A LONGITUDINAL STUDY OF BAYOU VIRUS, HOSTS, AND HABITAT

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Abstract. What is currently known about the ecology of North American hantaviruses has come largely from studies on Sin Nombre virus (SNV). We conducted a longitudinal study of Bayou virus (BAYV), the second-leading agent of hantavirus pulmonary syndrome in the United States. Antibodies to hantavirus were detected from Oryzomys palustris (most commonly infected species), Sigmodon hispidus, Peromyscus leucopus, Reithrodontomys fulvescens, and Batomys taylori. However, only O. palustris had viral RNA in tissues and excreta, suggesting that antibodies detected in other species may have resulted from spill-over infection. Seroprevalence rates averaged around 16% for O. palustris and varied seasonally. The heaviest males exhibited the highest levels of seroprevalence. Seroprevalence was higher in coastal prairie (20.0%) than old-fields (10.5%) and was associated with host abundance. These patterns are similar to those of SNV and can be used in identification of potentially at-risk areas.

INTRODUCTION

Hantaviruses are rodent-borne zoonotic agents that cause hemorrhagic fever with renal syndrome (HFRS) in humans throughout Europe and Asia.1 In the Americas, hantaviruses cause an often-fatal cardiopulmonary syndrome, hantavirus pulmonary syndrome (HPS).2 At least 29 hantavirus strains have been identified worldwide (12 in North America), each with a primary rodent host species from the family Muridae.3 Transmission of hantaviruses to humans is thought to occur by inhalation of infectious aerosols,4 whereas it is believed that viral transmission between rodents occurs via biting during aggressive encounters5 because an animal in which the virus establishes a persistent infection sheds virus in urine, feces, and saliva.6 Because there is as yet no cure for HFRS or HPS, prevention is our main defense against illness, but prevention is only as effective as our understanding of viral persistence, maintenance, and transmission in a dynamic host population.

Previous studies of the natural history of the New World hantaviruses have primarily focused on Sin Nombre (SNV, agent of the 1993 Four Corners HPS outbreak and major agent of HPS in the United States7), the natural history of the other 11 North American strains is largely unknown. One of the least understood of these is Bayou virus (BAYV, whose primary host is the marsh rice rat, Oryzomys palustris).8,9 However, BAYV ranks second to SNV among strains implicated in HPS in the United States,10 and it occurs in a region that currently is experiencing rapid urban development. Numerous large and growing human population centers are located within its geographic distribution (e.g., Houston, Galveston, Baton Rouge, New Orleans), making it of particular health concern to the >6.5 million people who live within its range. Nisbett and others11 suggested that it would be fruitful to study BAYV vis-à-vis SNV, a primary objective of our study.

Previous studies have endeavored to identify environmental variables that are correlated with, or predictive of, the presence or prevalence of SNV, its primary host (Peromyscus sp.), and/or cases of HPS. Antibody seropositivity in rodents and cases of HPS have been correlated with aspect, elevation, and vegetative cover, including changes to vegetation induced by El Niño Southern Oscillation (ENSO) precipitation dynamics. Specifically, HPS and seropositivity are associated with piñon-juniper woodlands at 1,800–2,500 m above sea level (ASL) on north-facing slopes during spring and summer following an ENSO.12–18 Although these natural history studies of hantavirus have suggested that landscape structure may play an important role in viral epidemiology, most of these studies did not quantify habitat composition and configuration explicitly7,12,19,20 or only examined habitat at a broad spatiotemporal scale.14,18,21,22 However, data from finer scales are needed for a clearer quantification of the role habitat plays in the abundance and distribution of hosts, viral prevalence, and, consequently, human risk.10 To gain further insight into the transmission of hantavirus in rodent populations and the ecological mechanisms that maintain these complex virus-host relationships, we conducted a longitudinal survey of rodents at the Peach Point Wildlife Management Area, Brazoria County, Texas. Herein we report the population fluctuations of the rodents and the virus harbored by the rodents. Male sex and high weight correlated with a high prevalence of antibody and RNA in O. palustris. Finally, we documented the shedding of virus in saliva and urine of O. palustris, which suggests three possible routes of transmission.

