Bayou virus detected in non-oryzomyine rodent hosts: an assessment of habitat composition, reservoir community structure, and marsh rice rat social dynamics

Tyla S. Holsomback1,2,3, Nancy E. McIntyre1, Richard A. Nisbett2, Richard E. Strauss1, Yong-Kyu Chu1, Alisa A. Abuzeineh1, Noé de la Sancha1, Carl W. Dick3, Colleen B. Jonsson4, and Brandon E. L. Morris5,6

1Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, U.S.A.
2Department of Global Health, University of South Florida, Tampa, FL 33612-3805, U.S.A.
3Department of Biochemistry and Molecular Biology, Southern Research Institute, Birmingham, AL 35255-5305, U.S.A.
4Department of Biology, Aquatic Station, Texas State University, San Marcos, TX 78666, U.S.A.
5Department of Zoology, Field Museum of Natural History, Chicago, IL 60605-2496, U.S.A.
6Department of Botany and Microbiology, Institute for Energy and the Environment, The University of Oklahoma, Norman, OK 73019-0245, U.S.A.

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ABSTRACT: In the United States, Bayou virus (BAYV) ranks second only to Sin Nombre virus (SNV) in terms of hantavirus pulmonary syndrome (HPS) incidents, having been confirmed in cases from Texas and Louisiana since its discovery in 1994. This study on BAYV infection among sympatric, non-oryzomyine rodents (“spillover”) in Freeport, TX, is the first to link patterns of hantavirus interspecific spillover with the spatiotemporal ecology of the primary host (marsh rice rat, Oryzomys palustris). Mark-recapture and/or harvest methods were employed from March 2002 through May 2004 in two macrohabitat types. Rodent blood samples were screened for the presence of IgG antibody to BAYV antigen by IFA after which Ab-positive blood, saliva, and urine were analyzed for the presence of viral RNA by nested RT-PCR. From 727 non-oryzomyine captures, five seropositive (but not viral RNA positive) individuals were detected: one each of Batomys taylori, Peromyscus leucopus, and Reithrodontomys fulvescens; and two Sigmodon hispidus. Spillover hosts were not associated with macrohabitat where O. palustris abundance, density, or seroprevalence was highest. Rather, spillover occurred in the macrohabitat indicative of greater overall disturbance (as indicated by grazing and exotic plant diversity) and overall biodiversity. Spillover occurred during periods of high seroprevalence detected elsewhere within the study region. Spillover locations differed significantly from all other capture locations in terms of percent water, shrub, and grass cover. Although greater habitat and mammal diversity of old-fields may serve to reduce seroprevalence levels by tempering intraspecific contacts between rice rats, greater diversity also may create an ecologically opportunistic setting for BAYV spillover. Impacts of varying levels of disturbance and biodiversity on transmission dynamics represent a vastly uncharacterized component of the evolutionary ecology of hantaviruses. Journal of Vector Ecology 34 (1): 9-21. 2009.

Keyword Index: Bayou virus (BAYV) spillover, Oryzomys palustris, virus-host specificity, host switching, marsh rice rat.

INTRODUCTION

Following human deaths in the southwestern U.S. in 1993 and with the discovery that Sin Nombre virus (SNV; carried by the ubiquitous deermouse, Peromyscus maniculatus) was the causative agent (Nichol et al. 1993), hantaviruses (and Hantavirus Pulmonary Syndrome or HPS) became duly acknowledged throughout the Americas as menacing panzootics. From that time onward, N. American researchers from a variety of scientific disciplines have engaged in numerous transcontinental serosurveys of small mammal communities, focusing mainly on genetic and serologic characterizations, host associations, and the phylogeographic dynamics of SNV (Drebot et al. 2001, Monroe et al. 1999, Spiropoulou et al. 1994). At present, much of what is surmised about the N. American hantavirus assemblage has been extrapolated from information obtained from the initially studied system of SNV and the deermouse, the viral and rodent species to which most HPS cases in the U. S. and Canada have been attributed.

Even with an extraordinary compilation of data from hantaviral field and laboratory studies, the modes of transmission, among rodents and from rodent to human, have not been firmly substantiated. Cumulative evidence supports a preponderate transmission dynamic of subcutaneous inoculation of infectious bodily fluids during antagonism between older and perhaps socially dominant males (Glass et al. 1988, Klein 2003). Infection in rodents may be contracted secondarily by inhalation, ingestion, or possibly mucosal absorption of mature virions present in saliva and its aerosols (Orellana 2003); indirect exposure, as rodents navigate through a heavily soiled and virus-contaminated environmental matrix, also has been suggested (Kallio et al. 2006, Sauvage et al. 2003). Indirect transmission of this nature could have far-reaching consequences, particularly among communities with species that scent mark their territorial boundaries with urine and/or feces, and among individuals that engage in olfactory exploration while attempting to secure a breeding home range. The
durability of hantaviruses outside their mammalian hosts is unknown, although Hutchinson et al. (1998) and Kallio et al. (2006) have shown support for external survivability of Black Creek Canal virus (BCCV) and Puumala virus (PUUV), respectively. Comparably virulent RNA viruses transmitted normally by some form of social or physical contact have evolved the capacity for prolonged survival in liquid media, the soil strata, or forest litter, such as rabies virus and various arenaviruses and enteroviruses (Garnett and Antia 1994). It is widely believed that hantaviruses cause no discernible, deleterious effects in their rodent hosts with respect to fecundity or longevity (Bernshtein et al. 1999, Schmaljohn and Hjelle 1997), despite studies which entertain a re-evaluation of the reigning paradigm of the benign nature of hantaviral infection in rodents (Douglas et al. 2001, Kuenzi et al. 1999).

