

Geographical distribution of rodent-associated hantaviruses in Texas

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ABSTRACT: The purpose of this study was to increase our knowledge of the geographic distribution and natural host range of hantaviruses in Texas, southeastern New Mexico, and Mexico. Blood samples from 3,225 wild rodents, representing 34 species, were tested for hantavirus antibody (IgG), using an enzyme-linked immunosorbent assay. Hantavirus antibody was found in one or more rodents from each of 13 counties in Texas, Otero County in southeastern New Mexico, and Mexico State (central Mexico). The 133 antibody-positive rodents included seven *Peromyscus* species (*P. attwateri*, *P. boylii*, *P. hylocetes*, *P. leucopus*, *P. maniculatus*, *P. melanotis*, and *P. pectoralis*), *Sigmodon hispidus*, *Oryzomys palustris*, two *Reithrodontomys* species (*R. fulvescens* and *R. megalotis*), *Neotoma albigula*, and *Perognathus merriami*. This study provides further evidence that rodent-associated hantaviruses are geographically widely distributed in Texas. The discovery of antibody in *P. hylocetes* and *P. melanotis* is evidence that peromyscine rodents in Mexico are naturally associated with viruses belonging to the genus *Hantavirus*. **Journal of Vector Ecology 26(1): 7-14. 2001.**

Keyword Index: Hantavirus, *Peromyscus*, Texas, Mexico

INTRODUCTION

Specific rodents, usually one or two closely related species, are the principal hosts of the hantaviruses (family Bunyaviridae, genus *Hantavirus*) for which natural host relationships have been well characterized. The hantaviruses known to occur in North America include Seoul (Childs et al. 1987, Glass et al. 1994), Prospect Hill (Lee et al. 1982, Yanagihara et al. 1987), Isla Vista (Song et al. 1995), Bloodland Lake (Hjelle et al. 1995b), Sin Nombre (Childs et al. 1994, Monroe et al. 1999, Nichol et al. 1993), Monongahela (Song et al. 1996), New York (Hjelle et al. 1995c, 1995d), Blue River (Morzunov et al. 1998), El Moro Canyon (Hjelle et al. 1994), Rio Segundo (Hjelle et al. 1995a), Black Creek Canal (Rollin et al. 1995), Muleshoe (Rawlings et al. 1996), and Bayou (Ksiazek et al. 1997, Torrez-Martinez et al. 1998). Seoul virus is an Old World hantavirus that probably was introduced into the Americas in association with its principal host, *Rattus norvegicus* (Norway rat). The 12 other viruses are naturally associated with rodents indigenous to the Americas (specifically, murid rodents belonging to the

subfamily Sigmodontinae or Arvicolinae). Four of these viruses (Sin Nombre, New York, Black Creek Canal, and Bayou) are known to cause hantavirus pulmonary syndrome (HPS), a severe and oftentimes fatal human disease.

The diverse environment of Texas supports a resident fauna of 27 sigmodontine and three arvicoline species (Davis and Schmidly 1994). The hantaviruses known to occur in Texas are Sin Nombre, El Moro Canyon, Muleshoe, and Bayou. Sin Nombre virus infection was found in *Peromyscus maniculatus* (deer mouse) and *Peromyscus leucopus* (white-footed mouse) in Castro, Elko, Deaf Smith, Loving, and/or Moore counties (Monroe et al. 1999, Rawlings et al. 1996); Muleshoe virus was discovered in association with *Sigmodon hispidus* (hispid cotton rat) collected near the town of Muleshoe in Bailey County (Rawlings et al. 1996); Bayou virus infection was detected in *Oryzomys palustris* (marsh rice rat) collected from Jefferson County (Torrez-Martinez et al. 1998); and El Moro Canyon virus infection was found in *Reithrodontomys megalotis* (western harvest mouse) and *P. maniculatus* captured in Bailey County (Rawlings

et al. 1996). Other evidence that hantaviruses occur in Texas includes 12 HPS cases: one case each from Angelina, Castro, Deaf Smith, El Paso, Gaines, Hunt, Kleberg, Potter, Randall, and Taylor counties, and two cases from Jefferson County (Alexander 1999, Rawlings et al. 1996, Ray et al. 1998, Torrez-Martinez et al. 1998).

The primary objective of the present study was to increase our knowledge of the geographic distribution and natural host range of hantaviruses associated with rodents native to Texas. A secondary objective was to extend our knowledge of the rodent host associations of hantaviruses in southeastern New Mexico and Mexico.