MATERIALS AND METHODS

Study site. We conducted our study at the 4,174.5–ha Peach Point Wildlife Management Area in Brazoria County, Texas (~100 km south of Houston), where rodents with antibodies to hantavirus previously have been detected.23 Work was initiated in March 2002 and concluded in May 2004. The topography of this area is low and relatively constant, ranging from 0 to 5 m ASL, and is composed of brackish to saline coastal prairie that grades farther inland to slightly higher ground with freshwater marshes, old-fields, and trees. The area experiences periodic tropical storms, managed burns, and cattle grazing. The terrain is periodically flooded during heavy rains or tropical storm surge. Unlike sites for previous work on SNV, this is an environment that is relatively unaffected by ENSO and experiences no large changes in elevation. Average precipitation is 133.35 cm/yr. Average high temperatures range from 18°C in winter to 33°C in summer, and average lows do not fall below freezing even in winter (8°C).

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Rodent sampling. Rodents were live-trapped on 4 grids placed adjacent to water (each with 100-113 Sherman live-traps [H.B. Sherman Traps, Inc., Tallahasee, FL] at 10-m intervals, placed in approximately 5 lines of 20 traps as permitted by variations in shoreline geography) for 4–6 consecutive nights per season during 3 years (May, August, December 2002; March, May, August 2003; May 2004). Grids 1–2 were in old-field habitat whereas Grids 3–4 were in coastal prairie. Grids were separated by at least 1 km to represent individual populations. Captured rodents were toe-clipped or injected with a Passive Integrated Transponder (Biomark, Inc., Boise, ID) for unique individual identification, blood (0.5–1.0 mL) was collected from the retro-orbital plexus following the protocol of Mills and others, and demographic and morphometric data were recorded. Saliva was collected by swabbing the mouth, and urine was collected by swabbing the urine left inside a clean zip-lock bag into which rodents were placed for handling. Blood, saliva, and urine samples were each placed directly into 1 mL of minimum essential medium containing 10% fetal bovine serum and 100 μg/mL and 100 units/mL, respectively, of penicillin and streptomycin (Fisher Scientific, Atlanta, GA). All samples were stored immediately in a liquid-nitrogen tank and transported to the Museum of Texas Tech University, where they were stored in a –70°C freezer until analysis. Rodents were released immediately at the site of capture. Live-trapping and mark-recapture methods allow for the collection of serological data without altering rodent population structure. Rodents were trapped simultaneously on separate traplines (25 traps/trapline, with traps placed at 10-m intervals) for harvest of lung, kidney, liver, spleen, and heart tissues. Traplines were placed at least 200 m from grids; we never captured a grid animal on a trapline. Traplines were also run in March 2002 (2 months before grids were established) in a pilot survey to assess rodent species diversity and distribution. Standardized procedures were followed for the humane capture, handling, marking, and bleeding of rodents (Texas Tech University Animal Care and Use Committee permit 03049-08); transport of biohazardous materials; and preservation of human safety.7

Immunofluorescent assay. IgG antibodies to hantavirus antigens were detected by immunofluorescent assay (IFA), following the method of Chu and others.24 Samples were tested initially at a dilution of 1:32. End-point titers were determined for all positive samples. The specificity of the test was confirmed in each experiment by both positive and negative controls. The positive controls we used BAYV-infected cells, and for the negative controls we used Vero E6 cells.

Extraction of RNA and nested RT-PCR. Very little is actually known for hantaviruses with respect to the presence and absence of viral RNA; our study is one of the first attempts to correlate viral RNA and antibody presence. Total RNA from blood, saliva, and urine samples from antibody-positive animals was extracted and amplified by nested RT-PCR as described by Chu and others.25 For blood samples, a clot from 100 μL of collected blood was ground in a 1.5-mL microfuge tube containing 1 mL of Trizol (Invitrogen, Bethesda, MD) using a disposable tissue grinder (Fisher, Atlanta, GA). The manufacturer’s protocol of extracting total RNA was followed. To extract RNA from saliva and urine, we used the RNA extraction kit and protocol from Epicentre (Madison, WI). Extracted RNA was stored in 10 μL of RNase-free distilled water and subjected to RT-PCR with outer generic primers from the G2 region of the M segment using a one-step RT-PCR kit (Invitrogen, San Diego, CA). The sequence of the forward outer generic primer was 5'-GAYACAGCHCATGGTDDG-3' and the reverse outer generic primer was 5'-CCHAGIAGCCAYTCCWCGA-3'. The M segment generic primers were selected from the consensus regions shown in an alignment from North and South American hantaviruses. The size of the PCR amplicon derived from the G2 region of the M segment is 618 nucleotides which includes primers. The sequence of the forward inner generic primer was 5'-HYTRGGICAYTGATGATGATG-3' and the reverse inner generic primer was 5'-TGRAWAGARTCYYTWGTDCCAT-3'. RNA was reverse transcribed for 45 min at 45°C and then subjected to PCR for 35 cycles (30 seconds at 94°C, 30 seconds at 50°C, 45 seconds at 72°C) using a one-step RT-PCR kit (Invitrogen, San Diego, CA). Two microliters of each amplicon were further amplified by nested PCR for an additional 35 cycles (30 seconds at 94°C, 30 seconds at 55°C, 45 seconds at 72°C) with the M segment inner generic primers using the PCR Core Kit (Roche, Penzberg, Germany). Amplicons were analyzed in a 1.2% agarose gel in Tris-Acetate-EDTA buffer and visualized by ethidium bromide staining.