Transmission of hantavirus from specific (primary) hosts to non-specific secondary (reservoir) hosts (“spillover”) is speculated to occur frequently among natural populations of rodents living in sympathy or syntopy (Monroe et al. 1999), particularly during periods of high rodent population density (Mills et al. 1997) and/or seroprevalence; these conditions also are believed to act synergistically to accelerate viral spreading throughout the rodent community due to increased rodent-to-rodent contacts (Hjelle and Yates 2001). Ironically, compelling evidence has not been produced for a clear covariation between rodent host densities and seroconversion incidence (Abbott et al. 1999, Biggs et al. 2000). Hantavirus spillover in nature is perceived as inconsequentially common for the most part because non-primary host cells are assumed to be incompatible with viral colonization-induced persistence (precluded by the ancient virus-carrier union of coevolutionary specificity and micro-adaptation). Therefore, spillover hosts may not be hosts at all or perhaps fleeting hosts at best, as their immune systems expunge the viruses rapidly; thus with regards to the cycle of viral trafficking and maintenance within rodent and human communities, their expected contribution should be insignificant. No information is available on the potential impacts of non-primary hosts functioning minimally as short-term transporters and translocaters of hantaviruses. However, these roles are worthy of investigation, as there are several examples of how prolonged association of hantavirus infection in a secondary host can lead eventually to interspecific host switching. For these reasons, the importance of spillover hosts, and the elucidation of the conditions under which spillover occurs, should not be underestimated.

In North America to date, no published works have investigated hantavirus spillover in rodents explicitly, to attempt quantification of relative frequencies of occurrence, to characterize possible spatiotemporal reservoir-habitat associations, or to assess possible impacts on transmission rates between rodents and from rodents to humans or alterations in virus evolution. Czech Republic researchers have produced genetic evidence for Dobrava virus (DOBV) spillover from the yellow-necked field mouse (Apodemus flavicollis) to other rodents (Weidmann et al. 2005) and in Sweden, Klingström et al. (2002) have demonstrated secondary host infection of PUUV in artificially and naturally infected rodents. The previous two examples notwithstanding, mostly known information about hantavirus spillover (among both wild-trapped and lab-reared rodents) is anecdotal, reported sporadically as ancillary data from studies with widely varied emphases (Abbott et al. 1999, Childs et al. 1994, Hjelle and Yates 2001, Jay et al. 1997, Kuenzi et al. 2001, Levis et al. 1998, Mills et al. 1997; 1999, Rawlings et al. 1996, Rhodes et al. 2000, Rowe et al. 1995, Schmaljohn and Hjelle 1997, Ulrich et al. 2002).

As part of a broader ecological study of BAYV and the marsh rice rat (McIntyre et al. 2005), our objectives here were to detect and describe spillover hosts, and to ecologically characterize spillover sites in comparison with non-spillover sites at our study location. Moreover, our intent was to ascertain how rice rat demographic or behavioral features, host community structure, or specific habitat variables might predict areas where secondary host infection is likely to occur.

MATERIALS AND METHODS

Study site
Rodent trapping and habitat assessment were conducted seasonally at the Peach Point Wildlife Management Area (PPWMA) (UTM: 15-3202562-262435) in Brazoria County, TX, from March 2002 through May 2004. Representative of the Gulf Coast Prairies and Marshes Ecoregion, PPWMA is located approximately 100 km south of Houston, encompasses 4174.5 ha, and is bordered southeasterly by the Gulf Coast Intracoastal Waterway (GCIW). Topographically, the landscape is relatively flat and low (0–5 m ASL) with clay soils, and is composed of low-lying assemblages of brackish to saline coastal prairies, grading farther inland to upland habitats of freshwater marshes and old-fields with some trees. The area experiences episodic tropical storms, resulting in inundation of areas near the GCIW. Precipitation occurs throughout the year (with 60% falling between April and September), and average precipitation is 133.35 cm/yr (data from the National Oceanic and Atmospheric Administration Freeport 2NW weather station, located ~10 km from PPWMA; http://www.noaa.gov/). Average high temperatures range from 18° C in winter to 33° C in summer; average annual lows are above freezing (8° C) even in winter.