MATERIALS AND METHODS

Rodents

Blood samples from 3,225 wild rodents (representing 22 sigmodontine and one arvicoline species in the family Muridae, and 11 species in the family Heteromyidae) were tested for hantavirus antibody. The geographical distribution of the rodents was 2,954 from 23 counties in Texas, 250 from Otero County in southeastern New Mexico, and 21 from Mexico. All of the animals included in this survey were identified to species level by mammalogists at Texas Tech University.

The majority (1,677 or 52%) of the rodents were collected in 1997-1999 by scientists and students at Texas Tech University as part of a vertebrate faunal survey of lands owned or managed by the Texas Parks and Wildlife Department. The 1,277 other rodents from Texas were collected in 1995-1996 for a study on the ecology of rodents indigenous to Galveston County, Texas (Hice 1996, Hice and Schmidly 1999). The 2,954 rodents from Texas were collected in a variety of habitats: coastal prairie (Brazoria, Galveston, and Matagorda counties), Chihuahuan desert (Brewster, Dimmitt, La Salle, and Presidio counties), juniper grassland (Kerr and Kimble counties), mesquite grassland (Mason County), midgrass prairie (Bailey, Cottle, and Lubbock counties), montane pine-oak forest (Jeff Davis County), and hardwood bottomland (Anderson, Bowie, Cass, Harrison, Lamar, Leon, Morris, Shelby, and Titus counties).

The 250 animals from New Mexico were collected in 1998 as part of a vertebrate faunal survey of Fort Bliss (United States Department of Defense). The 21 animals from Mexico were collected by scientists from Texas Tech University and the Universidad Nacional Autonoma de Mexico. Eighteen of the 21 Mexican specimens were collected near San Bartolo Morelos, a

small rural community in Mexico State. The three other Mexican rodents were collected from Ocampo in the state of Coahuila. San Bartolo and Ocampo are located approximately 100 km west-northwest and 950 km northwest of Mexico City, respectively.

Antibody assay

The blood samples were tested for antibody (IgG) reactive against Caño Delgadito (CDG) virus, using an enzyme-linked immunosorbent assay (ELISA) described previously (Fulhorst et al. 1997). CDG virus is a New World hantavirus and highly cross-reactive in the ELISA with Sin Nombre and Black Creek Canal viruses, and other sigmodontine rodent-associated hantaviruses (Fulhorst et al. 1997). The test and control (comparison) antigens were sonicated, detergent (t-Octylphenoxypolyethoxyethanol [Triton X-100; Sigma Chemical Co., St. Louis, MO]) extracts of Vero E6 cell monolayers. The test antigen was prepared from a Vero E6 cell monolayer infected with the CDG virus prototype strain VHV-574 (Fulhorst et al. 1997). The control antigen was prepared from an uninfected Vero E6 cell monolayer in a manner quantitatively identical to that used to prepare the test antigen. The antigens were diluted in 0.01 M phosphate-buffered saline, pH 7.40, and coated onto 96-well U-bottom polyvinyl-chloride flexible assay plates (Becton Dickinson Labware, Oxnard, CA). Serial fourfold dilutions (from 1:80 through 1:5,120) of each blood sample were tested against the test and control antigens. Bound IgG was detected by using a mixture of goat anti-Rat IgG peroxidase conjugate and goat anti-*Peromyscus leucopus* IgG peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD; catalogue no. 14-16-06 and 14-33-06, respectively) in conjunction with the ABTS (2,2'-azino-di[3-ethyl-benzthiazoline sulfonate (6)]) Microwell Peroxidase Substrate System (Kirkegaard and Perry Laboratories, catalogue no. 50-62-00). Optical densities (OD) at 410 nm (reference = 490 nm) were measured with a Dynatech MRX II microplate reader (Dynatech Industries, Inc., McLean, VA). The adjusted OD (OD_{adjusted}) of a blood sample-antigen reaction was the optical density of the well coated with the test antigen less the OD of the corresponding well coated with the comparison antigen. A blood sample was considered to be antibody-positive if the OD_{adjusted} at 1:80 and the OD_{adjusted} at 1:320 both were ≥ 0.200 , and the sum of the OD_{adjusted} for the series of fourfold dilutions (from 1:80 through 1:5,120) was ≥ 0.750 . The antibody titer of a positive sample was the reciprocal of the highest dilution for which the OD_{adjusted} was ≥ 0.200 .

