

ECOLOGICAL CORRELATES OF SEROLOGICAL STATUS FOR BAYOU VIRUS IN *Oryzomys palustris* (RODENTIA: SIGMODONTINAE)

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ABSTRACT: Home range size and habitat use of seropositive *Oryzomys palustris*, primary host for the Bayou strain of hantavirus, were compared to that of seronegative individuals from March 2002 - August 2003 in a longitudinal field population study. There were significant macrohabitat associations with serological status, but selection for most microhabitat variables did not differ between seropositive and seronegative animals. Seropositive adult males moved farther (had larger home ranges) than did seronegatives and were larger in terms of mass and testes size. These patterns may reflect the influence of infection on rodent ecology in terms of dominance and access to preferred habitats.

RESUMEN: Correlaciones ecológicas del estatus serológico para el virus Bayou en *Oryzomys palustris* (Rodentia: Sigmodontinae). Durante dos años consecutivos (marzo 2002 a agosto del 2003), estudiamos la ecología básica de *Oryzomys palustris*, el reservorio natural de la cepa Bayou de Hantavirus, bajo la hipótesis de que el estado serológico afectaba dos variables demográficas: el tamaño del ámbito de hogar (home range) y el uso de hábitat. Encontramos asociaciones significativas entre uso del macrohábitat y el estado serológico, pero no se encontraron diferencias en el uso del microhábitat entre animales seropositivos y seronegativos. Sin embargo, machos adultos serológicamente positivos tuvieron ámbitos de hogar más amplios que los seronegativos, además de ser más grandes en términos de masa corporal y tamaño de sus testículos. Sugerimos que estos patrones pueden reflejar la influencia de la infección sobre la ecología de roedores, posiblemente en función de dominancia social y acceso a hábitats preferidos.

Key words. BAYV. Habitat selection. Hantavirus. Home range size. Texas.

Palabras clave. BAYV. Hantavirus. Selección de hábitat. Tamaño del radio de acción. Texas.

INTRODUCTION

Hantaviruses cause human illnesses throughout much of the world, with each hantavirus being associated with a primary host rodent species (Schmaljohn and Hjelle, 1997). Although most studies have failed to reveal effects of infection in terms of most variables, such as respiratory function (O'Connor et al., 1997) or survivorship (Douglass et al., 2001) in adult *Peromyscus maniculatus*, physical condition in *Myodes glareolus* (Yanagihara et al., 1985), and maturation rates, survival, and fecundity in adult *Rattus norvegicus* (Childs et al., 1989), there is an emerging body of evidence suggesting that hantaviruses have subtle but significant effects on their hosts. For example, studies have revealed that infected animals show lower survival rates for adult *Myodes glareolus* (Kallio et al., 2007) and neonatal *Mus musculus* (Tsai et al., 1982; Kim and McKee, 1985), lower body mass in juvenile *Peromyscus maniculatus* (Borucki et al., 2000), and slower weight gain in juvenile *Rattus norvegicus* (Childs et al., 1989).

Such effects typically have become apparent only after examining long-term (i.e., multi-year) datasets from field studies, indicating the complexity of hantavirus-host relationships in nature. Our understanding of the influence of the environment on viral presence and prevalence and, ultimately, on human disease risk is still incomplete. However, it has been hypothesized that ecological disturbance may play a role in the emergence of hantaviruses as human pathogens (Ruedas et al., 2004; Sauvage et al., 2007); therefore, given the accelerating pace of anthropogenic changes to the environment, it is imperative that ecological variables and/or drivers be identified that are associated with such risk. Previous studies examining ecological variables (e.g. Calisher et al., 1999; Mills et al., 1999; Root et al., 1999; Biggs et al., 2000; Boone et al., 2000; Yates et al., 2002; Armién et al., 2004; Olsson et al., 2005; Suzán et al., 2006) have typically focused on a macroscopic scale, identifying regional variables associated with hantavirus presence and prevalence in host populations. From these