Mark-recapture grids for antibody (IgG) analysis
Field collection protocols were approved by the Texas Tech University Animal Care and Use Committee (permit #01134BX) prior to the onset of this study. Trapping and sampling procedures were sanctioned by, and scientific collection permits were secured from, the Texas Parks and Wildlife Department (permits #APR-0498-944 and #SPR-0504-381). To characterize the rodent community, a pilot study was conducted in mid-March 2002, when species
diversity, distributions, and antibody status of rodents were determined using standard small mammal capture and harvest methods (Jones et al. 1996, Mills et al. 1995a). From those data, two simultaneous yet spatially independent trapping designs were implemented in May 2002. Rodents were live-trapped on four capture-mark-release-recapture grids (each ~7100 m², ca. 100 traps each) in both macrohabitat types (two in coastal prairie, two in old-field) for four to six consecutive nights per each of four seasons (mid-March, late May, late August, mid-December). Grid shapes, dimensions, and trap numbers varied slightly due to waterline constraints. Grids were separated by >1 km to demarcate individual rodent populations. Specimens were live-captured using Sherman 7.8 x 9.3 x 23.5 cm folding galvanized aluminum traps (H. B. Sherman Traps, Inc., Tallahassee, FL) placed singly on terra firma (exception: affixed to floating rafts on Grids 3 and 4 when inundated; see Abuzeineh et al. 2007 for descriptions) at 10-m intervals (Jones et al. 1996). Slightly before dusk, traps were baited with rolled oats and peanut butter. Traps were checked immediately after dawn to minimize stress-induced deaths resulting from hypothermia, starvation, and/or dehydration, and traps remained closed during the day to avoid diurnal capture mortalities. New plastic zip-top bags were used to contain each animal, and handling time was kept to a minimum while processing each animal individually at the site of capture. Rodents were marked by a unique numeric or alpha-numeric identifier (either by toe-clipping/ear-punching, or by intrascapular, subdermal Passive Integrated Transponder [PIT] tag insertion; Biomark, Inc., Boise, ID). Data collected for each individual rodent included capture status (new or re-capture), its unique identifier, species, trap station, age (juvenile, subadult, adult), sex, weight (using a Pesola spring scale), and reproductive status (testes position: abdominal, inguinal, or descended; vaginal condition: perforate or closed; pregnancy status: pregnant, recent parturition, or lactating). Recorded also were the presence, location, and severity of wounds and scars, along with any obvious physical abnormalities. Capture coordinates were recorded using a handheld global positioning system (GPS). Buccal cells and saliva were collected with a sterile cotton-tipped swab and placed immediately into a sterile 2-ml O-ring capped cryovial of Minimum Essential Medium containing 10% fetal bovine serum and an antibiotic mixture of penicillin [10 units/ml]/streptomycin [10 μg/ml] (Fisher Scientific, Atlanta, GA); a urine sample, when available, was collected from inside the containment bag and handled in the same manner as saliva. Using a sterile Pasteur pipet, a blood sample of several drops (0.1-0.5 ml) was extracted aseptically from the retro-orbital sinus and delivered to a sterile cryovial, after which the animal was released at the site of capture. Condition upon release was noted and recorded. All samples were stored on ice while processing animals in the field, and afterwards samples were contained within liquid nitrogen. Animals recaptured during subsequent trapping seasons were evaluated, weighed, and resampled to monitor any changes in body weights and conditions, reproductive conditions, and infection statuses. Standardized procedures for humane capture, handling, toe-clipping/ear-punching, and bleeding of rodents, as well as human safety precautions, were employed (Mills et al. 1995b). Between each processed animal, all nondisposables (instruments, contact surfaces) were decontaminated with a 10% hypochlorite solution and/or flame-sterilized. Samples were archived in the Natural Science Research Laboratory at the TTU Museum in an ultracold freezer (-80°C) until transfer to the Southern Research Institute.

Rodent harvest traplines for rRNA detection (hereafter referred to as “traplines”) Traplines were run in locations on the PPWMA that were spatially separate from but reflective of the four mark-recapture grids in terms of plant species composition. Individual traplines of 50 stations, with traps spaced 10 m apart, were run for four to six nights/season. Locations of traplines varied seasonally and depended upon disturbance regimes and area access. A GPS unit was used to record the coordinates at each station where a rodent was captured. Disposable plastic bags were used to transport traps containing captured rodents to a main processing site.

At a well-ventilated processing site within the PPWMA, all trapline captures were catalogued, bled from the retro-orbital plexus, and euthanized in a Nalgene container by ether inhalation (TTU-ACUC permits 01134BX and 03049-08). The euthanasia container was decontaminated between processed animals by a two-part method: first, it was soaked for 15 min in a 10% hypochlorite solution, then the container was sprayed with 70% ethanol, remaining unused until all remnants of liquid had volatilized. Following blood extraction, each animal was processed as a standard museum specimen (see Jones et al. 1996). Proper precautions were followed during all aspects of animal collection, transport, and processing (Mills et al. 1995a, 1995b). To prevent cross-contamination, a new pair of latex gloves was worn for each animal, and all necropsy utensils and contact surfaces were decontaminated with a 10% hypochlorite solution between processed individuals. Prior to their replacement and use, traps were submerged for 30 min in a 10% hypochlorite solution for disinfection, rinsed thoroughly with water, and then placed upright in the sun and wind for evaporation of any remaining liquid and for UV sterilization.

