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Review Article

Species Identity Supersedes the Dilution Effect Concerning Hantavirus Prevalence at Sites across Texas and México

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Abstract

Recent models suggest a relationship exists between community diversity and pathogen prevalence, the proportion of individuals in a population that are infected by a pathogen, with most inferences tied to assemblage structure. Two contrasting outcomes of this relationship have been proposed: the "dilution effect" and the "amplification effect." Small mammal assemblage structure in disturbed habitats often differs from assemblages in sylvan environments, and hantavirus prevalence is often negatively correlated with habitats containing high species diversity via dilution effect dynamics. As species richness increases, prevalence of infection often is decreased. However, anthropogenic changes to sylvan landscapes have been shown to decrease species richness and/or increase phylogenetic similarities within assemblages. Between January 2011 and January 2016, we captured and tested 2406 individual small mammals for hantavirus antibodies at 20 sites across Texas and México and compared differences in hantavirus seroprevalence, species composition, and assemblage structure between sylvan and disturbed habitats. We found 313 small mammals positive for antibodies against hantaviruses, evincing an overall prevalence of 9.7% across all sites. In total, 40 species of small mammals were identified comprising 2 taxonomic orders (Rodentia and Eulipotyphla). By sampling both habitat types concurrently, we were able to make real-world inferences into the efficacy of dilution effect theory in terms of hantavirus ecology. Our hypothesis predicting greater species richness higher in sylvan habitats compared to disturbed areas was not supported, suggesting the characteristics of assemblage structure do not adhere to current conceptions of species richness negatively influencing prevalence via a dilution effect.

Key words: biotic homogenization; disturbance; EIDs; habitat; Hantaviruses; rodents

Background

Anthropogenic habitat disturbance continues to increase worldwide, leaving few areas unaffected by human alterations (Burney and Flannery 2005; Johnson et al. 2013). Invasive urbanization and land use create changes in ecosystem functioning (Alberti 2005) and fragmented habitats and lead to declines in the presence of native habitat specialists (Trentanovi et al. 2013). These disturbances can have severe impacts on biological diversity by altering ecological relationships (Foley et al. 2005) and modifying natural host-pathogen dynamics (Keesing et al. 2006), potentially leading to the emergence of infectious diseases in humans and wildlife (Gottdenker et al. 2014; Hjelle and Torres-Pérez 2010; Mills et al. 2010). Although the study of emerging infectious disease (EID) dynamics from zoonotic origins is challenging, recent advances promise great strides in this area of research. Current advancements in understanding these relationships consider whole ecological communities where zoonotic pathogens occur, the interactions facilitating pathogen persistence within them, and how assemblage characteristics contribute to outbreaks of human disease (Johnson et al. 2015; Suzán et al. 2015).

Recent models indicate that a relationship exists between community diversity and pathogen prevalence (i.e., the proportion of individuals that are infected by a pathogen) with most of the inferences referring to the assemblage level (i.e., groups of species of the same taxon). Species richness, relative abundance, pathogen specificity, and host species interactions (both intra-/interspecies) within assemblages likely have complex roles in modulating pathogen levels within local assemblages (Milholland, in press; Zargar et al. 2015). Two contrasting outcomes of this relationship have been proposed: the "dilution effect" and the "amplification effect" (Keesing et al. 2006; Ostfeld and Keesing 2000; Pagán et al. 2012; Zargar et al. 2015). In the dilution effect, species diversity in an assemblage (*sensu*, Fauth et al. 1996) presumably reduces disease prevalence and transmission events (Ostfeld and Keesing 2000, 2012; Zargar et al. 2015). Dilution effects may be present when a sufficient number of poor hosts for the propagation of a pathogen (i.e., noncompetent hosts) become infected and are unable to transmit it other individuals (Cohen et al. 2016; Keesing et al. 2010; McGill et al. 2006). This general assumption for the decrease in disease prevalence is often attributed to species richness alone (Calisher et al. 2002; Clay et al. 2009). However, assemblages comprised of many phylogenetically related species, or species with high pathogen competency, can increase, or amplify, infection transmission, thus creating a positive correlation of pathogen prevalence with diversity (Clay et al. 2009). In this case, richness can be relatively large, and phylogenetically related species can, in turn, create an amplification effect due to species diversity (Han et al. 2016; Huang et al. 2016; Milholland et al. 2018; Rubio et al. 2014).

Both models of disease presence have been supported with experimental and observational evidence, particularly in the case of vector-borne diseases (e.g., Lyme disease) (Dizney and Ruedas 2009; LoGiudice et al. 2003; Ostfeld and Keesing 2012). No consensus on the generality of each model has been reached, because

the mechanisms that govern the relationship between diversity and pathogen prevalence are still not fully understood. For example, with hantaviruses (zoonotic agents responsible for hantavirus pulmonary syndrome in the Americas and hemorrhagic fever with renal syndrome in Eurasia), the phylogenetic relationship among species comprising the assemblage plays a crucial role in hantavirus transmission and maintenance as host-switching between related species and pathogen persistence are intimately entwined (Bohlman et al. 2002; Levis et al. 1998; Milholland et al. 2018; Monroe et al. 1999; Morzunov et al. 1998; Nemirov et al. 2010; Ramsden et al. 2009).

Regionally distinct communities of a given taxon can also be influenced by metacommunity dynamics (Holyoak et al. 2005), which, in turn, shape EID persistence and transmission dynamics at landscape scales (Dearing and Dizney 2010; Keesing et al. 2010; Suzán et al. 2015), where prevalence of EID is often associated with the condition of the habitat (Daszak et al. 2001; Gottdenker et al. 2014; Jones et al. 2008; Patz et al. 2004). Small mammal assemblage structures in disturbed habitats often differs from assemblages in sylvan environments (Murphy and Romanuk 2014; Rubio et al. 2014). Anthropogenic changes to sylvan landscapes have been shown to decrease species richness and/or increase phylogenetic similarities of assemblages across spatial scales (Olden and Rooney 2006). These anthropogenic effects are not necessarily changes in number of species at a site, but may also influence the identity of the species (Suzán et al. 2015). As a result, species homogenization in disturbed sites can produce a replacement of specialist species with more adaptive, generalist species and can potentially have highly profound ecological consequences (McKinney 2006; Olden et al. 2004), including the spread and maintenance of EIDs (Previtali et al. 2010).