studies, the likelihood of detecting hantavirus antibodies in hosts has been correlated with rodent density, topography, and vegetation at a macroscopic scale (Root et al., 1999; Biggs et al., 2000; Boone et al., 2000; Glass et al., 2000; Olsson et al., 2002; Yates et al., 2002; Olsson et al., 2005; Suzán et al., 2006), but there are relatively few published studies examining possible ecological associations of host or hantavirus presence or prevalence at a finer scale of resolution, such as the scale of an individual host's territory (Abbott et al., 1999; Lozada et al., 2000; Gottesman et al., 2004). A consideration of scale is crucial, however, to our understanding of the environmental variables associated with disease risk (Giuggioli et al., 2005) because there may be differences in the relative influences of landscape vs. local variables (Orrock et al., 2000; Suzán et al., 2006) and host abundance vis-à-vis host seroprevalence in seeking to explain seasonal patterns in primary human cases (Davis et al., 2005). We therefore took a multi-scaled approach in examining hantavirus-host relationships at macrohabitat and microhabitat scales.

By examining patterns of host movement (i.e., home range size) and habitat selection (defined here as greater use of habitat types than would be expected based upon the availability of habitat types on the landscape; Johnson, 1980) and relating these patterns to the serological status of hosts, we attempt to gain a more complete understanding of viral-host relationships. Moreover, certain morphological traits in rodent hosts (e.g. body mass, testes size) are associated with factors that may alter an individual's risk of infection, such as aggression or ranging (Escutenaire et al., 2002; Hinson et al., 2004). Thus, identification of both ecological and morphological traits associated with viral presence and prevalence in a host population is necessary in determining not only why certain hosts within a population are more susceptible to infection than are others, but also which factors may facilitate or inhibit viral transmission and influence other aspects of infectious disease ecology and epidemiology (Abbott et al., 1999; Giuggioli et al., 2005).

The rodent genus *Oryzomys* is a large, diverse, and geographically widespread group distributed primarily in the Neotropics (Musser and Carleton, 2005), but with one species (*O. palustris*) extending northward into the United States (Hall, 1981). Although there is some evidence for coevolution of hantaviruses and their rodent hosts (Plyusnin and Morzunov, 2001; Yates et al., 2002), recent studies have revealed interesting deviations from this pattern. Chu et al. (2008) described Playa de Oro virus (OROV), a new hantavirus from western Mexico, from both *O. couesi* and *Sigmodon mascotensis*. Although *O. couesi* is the apparent primary host for OROV, phylogenetic analyses indicated that OROV clusters with Catacamas (also from *O. couesi*), Bayou (*O. palustris*), and Black Creek Canal and Muleshoe viruses (both from *S. hispidus*). Thus, the evolutionary and geographic associations of hantaviruses with the northern-most oryzomyines and other sympatric rodent species is of increasing interest, and identifying ecological characteristics of the viruses and their rodent hosts may be of particular interest in providing information that may help to explain departures from the general hantavirus-host coevolutionary pattern.

We compared patterns of movement and habitat use between seronegative and seropositive host rodents in a natural setting at a macrohabitat scale (whole trapping grids, ~7600-8100 m²), and then compared these to patterns of overall habitat availability at a finer (3-m radius from individual traps) scale. Our focus was on Bayou virus (BAYV) and its primary host, *Oryzomys palustris*, which has been linked to Hantavirus Pulmonary Syndrome in the southern U.S. (Torrez-Martinez et al., 1998).

MATERIALS AND METHODS

Our study was conducted at the Peach Point Wildlife Management Area (PPWMA) in Brazoria County, Texas (Fig. 1). Located just inland of the Gulf Coast Intracoastal Waterway, the 4174.5-ha PPWMA consists of two macrohabitats: treeless coastal prairies and uplands with some trees. All portions of the study area were <5 m in elevation.

Simultaneous habitat and rodent population sampling took place seasonally (March, May, August, December) from March 2002-August 2003 on four mark-recapture trapping grids. Each grid consisted of ~100 Sherman-live traps (H.B. Sherman Traps, Tallahassee, Florida, USA) spaced approximately at 10-m intervals, which defined roughly rectangular trapping grid areas of between 7600 and 8100 m² in area, with variation due to shoreline topography and storm flooding. Grids were placed adjacent to open water, a component of preferred habitat for *O. palustris* (Wolfe 1982, 1985), and were separated by at least 1 km. Traps were opened for 4-6 nights/season in both macrohabitat types (2 replicate grids per macrohabitat). Grid-captured rodents were toe-clipped (in 2002) or injected with a Passive Integrated Transponder (Biomark, Inc., Boise, Idaho, USA) (in 2003) for individual identification; after demographic data were recorded