Cloning, sequencing, and genetic analyses. PCR amplicons were extracted from agarose gels using the glass-bead-based DNA extraction kit (BIO101, La Jolla, CA) and cloned into pGEM-T (Promega, Madison, WI). T7 and SP6 primers were used to sequence both directions of the closed PCR amplicons. At least 3 clones of each amplicon were sequenced using the Big Dye version 3.0 terminator sequencing system (ABI, Foster City, CA) as described by the manufacturer. The nucleotide and deduced amino acid sequences of the m segment viral genome from the rodent samples were compared with several New World hantaviruses using the Align X program of Vector NTI software (Informax Inc., Bethesda, MD). Phylogenetic analysis was performed using maximum likelihood program in PAUP software version 4.0 b10 (Sinauer Associates Inc., Sunderland, MA). In maximum likelihood analysis, phylogenetic trees were obtained by the heuristic search method using weighting schemes of 4:1 in transitions over transitions. Gaps were treated as missing data or as a fifth character. Bootstrap confidence limits were obtained by 1,000 heuristic search repetitions.

Habitat assessment. Habitat structure was assessed within a 3-m-radius circle centered around each rodent trap. Habitat structure was assayed in terms of composition (vegetation type and height for woody and herbaceous species, percent ground cover of 10 mutually exclusive categories [grass, forb, shrub, tree, duff, bare ground, water, reed, sedge, and other], number of trees, tree height) and configuration (distance to nearest woody vegetation, distance to nearest anthropogenic structure [e.g., road, building]), and distance to water, following standardized protocols.26–29 Percent cover variables were arcsine-square root transformed for analysis.

Statistical analyses. Relationships among ecological parameters and viral prevalence and dynamics were then assessed. χ² tests were used to assess differences in serological status by date and weight class; Fisher’s exact test (2×2 χ²) was used to compare serological status by macrohabitat type and sex. Stepwise multiple regression with Pearson correlations was used to determine the nature of trends between seropositivity and habitat variables. Differences in average vegetation
height between macrohabitats were detected using Satterthwaite’s t test for samples with unequal variances; differences in height by date were evaluated with analysis of variance. All statistical analyses were performed with SAS version 8.2.

RESULTS

Prevalence of antibodies to hantavirus antigens and RNA prevalence. Over a 26-month period, 1277 rodents from 5 species were captured, marked, and released on the 4 trapping grids; an additional 327 were harvested from traplines. The 1,277 mark-recapture rodents consisted of 549 Oryzomys palustris, 176 Sigmodon hispidus, 369 Reithrodontomys fulvescens, 16 Peromyscus leucopus, and 167 Baiomys taylori. Among these, 93 (7.3%) individuals were antibody-positive and 67 (5.2%) were RNA-positive (Table 1). Seroconversion was observed in 9 O. palustris and 1 R. fulvescens (Table 2). Of the seropositive individuals, 88 were O. palustris, and 67 of these had viral RNA in the blood. Viral RNA also was detected in 52.9% of saliva samples and 35.3% of urine samples from (seropositive) O. palustris. Antibodies to BAYV also were detected in 2 (1.1%) S. hispidus, 1 (0.3%) R. fulvescens, 1 (6.2%) P. leucopus, and 1 (0.6%) B. taylori; however, we did not detect BAYV RNA from these species, suggesting that the antibodies were a result of a nonproductive persistent infection or spill-over that stemmed from interactions with the major BAYV reservoir, O. palustris.