Often, anthropogenic habitat disturbance decreases assemblage diversity by extirpating dietary or microhabitat specialists (Mills et al. 2010) while increasing abundance of generalist species, many of which are reservoirs of hantaviruses (Calisher et al. 2007; Dearing and Dizney 2010; Lehmer et al. 2008; Mills et al. 2007; Rubio et al. 2014). Moreover, highly disturbed sites can also impede the survival of wild, native, and specialist rodent species, including infected hosts (Lehmer et al. 2008). These anthropogenically dominated sites pose risks (e.g., poison, traps, domestic predators) and increase competition with human commensal rodent species, leaving a few species able to thrive in these environs (Dizney et al. 2010; Meerburg et al. 2009; Morand et al. 2015). A reduction in rodent diversity in disturbed areas may then increase the abundance of resilient, opportunistic hantavirus rodent reservoir species inhabiting species-poor areas, resulting in a greater potential for human-host interactions (Calisher et al. 2002; Clay et al. 2009; Rubio et al. 2014).

Sylvan, or markedly less disturbed, habitats presumably can maintain a lower assemblage seroprevalence associated with higher species richness via the dilution effect (Blasdell et al. 2011; Ostfeld and Keesing 2000). Comparing these natural sites to those anthropogenically modified habitats can provide a measure of the relationship of hantavirus prevalence with species diversity in the same area. The relationship between rodent assemblage structure of sylvan and disturbed sites, and differences in hantavirus seroprevalence between them, will allow inference into correlations between habitat alteration, species diversity, and hantavirus infection (Dizney et al. 2010; Kuenzi et al. 2005).

The study of hantaviruses serves as a good model to address questions regarding the influence of species diversity on pathogen presence for the following reasons: (1) hantaviruses are thought to be directly transmitted between individuals in close associations; (2) hosts of hantaviruses appear to have no major negative impairments caused by infection; (3) hosts (e.g., rodents) are ubiquitous across the landscape, which (4) provides an avenue of inquiry across varying spatial scales.

Figure 1 Sites sampled across Texas and México where small mammals were trapped and tested for hantavirus antibodies between January 2011 and January 2016. At each site, sylvan and disturbed habitats were sampled concurrently.

Our research question was centered on determining if rodent assemblage structure differed between sylvan and disturbed sites at a given locality. We also assessed whether disturbed habitats supported greater assemblage-wide hantavirus seroprevalence as well as greater relative abundance and numerical dominance of hantavirus reservoir rodent species at these sites. Moreover, if species richness differs between sylvan and disturbed habitats, are differences in hantavirus seroprevalence more consistent with the dilution effect than the amplification effect? The objectives of this research were to: (1) determine the rodent assemblage structure in sylvan and disturbed habitats from the same locality in selected sites across a latitudinal gradient covering México and Texas; (2) determine hantavirus seroprevalence in rodents and other small mammals from each habitat type in each locality; and (3) compare seroprevalence in sylvan and disturbed habitats, considering the small mammal assemblage structure at each habitat. We hypothesized that sylvan habitats would have higher species diversity and lower dominance of reservoir species compared to disturbed habitats and that hantavirus prevalence would be higher in disturbed habitats.

Methods

Ethics Statement

Texas fieldwork was conducted with prior approval from Texas State University (IACUC nos. 1206-0113-02 and 201598,223), Texas A&M University (IACUC nos. 2014-0227 and 2016-0243), Texas Parks and Wildlife (TPWD-SPR-1112-1052), and United States Department of the Interior (BITH-2015-SCI-0016). Rodent sampling in México was approved by Secretaría de Medio Ambiente y Recursos Naturales (permit SGPA/DGVS/00,622/11).

Table 1 List of sites following a north-south latitudinal gradient through Texas and México where small mammals were sampled for hantavirus prevalence

Country	State	Site	Geographic location	Ecoregion
United States	Texas	Gus Engeling WMA	31°57′32″N 95°53′28″W	East Central Texas Plains—Post Oak Savannah
		Big Thicket National Preserve	30°32′22″N 94°20′25″W	Piney Woods
		Mason Mountain WMA	30°50′12″N 99°13′23″W	Edwards Plateau
		San Marcos	29°53′18″N 97°56′47″W	Edwards Plateau-Blackland Praries Interface
		Tejas Ranch	29°46′30″N 97°22′53″W	East Central Texas Plains—Floodplains and Low Terraces
		Chaparral WMA	28°19′53″N 99°25′13″W	Southern Texas Plains
		Las Palomas WMA	26°18′48″N 97°30′27″W	Western Gulf Coastal Plain
México	Chihuahua	Janos	30°54'7″N 108°25'17″W	Sierra Madre Occidental
	Tamaulipas	Alta Cima	23°3′38″N 99°12′14″W	Great North American Plains
		Gómez Farías	23°3′56″N 99°10′8″W	North Gulf Coast Plains
		San Jose	23°2′47″N 99°13′53″W	Sierra Madre Oriental
	Hidalgo	Chilcuatla	20°19′29.892″N 99°13′39″W	Central Volcanic Belt
	Veracruz	Coatepec	19°27′54″N 96°59′56″W	South Gulf Coastal Plain
	Morelos	Yautepec	18°47′57″N 99°3′51″W	Sierra Madre del Sur
		Zacualpan	18°49′22″N 98°45′59″W	Sierra Madre del Sur
		Tepalcingo	18°35′28″N 98°58′57″W	Sierra Madre del Sur
		Miacatlán	18°46′10″N 99°23′16″W	Sierra Madre del Sur
		Puente de Ixtla	18°27′51″N 99°15′25″W	Sierra Madre del Sur
		Tepoztlán	18°47′59″N 99°4′6″W	Sierra Madre del Sur
		Tetela del Volcan	18°54′53″N 98°41′35″W	Sierra Madre del Sur

At each site, equal trapping efforts compared sylvan and disturbed (e.g., peridomestic) habitats; WMA, wildlife management area.