RESULTS

Antibody (IgG) reactive with CDG virus was found in 133 (4.1%) of 3,225 rodents (Table 1). The geographic distribution of the antibody-positive animals was 127 from Texas, two from Otero County in southeastern New Mexico, and four from Mexico.

Texas

Antibody was found in 13 of the 21 sigmodontine species collected from Texas. The antibody-positive animals included one (1.8%) of 55 *Neotoma albigula* (white-throated woodrat), 38 (12.5%) of 305 *O. palustris*, six (3.8%) of 158 *Perognathus merriami* (Merriam's pocket mouse), 10 (4.9%) of 206 *Peromyscus attwateri* (Texas mouse), 15 (5.4%) of 277 *Peromyscus boylii* (brush mouse), three (3.4%) of 89 *P. leucopus*, one (3.3%) of 30 *P. maniculatus*, three (2.5%) of 118 *Peromyscus pectoralis* (white-ankled mouse), one (0.9%) of 108 *Reithrodontomys fulvescens* (fulvous harvest mouse), three (9.1%) of 33 *R. megalotis*, and 48 (3.6%) of 1,351 *S. hispidus* (Table 1).

The antibody-positive rodents were from 13 counties: Morris (northwestern Texas), Cottle and Lubbock (northern Texas), Kimble and Kerr (central Texas), Jeff Davis and Brewster (southwestern Texas), Mason, Dimmit, and La Salle (southern Texas), and Brazoria, Galveston, and Matagorda (Gulf Coast) (Table 2, Figure 1). Antibody was found in none of the 108 rodents collected from 10 other counties: Anderson, Bailey, Bowie, Cass, Harrison, Lamar, Leon, Presidio, Shelby, and Titus.

The crude seroprevalence at the antibody-positive sites in Texas ranged from 1/112 (0.9%) in Kimble County to 2/10 (20.0%) in Lubbock County (Table 2). The species-specific seroprevalence at the antibody-positive sites ranged from 1/85 (1.2%) in *S. hispidus* (Virginia Point, Galveston County) to 5/9 (55.6%) in *O. palustris* (Peach Point Wildlife Management Area, Brazoria County) (Table 2).

Antibody was found in two or more species at each of five trapping sites: Matador Wildlife Management Area (Cottle County), Virginia Point and Galveston Island (Galveston County), Kerr Wildlife Management Area (Kerr County), and Elephant Mountain Wildlife Management Area (Brewster County) (Table 2). Antibody was found in only one species at each of the nine other antibody-positive counties: Morris, Kimble, Jeff Davis, Brewster, Brazoria, Matagorda, Mason, Dimmit, and La Salle (Table 2).

Southeastern New Mexico

Hantavirus antibody was detected in one (2.3%) of 44 *N. albigula*, one (9.1%) of 11 *R. megalotis*, and none of 32 *Chaetodipus* spp. (18 *C. eremicus*, one *C. hispidus*, and 13 *C. intermedius*), 62 *Dipodomys* spp. (34 *D. merriami*, 27 *D. ordii*, and one *D. spectabilis*), 25 *Neotoma micropus*, eight *Onychomys* spp. (one *O. arenicola* and seven *O. leucogaster*), 17 *Perognathus* spp. (two *P. flavescens* and 15 *P. flavus*), 35 *Peromyscus* spp. (10 *P. eremicus*, 12 *P. leucopus*, and 13 *P. maniculatus*), and 16 *S. hispidus* collected from Otero County, New Mexico.

Mexico

Antibody was found in two (25.0%) of eight *Peromyscus hylocetes* (southern wood mouse) and two (22.2%) of nine *Peromyscus melanotis* (dark-eared mouse) collected near San Bartolo Morelos. Hantavirus antibody was found in none of one *P. eremicus* and two *D. merriami* collected from Ocampo.

DISCUSSION

The present study is the first extensive survey for hantavirus infection in rodents native to Texas. The results increase the known geographic distribution of hantavirus infections in sigmodontine rodents in Texas from seven to 20 counties. Collectively, more than one million people reside in these 20 counties (Anonymous 1996). Thus, there is a substantial human population in Texas at risk of infection with sigmodontine rodent-associated hantaviruses.

