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Survey for Antibody to Hantaviruses in Tamaulipas, México

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ABSTRACT: Wild rodents (n=248) were trapped in two ecologically distinct sites at El Cielo Biosphere Reserve in the state of Tamaulipas, Mexico, during the summer of 2003. Samples from 199 individuals were tested for *Hantavirus* antibodies by an indirect enzyme-linked immunosorbent assay (ELISA). *Hantavirus* antibodies to recombinant *Sin Nombre virus* nucleocapsid protein were found in seven rodents (3.5%) of a single species, *Peromyscus levipes*. Antibody-positive rodents were found only in the Cloud Forest site, which had lower rodent species diversity than the Tropical Subdecidous Forest site. Although the identity of the virus in *P. levipes* remains to be determined, our study provides further evidence that *Hantavirus* antibody–positive individuals are prevalent in the rodent fauna of Mexico. This is the first survey for *Hantavirus* antibodies in the rodent fauna of Tamaulipas and the first report of *P. levipes* as a potential host for a *Hantavirus*.

Key words: Community composition, *Hantavirus*, México, *Peromyscus*, sigmodontine rodents, Tamaulipas.

Hantavirus pulmonary syndrome (HPS) is a zoonosis caused by certain members of the genus *Hantavirus* (family *Bunyaviridae*). Hantavirus pulmonary syndrome in humans is a severe, and often fatal, disease known throughout the New World, with cases reported in Argentina, Bolivia, Brazil, Canada, Chile, Panama, Paraguay, Uruguay, and the United States, with the highest frequency of occurrence in the southwestern United States (Yates et al., 2002). Globally, hantaviruses occur in close association with rodents and shrews of the families Cricetidae and Soricidae (Schmaljohn and Hjelle, 1997), and New World Hantaviruses have been detected mostly in reservoir rodent species of the subfamily Sigmodontinae. Rodents with *Hantavirus* antibodies have been detected in Peru, Venezuela, Costa Rica, Honduras, and Mexico, although HPS in humans has not been documented in these countries (Yates et al., 2002; Milazzo et al., 2006). *Sin Nombre virus* (SNV) is the major cause of HPS in the United States and Canada, where the North American deermouse (*Peromyscus maniculatus*) is the primary rodent reservoir (Schmaljohn and Hjelle, 1997). The broad geographic distribution of this rodent species extends into Mexico, and one individual with antibodies to a *Hantavirus* has been reported from central Mexico (Suzan et al., 2001). In Mexico, eight rodent species with antibody-positive individuals have been identified so far: the western harvest mouse (*Reithrodontomys megalotis*; Hjelle et al., 1995), Sumichrast's harvest mouse (*Reithrodontomys sumichrast*), the North American deermouse (Suzan et al., 2001), the black-eared mouse (*Peromyscus melanotis*), the transvolcanic deermouse (*Peromyscus hylocetes*; Mantooth et al., 2001), the southern pygmy mouse (*Baiomys musculus*), Coues's rice rat (*Oryzomys couesi*), and the west Mexican cotton rat (*Sigmodon mascotensis*; Chu et al., 2008). *El Moro Canyon virus*, which also occurs in the western United States, has been identified in *R. megalotis* (Hjelle et al., 1995; Monroe et al., 1999), whereas the Playa de Oro virus, a newly described

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Hantavirus, is associated to *O. couesi* and *S. mascotensis* in western Mexico (Chu et al., 2008).

Mexico has a diverse mammalian fauna of approximately 525 species, and rodents represent almost half (44.8%) of these species (Ceballos and Oliva, 2005). Based on this species diversity, it is likely that there are many unrecognized species that may harbor different hantaviruses in this country (Suzan et al., 2001; Chu et al., 2008). Moreover, 12 species of rodents present in Mexico have tested positive for antibody to the *Hantavirus* along the northeastern border (Mantooth et al, 2001). Because the abiotic environment and rodent assemblages of northeastern Mexico are similar to those of areas in the adjacent United States, we expected that the virus may be circulating in Mexico as well, but no report of a *Hantavirus* exists for the bordering states (Chihuahua, Coahuila, Nuevo Leon, and Tamaulipas, Mexico). Because environmental conditions in the state of Tamaulipas support a mammalian faunal mix of nearctic and neotropic taxa (Alvarez, 1963; Ceballos and Oliva, 2005), there is the possibility that rodent species of neotropic affinities could harbor any of the hantaviruses found in more tropical areas, such as the Catacama virus present in *O. couesi* from Honduras (Milazzo et al., 2006). The objective of our research was to conduct serologic tests for antibodies against hantaviruses on wild rodents from Tamaulipas, Mexico, and to provide information on the distribution and prevalence of these viruses at a biosphere reserve from this state.