Study Sites

Twenty sites were chosen across the state of Texas, United States and the Méxican states of Chihuahua, Hidalgo, Morelos, Tamaulipas, and Veracruz (see Figure 1 and Table 1). Study sites (listed below) follow a north-to-south latitudinal gradient, encompassing a vast region beginning in northeast Texas and concluding in the southern portion of Morelos in central México, thus covering both neartic and neotropical sites. Texas sites included: Gus Engeling Wildlife Management Area (WMA); Big Thicket National Preserve (NP); Mason Mountain WMA; areas within and around the city of San Marcos; Tejas Ranch; Chaparral WMA; and Las Palomas WMA. Sites in México included: Janos, Chihuahua; Alta Cima, Gómez Farías, and San Jose in the state of Tamaulipas; Chilcuatla, Hidalgo; Coatepec, Veracruz; and Yautepec, Zacualpan, Tepalcingo, Miacatlán, Puente de Ixtla, Tepoztlán, and Tetela del Volcan in the state of Morelos. Unique sylvan and disturbed transects were selected and sampled concurrently at each site between January 2011 and January 2016. Habitats with the most historically natural conditions were described as sylvan, and habitats exhibiting visible recent anthropogenic modifications or with existing structures (e.g., buildings, roads, and barns) were considered disturbed.

Rodent Trapping

Using curvilinear transects, small mammals were trapped with (400–500) Sherman live-traps (H. B. Sherman Traps) spaced approximately 5 m apart and baited with rolled oats, peanut butter, and imitation vanilla. Concurrent trapping effort for both habitats occurred for 3 consecutive nights as weather and logistics allowed. All captured rodents were humanely killed with sedation by respiratory inhalation of isoflurane followed by cervical dislocation and were necropsied in the field according to appropriate use and safety protocols (Kelt et al. 2007, 2010; Leary et al. 2013; Mills et al. 1995; Sikes et al. 2011). From each individual, tissues (i.e., blood, heart, lung, liver, kidney, spleen, and articulating joint) were flash frozen in liquid nitrogen then stored at –80°C or placed in 95% ethanol (i.e., articulating joint and spleen) for future analyses. Skull and pelt voucher specimens were also collected and are currently held at Texas State University, San Marcos, TX, or at the Biodiversity Research and Teaching Collections at Texas A&M University, College Station, TX. Voucher specimens collected in Morelos are held at Colección de Mamíferos del CIByC, Universidad Autónoma del Estado de Morelos.

Rodent Species Identification

Trapped rodents were identified in the field. However, juvenile and subadult *Peromyscus* species are notoriously difficult to differentiate (McDaniel et al. 1983). Using skull vouchers, suspect *Peromyscus* were identified to species based on skull measurements and occlusal surface characteristics (Hall 1981). If rodent species identification remained equivocal, we resorted to genetic identification of specimens. DNA was extracted from frozen tissue samples following manufacturer protocol from the Qiagen DNeasy Blood and Tissue Kit (Qiagen Inc.) and stored at –30°C. The mitochondrial *Cytb* gene was amplified and sequenced for each specimen using two sets of overlapping primers: (1) MVZ05 forward (5′- CGA AGC TTG ATA TGA AAA ACC ATC GTT G -3′) (Smith and Patton 1993) and P3′ reverse (5′- TCT CTC CGG TTT ACA AGA CCA AAG T -3′); and (2) LGL 765 forward (5′- GAA AAA CCA YCG TTG TWA TTC AAC T -3′) (Bickham et al. 2004) and 752 reverse (5′- GCA GGA GTG TAA TTA TCG GGG TCT -3′). These primer sets were modified from C. W. Edwards and R. D. Bradley (2002) with denaturation at 94°C for 1 min, followed by 35 cycles of 30 sec at 94°C, 1 min at 50°C, and 70 sec at 72°C. Sequences were aligned and consensus sequences were generated from forward and reverse sequences for every specimen in Geneious 8.1.7 (Biomatters Ltd.),

Table 2 List of small mammals captured and tested for hantavirus antibodies across sites in Texas and México between January 2012 and January 2016

Order/family	Subfamily	Species	N	TSI
Rodentia/Cricetidae	Neotominae	*Baiomys musculus*	24	0
Rodentia/Cricetidae	Neotominae	*Baiomys taylori*	60	6
Rodentia/Cricetidae	Neotominae	*Hodomys alleni*	1	0
Rodentia/Cricetidae	Neotominae	*Neotoma albigula*	8	4
Rodentia/Cricetidae	Neotominae	*Neotoma floridana*	26	10
Rodentia/Cricetidae	Neotominae	*Neotoma mexicana*	4	0
Rodentia/Cricetidae	Neotominae	*Neotoma micropus*	14	5
Rodentia/Cricetidae	Neotominae	*Ochrotomys nuttali*	41	2
Rodentia/Cricetidae	Neotominae	*Onychomys arenicola*	87	19
Rodentia/Cricetidae	Neotominae	*Onychomys leucogaster*	15	2
Rodentia/Cricetidae	Neotominae	*Peromyscus attwateri*	23	2
Rodentia/Cricetidae	Neotominae	*Peromyscus boylii*	7	1
Rodentia/Cricetidae	Neotominae	*Peromyscus difficilis*	41	11
Rodentia/Cricetidae	Neotominae	*Peromyscus furvus*	7	0
Rodentia/Cricetidae	Neotominae	*Peromyscus gossypinus*	95	9
Rodentia/Cricetidae	Neotominae	*Peromyscus leucopus*	380	16
Rodentia/Cricetidae	Neotominae	*Peromyscus levipes*	136	4
Rodentia/Cricetidae	Neotominae	*Peromyscus maniculatus*	117	41
Rodentia/Cricetidae	Neotominae	*Peromyscus melanophrys*	66	16
Rodentia/Cricetidae	Neotominae	*Peromyscus mexicanus*	15	0
Rodentia/Cricetidae	Neotominae	*Peromyscus ochraventer*	56	2
Rodentia/Cricetidae	Neotominae	*Peromyscus pectoralis*	35	2
Rodentia/Cricetidae	Neotominae	*Peromyscus species*	25	0
Rodentia/Cricetidae	Neotominae	*Reithrodontomys fulvescens*	50	1
Rodentia/Cricetidae	Sigmodontinae	*Oryzomys couesi*	3	0
Rodentia/Cricetidae	Sigmodontinae	*Oryzomys paulustris*	3	0
Rodentia/Cricetidae	Sigmodontinae	*Oryzomys species*	3	0
Rodentia/Cricetidae	Sigmodontinae	*Sigmodon hispidus*	200	23
Rodentia/Cricetidae	Sigmodontinae	*Sigmodon toltecus*	25	0
Rodentia/Heteromyidae	Perognathinae	*Chaetodipus hispidus*	55	1
Rodentia/Heteromyidae	Perognathinae	*Chaetodipus penicillatus*	83	11
Rodentia/Heteromyidae	Dipodomyinae	*Dipodomys merriami*	255	56
Rodentia/Heteromyidae	Dipodomyinae	*Dipodomys ordii*	9	0
Rodentia/Heteromyidae	Dipodomyinae	*Dipodomys spectabilis*	110	28
Rodentia/Heteromyidae	Heteromyinae	*Liomys irroratus*	109	6
Rodentia/Heteromyidae	Perognathinae	*Perognathus flavus*	45	6
Rodentia/Heteromyidae	Perognathinae	*Perognathus merriami*	8	0
Rodentia/Muridae	Murinae	*Mus musculus*	75	8
Rodentia/Muridae	Murinae	*Rattus rattus*	19	6
Rodentia/Sciuridae	Sciurinae	*Glaucomys volans*	6	0
Rodentia/Sciuridae	Xerinae	*Spermophilus variegatus*	3	1
Eulipotyphla/Soricidae	Soricinae	*Cryptotis parva*	62	5