Seasonal variation of rodents and prevalence of BAYV. Hantavirus antibody and genomic RNA was noted in O. palustris throughout the study period. The virus was temporally dynamic: seroprevalence in O. palustris varied from 3.2% (December 2002) to 28.3% (August 2003) (Figure 1). These values are similar to the rates and dynamics of SNV and were positively associated with rodent abundance. Most species experienced fluctuations in abundance, particularly in December 2002, when O. palustris increased 1.5- to 1.7-fold, S. hispidus increased 2.6- to 2.8-fold, R. fulvescens increased 8- to 15-fold, and B. taylori increased 6- to 12-fold. (Numbers of P. leucopus were consistently very low.) These numbers decreased to pre-December levels by March 2003. However, seroprevalence levels fluctuated only in O. palustris as rates of infection were relatively low in the other species.

Sex and body size patterns of seroprevalence and RNA prevalence. Strong sex and size biases in BAYV infection in O. palustris were observed. We captured 305 males (74 seropositive, 24.3%) and 244 females (14 seropositive, 5.7%); males were significantly more likely to be seropositive than

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<th>Bd</th>
<th>Sa</th>
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females (Fisher’s exact test $F = 218, P = < 0.0001$). Furthermore, only 19 of 61 males in the 46–55 g body weight range were antibody-positive, whereas 29 of 66 in the 56–65 g range and 20 of 28 in the ≥ 66 g range were seropositive, meaning that the heaviest males exhibited the highest levels of seroprevalence ($\chi^2 = 12.65, P = 0.0018$). Whereas the total RNA prevalence was less than that for antibody prevalence in males (59 of 305, 19.3%), it was more than 5 times higher than that detected in females (8 of 244, 3.3%).

**Antibody and RNA conservation among recaptured rodents and virus shedding in saliva and urine.** Among the 1,277 captured, marked, and released rodents from the four trapping grids, 549 (43.0%) were *O. palustris*. Of these, 45 (8.2%) were recaptured between months over the course of the study. Nine of these animals (20%) seroconverted and seven (15.6%) maintained antibody to BAYV over the duration of the study (Table 2). The remaining *O. palustris* had no detectable antibody. Four of the 9 seroconverted rodents were RNA-negative in blood, saliva, and urine. Among the remaining 5 RNA-positive rodents, only 1 animal had RNA in its saliva and urine (however, we did not obtain a urine sample from 2 of the 5). Among the 7 recaptured seropositive *O. palustris*, 3 exhibited RNA conversion in blood, and 3 had viral RNA in saliva but not urine (Table 2). We screened for the presence of viral RNA from both saliva and urine samples from 34 seropositive *O. palustris*. Of these, 8 had no viral RNA in blood, saliva, or urine. However, 18 (52.9%) had viral RNA in saliva, 12 (35.3%) had viral RNA in urine, and 7 (20.6%) had viral RNA in blood (Table 2).

**Sequence and phylogenetic similarity to other American hantaviruses.** The G2 region of the M segment PCR amplicon from blood samples from 6 *O. palustris* was cloned, sequenced, and compared with representatives from both North and South American hantaviruses. The 6 virus sequences isolated showed 7–8% nucleotide sequence divergence and 1–3% amino acid differences with BAYV (GenBank L36930, which was from a fatal HPS case in Louisiana) (Table 3). BAYV appears within a “North American” clade, distinct from the South American group of hantaviruses (Figure 2). BAYV is harbored by an oryzomyine rodent that is likely of Central American origin. Oryzomyines radiated primarily southward, where a number of species harbor a variety of the South American hantaviruses. Thus, the notion that viral phylogenies faithfully reflect host rodent phylogenies is not exact, or more precisely is only true “within continents” and not within the Western Hemisphere as a whole.

**Habitat influence on seroprevalence and RNA prevalence.** The 4 trapping grids represented a gradient of woody vegetation cover, ranging from no trees and very few and small shrubs at Grid 3, to more and taller shrubs at Grid 4, to early successional old-field with trees and shrubs at Grid 1, to later-seral-stage old-field at Grid 2 (Table 4). Not surprisingly, there were significant differences in vegetation height by macrohabitat (t = –4.57, $P = 0.0027$) and season ($F = 7.10, P = 0.0346$), with taller vegetation occurring on Grids 1 and 2 and during the warmer months. Rodent abundance and seroprevalence varied by trapping grid (Grid 1: 5 of 40, 12.5%; Grid 2: 5 of 54, 9.2%; Grid 3: 4 of 183, 24.6%; Grid 4: 9 of 75, 12.0%). Some macrohabitat differences in seroprevalence were detected: levels were much higher in coastal prairie (Grids 3 and 4 collectively, 20.0%) than in old-fields (Grids 1 and 2 collectively, 10.5%) (Figure 3). Some other habitat variables, being correlated with macrohabitat type, also revealed relationships. For example, there was a significant negative relationship between seroprevalence and vegetation height ($F = 3.44, P = 0.0013$), and number of trees ($R = –0.16$). There were slight but significant negative associations between seroprevalence and percent woody vegetation cover (trees: $R = –0.17$; shrubs: $R = –0.07$), and a positive association with grass cover ($R = 0.17$) ($P < 0.05$ for all Pearson’s R-values). Seropositive mice were located significantly closer to water ($R = 0.29$) and farther from trees ($R = –0.21$) than seronegatives. However, there was no effect of the proximity of anthropogenic structures ($R = 0.00$).