Antibody (IgG) determination by immunofluorescence microscopy (IFA) Assays using rodent excreta and tissues were conducted at the Southern Research Institute and adhered to BSL3 practices and CDC regulations. The analyses described herein were developed and validated by Chu et al. (1995, 2003). Acetone fixed and gamma-ray irradiated BAYV-infected Vero E6 cells on a spotted glass slide were used as antigen. To detect antibody, 40 μl of an initial serum dilution of 1:16 from each rodent were added to a separate well of the antigen slide. Slides were incubated for 30 min at 37°C in a moist chamber, washed twice with phosphate buffered saline (0.01M PBS, pH 7.4) for 5 min, and rinsed with distilled water. Slides were air-dried, 25 μl of FITC- 
labeled anti-mouse IgG (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) were added, slides were incubated for 30 min, and then washed as described previously. After mounting with media (90% glycerol in PBS buffer), slides were observed under a fluorescence microscope (Axioskop; Zeiss, Oberkochen, Germany) for detectable presence of IgG. To determine end-point antibody titer, 1:16 diluted antibody positive samples were further diluted with a two-fold serial dilution, and each dilution was processed as described previously. Positive control samples (i.e., pooled plasma from rodents confirmed to have anti-BAYV IgG and negative control samples (i.e., pooled plasma from confirmed BAYV-naïve rodents) were included for comparisons.

Tissue extraction of total RNA, nested reverse transcriptase–polymerase chain reaction (RT-PCR) for vRNA detection

Total RNA from Ab-positive and Ag-suspected rodent tissues was extracted and amplified by nested RT-PCR using validated protocols (Chu et al. 1995, 2003). Specific details of the RT-PCR methods can be found in McIntyre et al. (2005).

Habitat assessment

Habitat composition was quantified at three spatial scales consisting of concentric circles 0.25 m, 1 m, and 3 m in radius centered on each trap station. Descriptors included vegetation height and type for woody and herbaceous species, percent ground cover of 11 mutually exclusive categories (G=grass, F=forb, B=bare ground, L=litter/detritus, T=tree, S=shrub, WD=woody debris (downed wood), V=vine, CP=cowpie, H=water, R=reed), number of plant species, and number of trees, following standardized protocols (refs. in McIntyre et al. 2005). Each habitat descriptor was coded individually for a total of 40 distinct variables. The vegetation classes, scaled equally, were converted to a correlation matrix.

Statistical analysis

From the original 40 ecological variables, two (% cowpie at 0.25 m and at 1 m) were excluded from the analysis due to variance, and one (number of trees) was eliminated because of missing data. Discriminant function analysis (DFA) was performed using 37 habitat characters as independent variables to determine whether spillover sites (seropositive, non-Oryzomys capture) could be discriminated from non-spillover sites (all other capture types, each assigned a unique numeric code) for all traps for the duration of the study. For two predefined groups, a single function (DF1) characterizes the discrimination; the second function (DF2) describes only within-group variation but permits the discriminant scores to be portrayed by scatterplots. Loadings (correlations of variables with the discriminant function) were estimated as vector correlations. Multivariate analysis of variance (MANOVA) was used to assess whether or not the spillover sites differed significantly from the non-spillover sites. To avoid the strict assumptions of MANOVA (multivariate normality, homogeneity of within-group variances and covariances; Tabachnick and Fidell 2007), levels of statistical significance (p-values) were assessed by random permutation (Manly 1997) using 1,000 iterations. The DFAs and MANOVAs were performed twice: once using all trap locations (including no capture traps); and once for spillover sites versus non-spillover sites. Due to the extreme skewness of sample sizes (i.e., very few spillover stations compared to all other trap stations), no capture stations were eliminated from the analyses; capture outliers were included. Data analyses were executed using functions written in Matlab*, version 6.5, release 13.0.1 (Mathworks, Inc.; http://www.faculty.biol.ttu.edu/strauss/Matlab/Matlab.htm).

RESULTS

Summary of plant and animal community by macro-habitat

In this study, vegetative and rodent species composition differed between the two macrohabitat types. Generally, coastal prairie (Grids 3, 4) was less heterogeneous than old-field (Grids 1, 2) in terms of both horizontal and vertical architecture. Table 1 summarizes the main characteristics of each grid by type and average number of plant and terrestrial small mammal species, as well as the numerically dominant plant (in terms of percent cover averaged over the entire study period) and rodent species, and other defining grid features. Note the similarities of replicate grids 1 and 2 (old-field habitat), the similarities of replicate grids 3 and 4 (coastal prairie), and the differences between the two (e.g., the numerically dominant rodent species by grid). Overall small mammal and plant species richness and evenness decreased from Grid 2 ≥ 1 > 4 ≥ 3.

