We observed interannual variations in captures and seroprevalence (Figure 3; Appendix S1: Figure S2). Such variations in rodent captures have been linked to environmental events that cause drastic landscape changes, such as vegetation flowering, forest fires, and climate anomalies (e.g., ENSO), among others, especially at higher latitudes (Mölle et al., 2022; Olsson et al., 2002). Therefore, despite the high number of captures and blood samples for species such as *Pe. maniculatus*, the 6-year period of hantavirus serological data available in NEON does not allow robust time-series analyses capturing signals of long-term processes (Hyndman & Athanasopoulos, 2018; Pankratz, 1983). The patterns observed in our analysis, however, allowed us to identify correlations and seasonal signals of hantavirus dynamics, useful to guide epidemiological surveillance.

Our study identified a consistent intra-annual trend of increased rodent captures during late spring and summer (May to August) across all data groups (All rodents, *Pe. maniculatus*, *Pe. leucopus*), followed by a sharp decline in winter (November to March) (Figure 4). This pattern partially aligns with the dynamics of *Peromyscus* species described in North America (Karunarathna et al., 2024). Unlike previous studies, we did not observe a robust peak in abundance during the fall (October–November) (Sullivan & Sullivan, 2004; Wilder & Meikle, 2006). Nevertheless, the compiled monthly values present a high variability that hampers the identification of clear trends in some months (Appendix S1: Figure S4). This variability may be attributed to the broad geographical coverage of the NEON program, spanning from Puerto Rico to Alaska, which may introduce greater variability between populations at different latitudes and longitudes. In contrast, studies conducted in more localized areas are subject to more uniform environmental conditions and lower seasonal variability (Karunarathna et al., 2024).

In the NEON database, the sharp decline in the number of rodent captures during fall and winter primarily reflects lower fieldwork efforts in response to lower capture success. Even though the NEON protocol aims to have consistent capture efforts, it is assumed that it will be reduced during periods with challenging field conditions, considering personnel safety, logistic constraints, and the impacts of field captures on animal welfare (Paull et al., 2023). Captures in December occurred only in states with temperate weather or mild winter conditions, such as California, Puerto Rico, and Florida, while those in January and February were exclusively collected from California. Consequently, blood sampling also decreased, resulting in only 43 blood samples in January, all from 2018, with just one seropositive sample (Figure 4). Therefore, the information on hantavirus dynamics during winter remains limited, hampering our understanding of seasonal patterns (Bagamian, Douglass, et al., 2012).

The temporal patterns of seroprevalence exhibited inconsistent intra- and interannual trends, with a general bimodal pattern and some extreme peaks (Figure 4; Appendix S1: Figures S1 and S2). Seroprevalence is greatly influenced by sample sizes, often resulting in extreme values with lower robustness. For instance, in November 2018 and in late 2015, fewer than 10 *Pe. maniculatus* were captured and sampled, potentially misrepresenting infection expressed as 100% seroprevalence in 2018 and 0% in 2015.

While no temporal association was found between captures and seroprevalence when considering all rodents collectively, such an association was observed in *Pe. leucopus* and in *Pe. maniculatus*, where abundance

(captures) predicted seroprevalence. The association between population abundance and hantavirus transmission is inconsistent in the literature, with studies reporting no association (Clay et al., 2009; Pearce-Duvet et al., 2006), positive association (Boone et al., 1998; Douglass et al., 2007), or negative association (Calisher et al., 1999; Douglas et al., 2021; Semmens et al., 2001). These associations should consider the diagnostic tests used and whether the test indicates current or previous infection (Banther-McConnell et al., 2024; Lewis, 2005). NEON's hantavirus diagnosis relies on a serological test (ELISA IgG), which does not necessarily indicate hantavirus viremia (Klingström et al., 2002; Thibault, 2022) and therefore does not inform about the time of infection, complicating temporal-trend interpretations. Molecular virus detection may also fail to identify the time of transmission, as RNA can persist in blood for 4–6 months postinfection (Bagamian et al., 2013) and vary depending on the organ analyzed (Botten et al., 2002; Quizon et al., 2022). Future research should explore the establishment and duration of hantavirus antibodies in wild rodents to improve inferences about the time of infection.