We conducted fieldwork at El Cielo Biosphere Reserve (ECBR) in southwestern Tamaulipas, Mexico (23°03′42″N, 99°12′18″W; Fig. 1), during the summer (May to August) of 2003. The eastern-facing slopes at ECBR have a pronounced (200–1,800 m) elevational gradient. We sampled two ecologically contrasting sites, each in a different vegetation zone (Fig. 1), one in Tropical Subdecidous Forest (TSDF; Los Cedros; 320 m) and one in Cloud Forest (CF; San Jose; 1,320 m). A detailed description of the sites appears in Puig and Bracho (1987). At each site, we sampled all microhabitats, using transects of varying size, with 180 to 200 Sherman live traps, set 10 m apart and baited with rolled oats. Total number of trapping nights was 31. Handling of captured rodents and collection of blood samples followed the Centers for Disease Control and Prevention standards (Mills et al., 1995). Not all captured individuals were sampled for blood, and recaptured individuals were not resampled.

Serologic testing was conducted at the Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaría de Salud, México. All available whole-blood samples from rodents were tested for immunoglobulin G (IgG) antibodies, using an SNV, recombinant, nucleocapsid protein antigen by an indirect enzyme-linked immunosorbent assay (ELISA), according to a standardized protocol (Feldman et al., 1993). Blood specimens obtained from Nobuto strips (Advantec MFS, Inc., Pleasanton, California, USA) were initially rehydrated in 0.01 M phosphate-buffered saline (PBS), pH 7.4, then diluted 1:25 in 5% skim milk in 0.01 M PBS, pH 7.4, with 0.5% Tween-20, and subsequently diluted to 1:100 through 1:6,400 in four-fold dilutions in microtiter plates. Samples were tested against recombinant, nucleocapsid antigen and a recombinant, control antigen. A conjugate mix of anti–*Rattus norvegicus* and anti–*Peromyscus leucopus* (heavy and light chains) IgG was used to detect bound immunoglobulin (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Maryland, USA). Adjusted optical densities (OD) for each dilution were calculated by subtracting the OD 410 nm of the control antigen from the OD 410 nm of the SNV antigen. A serum specimen was considered SNV-positive if its titer was 400 nm or its sum-adjusted OD was 0.95. Serologic tests detect the presence of an active immune response to