Seroprevalence and mammal species richness by macro-habitat

Throughout the study period and in both macrohabitat types, richness of small mammal species varied inversely with BAYV seroprevalence levels (Figure 1). In old-field habitat (Grids 1, 2), the mean number of small mammal species was more than double (5.5 vs 2.5) the number of species found in coastal prairie habitat (Grids 3, 4). Levels of seroprevalence in old-field were almost half the levels in coastal prairie habitat (10.5% and 20.0%, respectively).

Detection of spillover rodents

From approximately 14,000 trap nights, 476 non-oryzomyine rodents (230 Reithrodontomys fulvescens, fulvous harvest mouse; 119 Sigmodon hispidus, hispid cotton rat; 120 Baiomys taylori, northern pygmy mouse; 7 Peromyscus leucopus, white-footed mouse) were captured, marked, and released on the four live-capture grids; from approximately 7000 trap nights, an additional 251 non-oryzomyines were collected from the traplines: 139 R. fulvescens; 57 S. hispidus; 46 B. taylori; 9 P. leucopus. Anti-BAYV IgG was detected in S. hispidus (n = 2, 1.1%), P. leucopus (n = 1, 6.2%), B. taylori (n = 1, 0.6%), and R. fulvescens (n = 1, 0.3%). Non-primary host rodents tested negative for the presence of vRNA.
Table 1. Main characteristics of mark-recapture grids.

<table>
<thead>
<tr>
<th>Grid</th>
<th>Macrohabitat type</th>
<th>Mean number of plant species</th>
<th>Richness of terrestrial small mammals</th>
<th>Dominant plant species*</th>
<th>Numerically dominant rodent species</th>
<th>Other grid habitat features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Old-field</td>
<td>34</td>
<td>5a</td>
<td><em>Spartina spartinae</em></td>
<td>Reithrodontomys fulvescens</td>
<td>Freshwater pond Chinese tallow Grazed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Iva annua</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Sapium sebiferum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Old-field</td>
<td>37</td>
<td>6b</td>
<td>Rubus trivialis*</td>
<td>Baiomys taylori</td>
<td>Freshwater pond Most woody diversity and stature Grazed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Iva annua</em></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Sapium sebiferum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Coastal prairie</td>
<td>10</td>
<td>2c</td>
<td>Distichlis spicata</td>
<td>Oryzomys palustris</td>
<td>Shared watershed with Grid 4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Batis maritima</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Spartina patens</em></td>
<td></td>
<td>Low vegetative diversity Ungrazed; ground saturated to inundated</td>
</tr>
<tr>
<td>4</td>
<td>Coastal prairie</td>
<td>11</td>
<td>3d</td>
<td>Borrichia frutescens</td>
<td>Oryzomys palustris</td>
<td>Shared watershed with Grid 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Spartina patens</em></td>
<td></td>
<td>Some shrubs, forbs, litter Ungrazed; ground damp to dry</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Iva frutescens</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Based on cover, listed in descending order (see McIntyre et al. 2005 for complete list).
*Baionys taylori, Cryptotis parva, Oryzomys palustris, Reithrodontomys fulvescens, Sigmodon hispidus.
*O. palustris, *S. hispidus.
*exotic species.

**Ecological differentiation of spillover from other capture sites**

Scatter diagram results of the DFA are shown in Figure 2A. Dense clustering of points towards the center-left characterizes trap stations (as described by the 37 habitat variables) where rodents were captured frequently; points diverging to the right in the figure represent stations where rodents were captured rarely. Figure 2A has been reproduced (with non-spillover capture points replaced by the polygon, left) to facilitate discernment of the degree of overlap between the five spillover locations (semi-triangle, right) and all other capture locations (Figure 2B). The dotted circle represents where two spillover hosts were captured at a trap station characterized by identical ecological variables (i.e., scores for both spillover captures are the same: +4.5539, –1.2103). The correlations (loadings; Figure 3) between the ecological variables (y; y-axis) and the discriminant function (x-axis) indicate that the best habitat predictors to distinguish spillover sites from non-spillover sites are percent water cover at 0.25 m (v12), at 1 m (v22), and at 3 m (v33), and percent shrub cover at 1 m (v19) and at 3 m (v29); percent grass cover at 0.25 m (v4), at 1 m (v14), and at 3 m (v24) are correlated negatively with spillover rodents. Based on the randomized MANOVA, spillover sites differed significantly from all other capture sites (F = 4.9793, p = 0.020).