The use of CDG virus as the sole test antigen in the ELISA may have resulted in a failure to detect low-titered antibody against Prospect Hill virus or other hantaviruses antigenically distantly related to CDG virus. Similarly, the mixture of anti-rat IgG and anti-*Peromyscus leucopus* IgG peroxidase conjugates may have failed to detect hantavirus-specific IgG in the bloods from pocket mice, kangaroo rats, and other heteromyid rodents. Thus, the results of the present study likely underestimate the true prevalence of infection in the 3,225 rodents tested for antibody. Nevertheless, the present study provides strong evidence for hantavirus infection in a broad array of rodents native to Texas.

Specific rodents (usually one or two closely related species) are the principal hosts of the hantaviruses for which natural host relationships have been well characterized. The occurrence of antibody in multiple sympatric species (e.g., *O. palustris* and *S. hispidus* on Galveston Island) could represent the coexistence of multiple hantaviruses. Alternatively, hantavirus

Table 1. Frequency of antibody titers, by species.

Species	Antibody titer				Antibody prevalence*	
	<320	320	1,280	>5,120		
<i>Baiomys taylori</i>	7	—	—	—	0/7	(0.0%)
<i>Chaetodipus spp.</i> †	164	—	—	—	0/164	(0.0%)
<i>Dipodomys spp.</i> ‡	120	—	—	—	0/120	(0.0%)
<i>Microtus pinetorum</i>	1	—	—	—	0/1	(0.0%)
<i>Neotoma albigula</i>	54	—	—	1	1/55	(1.8%)
<i>Neotoma floridana</i>	2	—	—	—	0/2	(0.0%)
<i>Neotoma mexicana</i>	7	—	—	—	0/7	(0.0%)
<i>Neotoma micropus</i>	56	—	—	—	0/56	(0.0%)
<i>Onychomys arenicola</i>	3	—	—	—	0/3	(0.0%)
<i>Onychomys leucogaster</i>	12	—	—	—	0/12	(0.0%)
<i>Oryzomys palustris</i>	267	12	14	12	38/305	(12.5%)
<i>Perognathus flavescens</i>	2	—	—	—	0/2	(0.0%)
<i>Perognathus flavus</i>	15	—	—	—	0/15	(0.0%)
<i>Perognathus merriami</i>	152	2	—	4	6/158	(3.8%)
<i>Peromyscus attwateri</i>	196	2	1	7	10/206	(4.9%)
<i>Peromyscus boylii</i>	262	2	3	10	15/277	(5.4%)
<i>Peromyscus eremicus</i>	46	—	—	—	0/46	(0.0%)
<i>Peromyscus gossypinus</i>	25	—	—	—	0/25	(0.0%)
<i>Peromyscus hylocetes</i>	6	—	—	2	2/8	(25.0%)
<i>Peromyscus leucopus</i>	86	—	—	3	3/89	(3.4%)
<i>Peromyscus maniculatus</i>	29	1	—	—	1/30	(3.3%)
<i>Peromyscus melanotis</i>	7	1	1	—	2/9	(22.2%)
<i>Peromyscus nasutus</i>	16	—	—	—	0/16	(0.0%)
<i>Peromyscus pectoralis</i>	115	1	—	2	3/118	(2.5%)
<i>Reithrodontomys fulvescens</i>	107	—	—	1	1/108	(0.9%)
<i>Reithrodontomys megalotis</i>	30	—	—	3	3/33	(9.1%)
<i>Reithrodontomys montanus</i>	2	—	—	—	0/2	(0.0%)
<i>Sigmodon hispidus</i>	1,303	21	19	8	48/1,351	(3.6%)
TOTAL	3,092	42	38	53	133/3,225	(4.1%)

* No. antibody-positive/total no. tested (% positive).

† *Chaetodipus spp.* includes 18 *C. eremicus*, 45 *C. hispidus*, 36 *C. intermedius*, 57 *C. nelsoni*, and eight *C. penicillatus*.

‡ *Dipodomys spp.* includes 76 *D. merriami*, 42 *D. ordii*, and two *D. spectabilis*.

Table 2. Antibody prevalence in rodents collected from Texas, by county (site).