Total sampling effort was 16 875 trapnights. Mammals are arranged alphabetically according to taxonomic identification (Wilson and Reeder 2005). N, number of individuals collected and tested for hantavirus antibodies; TSI, total number of seropositive individuals.

and compared to sequences from the National Center of Biotechnology Information GenBank database.

Identifying Seropositive Rodents

Blood samples were collected using Nobuto strips (Advantec Inc.) and used for enzyme-linked immunosorbant assay (ELISA) techniques in the laboratory. Blood samples on Nobuto strips were dried in sunlight to inactivate infectious virus before performing the ELISAs. Initial ELISA testing was done at the Arthropod-Borne Infectious Diseases Laboratory, Colorado State University, and completed at the Department of Biology, Texas State University. Because antibodies to Sin Nombre virus nucleocapsid antigen are cross-reactive with several hantaviruses (Schountz et al. 2014), antibodies to specific viral species were not determined. Under BSL-2 conditions, with BSL-3 precautions (CDC 1994), dried Nobuto strips were placed in separate microfuge tubes and rehydrated into 1:5 dilutions overnight at 4°C in 500 μL elution buffer (sterile filtered Dulbecco's phosphate buffered saline [DPBS], 0.5% bovine serum albumin, and 1.0% penicillin/streptomycin). Recombinant Sin Nombre virus nucleocapsid antigen was diluted to 1 μg/mL in DPBS and 100 μL dispensed into wells of a 96-well polyvinylchloride plate (Falcon) (Schountz et al. 2014). Plates were incubated overnight at 4°C, washed (3×) with DPBS-Tween 20, and blocked with 150 μL/well of 0.25% porcine skin gelatin (Sigma-Aldrich Corp.) in DPBS (pH 7.4) for at least 1 h. Dilutions in microfuge tubes were heat inactivated at 60°C for 30 min as a precaution to further inactivate any hantaviruses without

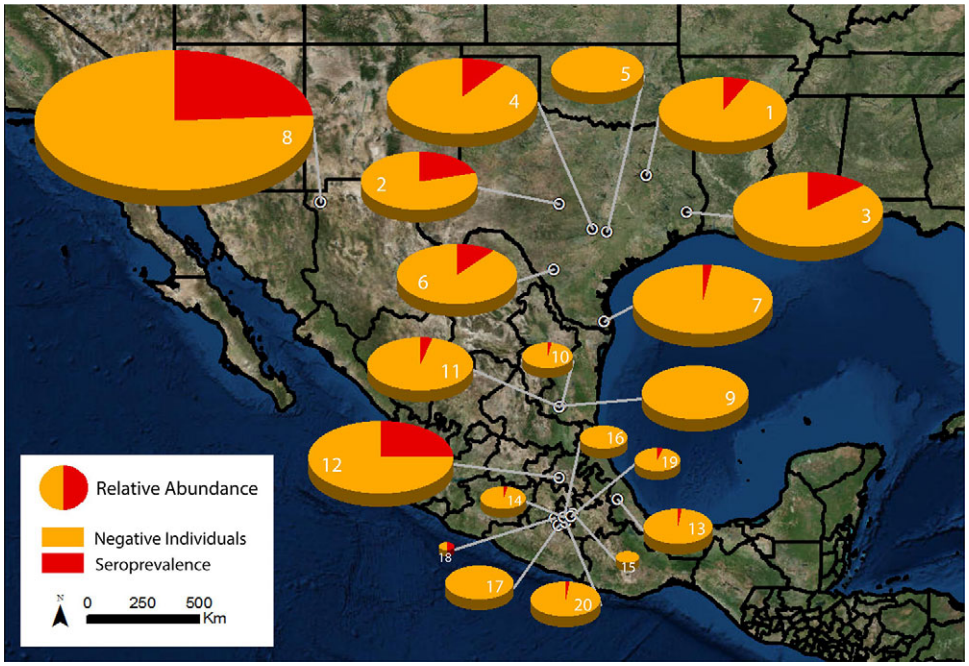

Figure 2 Sites sampled across Texas and México where small mammals were trapped and tested for hantavirus antibodies between January 2011 and January 2016. At each site, sylvan and disturbed habitats were sampled concurrently. Circle size represents abundance (N) of small mammals at each site. Red wedges show the proportion of individuals seropositive for hantavirus antibodies at each site. Sites numbers are listed in white and follow a north-south latitudinal gradient (see Figure 1).

damaging IgG antibodies. Samples were further diluted (1:100) in microfuge tubes by adding 475 µL to 25 µL of sample. Plates were washed (3×) with DPBS-Tween 20, and 100 µL of each sample, a positive control, and a negative control were added and incubated at ambient temperature for one hour. Plates were washed (4×) with DPBS-Tween 20 and 100 µL of 1:5,000 purified Recomb (Thermo Scientific) protein A/G horseradish peroxidase dilute in DPBS was added to wells and incubated at ambient temperature for 45 minutes. Plates were again washed (4×) with DPBS-Tween 20, and 100 µL of activated 2,2′–azinobis(3-ehylbenzthiazolinesulfonic acid was added to wells for 15 min. Absorbance in each well was recorded at 405 nm (BioTek PowerWave XS, BioTek Instruments, Inc.) where positive samples were determined as 0.20 optical density (OD) units greater than the negative control (Schountz et al. 2007).