![Figure 1](image.png)

**Figure 1.** Number of *O. palustris* captured (white bars) with number of seropositives (gray bars) by date.
**Figure 2.** Phylogenetic trees of PCR amplicon from rodents captured at Peach Point Wildlife Management Area, Texas (“PP” entries), and other hantaviruses. Numbers on branches indicate bootstrap support.

**Table 4: Vegetative characteristics of the four trapping grids**

<table>
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<tr>
<th>Macrohabitat</th>
<th>Grid 1 Old-field</th>
<th>Grid 2 Old-field</th>
<th>Grid 3 Coastal prairie</th>
<th>Grid 4 Coastal prairie</th>
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<tr>
<td>No. plant species</td>
<td>34</td>
<td>37</td>
<td>10</td>
<td>11</td>
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<tr>
<td>Height (range) (cm)</td>
<td>123.12</td>
<td>88.35</td>
<td>45.51 (31.25–54.42)</td>
<td>41.37 (25.54–49.93)</td>
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<td>Grass (range)</td>
<td>58.62 (57.29–63.75)</td>
<td>50.62 (47.82–58.77)</td>
<td>87.34 (86.20–88.44)</td>
<td>59.82 (57.63–61.63)</td>
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<tr>
<td>Forb (range)</td>
<td>18.94 (17.26–20.98)</td>
<td>25.26 (16.05–29.27)</td>
<td>0.025 (0–0.05)</td>
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<tr>
<td>Shrub (range)</td>
<td>1.68 (1.63–1.79)</td>
<td>0.13 (0.11–0.13)</td>
<td>0.48 (0.47–0.48)</td>
<td>29.20 (29.00–29.70)</td>
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<td>Tree (range)</td>
<td>1.88 (1.67–2.06)</td>
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<td>Duff (range)</td>
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<td>Bare ground (range)</td>
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<td>Reed (range)</td>
<td>6.75 (6.34–8.05)</td>
<td>1.51 (0.86–2.14)</td>
<td>0.03 (0–0.05)</td>
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<td>Sedge (range)</td>
<td>0.88 (0.82–1.04)</td>
<td>3.01 (2.52–3.52)</td>
<td>6.51 (6.16–7.68)</td>
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<td>9.63 (6.16–7.68)</td>
<td>16.42 (3.88)</td>
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<td>9.06</td>
</tr>
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Means (for plant height and percent ground covers) are given as percentages and are averaged over all time periods.

**Dominant plant species** (based on cover), listed in decreasing order:

- *Spartina spartinae*
- *Rubiis trivialis*
- *Distichlis spicata*
- *Borrichia frutescens*
- *Iva annua*
- *Spartina patens*
- *Batis maritima*
- *Spartina patens*
- *Salicornia spp.*
- *Scirpus robustus*
- *Ambrosia trifida*
- *Sambucus canadensis*
- *Borrichia frutescens*
- *Verbena brasiliensis*
- *Juncus effusus*
- *Scirpus robustus*
- *Juncus effusus*
- *Puccinellia distichophylla*
- *Rumex crispus*
- *Ambrosia trifida*
- *Cardiospermum halicacabum*
- *Juncus effusus*