County	Site†	Species*									Total
		Opal	PGmer	PMatt	PMman	PMpec	PMspp‡	Reithspp§	Shisp	Others¶	
Brazoria		5/9	—	—	0/3	—	0/8	0/9	—	0/1	5/30
Brewster	B 1	—	2/59	—	—	—	0/33	—	0/1	0/87	2/180
Brewster	B 2	—	2/38	—	0/7	1/21	0/21	—	0/2	0/58	3/147
Cottle		—	—	2/18	1/6	—	3/25	1/5	0/4	0/12	7/70
Dimmit		—	2/58	—	—	—	0/13	0/1	0/35	0/56	2/163
Galveston	G 1	13/126	—	—	—	—	—	0/37	42/1,125	—	55/1,288
Galveston	G 2	19/163	—	—	—	—	—	0/11	1/85	—	20/259
Jeff Davis		—	—	—	—	—	15/277	0/13	0/6	0/8	15/304
Kerr		—	—	7/116	—	1/7	—	—	0/7	0/2	8/132
Kimble		—	0/1	1/55	—	0/47	—	—	0/9	—	1/112
La Salle		—	0/1	—	—	—	—	—	2/26	0/14	2/41
Lubbock		—	—	—	—	—	—	2/10	—	—	2/10
Mason		—	—	0/17	—	1/43	0/2	—	0/1	—	1/63
Matagorda		1/7	—	—	—	—	0/6	0/4	0/5	0/5	1/27
Morris		—	—	—	—	—	0/1	0/1	3/18	—	3/20
Others		—	0/1	—	—	—	0/44	0/41	0/11	0/11	0/108
Total P		38/305	6/158	10/206	1/16	3/118	18/430	3/132	48/1,335	0/254	127/2,9545

* Opal = *Oryzomys palustris*, PGmer = *Perognathus merriami*, PMatt = *Peromyscus attwateri*, PMman = *Peromyscus maniculatus*, PMpec = *Peromyscus pectoralis*, Other PMspp = other *Peromyscus* spp., Reithspp = *Reithrodontomys* spp., and Shisp = *Sigmodon hispidus*. Values are the no. antibody-positive/no. tested.

† Hantavirus antibody was found in rodents collected from two sites each in Brewster and Galveston counties. B1 = Black Gap, B2 = Elephant Mountain, G1 = Galveston Island, G2 = Virginia Point.

‡ Other *Peromyscus* spp. includes 276 *P. boylii* from Brazoria (n = 17) and Jeff Davis (259) counties; 36 *P. eremicus* from Brewster County; one *P. gossypinus* from Morris County; 57 *P. leucopus* from Brazoria (eight), Brewster (one), Cottle (25), Dimmitt (13), Jeff Davis (two), Mason (two), and Matagorda (six) counties; and 16 *P. nasutus* from Jeff Davis County.

§ *Reithrodontomys* spp. includes 69 *R. fulvescens* from Brazoria (n = nine), Cottle (five), Dimmitt (one), Galveston (48), Jeff Davis (one), Matagorda (four) and Morris (one) counties; and 22 *R. megalotis* from Jeff Davis (10), Lubbock (10) counties; and two *R. montanus* from Jeff Davis County.

¶ Others includes six *Baiomys taylori*, 132 *Chaetodipus* spp., 52 *Dipodomys* spp., 11 *Neotoma albigula*, seven *Neotoma mexicana*, 28 *Neotoma micropus*, two *Onychomys arenicola*, and five *Onychomys leucogaster*.

antibody in multiple sympatric rodent species may represent inter-specific virus transmission of a single hantavirus. Further study is needed to determine the identity of the hantavirus(es) associated with each of the antibody-positive species at each of the antibody-positive localities.