Fig. 1. Map of study site: Peach Point Wildlife Management Area (PPWMA) in Brazoria County, Texas, USA.

rodents were released at the site of capture. Aging of animals into one of three categories (adult, sub-adult, or juvenile) was based on multiple criteria including weight (juvenile: <30 g; sub-adult: 31-49 g; adult: >50 g), pelage color, and reproductive condition (position of testes, condition of vaginal opening), based on Hall (1981) and Wolfe (1985). Grid-captured rodents were used for antibody, home range, and habitat selection analyses. Confirmation of viral presence came from tissues harvested from animals captured on separate traplines, usually placed at least 0.5 km away from grids and consisting of 50 traps spaced every 10 m. Trapline captures were taken to an open-air processing center and euthanized with ether. All protocols for capture, handling, and euthanasia of rodents were approved by the Texas Tech University Animal Care and Use Committee (permit 03049-08). Standardized protocols from Mills et al. (1995) were followed regarding the handling of biohazardous materials and preservation of human safety.

Assays using rodent excreta, blood, and tissues were conducted at the Southern Research Institute (Birmingham, Alabama, USA) and adhered to BSL2 and BSL3 practices and CDC regulations. To detect anti-Bayou virus (BAYV) IgG, BAYV-infected Vero E6 cells (ATCC, CRL 1586) were used. Briefly, a monolayer of Vero E6 cells was inoculated with BAYV after which the virus was cultured for 10 days in a tissue-culture flask with DMEM medium (Invitrogen, Bethesda, Maryland, USA) containing 10% FBS and 100 units/mL penicillin and 100 µg/mL of streptomycin. At 10 days post-inoculation, cells were trypsinized and resuspended in culture medium and transferred onto a spotted glass slide (Cel-line, Newfield, New Jersey, USA). The following morning, infected cells on spotted glass slides were washed with phosphate buffered saline (PBS), dried in a biological safety cabinet at the BSL3 containment laboratory, fixed with pure ice-cold acetone, and irradiated with ⁶⁰Co for four hours in a container packed with dry ice. Slides of deactivated antigen were kept at -80°C in a BSL2 laboratory until used.

To evaluate antibody prevalence among collected rodents, an initial serum dilution of 1:32 in PBS was used. Antibody-positive sera were tested further to determine reciprocal endpoint titers with 2-fold serial dilutions as described in Chu et al. (1995, 2003). Briefly, 40 µL of each diluted serum sample from each rodent were added to individual wells of the antigen slide and incubated for 30 minutes at 37°C in a moist chamber. Slides were washed twice for 5 minutes with PBS buffer and rinsed briefly with distilled water. Slides were then

air-dried inside a biological safety cabinet, after which 25 µL of diluted fluorescein isothiocyanate (FITC)-labeled anti-mouse IgG (KPL, Gaithersburg, Maryland, USA) in PBS buffer were added. Slides were incubated, washed, and dried as described previously and then mounted with appropriate media (90% glycerol in PBS buffer). A fluorescent microscope (Axioscope; Zeiss, Oberkochen, Germany) was used to visualize the presence (fluoresced circles) or absence (no fluorescence) of anti-BAYV IgG in rodent samples. Positive control serum from rodents confirmed to have anti-BAYV IgG and negative control serum from uninfected laboratory Balb/c mice were included for comparison.

Total RNA from blood and tissues derived from antibody-positive and some randomly selected rodents was extracted and amplified by nested RT-PCR as described in Chu et al. (1995, 2003). Briefly, 0.1 g of tissue was ground in a 1.5-mL microfuge tube containing 1 mL of Trizol (Invitrogen, Bethesda, Maryland, USA) using a disposable tissue grinder (Fisher Scientific, Atlanta, Georgia, USA) and following the manufacturer's protocol for extraction of total RNA. To extract RNA from blood samples, clots were transferred to a 1.5-mL microfuge tube containing 1 mL of Trizol and ground as above. To extract RNA from urine and saliva, an RNA extraction kit (Epicentre, Madison, Wisconsin, USA) was used, following the manufacturer's protocol. Extracted RNA was diluted with 10 µL of DEPC-treated 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, California, USA). The sequence of the forward outer generic primer was 5'-GAYACAGCHCATGGIGTDGG-3' and the reverse outer generic primer was 5'-CCHAGIAGCCAYTCWCCWGA-3'. The PCR program for reverse transcription of RNA was one cycle of 45 minutes at 45°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 45 seconds at 72°C. The G2 region of the M-segment generic primers were selected from consensus regions in alignment among six different American hantaviruses with the following GenBank accession numbers: Andes (AF004660); Bayou (L36929); Black Creek Canal (L39949); Blue River (AF030552); El Moro Canyon RM-97 (U11427); and Sin Nombre Convict Creek 107 (L33683). For nested PCR, 2 µL of each amplicon were further amplified using inner generic primers from the G2 region of the M-segment using a PCR core kit (Roche, Penzberg, Germany). The nested PCR pro-