Assemblage Structure and Seroprevalence Data Analyses

We used capture data and ELISA results to compare assemblage structure and hantavirus seroprevalence between the 2 habitat types at each site and collectively across Texas and México. Assemblage descriptors included the following: (SRPV) = the prevalence of assemblage-wide hantavirus antibody-positive individuals captured in each habitat per site; (N) = the number of individuals of each species captured in each habitat per site (i.e., species abundance); (S) = species richness included the number of different species captured in each habitat per site; and (PIE), Hurlbert's probability of interspecies encounters (Hurlbert 1971) as a measure of assemblage evenness. To account for imperfect detections (Kellner and Swihart 2014) across sites, the Chao estimate and the abundance coverage-based estimate of species richness were calculated using the Species Prediction and Diversity Estimation (Chao 1984; Chao and Lee 1992). These estimated values were compared to the raw species richness to determine if any difference exists for use in the overall statistical analysis.

It is important to note that SRPV estimates are calculated as the average of within-species prevalence in each assemblage, providing a more accurate representation of the contribution of each species to overall assemblage prevalence than would a single, collective percentage. Furthermore, estimates of PIE provide a numerical description of the proportions of each species distributed throughout each assemblage. Seroprevalence and assemblage dominance descriptors were initially compared using paired Student's *t* tests. All sites were compared regarding SRPV and N. However, the ordinate rank of the most abundant host, the number of infected dominant host individuals, and the relative proportion of the dominant host species within the assemblage (DR) was calculated for sites with $S > 1$, and sites where hantavirus antibodies were detected.

We also utilized a Generalized Linear Mixed Effect Model to make inferences regarding habitat type and host genera on the likelihood of hantavirus infection among individuals. Each rodent capture was treated as a binary data point for infection serostatus as determined by ELISA. In our model, site was treated as a random effect while habitat and genus remained as fixed factors using the "glmmML" package in R.

Results

Capture Totals and Hantavirus Prevalence

Total sampling effort included 16 875 trapnights from January 2011 to January 2016 across 13 different ecoregions (Table 1) resulting in the capture of 2406 individual small mammals. In

Table 3 Total number of small mammals collected and tested for hantavirus antibodies from sites across Texas and México

Country	State	Site	N	S	PIE	TSI	SRPV	DR	Rank	#Positive	Dominant Host Species
United States	Texas	Gus Engeling WMA-Disturbed	71	10	0.81	3	6.48	0.38	1	1	*Sigmodon hispidus*
		Gus Engeling WMA-Sylvan	80	8	0.84	10	17.20	0.26	1	3	*Peromyscus gossypinus*
		Mason Mountain WMA-Disturbed	60	5	0.54	1	10.00	0.03	4	1	*Neotoma micropus*
		Mason Mountain WMA-Sylvan	64	7	0.72	4	17.55	0.27	2	2	*Peromyscus attwateri*
		Big Thicket National Preserve-Disturbed	122	8	0.78	15	38.51	0.03	5	2	*Ochrotomys nuttalli*
		Big Thicket National Preserve-Sylvan	81	7	0.78	13	17.31	0.35	1	5	*Peromyscus gossypinus*
		San Marcos-Disturbed	135	9	0.79	22	13.91	0.36	1	13	*Sigmodon hispidus*
		San Marcos-Sylvan	72	6	0.39	0	0.00	N/A	N/A	N/A	N/A
		Tejas Ranch-Disturbed	41	6	0.42	0	0.00	N/A	N/A	N/A	N/A
		Tejas Ranch-Sylvan	36	4	0.30	2	1.67	0.83	1	2	*Peromyscus leucopus*
		Chaparral WMA-Disturbed	102	7	0.63	9	12.60	0.19	2	5	*Sigmodon hispidus*
		Chaparral WMA-Sylvan	30	5	0.76	2	6.67	0.40	1	1	*Peromyscus leucopus*
		Las Palomas WMA-Disturbed	136	8	0.68	3	7.18	0.20	2	2	*Liomys irroratus*
		Las Palomas WMA-Sylvan	41	4	0.62	1	8.33	0.07	3	1	*Neotoma micropus*
México	Chihuahua	Janos-Disturbed	26	6	0.74	11	41.67	0.46	1	6	*Onychomys arenicola*
		Janos-Sylvan	682	13	0.80	140	23.53	0.37	1	56	*Dipodomys merriami*
México	Tamaulipas	Alta Cima-Disturbed	64	5	0.72	0	0.00	N/A	N/A	N/A	N/A
		Alta Cima-Sylvan	49	3	0.55	0	0.00	N/A	N/A	N/A	N/A
		Gómez Farías-Disturbed	10	4	0.78	1	6.25	0.40	1	1	*Peromyscus pectoralis*
		Gómez Farías-Sylvan	13	1	N/A	1	7.69	1.00	1	1	*Peromyscus pectoralis*
		San Jose-Disturbed	73	3	0.27	1	0.54	0.85	1	1	*Peromyscus levipes*
		San Jose-Sylvan	28	3	0.64	4	11.11	0.43	1	2	*Peromyscus levipes*
México	Hidalgo	Hidalgo-Disturbed	93	9	0.84	37	29.24	0.24	1	14	*Peromyscus maniculatus*
		Hidalgo-Sylvan	99	5	0.72	28	20.18	0.39	1	14	*Peromyscus maniculatus*
México	Veracruz	Veracruz-Disturbed	18	6	0.76	1	2.08	0.44	1	1	*Mus musculus*
		Veracruz-Sylvan	26	5	0.73	0	0.00	N/A	N/A	N/A	N/A
México	Morelos	Tepoztlán-Disturbed	15	6	0.86	1	4.17	0.27	1	1	*Mus musculus*
		Tepoztlán-Sylvan	4	2	0.67	0	0.00	N/A	N/A	N/A	N/A
		Tetela Del Volcan-Disturbed	4	3	N/A	0	0.00	N/A	N/A	N/A	N/A
		Tetela Del Volcan-Sylvan	1	1	N/A	0	0.00	N/A	N/A	N/A	N/A
		Yautepec-Disturbed	5	2	0.60	0	0.00	N/A	N/A	N/A	N/A
		Yautepec-Sylvan	16	5	0.81	0	0.00	N/A	N/A	N/A	N/A
		Puente de Ixtla-Disturbed	23	3	0.63	0	0.00	N/A	N/A	N/A	N/A
		Puente de Ixtla-Sylvan	20	4	0.72	0	0.00	N/A	N/A	N/A	N/A
		Miacatlán-Disturbed	1	1	N/A	0	0.00	N/A	N/A	N/A	N/A
		Miacatlán-Sylvan	1	1	N/A	1	100.00	N/A	N/A	1	*Liomys irroratus*
		Zacualpan-Disturbed	13	3	0.72	1	8.33	0.31	2	1	*Liomys irroratus*
		Zacualpan-Sylvan	6	2	0.60	0	0.00	N/A	N/A	N/A	N/A
		Tepalcingo-Disturbed	34	5	0.80	1	3.33	0.18	4	1	*Peromyscus levipes*
		Tepalcingo-Sylvan	11	5	0.78	0	0.00	N/A	N/A	N/A	N/A