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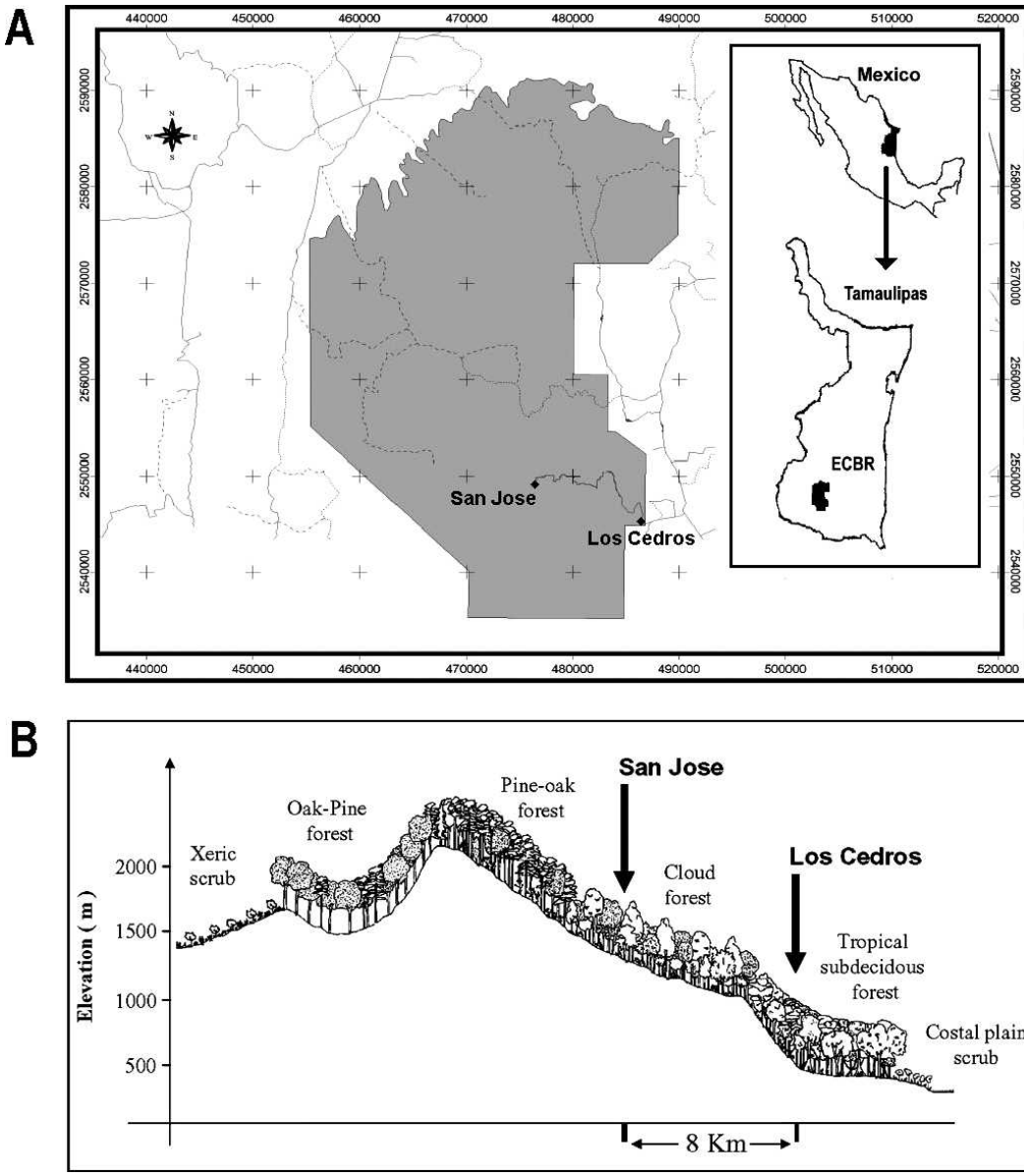


FIGURE 1. (A) Location of El Cielo Biosphere Reserve (ECBR) in the state of Tamaulipas, Mexico. Insert shows the location of this state within Mexico. (B) Diagram of the vegetation change along the elevational gradient illustrating the distance and location of our sampling sites (modified after Puig and Bracho, 1987).

a *Hantavirus*, and positive findings with SNV antigens in the IgG ELISA indicated infection with North American hantaviruses. Antibodies to other hantaviruses are cross-reactive with SNV antigen.

With a total effort of 6,032 trap-nights (malfunctioning or disturbed traps were not included in the effort tally), we captured 248 individuals during 568 capture events (127 were captured only once and 121 were captured up to five times). Rodent communities were highly different in both composition (identity of species) and structure (number of species and relative abundance of each species), with no species shared between them, even

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TABLE 1. Composition, rank abundance distribution, and number of *Hantavirus* antibody–positive individuals for the two sampled small-mammal communities. Diversity index values for each site are TSDF: SHD = 1.5, S = 10, BP = 1.91, R = 7, and CE = 0.34; and CF: SHD = 0.90, S = 4, BP = 1.53, R = 2, and CE = 0.48. Abbreviations: SHD = Shannon Diversity; S = species richness; BP = Berger-Parker dominance; R = number of rare species; CE = Camargo evenness index; TSDF = Tropical Subdecidous Forest; CF = Cloud Forest.