gram consisted of 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C, and 45 seconds at 72°C. The sequence of the forward inner generic primer was 5'-HYTRGGICAYTGGATGGATG-3', and the reverse inner generic primer was 5'-TGRAAWGARTCYTWTGTDGCCAT-3'. Nested RT-PCR amplicons were analyzed by electrophoresis in 1.2% agarose gels in Tris-Acetate-EDTA (TAE) buffer followed by staining with ethidium bromide for visualization of nucleic acids.

Home ranges were estimated for adult male *O. palustris* using the 95% adaptive kernel method with CALHOME software (Forestry Sciences Lab, Fresno, California, USA). The kernel method is a commonly used nonparametric technique for estimating home range size (Worton, 1989) that is robust in handling outliers, autocorrelated samples, and multiple centers of activity (Kernohan et al., 2001). Because home range size is influenced by number of locations (i.e., recaptures) as well as animal sex and age, home ranges were estimated only for adult males that were recaptured at least twice (i.e., at least three captures total) within a given season; this conservative method avoids inclusion of other ages or sexes and non-residents or transients (since such individuals would likely not be recaptured more than once even when trapping occurred daily; Kie et al., 1996) and has been used to examine home ranges in a variety of animal species, including other rodents (Ribble and Stanley, 1998). This criterion would naturally limit sample size, but the sample would more accurately represent home range size of resident animals. Based on these consistent and conservative criteria, only one of the four trapping grids had sufficient recaptures (N = 36 adult males, ranging from 3-6 locations/individual) to estimate home range. Each individual's home range was estimated for each trapping period and averages were calculated separately for seropositive (N = 15) and seronegative (N = 21) animals. When using the 95% adaptive kernel method in CALHOME, a bandwidth (smoothing parameter) must be chosen to deal with potentially non-normal data (as would be the case for multiple centers of activity); different bandwidths are then compared using a least-squares cross-validation (LSCV) score, which is a measure of how well the bandwidth fits the data. The bandwidth is then reduced until the smallest LSCV score is achieved. We found that using the default bandwidth of 0 with a grid cell option of 30 (i.e., a 30x30 data matrix) resulted in the lowest LSCV score.

Most other ecological studies on hantavirus-host relationships have focused on large-scale patterns

(Root et al., 1999; Biggs et al., 2000; Boone et al., 2000; Glass et al., 2000; Olsson et al., 2002; Yates et al., 2002; Armien et al., 2004; Olsson et al., 2005; Goodin et al., 2006), but even within broad habitat types that are associated with higher viral prevalence, the distributions of hosts and viruses are not necessarily uniform due to microhabitat selection. Macrohabitat preferences are known for *O. palustris* (Wolfe, 1982, 1985; McIntyre et al., 2005), but habitat selection at a finer spatial scale has not been established for this species. We assessed microhabitat selection by comparing microhabitat use (i.e., at sites where animals were actually captured) versus availability of individual microhabitat categories based on the coverage of those categories in the surrounding area. Microhabitat composition (percent ground coverage of 10 mutually exclusive categories: grass, forb, bare ground, tree, shrub, litter [duff], vine, coarse woody debris, water, reed) was measured in a 3-m-radius circle centered on each successful trap site, representing habitat use (N = 264 trap sites in 2002, N = 282 in 2003), following the protocol of Bullock (1996). Microhabitat composition also was measured in 2002 around randomly selected unsuccessful trap sites (N = 132) to represent habitat availability. Using unsuccessful trapping locations to represent microhabitat availability represented a compromise between choosing sites at which to sample microhabitat availability completely at random (which could have included points in water, on roads, or other non-habitat sites) while maintaining a degree of confidence of where rodents did not occur on land (since they were not trapped at those sites). Because they were proportions, each microhabitat coverage percentage was arcsine-square root-transformed for analysis (Sokal and Rohlf, 1981). Microhabitat selection was compared between seropositive and seronegative *O. palustris* for both years. Fisher's exact test (a 2x2 χ^2 ; Sokal and Rohlf, 1981) was used to compare seroprevalence rates between macrohabitat types; significant χ^2 tests were followed by comparisons of use versus availability of individual microhabitat categories, and differences in microhabitat use between seropositive and seronegative individuals, with 95% confidence intervals (95% CI) (following the protocol in Neu et al., 1974). A correlation analysis was performed to determine the possible influence of mass on home range size, followed by a general linear model analysis of covariance (ANCOVA within PROC GLM in SAS; SAS Institute, 1999) to compare home range sizes between seropositive and seronegative males, controlling for potential effects of mass.