Mammals were concurrently trapped in distrubed and sylvan habitats at each site. Trapping occurred from January 2011 to January 2016 during 16 875 trapnights. N, relative abundance; S, species richness; PIE, Hurlbert's index of evenness; SRPV, percent hantavirus seroprevalence; TSI, total number of seroprevalent individuals; DR, dominance index of most abundant host in the assemblage; Rank, ordinate abundance rank of dominant host in the assemblage; #Positive, number of seropositive dominant host species. Sites are listed in a north-south latitudinal gradient.

Texas, 1009 rodents and 62 soricomorphs were collected with an additional 1335 rodents collected in México. In total, 40 species of small mammals were identified comprising 2 taxonomic orders (Rodentia and Eulipotyphla) including 4 and 1 families and 9 and 1 subfamilies, respectively (Table 2). In all, 304 small mammals tested positive for antibodies against hantaviruses (Table 2), thus evincing an overall prevalence of 9.7% across all sites. The distribution of prevalence was highly heterogenous ranging from sites without captured infected individuals (Alta Cima, Puente De Ixtla, Tetela Del Volcan, and Yautepec in México) to sites with high prevalence occurring at Chilcuatla (SRPV = 24.6%; Hidalgo, MX) and Janos (SRPV = 24.2%; Chihuahua, MX) (Figure 2; Table 3). Seropositive (number of individuals with antibodies detected/number of individuals within a species) *Peromyscus* species (*P. maniculatus* 28/61; *P. difficilis* 11/41; *P. melanophrys* 16/35) were the numerically dominant genus at the Chilcuatla site in both habitat types (Tables 2 and 3). Species richness also varied across all sites (Figure 3; Table 3) but was greatest at the Janos site (S = 13), which was numerically dominated by infected Heteromyid species (*Dipodomys merriami* 56/255; *D. spectabilis* 28/110; *Chaetodipus penincillatus* 11/83; *Perognathus flavus* 5/42; *C. hispidus* 1/22; *D. ordii* 0/9) followed by Cricetid species (*Onychomys arenicola* 19/87; *P. maniculatus* 13/47; *P. leucopus* 9/24; *O. leucogaster* 2/12; *Neotoma albigula* 4/8; *Baiomys taylori* 2/3). Additionally, we captured 62 *Cryptotis parva* with 5 individuals positive for hantavirus antibodies in Texas (Table 2). Student's *t* tests revealed no statistical differences in seroprevalence (SRPV $t_{19} = -0.99$, $P = 0.17$), capture abundance (N $t_{19} = -0.46$, $P = 0.33$), species richness (S $t_{17} = -1.26$; $P = 0.11$), or evenness distributions of species (PIE $t_{17} = -0.84$; $P = 0.20$) within assemblages between habitat types across all sites. Our Generalized Linear Mixed Effect Model (serostatus ~ habitat + genus) places higher emphasis on the likelihood of

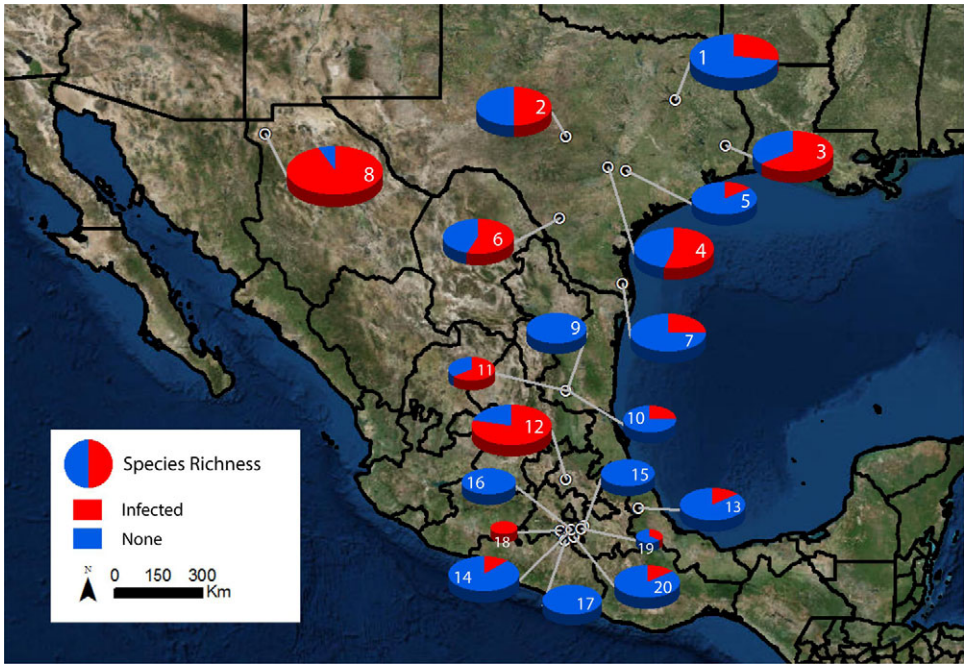

Figure 3 Sites sampled across Texas and México where small mammals were trapped and tested for hantavirus antibodies between January 2011 and January 2016. At each site, sylvan and disturbed habitats were sampled concurrently. Here, circle size is based on species richness (S) of small mammals captured at each site. Red wedges show the proportion of taxonomic genera seropositive for hantavirus antibodies at each site. Sites numbers are listed in white and follow a north-south latitudinal gradient (see Figure 1).