The negative correlation delay observed in *Pe. leucopus* (4–5 months), and the positive correlation delay in *Pe. leucopus* (9–11 months) and in *Pe. maniculatus* (2 months) suggests that, even though with different effects and at different lags, rodent abundance influences the transmission and immune response development. In *Pe. leucopus*, the capture peak observed from July to August (breeding season) predicts a rise in seroprevalence in the following spring (9–11 months lag) and a decreased seroprevalence observed from November to January (4–5 months lag). On the other hand, in *Pe. maniculatus*, the summer peak of captures predicted an increased seroprevalence in the following fall (September–November 2 months lag). Our findings align with previous studies that demonstrate an association between abundance and transmission (Bagamian, Douglass, et al., 2012; Pearce-Duvet et al., 2006). For instance, Luis et al. (2010, 2015) described a positive temporal lag of 8–16 months between the rise in rodent abundance and the subsequent rise in hantavirus seroprevalence.

The differing temporal lags between abundance and prevalence observed in *Pe. leucopus* compared with *Pe. maniculatus* may reflect distinct dynamics of hantavirus transmission. In general, both species of *Peromyscus* tend to present similar population dynamics across time (abundance, breeding season), highly dependent on resource availability, habitats, latitudes, vegetation structure, and climate, among others (Sullivan & Sullivan, 2022; Wang et al., 2008; Wilder & Meikle, 2006; Wolff, 1985). In both species, northern latitudes and higher altitudes may influence the range of temporal variability, while winter conditions decrease population survival (Wang et al., 2008). *Pe. maniculatus*, with larger geographical extension compared with *Pe. leucopus*, is exposed to larger ranges of latitudes, and therefore would tend to present higher dispersion of seasonal captures and seroprevalence values (Figure 4; Appendix S1: Figure S4) (Wang et al., 2008). This variability, together with potential interspecies differences in the establishment of a detectable immune response and persistence of antibodies in circulation (Schönrich et al., 2008), may explain in part the different time-lag associations of captures and seroprevalence between *Pe. maniculatus* and *Pe. leucopus*.

Sex, life stages, and body measurements

Our findings align with previous studies, confirming that adult rodents exhibit higher hantavirus seroprevalence (Douglass et al., 2007; Luis et al., 2015). Notably, we detected only one seropositive sample in a juvenile *Pe. truei*. The persistence of antibodies, which can remain in circulation for weeks, months, or permanently through a rodents' life (Engdahl & Crowe, 2020; Schönrich et al., 2008; Tischler et al., 2005), likely contributes to the higher seroprevalence in adults. This may reflect the cumulative persistence of antibodies rather than a lack of exposure during early life stages. Additionally, the juvenile stage represents a brief period of the rodents' life, potentially insufficient for developing a detectable immune response (Luis et al., 2010; Schönrich et al., 2008).

There is limited evidence regarding the effects of maternal hantavirus antibodies. An experimental study on Puumala hantavirus in *Myodes glareolus* suggested that maternal antibody transfer could delay the timing of hantavirus infection (Kallio et al., 2006). Consequently, the low hantavirus infection rates in juveniles could be attributed to early protection conferred by undetected maternal antibodies.

In terms of sex differences, our study found higher seroprevalence in males compared with females (Bagamian et al., 2013; Bennett et al., 1999), and a correlation between the presence of scrotal testes and seroprevalence (Figure 5; Appendix S1: Table S3). Sexually active rodents are typically more aggressive and exhibit greater spatial dispersal (Bagamian et al., 2013; Maroli et al., 2015), facilitating hantavirus transmission through increased direct contact and overlap between infected and uninfected individuals (Bagamian, Douglass, et al., 2012; Pearce-Duvet et al., 2006).