**Spillover demography: climatic-spatial-temporal characterizations**

Seropositive non-Oryzomys hosts were detected in three of the eight (37.5%) trapping sessions (Table 2). Interestingly, events of spillover occurred during 3 of the 4 periods of lowest cumulative monthly precipitation (Table 2). All five spillover rodents were captured on either the traplines (March 2002, 2003) or from Grid 2 (March and August 2003) (Table 3); no spillover was detected on Grid 1 (old-field habitat) or Grids 3 and 4 (coastal prairie habitat). Spillover hosts comprised three male adults, one male subadult, and one female adult (Table 3). In March 2002, two *S. hispidus* (both adult males) were harvested from the traplines. In March 2003, one *B. taylori* (a subadult male) was collected from a trapline, and one *P. leucopus* (an adult female) was captured and released on Grid 2. Also in March...
Figure 1. Prevalence of anti-BAYV antibody (line, right Y-axis) and mean number of known terrestrial small mammal species (columns, left Y-axis) in old-field (Grids 1, 2) and in coastal prairie (Grids 3, 4) habitat at the PPWMA, Brazoria County, TX, 2002-2004.

Figure 2. A) Scatter diagram depicting results of the discriminant function analysis for capture sites denoted as circles. All discrimination is along DF1. B) Polygon (left) represents all capture types other than spillover. Semi-triangle (right) shows the ecological occurrence of spillover (denoted as circles where sides of the semi-triangle connect). A plus sign (“+”) denotes the centroid. Dotted circle on semi-triangle represents an ecologically identical area where two spillover hosts were captured.
Table 2. Temporal occurrence of BAYV spillover and corresponding total precipitation by trapping session.

<table>
<thead>
<tr>
<th>TRAPPING SESSION</th>
<th>TOTAL PRECIPITATION (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>* March 2002</td>
<td>** 2.24</td>
</tr>
<tr>
<td>May 2002</td>
<td>10.21</td>
</tr>
<tr>
<td>August 2002</td>
<td>21.54</td>
</tr>
<tr>
<td>December 2002</td>
<td>19.35</td>
</tr>
<tr>
<td>* March 2003</td>
<td>** 3.40</td>
</tr>
<tr>
<td>May 2003</td>
<td>** 0.00</td>
</tr>
<tr>
<td>* August 2003</td>
<td>** 8.99</td>
</tr>
<tr>
<td>May 2004</td>
<td>10.11</td>
</tr>
</tbody>
</table>

* Spillover rodents captured.
** Lowest cumulative monthly precipitation levels across study period (represents 4:8).

Table 3. Host community demography of traplines and old-field grids combined by trapping session.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baiomys taylori</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>121</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Peromyscus leucopus</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Oryzomys palustris</td>
<td>40</td>
<td>47</td>
<td>11</td>
<td>63</td>
<td>27</td>
<td>36</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Reithrodontomys fulvescens</td>
<td>43</td>
<td>15</td>
<td>7</td>
<td>147</td>
<td>96</td>
<td>25</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Sigmodon hispidus</td>
<td>22</td>
<td>(2 A ♂)</td>
<td>9</td>
<td>8</td>
<td>53</td>
<td>28</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Trapline captures only (no grids).
Spillover trap sessions underlined.
Number of spillover individuals by species in parentheses; A = adult, S = subadult.

Table 4. BAYV primary host (Oryzomys palustris) abundance (for all grid and trapline captures combined) and seroprevalence by trapping session.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td># Sero +</td>
<td>8</td>
<td>16</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>17</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>Total #</td>
<td>40</td>
<td>109</td>
<td>54</td>
<td>99</td>
<td>67</td>
<td>126</td>
<td>113</td>
<td>25</td>
</tr>
<tr>
<td>% Sero +</td>
<td><strong>20.0</strong></td>
<td>14.7</td>
<td>3.7</td>
<td>4.0</td>
<td><strong>16.4</strong></td>
<td>13.5</td>
<td><strong>24.8</strong></td>
<td>12.0</td>
</tr>
</tbody>
</table>

*Only traplines sampled (no grids). Trapping session when spillover occurred denoted by underline. **Bold underline** indicates corresponding (and also highest) seroprevalence levels detected in O. palustris during study; these were also the periods of spillover detection.
2003 on Grid 2, one seronegative *R. fulvescens* (an adult male) was captured and released only to be recaptured five months later in August 2003, at which time seroconversion was determined. During this study, only one seropositive rice rat was captured on Grid 2. An adult male captured initially in May 2003, this rodent was recaptured in the same trapping station (a trap placed in the shallow end of a pond) three months later in August 2003; this adult male was shedding virus in blood and saliva both times he was captured. Both spillover rodents on Grid 2 were captured within 30 m and 60 m of the BAYV-positive rice rat, and both spillover rodents were captured within 10 m and 50 m of the pond. Additionally, Grid 2 was the only grid showing a consistent pattern of equivalence in the sex ratio of adult male and female rice rats (data not shown).