**Microhabitat**

- BCCV
- New York
- El Moro Canyon
- HTN 118
- SR 11
- Blue River
- SNV CC107
Field studies have suggested that horizontal transmission of hantaviruses occurs through aggressive encounters among animals, most frequently among older males. Our results would corroborate this hypothesis, as the highest proportion of infected animals was among heavier (and thus older) males. During such direct encounters, transmission may occur via two routes. One is through biting, whereby the virus is transferred via the saliva from an infected rodent to the bloodstream of an uninfected individual. Alternatively, the route of transmission may be via urine released and aerosolized during fighting, entering an uninfected individual’s respiratory pathway. In laboratory studies, Apodemus infected with Hantaan (HTN) shed virus in saliva, urine, and feces, with viral shedding occurring through urine up to one year after infection. Shedding of BCCV in excreta in S. hispidus was shown 5 months after infection. SNV has been found in P. maniculatus salivary glands 60 days and in kidney 7 months after infection but not in saliva or urine. In our study, we found that BAYV was shed in saliva and urine for at least 3 months in recaptured O. palustris, suggesting that excreta may play an important role in BAYV transmission among rodents. If so, then transmission may also occur through a third route that does not involve direct interactions among rodents. Areas with high rodent density may experience environmental “contamination” from excreta infected with shed virus. In such areas, uninfected individuals may become infected by coming into contact with excreta. Our data would support this premise because we documented the highest rates of seroprevalence in areas with the highest density of O. palustris. However, areas of high abundance are also areas where more aggressive encounters (and, thus, direct transmission) among rodents would be expected to occur. Further research would be necessary to tease apart the relative importance of these possible modes of transmission.

With respect to direct transmission, most efforts have focused on understanding what age and sex rodents transmit virus as well as identifying which aggressive behaviors might correlate with transmission. It has been well-documented that larger and older males are more likely to be seropositive. Likewise, our study of BAYV in O. palustris found that most of the seropositives were males and that the heaviest males were significantly more likely to be seropositive. Interestingly, in a transmission study of BCCV in S. hispidus in the laboratory, neither the age nor the sex of the rodents influenced susceptibility to infection. Similarly, Nisbet and others found that 86% of all SNV-seropositive peromyscines were males, whereas only 47% of the S. hispidus seropositives were males. It has been hypothesized that horizontal transmission related to intraspecific encounters is the primary means of hantavirus transmission among the reservoir hosts but it could be that other transmission dynamics are at play due to the nature of both interspecific interactions and intraspecific social behaviors. In our study, both males and females were in reproductive status throughout the year (data not shown). Continuous reproduction may be favorable for transmission of the virus among males in territorial disputes over access to females.

The presence and prevalence of SNV have been shown to be spatially heterogeneous and temporally dynamic. Like SNV, Bayou is spatially patchy and temporally dynamic. At-risk areas for BAYV are associated with high abundance of O. palustris, which is itself associated with coastal prairie macrohabitat. Because O. palustris is not a peridomestic species, however, human risk is relatively low. The importance of environmental heterogeneity on disease transmission has only recently been explored. Identifying associations between habitat parameters and seropositivity is a crucial step needed for effective implementation of protocols designed to minimize hantavirus-human encounters and viral emergence.

DISCUSSION

Transmission studies of several hantaviruses such as SNV, Seoul virus, and Black Creek Canal Virus (BCCV) in their respective reservoirs have shown that virus is transmitted horizontally. Field studies have suggested that horizontal transmission of hantaviruses occurs through aggressive encounters among animals, most frequently among older males. Our results would corroborate this hypothesis, as the highest proportion of infected animals was among heavier (and thus older) males. During such direct encounters, transmission may occur via two routes. One is through biting, whereby the virus is transferred via the saliva from an infected rodent to the bloodstream of an uninfected individual. Alternatively, the route of transmission may be via urine released and aerosolized during fighting, entering an uninfected individual’s respiratory pathway. In laboratory studies, Apodemus infected with Hantaan (HTN) shed virus in saliva, urine, and feces, with viral shedding occurring through urine up to one year after infection. Shedding of BCCV in excreta in S. hispidus was shown 5 months after infection. SNV has been found in P. maniculatus salivary glands 60 days and in kidney 7 months after infection but not in saliva or urine. In our study, we found that BAYV was shed in saliva and urine for at least 3 months in recaptured O. palustris, suggesting that excreta may play an important role in BAYV transmission among rodents. If so, then transmission may also occur through a third route that does not involve direct interactions among rodents. Areas with high rodent density may experience environmental “contamination” from excreta infected with shed virus. In such areas, uninfected individuals may become infected by coming into contact with excreta. Our data would support this premise because we documented the highest rates of seroprevalence in areas with the highest density of O. palustris. However, areas of high abundance are also areas where more aggressive encounters (and, thus, direct transmission) among rodents would be expected to occur. Further research would be necessary to tease apart the relative importance of these possible modes of transmission.

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