Table 4 Summary results for a Generalized Linear Mixed-Effect Model from small mammals captured and tested for hantavirus antibodies across sites in Texas and México between January 2012 and January 2016

Factor	β	SE	z	P
(Intercept)	−3.92	0.57	−6.94	**<0.001**
habitat SYLVAN	−0.20	0.19	−1.04	0.30
Chaetodipus	−0.24	0.59	−0.40	0.69
Cryptotis	1.02	0.73	1.40	0.16
Dipodomys	0.64	0.53	1.21	0.23
Glaucomys	−10.94	538.82	−0.02	0.98
Hodomys	−7.63	462.27	−0.02	0.99
Liomys	0.26	0.64	0.41	0.68
Mus	1.33	0.63	2.12	**0.03**
Neotoma	3.02	0.58	5.17	**<0.001**
Ochrotomys	0.58	0.90	0.64	0.52
Onychomys	0.54	0.57	0.95	0.34
Oryzomys	−9.99	389.97	−0.03	0.98
Perognathus	−0.08	0.66	−0.12	0.90
Peromyscus	0.93	0.47	1.98	**0.05**
Rattus	2.78	0.75	3.73	**<0.001**
Reithrodontomys	−0.14	1.13	−0.12	0.91
Sigmodon	1.68	0.55	3.06	**<0.01**
Spermophilus	3.16	1.40	2.26	**0.02**

Total sampling effort was 16 875 trapnights. Individual infection status was the response variable, with factors for genus and habitat (sylvan/disturbed) as fixed effects. Species are listed alphabetically and genera were compared to *Baiomys*. Site was treated as a random effect.

certain genera being infected with hantaviruses than habitat type (Table 4), where *Mus* (β = 1.33; $P = 0.03$), *Neotoma* (β = 3.02; $P < 0.001$), *Peromyscus* (β = 0.93; $P = 0.05$), *Rattus* (β = 2.78; $P < 0.001$), *Sigmodon* (β = 1.68; $P < 0.01$), and *Spermophilus* (β = 3.16; $P = 0.02$) having the highest likelihood of infection given our dataset.

Across all sites, only one (Janos) seems to follow traditional dilution effect dynamics showing a higher SRPV, lower S, and lower N in disturbed habitat when compared to sylvan (Table 2); however, the disproportionate sample size between the two habitat types may mask the true underpinnings of hantavirus maintenance in this locality. We found further evidence of dilution effect tendencies at Big Thicket NP, San Marcos, Chaparral WMA, and Hidalgo, where SRPV was greater in disturbed habitats when compared to sylvan. Yet at each of these sites species diversity appears to greater in disturbed areas (Table 2), which is not consistent with dilution effect predictors. Though dilution effects appear to be site-specific, the heterogeneity of SRPV across all sites and habitat types ($SRPV_{mean} = 9.7\%$; Figure 4), when concerted with species richness (S), assemblage evenness (PIE), and the dominance of host species (DR) (Table 2), suggests assemblage-wide prevalence and maintenance of hantaviruses across our study area do not conform to dilution effect dynamics.

Discussion

To our knowledge, this study represents the most extensive hantavirus serosurvey using standardized methods that compares prevalence between sylvan and disturbed habitat types at a large geographical scale. By sampling both habitat types concurrently, we were able to meet our research objectives and make real-world inferences into the efficacy of dilution effect theory in terms of hantavirus ecology. The hypothesis predicting greater species richness in sylvan habitats compared to disturbed areas was not supported (see also, Lehmer et al. 2008), suggesting the characteristics of assemblage structure (e.g., high species diversity = lower disease prevalence) do not adhere to current conceptions of species richness negatively

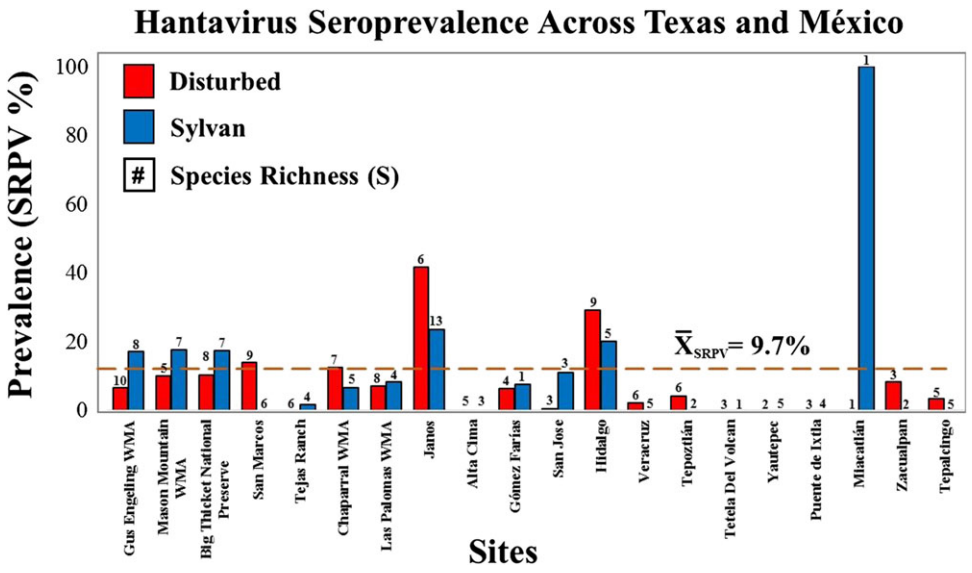

Figure 4 Bar graph showing hantavirus prevalence across sites sampled across Texas and México where small mammals were trapped and tested for hantavirus antibodies between January 2011 and January 2016. At each site, sylvan and disturbed habitats were sampled concurrently. Red bars indicate seroprevalence of small mammal assemblages sampled in disturbed habitats, while sylvan samples are displayed in blue. Black numbers at the top of each bar represent species richness of the assemblage. Orange dashed-line indicates the mean of seroprevalence across all sites.

influencing prevalence via a dilution effect (Clay et al. 2009; Calisher et al. 2002; Reusken and Heyman 2013). Additionally, some assemblages with high species diversity appear to amplify hantavirus prevalence, regardless of habitat type, and maintain infections through spillover dynamics. Furthermore, assemblages with high species richness can maintain hantavirus infection within assemblages of closely related species. Therefore, species identity and the phylogenetic relationship between each species comprising an assemblage is likely a strong driver for the persistence of hantavirus infection within small mammal populations.