Site	Species	Captured individuals	Blood samples	No. of Positives prevalence (%)
TSDF	*Peromyscus pectoralis*	104	62	0
TSDF	*Sigmodon toltecus*	37	34	0
TSDF	*Oryzomys couesi*	21	21	0
TSDF	*Liomys irroratus*	13	13	0
TSDF	*Baiomys taylori*	10	9	0
TSDF	*Oligoryzomys fulvescens*	6	6	0
TSDF	*Rattus rattus*	4	3	0
TSDF	*Reithrodontomys fulvescens*	2	2	0
TSDF	*Mus musculus*	1	1	0
TSDF	*Oryzomys rostratus*	1	1	0
CF	*Peromyscus levipes*	32	31	7 (23)
CF	*Peromyscus ochraventer*	12	11	0
CF	*Oryzomys chapmani*	4	4	0
CF	*Cryptotis obscura*	1	1	0

though they are only separated by only 8 km (Table 1). Ten species, representing ten genera, were captured in TSDF, whereas only four species from three genera were captured in CF. We obtained blood samples from 199 individuals that represented all captured species. Only seven individuals, all *Peromyscus levipes* (nimble-footed deermouse), were positive for *Hantavirus* antibodies (Table 1). Prevalence was sex-dependent: higher in males than in females (X^2 contingency test with Yates correction: $X^2=4.075$, df=1, $P=0.043$).

Our study provides the first evidence of *P. levipes* as host for a *Hantavirus* and the first record for the presence of a *Hantavirus* in Tamaulipas, Mexico. *Peromyscus levipes* is a closely related species to the brush deermouse (*Peromyscus boylii*; Bradley et al., 2007), the host of the Limestone Canyon virus (Sanchez et al., 2001), thus suggesting that this virus might be the one present in *P. levipes*. However, the genetic identification and characterization of the virus present in *P. levipes* remains to be done.

The contrasting characteristics of rodent communities between sites and their proximity raise interesting questions on the likely importance that composition and structure of rodent communities have in understanding the epidemiology of hantaviruses (Mills et al., 1999; Peixoto and Abramson, 2006). The exclusive detection of antibody-positive individuals in the most species-poor community (CF) agrees with results of seroprevalence in small mammal communities of the Azuero Peninsula in Panama (Ruedas et al., 2004). In small mammal communities that are rich in species, the efficiency of virus transmission may be low (Peixoto and Abramson, 2006). Although we did not capture any *P. levipes* individuals at the TSDF site, this species has been reported at other sites within the TSDF (Vargas-Contreras and Hernandez-Huerta, 2001), and it is a relatively abundant in the transition zone between CF and TSDF (Castro-Arellano, pers. obs.). Thus, this species can potentially come into contact with other rodent species at the TSDF and spread the virus; however, we did not record any positive individuals at our site in the TSDF. More detailed and extensive

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studies are needed to assess the role of community structure on *Hantavirus* prevalence at ECBR. This question is relevant because large-scale modeling studies predict a widespread occurrence of hantaviruses in Mexico, based on rodent-host distributions (Sanchez-Cordero et al., 1995), but on local scales, prevalence could be tied to the ecologic patterns of small mammal communities where the virus occurs (Peixoto and Abramson, 2006).

The prevalence of antibodies to a *Hantavirus* in *P. levipes* in Tamaulipas, Mexico, is comparable to that in sigmodontine hosts in areas that have experienced outbreaks of HPS (Ruedas et al., 2004). Although HPS has not been reported for Mexico, this may be due to a failure to recognize human cases rather than the absence of the disease (Suzan et al., 2001; Chu et al., 2008). Throughout its distribution *P. levipes* is associated with higher elevations (Ceballos and Oliva, 2005) and is absent from lower elevations at ECBR, where the largest human settlements exist. Nevertheless, high likelihood of contact between humans and *P. levipes* occurs at several small settlements at ECBR because this rodent readily enters human dwellings where it builds nests and scavenges food (Castro-Arellano, pers. obs.). Similarly, ecotourists use primitive camping facilities at ECBR. As such, the potential for contact between *P. levipes* and local residents, as well as visitors, should be monitored to assess potential human health risks. Similarly, many human settlements occur throughout the range of *P. levipes*, but information to assess human health concerns (seroprevalence in rodent hosts, small mammal community ecology patterns, and contact potential) is currently unavailable. A more complete evaluation of the presence of *Hantavirus* in Mexico, as well as knowledge of the ecology of sigmodontine reservoirs, is required to provide a comprehensive assessment of the public health risks associated with hantaviruses in the country.

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