Testes were measured (length x width in mm, as per Mills et al., 1995) in kill-lines-harvested males as a potential covariate with serological status, for larger testes are associated with increased testosterone levels and thence aggression and territorial defense in a wide variety of hantavirus host rodent species (Glass et al., 1988; Escutenaire et al., 2002; Hinson et al., 2004; Klein et al., 2004). Correlation analysis and ANCOVA were used to compare testes sizes (separate analyses for length and width) between seropositive and seronegative males, with mass as a covariate. A single analysis combining mass, home range, and testes sizes could not be conducted because testes were measured only on harvested animals, not ones in our mark-recapture study (from which home ranges were estimated). All statistical analyses were conducted in SAS version 8.2 (SAS Institute, 1999).

RESULTS

In total, 318 *O. palustris* (171 males, 147 females) were captured on the mark-recapture trapping grids over 14,179 trap-nights in 2002-03; an additional 135 (73 males, 62 females) were captured on the tissue-harvest traplines (6900 trap-nights). *O. palustris* was more abundant on the two coastal prairie trapping grids than the two upland grids (2002: 107 in coastal prairie vs. 64 in uplands; 2003: 121 vs. 26), and this pattern was driven by males: more males than females were captured in coastal prairie in both years (2002: 66 males, 41 females; 2003: 65 males, 56 females), with the reverse true for uplands (2002: 27 males, 37 females; 2003: 12 males, 14 females). A few individuals (20 males, 14 females) were recaptured between two consecutive trapping seasons, and 7 individuals (3 males, 4 females) were recaptured over three consecutive trapping seasons. Marked animals were recaptured on their own grids only, and no grid animals were captured on animal-harvest kill-lines.

As with abundance, seroprevalence rates were significantly higher in coastal prairie macrohabitat (51/228 [22.4%], sexes and years combined) than in uplands (9/90 [10.0%]) (Fisher's exact test: $F_1 = 96$, $P = 0.0196$). At the microhabitat scale, there was significant use of coarse woody debris and avoidance of areas dominated by forbs (Fig. 2A). Cover of

6 out of the 10 microhabitat variables examined did not vary by season; these 6 were either perennials (tree, vine) or non-growing (bare ground, litter, woody debris, water). Overlap in the 95% CI between seropositive and seronegative animals for most microhabitat variables indicated little difference in microhabitat selection with serological status (Fig. 2B). However, significant differences in microhabitat selection with serological status were detected for the three most commonly used microhabitat variables: seropositive rodents were significantly more likely to avoid grasses and forbs compared to seronegatives, which were more likely to select microhabitats dominated by coarse woody debris (Fig. 2B).