Conversely, pregnancy was negatively correlated with seroprevalence, indicating that nonpregnant females tended

to have higher values of seroprevalence. Although pregnancy involves close contact with multiple males (Cornejo-Latorre et al., 2021), which would typically suggest higher seroprevalence, our results may be explained by the short gestation period in *Peromyscus* species, lasting 24–27 days (Wilson & Ruff, 1999), probably insufficient for developing a detectable immune response postinfection during mating. Additionally, NEON's sampling protocol excludes blood collection from females in advanced pregnancy (Paull et al., 2023), limiting our ability to assess the impact of pregnancy on hantavirus infection.

In both *Pe. leucopus* and *Pe. maniculatus*, seropositivity was positively associated with body mass, though not with hind foot length or total body length (Figure 5; Appendix S1: Table S5). Given that most captured rodents were adults, body mass likely reflects body condition and, to a lesser extent, age-related changes. Thus, we infer that heavier, more robust, sexually active, and potentially more aggressive rodents are more susceptible to hantavirus exposure (Bagamian, Towner, et al., 2012). Consistent with previous studies, these findings also suggest that SNV infection has minimal impact on the fitness of *Peromyscus* species (Abbott et al., 1999; Mills et al., 1998; Quizon et al., 2022).

In addition to body mass, ear length also differed between seropositive and seronegative individuals in *Pe. maniculatus*. The reliability of this difference, however, remains uncertain due to the smaller sample size and the high variability in measurements (Figure 5). This low precision may be attributed to the challenges in field measurements given the small size of the ears and the influence of individual researcher skills.

Future directions

The NEON program delivers one of the largest datasets of standardized hantavirus surveillance in small mammals in the United States and the world, providing a unique opportunity to study hantavirus dynamics in nature. The discontinuation of the NEON hantavirus program in 2019 limits its contribution to the understanding of long-term processes and phenomena that occur gradually, including the effects of climate change and interannual transmission dynamics within the hantavirus system. Fortunately, NEON maintains a sample biorepository, providing a source of rodent blood for further testing to identify hantavirus infections. In addition, NEON databases provide valuable data on the ecology and dynamics of several pathogen hosts.

The NEON hantavirus program, as well as most of the scientific studies reviewed, lacks a robust number of samples during fall and winter seasons, generating a significant gap for the understanding of temporal patterns of infection and transmission. We recommend the implementation of hantavirus surveillance studies that aim to increase sample collection during fall and winter seasons and compensate for the systematic lack of evidence for such periods. These programs should consider animal welfare and availability of resources.

Finally, the identification of specific hantaviruses circulating would give crucial information about the risks for human health and the epidemiological role of each rodent host. Not all hantaviruses have known zoonotic potential, and new hantaviruses are periodically described. The implementation of additional analytical tests and specimen collection (e.g., organs) to characterize hantaviruses would enable deeper studies regarding hantavirus ecology and evolution.

CONCLUSIONS

Hantaviruses have a complex dynamic that involves multiple hosts and pathogenic and non-pathogenic viral strains. We found that multiple hosts were exposed to hantavirus and identified new hosts and new areas of hantavirus circulation, highlighting the value of extended, permanent, and broad surveillance programs. Rodent captures presented an inconsistent pattern across years, with a typical peak in summer. Seroprevalence exhibited a bimodal trend, although it was biased by the lower sample size during winter months. Seroprevalence and captures presented great variability across species, time, and geographical locations, revealing the potential relevance of fine-scale features and the long-term gradual processes involved. This article advances our understanding of hantavirus circulation in wildlife across the United States and expands the current list of plausible hantavirus hosts.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Original data were obtained from NEON (National Ecological Observatory Network (NEON), 2023a, 2023b).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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