Antibody seroprevalence to hantaviruses in rodents from Reserva de la Biosfera Sierra de Huatla, Morelos

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Hantavirus Pulmonary Syndrome (HPS) is a rare disease caused by New World viral species of the genus *Hantavirus* (Family *Buyaviridae*). It has a high mortality rate (close to 40%; Ramos 2008) and has been detected in countries in North America (United States and Canada), Central America (Costa Rica, Honduras, and Panama), and South America (Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay), where most cases have been caused by the Sin Nombre and Andes viruses (Hjelle et al. 1995, Vincent et al. 2000, Yates et al. 2002, Milazzo et al. 2006). More than 20 viral types of *Hantavirus* have been described for the New World, and almost 50% of them are related to HPS. Hantaviruses are highly species specific and typically have a single primary host and a small number of secondary host species. In the Americas, the primary hosts are usually rodents from the family Cricetidae, most of which are species of the subfamily Neotominae (Schmaljohn and Hjelle 1997). Although rodents with antibodies to hantaviruses have been detected in Mexico, HPS has not been documented in this country (Yates et al. 2002, Vado-Solís et al. 2003, Milazzo et al. 2006).

Fourteen cricetid rodent species with antibody-positive individuals have been reported in Mexico: *Baiomys musculus*, *Habromys similis*, *Habromys sp.*, *Oryzomys couesi*, *Peromyscus aztecus*, *P. beatae*, *P. hylodectes*, *P. levipes*, *P. maniculatus*, *P. melanotis*, *Reithrodontomys fulvescens*, *R. megalotis*, *R. sumichrasti*, and *Sigmodon mascotensis* (Hjelle et al. 1995, Mantooth et al. 2001, Suzán et al. 2001, Chu et al. 2007, Ramos 2008, Castro-Arellano et al. 2009). However, the identity of the hosted virus is unknown for most of these species. At present, the only identified viral types are the Moro Canyon virus in *R. megalotis* (Mantooth et al. 2001) and...
the Playa de Oro virus found in *O. couesi* and *S. mascotensis* (Chu et al. 2007), neither of which is known to be pathogenic to humans. Risk for hantavirus infection to humans is distributed heterogeneously with higher probabilities, either for *Hantavirus* or other rodent-borne pathogens, occurring in rural or suburban areas where natural habitats have been altered by human activities (Patz et al. 2004). This risk distribution is likely due to the ubiquitous presence of invasive and generalist rodent species after habitat transformation, especially in areas with a high degree of fragmentation (Vado-Solis et al. 2003, Suzán et al. 2008).

Mexico has one of the highest diversities of mammals in the world, with 525 species, 235 of which (44.8%) belong to the order Rodentia (Ceballos and Oliva 2005). These values could be underestimated if we consider those cryptic species detected only by molecular markers with high genetic divergence (González-Cózatl et al. 2009). In addition, the high turnover rate (beta diversity) present in Mexico creates a mosaic of species with restricted distribution and a high probability of being potential reservoirs for pathogens such as hantaviruses.

*Peromyscus maniculatus* is the primary reservoir of the Sin Nombre virus (SNV), however it has been confirmed that SNV-like hantaviruses are widely distributed in North America. There are also other peromyscine-borne hantaviruses that are associated with HPS, such as the New York and Monongahela viruses (Monroe et al. 1999). Considering that Mexico has 49 species of the genus *Peromyscus* (Mussser and Carleton 2005) and that several peromyscine-borne hantaviruses are known from North America, many of these rodents species in Mexico probably harbor hantaviruses (Suzán et al. 2001, Castro-Arellano 2009). *Hantavirus*-infected individuals from 12 rodent species have been found in Texas, and their ranges extend south to Mexico (Mantooth et al. 2001). Similarly, there are records of the Catacama virus in *Oryzomys couesi* from Honduras (Milazzo et al. 2006) and reports of seropositive individuals of *Reithrodontomys mexicanus* from Costa Rica (Hjelle et al. 1995) and *Oligoryzomys fulvescens* from Panama (Vincent et al. 2000), all neotropical species with a wide distribution in Mexico. Detection of hantaviruses both north and south of Mexico suggests that hantaviruses should be present throughout the country. Therefore it is necessary to investigate their distribution and prevalence in Mexican rodents. We performed serologic tests to detect *Hantavirus* antibodies in wild rodents from the dry forest of the state of Morelos, Mexico, and we provide information about the distribution and prevalence of these viruses in the Reserva de la Bósfera Sierra de Huautla (RBSH), a protected area located in the southernmost part of the state of Morelos.