Seropositive males averaged significantly larger home ranges ($N = 16$, mean = 676.6 m², range: 150-1800 m²) than did seronegative males ($N = 20$, mean = 574.8 m², range: 3.6-1110 m²) (ANCOVA: $F_1 = 4.36$, $P = 0.0486$), even though the average number of capture locations did not differ greatly between the two groups (seropositive: 4.2 locations/individual; seronegative: 4.0). The home range pattern was not driven by mass (ANCOVA: $F_1 = 1.01$, $P = 0.3270$), for mass and home range size were not significantly correlated ($r^2 = 0.19$, $P = 0.3484$). However, body mass and testes size in adult males were significantly correlated (testis length: $r^2 = 0.40$, $P = 0.0005$; testis width: $r^2 = 0.24$, $P = 0.0419$). Furthermore, seropositive males had larger testes than did seronegative males (ANCOVA_{length}: $F_1 = 3.01$, $P = 0.0008$; ANCOVA_{width}: $F_1 = 1.72$, $P = 0.0001$). Seropositive males also were heavier on average than seronegative animals (seropositive: mean = 56.6 g; seronegative: mean = 40.2 g), but the pattern of larger testes in seropositives held true even when controlling for body size: the difference was most pronounced in the heaviest males (Table 1).

DISCUSSION

The presence of antibodies for BAYV was associated with both macrohabitat and rodent abundance: more *O. palustris* of both sexes were captured in coastal prairie than in up-

ECOLOGICAL CORRELATES OF BAYOU VIRUS

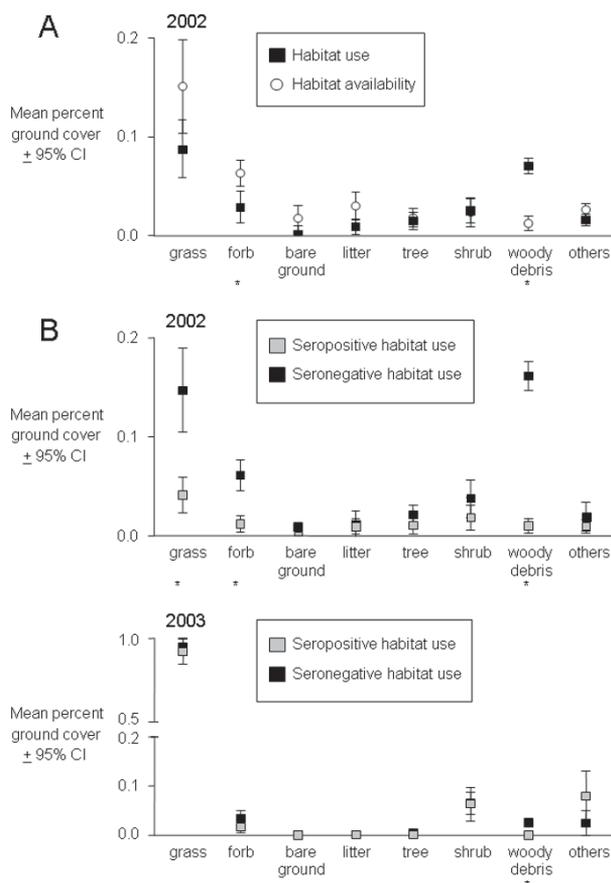


Fig. 2. A) Mean + 95% CI percent ground covers by microhabitat type for microhabitat use by *Oryzomys palustris* regardless of serological status (black squares) compared to microhabitat availability (white circles), with seasons pooled. Asterisks indicate significant lack of overlap in 95% CI. B) Mean + 95% CI percent ground covers by microhabitat type for seropositive (grey squares) and seronegative (black squares) *O. palustris*, with seasons pooled, for 2002 (top) and 2003 (bottom; note split Y-axis). Asterisks indicate significant lack of overlap in 95% CI. N_{2002} = 12 seropositive animals and 12 seronegative animals (microhabitat use) and 24 randomly selected unsuccessful trapping locations (microhabitat availability). N_{2003} = 100 seropositive animals and 182 seronegative animals.

Table 1

Testes measurements by weight class for seropositive (sero+) and seronegative (sero-) adult males of *Oryzomys palustris*.

Measurement	Weight class					
	46 – 55 g		56 – 65 g		≥ 66 g	
N	Sero+ 5	Sero- 11	Sero+ 9	Sero- 10	Sero+ 4	Sero- 1
Average testis LxW (mm)	11.0 x 5.6	11.1 x 6.5	11.9 x 5.7	11.9 x 6.6	14.0 x 6.25	12.0 x 8.0
Maximum testis LxW (mm)	13.0 x 7.0	12.0 x 7.0	16.0 x 8.0	12.0 x 8.0	18.0 x 8.0	12.0 x 8.0