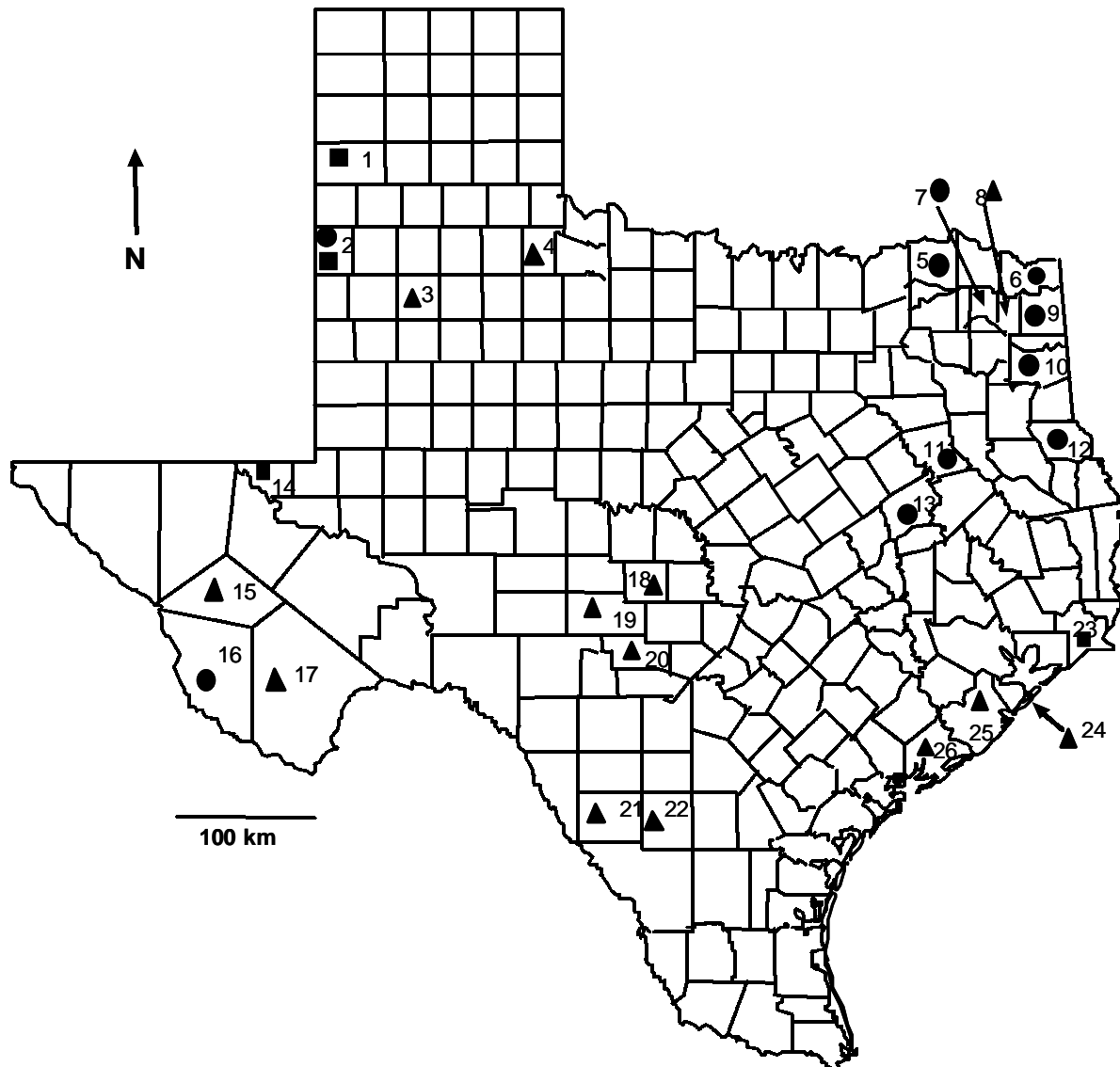
Prior to the present study, evidence for the existence of hantaviruses in Mexico was limited to the recovery of hantavirus-specific RNA from two *R. megalotis* collected from the State of Zacatecas in central Mexico (Hjelle et al. 1995a) and the discovery of hantavirus antibody in *P. maniculatus* and *R. megalotis* collected from a mountainous region south of Mexico City (G. Susan, personal communication). The discovery of hantavirus antibody in *P. hylocetes* and *P. melanotis* collected from San Bartolo Morelos is further evidence that *Peromyscus* species in Mexico

are naturally associated with hantaviruses. Whether the hantavirus associated with *P. hylocetes* and *P. melanotis* is a Sin Nombre(-like) virus or some other hantavirus remains to be determined.

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Figure 1. Map of Texas counties: 1 = Deaf Smith, 2 = Bailey, 3 = Lubbock, 4 = Cottle, 5 = Lamar, 6 = Bowie, 7 = Titus, 8 = Morris, 9 = Cass, 10 = Harrison, 11 = Anderson, 12 = Shelby, 13 = Leon, 14 = Loving, 15 = Jeff Davis, 16 = Presidio, 17 = Brewster, 18 = Mason, 19 = Kimble, 20 = Kerr, 21 = Dimmit, 22 = La Salle, 23 = Jefferson, 24 = Galveston, 25 = Brazoria, 26 = Matagorda. The filled squares represent hantavirus-positive sites from previous studies; filled triangles and filled circles represent sites in the present study that were antibody-positive and antibody-negative, respectively.



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