Host community demography, seroprevalence, and spillover

Collectively, from all traplines and grids, non-*Oryzomys* species constituted roughly half (48.4%) of the total captures in this study. Although total captures of *Oryzomys* and non-*Oryzomys* were approximately 1:1 ± 0.016 (774 and 727, respectively), the distribution of the groups according to trapping method was not the same. Non-*Oryzomys* species represented 44.2% of the total number of grid captures, but accounted for 59.0% of the total trapline captures. Species demographics and BAYV-seroprevalence varied by macrohabitat and season (McIntyre et al. 2005). Consistently, however, rice rat abundance and seroprevalence were lower in old-field (35 individuals per grid and 10.5%, respectively) than in coastal prairie (129 individuals per grid and 20.0%, respectively) habitat. BAYV interspecific spillover appears to occur generally when primary host seroprevalence, but not necessarily when primary host abundance, is greatest (Table 4, Figure 4). Moreover, spillover does not necessarily occur where rice rat abundance is greatest (Tables 3 and 4). For example, no spillover occurred on the traplines or the old-field grids during May or December 2002, despite relatively high capture rates of *O. palustris* (Figure 4).

DISCUSSION

Within our study site, the terrestrial small mammal community is comprised largely of one herbivore (*Sigmodon*), one herbivore-granivore (*Baiomys*), two omnivores (*Peromyscus* and *Reithrodontomys*), one insectivore (*Cryptotis*; data not shown), and one carnivore-insectivore (*Oryzomys*). Typically, graminivores occupy more open habitats whereas the other species occur in habitats with greater vertical cover (Davis and Schmidly 1994). The most abundant taxon in this coastal prairie habitat, *Oryzomys* is known to shift its primary diet of carnivory to exploit “substitutable resources” (Dunning et al. 1992) based on seasonal availability (e.g., seeds and succulent plant parts [Svihla 1931], and also arthropods and herbaceous dicots [Kincaid and Cameron 1982]). At certain times of the year, the rice rat may consume large quantities of fungi from the family *Endogonaceae* (Negus et al. 1961). Versatility in the alternate foraging economics of *O. palustris* creates seasonal habitat and diet overlap with coexisting populations of *S. hispidus* and *R. fulvescens*.

Like other directly transmitted mammalian viruses, hantaviruses elicit a marked propensity for both physical contact and aggressiveness in their rodent hosts (Escutenaire et al. 2002, Hinson et al. 2004, see also Klein 2003). Norway rats (*Rattus norvegicus*) infected by Seoul virus (SEOV) demonstrate several infection-mediated markers for transmission facilitation, such as increased hostility and wounding coincident with a diminished subordinate status (Glass et al. 1988, Klein et al. 2004). Serostatus is not the only perceptible difference between infected and uninfected rice rat males. Compared with their seronegative counterparts, BAYV-seropositive male rice rats often are larger bodied, heavier, and have larger testes (McIntyre et al., in press). Males negative for anti-BAYV antibodies are smaller in mass, display shorter movement distances, and maintain smaller home range sizes than seropositive males (McIntyre et al., in press). In some male vertebrates, body and testis size are androgen-based status signals strongly predictive of rank (Adkins-Regan 2005, Gomendio et al. 1998), because the larger these physical characteristics, the more likely the male should be able to travel his home range, marshal his territory, fend off would-be “sneakers,” and generally defeat smaller contestants. Rice rats often do not exist in stable, familiar groups for extended periods of time (inferred from their non-affiliative, frequently dispersive nature; but see Abuzeineh et al. 2007). Therefore, an enforced hierarchical system of dominant-subordinate relationships within populations would be unnecessary. Obvious exceptions to this generalized scenario could occur during prolonged periods of high population density and extensive home range overlap, which can lead to short-term, semi-communal conditions, or perhaps when a population remains relatively unchanged for a lengthy duration, in which case both habituation and tolerance might play important roles in obviating potential conflicts between increasingly familiar rodents. In our study, Grid 2 rice rat demography showed little flux over time, and although abundances of each age and sex class were low, relative population stability could play a role in the enforcement of dominance-subordinance statuses and resultant BAYV spillover.

Our 26-month assessment of the BAYV-marsh rice rat relationship in southern Texas does not support the pervasive assumption that hantaviral spillover is common among coexisting species of rodents. Rather, the data reveal a relatively low incidence of secondary host infection even in the hispid cotton rat - a highly aggressive, non-oryzomyine sigmodontid ecologically and phylogenetically closer to the rice rat (Cameron and Kruchek 2005, Weksler 2003) than members of the heteromyine-neotomine-peromyscine rodent complex. Equally important, our data do not lend support to the requisite mechanism for spillover—high rodent density, especially high primary host density. This is not to say that under different ecological or anthropogenic stresses, aberrant host community structure or dynamics, or modifications in virus-host specializations, BAYV-spillover
Figure 3. Correlations (loadings) between the discriminant function (X-axis) and habitat composition variables (Y-axis). Codes for the 37 habitat variables are as follows:

**Average plant height in cm @:** 0.25 m=1, 1 m=2, 3 m=3
G=grass, F=forb, B=bare ground, L=litter/detritus, T=tree, S=shrub, WD=woody debris (downed wood), V=vine, CP=cowpie, %H=water, %R=reed (*Juncus effusus*).