In our study, cricetid rodents of the subfamily Neotominae had the most individuals testing positive for antibodies to hantaviruses. However, 6 sites were dominated by heteromyid species (Table 2) representing 3 subfamilies (Dipodomyinae, Heteromyinae, and Perognathinae) with 84, 6, and 18 testing positive for hantavirus antibodies, respectively. Detections of hantavirus seropositive heteromyids are often dismissed as spillover occurrences due to narrow focus on specific host species. Here, and elsewhere, we find cases in which heteromyids appear to be directly associated with hantavirus prevalence and maintenance (Table 2) (Mills et al. 1997; Torres-Pérez et al. 2010; Arellano et al. 2012; Milholland et al. 2018). For example, *Liomys irroratus* appears to significantly influence the presence of hantaviruses in Las Palomas WMA and Zacualpan (Table 2), though, given the capture abundance, this species is not necessarily the dominant component of the assemblage. Moreover, *L. irroratus* (n = 2/15) was found to be hantavirus positive in Hidalgo, México where this species was fourth in ordinate rank in relative abundance of the most frequently captured host. Furthermore, within the same assemblage, 1 of 3 *Perognathus flavus* had hantavirus antibodies while ranking 7th in the assemblage. This supports the notion that hantaviruses can be maintained in phylogenetically diverse assemblages with high species richness.

This leads to questions regarding community assembly, hantavirus maintenance, and spatial scale. At what spatial scale do we find differences in species richness, phylogenetic composition, and dominance components of rodent host assemblages? Understanding the spatial scale at which differences in assemblage species composition become apparent is critical (Suzán et al. 2015) to effective surveillance and prevention of zoonotic disease. Ecological structure of species assemblages at certain localities may vary across seasons or conditions, especially if the species comprising the assemblages share similar habitats, utilize resources in similar ways, or are in competition for these resources (McKinney and Lockwood 1999). The persistence and transmission dynamics of hantaviruses across Texas and México may rely on interactions between infected and naïve individuals at various spatial scales and from interactions between adjacent assemblages, or meta-communities (Suzán et al. 2015) and be dependent upon host species distributions and their infection competency and potential (Johnson et al. 2015; Han et al. 2016; Milholland et al. 2018).

As anthropogenic influences increase patchiness of disturbed and sylvan habitats, we likely see fluxes of species movements in and among patches attracted to novel or limited resources (e.g., shelter and ephemeral sustenance). Thus, overall disturbed areas might experience reduced richness and the presence of more generalist species than sylvan; however, temporal fluxes could cause this pattern to disappear on occasion as species move into and out of disturbance and patches (Suzán et al. 2015). Biotic homogenization, or a process by which adjacent communities become similar over time (Baeten et al. 2012), is often driven by habitats influenced (e.g., disturbed) by anthropogenic manipulations (Foley et al. 2005; McKinney 2006; Trentanovi et al. 2013). This disturbance alters available environmental resources and often favors generalists host species with large home ranges, and/or highly adaptable exotic species which could serve as novel hosts (McKinney and Lockwood 1999; Trentanovi et al. 2013). Additionally, as human densities increase, natural habitats are becoming increasingly sparse with little sylvan areas remaining (Foley et al. 2005). Though urban disturbance can create artificial microhabitats increasing localized species richness (McKinney 2006), immigrating species are often generalist species with high zoonotic

potential and competency, increasing the frequency of human-wildlife interactions and the risk of spreading EIDs (Han et al. 2016).

Conclusions

Between January 2011 and January 2016, we captured and tested 2406 individual small mammals for hantavirus antibodies at 20 sites across Texas and México and compared differences in hantavirus seroprevalence, species composition, and assemblage structure between sylvan and disturbed habitats. We found 313 small mammals positive for antibodies against hantaviruses, with high heterogeneity across all sites. Though cricetid rodents appear to play a central role as hantavirus hosts, we found heteromyid species to be dominant contributors to hantavirus maintenance at 4 sites. Additionally, our study has shown increased species diversity is not necessarily the driver of decreased hantavirus prevalence and habitat disturbance has little predictive value in estimating prevalence. Instead, our data suggest the species identity of potential hosts comprising the assemblage, their zoonotic potential (Han et al. 2016), and their relative abundance provide a foundational contribution to assemblage-wide hantavirus prevalence.

The serological method we employed does not allow the identification of the specific hantavirus species causing the infections. Therefore, further molecular analyses of hantavirus infections in rodents from all study areas would help elucidate which hantaviruses species are harboring in any given locality. This information would allow to deepen the study of the dynamics of hantavirus transmission and maintenance in small mammal assemblages across Texas, México, and elsewhere.

Finally, as unabated urbanization continues, nonrandom specialized species extinction and/or replacement by generalists (McKinney 2006; McKinney and Lockwood 1999) is altering natural ecological functions (Trentanovi et al. 2013), community assembly (Suzán et al. 2015), and infectious disease (i.e., hantavirus) dynamics (Han et al. 2016; Ruesken and Heyman 2013), requiring intensive efforts to understand EID dynamics across spatial scales (Johnson et al. 2015; Milholland et al. 2018). Though it has been shown that habitat preservation and conservation decrease the expansion of invasive host species and can increase species diversity (Abadie et al. 2011; Jones et al. 2008), we find here truly sylvan habitat may be nonexistent in our study area and/or dilution effects are limited to site/habitat-specific assemblage characteristics. If the accuracy of the former scenario is true, these data provide urging support for increased land conservation efforts across Texas and México, which may help decrease the potential of EID reemergence in this region.

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