lands, and the abundance of seropositive rodents was likewise higher in coastal prairie. Furthermore, more males were captured than females in coastal prairie. Seropositive *O. palustris* of both sexes were captured more often in areas lacking cover from woody debris at both the macrohabitat and microhabitat scales. Using baited traps is standard protocol in trapping small mammals and ensures higher trap success and sample size, but use of baited traps may have influenced an animal to move into a microhabitat where it otherwise would not have occurred; more research into microhabitat selection by disease hosts is warranted. Although more seropositive animals were captured in one macrohabitat type (coastal prairie) than the other (uplands), this relationship is likely a function of higher host abundances in coastal prairie than in uplands. A similar density-dependent relationship between host abundance and seroprevalence has been documented for other hantavirus-host systems (Kuenzi et al., 1999; Biggs et al., 2000; Boone et al., 2002).

Home range size was not influenced by mass, which is not typical for mammals and may be a consequence of examining only adult males and use of baits to obtain animal locations. Relative to the size of our trapping grids (7600–8100 m²), home range sizes were much smaller (3.6–1800 m²); indeed, several home ranges were contained within a grid, and these home ranges did occasionally overlap, which has important implications on horizontal transmission. We endeavored to include only resident animals in our home range estimations by using a method that culled transient individuals from the capture distribution—we only analyzed those individuals for whom at least three locations were known. This protocol ensured that estimated home ranges were conservative and did not overestimate the potential for interspecific interaction.

Although horizontal transmission of hantaviruses may occur by environmental contamination from excreta containing virions (Kallio et al., 2006), the chief mode of transmission is thought to occur primarily by exchange of fluids (saliva or saliva aerosols;

Padula et al., 2004) during aggressive encounters, mainly between males (Abbott et al., 1999; Calisher et al., 1999; Mills et al., 1999; Nisbett et al., 2001; Escutenaire et al., 2002; Hinson et al., 2004). Therefore, it was not surprising that seropositive males exhibited traits associated with higher aggression, namely increased ranging and larger testes (Glass et al., 1988; Escutenaire et al., 2002; Hinson et al., 2004; Klein et al., 2004), which would account for the larger home ranges seen in seropositives. And although seropositive animals were larger than seronegatives, patterns of home range size with serological status were not significantly associated with mass, indicating that the ranging patterns we observed were not driven simply by body size. At PPWMA, ranging may be hindered at times by woody debris, large piles of which are swept in by tropical storms. Although rodents often seek out cover, large amounts may actually be barriers to movement because they disrupt existing rodent paths, escape routes, or territories, which may account for avoidance of areas with debris. Moreover, in our experience at PPWMA, woody debris served as primary cover for rattlesnakes (*Crotalus atrox*), which prey on rodents.

For infectious agents such as hantaviruses, natural selection by the virus may induce changes in the ecology and/or behavior of infected host animals in such a way as to facilitate viral transmission. Such selective pressures have been demonstrated for hosts of endoparasites, resulting in differences in host habitat selection and behavior with infection (Moore, 1995; Hurd, 2003). A similar mechanism may be at work on hantavirus-host relationships in terms of aggression or ranging (Hinson et al., 2004). Since viral transmission is affected by interspecific encounters, alterations to rodent behavior could elicit a shift in the dominance structure of a rodent population, which could in turn affect the spatiotemporal patterns of seroprevalence. For example, in their meta-analysis of spatial clustering of hantavirus-infected rodents from long-term studies in the United States, Root et al. (2005) concluded that overlapping use of space by

hosts (e.g. during high population density) is an important factor in horizontal transmission of hantaviruses. This is an area that deserves closer examination in future studies. Moreover, it should be noted that being seropositive simply indicates past viral exposure and is only partially reliable in indicating current viral status of the host (Boone et al., 2002). If behavioral alterations hinge upon actual infection, then confirmation of viral presence rather than serological status would be preferred in future work on this topic.

Our study provides empirical support for Bayou virus being associated with certain ecological variables at different spatial scales: macrohabitat and microhabitat. Establishing the existence of such indicators should be a priority to facilitate future studies of virus-host relationships (Deter et al., 2008), particularly in a multi-scaled approach (e.g. Suzán et al., 2006). Continued focus on host ecology such as movement patterns and habitat selection may help rectify our currently incomplete understanding of transmission and persistence of hantavirus infections in wild host populations.

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