**Percent ground cover @ 0.25 m:** %G=4, %F=5, %B=6, %L=7, %T=8, %S=9, %WD=10, %V=11, %H=12, %R=13

**Percent ground cover @ 1 m:** %G=14, %F=15, %B=16, %L=17, %T=18, %S=19, %WD=20, %V=21, %H=22, %R=23

**Percent ground cover @ 3 m:** %G=24, %F=25, %B=26, %L=27, %T=28, %S=29, %WD=30, %V=31, %CP=32, %H=33, %R=34

**Number of plant species @:** 0.25 m=35, 1 m=36, 3 m=37

Figure 4. Graphic representation showing proportion of rodent community consisting of the marsh rice rat, *Oryzomys palustris* (solid line) and fluctuations in rice rat seropositivity (dashed line) over time. Columns represent non-*Oryzomys* species collectively with number of captures combined for all 4 grids and all traplines by trapping session. Asterisk (*) indicates trapping session when spillover was detected.
cannot be amplified. Also noteworthy is that we presumed non-oryzomyines exposed to BAYV infection had an equal trapability to unexposed non-oryzomyines and that there was no infection-induced mortality in non-primary host species.

The population biology of hantavirus-host interactions involves the dynamics of the virus both within and between host rodents. Most host populations present themselves as erratic, unstable environments for hantavirus survival in terms of seasonal fluctuations in demography, density, and longevity. Moreover, because individual host immune responses vary, hantaviruses must be able to relocate facultatively to the next host before the occupied host either defeats persistence or dies. In other words, viral host specificity should be a common adaptation to evade host immunosurveillance and secure hantavirus infection persistence, and thus reproduction and survival. Evolution of the exclusive host-virus relationship most likely is affected by a variety of genetic, physiologic, and behavioral host traits (Poulin 1998). Specific rodent hosts ideally will be the most abundant small mammal in their respective community, retain the ability to shed live virus throughout their lifespan, and engage in frequent physical contact with other rodents. Taken to the extreme, host specificity can lead to high risks of local extinction if the primary host population crashes and the virus is unable to survive in sympatric species (or in some kind of external media). To reduce the threat of extinction in volatile rodent populations, some hantaviruses may have been selected to utilize a broader array of mammalian hosts. RNA viruses like hantaviruses typically have high mutability and broad host ranges, both of which facilitate not only rapid exploitation responses to environmental or host behavioral changes, but also preliminary infection of a new host followed by adaptation to the new host (Woolhouse et al. 2005).

Several authors have investigated spillover as it relates to taxonomic distance (e.g., inter-specific, -generic, and -familial host switches from primary hosts of the Old and New Worlds; Monroe et al. 1999, Rowe et al. 1995, Ulrich et al. 2002). Purportedly unable to manifest productive carrier states, spillover hosts are believed to have virtually no impact on the dispersal or maintenance of hantaviruses or their related diseases, despite numerous examples of how prolonged spillover host-virus associations can lead to host switching between species of mammals (Bohlmann et al. 2002, Levis et al. 1998, Vapalahti 1999). Clearly, some secondary hosts sustain more than mere seropositivity, leading to a decrease in host specificity.

Intrasexual, intraspecific aggression seems to be the predominant mode of BAYV transmission in the wild. Natural vagility (Smith and Vrieze 1979) and life longevity (Bloch and Rose, unpub. data) in the rice rat could promote dissemination of BAYV within the rodent community as well as to adjacent locales. Greater biodiversity, ecological proximity, and interference competition can create a setting for interspecific spillover from BAYV-infected rice rat males. Ecological correlates of percent water (particularly during low rainfall periods), shrub, and grass cover may be useful habitat compositional indicators for the predictive occurrence of BAYV-spillover hosts. Long-lived, virus-shedding, adult males may dominate preferred habitat (e.g., sources of water and cover), where they also may transmit infection to individuals of other species. Morphological (increased size) and behavioral (increased ranging) correlates of male seropositivity (McIntyre et al., in press) provide plausible explanations for how BAYV-spillover from the rice rat may transpire in the absence of any other overt or acute competitive pressures (e.g., over nest sites or predator escape routes). Allogrooming could provide another avenue by which the virus is trafficked within and between the species: McIntyre et al. (2005) documented salivary shedding of Bayou vRNA by *O. palustris* for up to several months. Aggression-associated reproductive pressures might play a lesser role in BAYV transmission in lower distributional latitudes like southeast Texas, where milder climatic and more favorable ecologic conditions often promote year-round breeding in the rice rat (Wolfe 1985). But how BAYV-spillover may happen does not explain why it happens. Does hantavirus spillover in rodents generally occur by happenstance? Or is it an evolutionarily-based “safety net” for survival – a virus-imposed selective force analogous to bet-hedging, in case the primary carrier species is extirpated? As with any biological or ecological phenomena, chance events can be significant. Although it is improbable that chance events are the primary mechanism for successful hantavirus transmission to the appropriate and intended host rodent, even unlikely spillover may have substantive end results. Accordingly, selected instances of hantavirus spillover ultimately may turn out to be neither random in process nor unimportant in product.

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