Fieldwork was done on the outskirts of the town El Limon, located in the RBSH, Morelos, Mexico (Fig. 1). Dominant vegetation corresponded to tropical deciduous forest. The region is characterized by, among other things, a high human emigration rate that has caused the
abandonment of areas previously used as crop-land. Sampling formed part of a larger project aimed at comparing biodiversity among areas with different times since abandonment. To obtain rodent samples, we did a total of 4 sampling periods, with 2 trapping nights each, during dry and rainy seasons (July and December 2006, and April and July 2007). Sherman traps were set in quadrats of 30 \times 50 \text{ m} (4 lines of 10 traps each) on 8 sites differentiated by the time (age) since abandonment (2 sites by age: 0–4, 5–8, 9–17, and 18–37 years) plus 2 sites that were never used for tillage (controls). Handling of rodents and collection of blood samples followed the standards that the Centers for Disease Control and Prevention recommended for rodents potentially infected with hantaviruses (Mills et al. 1995a, 1995b). We obtained a blood sample (0.1 mL) from each rodent by using a nobuto strip (Nobuto Filter Strips, Advantec MFS Inc., Pleasanton, CA). Collected individuals were prepared as voucher specimens and deposited at the Mammal Collection of Centro de Investigación en Biodiversidad y Conservación (CMC) of Universidad Autónoma del Estado de Morelos.

Serologic tests were performed at the Instituto de Diagnóstico y Referencia Epidemiológicos (InDRE), Secretaría de Salud de México. All blood samples were tested for immunoglobulin \text{G} (\text{IgG}) antibodies using an antigen of SNV nucleocapsid recombinant protein through enzymatic immunoassay (ELISA) according to a standardized protocol (Feldman et al. 1993). Blood samples were initially rehydrated with a phosphate IX on 0.01 M (PBS) buffer solution at pH 7.4, then diluted 1:25 on Blocking Buffer (PBS IX 0.01 M, pH 7.4 with skim milk at 5\% and Tween 20 at 0.1\%), and finally diluted 1:100 through fourfold dilutions up to 1:6400 on microtitulation plates. Samples were tested against the nucleocapsidic recombinant antigen and a control recombinant antigen. A conjugate mixture of IgG anti-

\textit{Rattus norvegicus} and anti-\textit{Peromyscus leucopus} (heavy and light strands) was used to detect linked immunoglobulins (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD). Adjusted optical densities (OD) for each solution were calculated by subtracting the OD410 of the control antigen from the OD410 of the Sin Nombre Virus (SNV) antigen. A specimen serum was considered SNV-positive if the reading value was higher than 3 times the standard deviation of the cut-off positive control. Serologic tests detect the presence of an active immune response to hantaviruses; positive individuals found with the SNV antigen on the IgG ELISA indicate infections with North American hantaviruses. Antibodies with other hantaviruses are cross-reactive with SNV antibodies.

Of the 3200 trap-nights, we collected 153 mice representing 6 species, 5 genera, and 2 families (Heteromyidae and Cricetidae; Table 1). Museum numbers corresponding to collected specimens are as follows: \textit{Liomys irroratus} CMC 1713–1745, 1757, 2073–2093, 2103–2106, 2324–2357; \textit{Baiomys musculus} CMC 1746–1756, 2094, 2107–2112, 2358–2363; \textit{Peromyscus levispes} CMC 1758, 2095; \textit{P. melanophrys} CMC 1759–1766, 2096–2097, 2113, 2363; \textit{Reithrodontomyys fulvescens} CMC 1767–1777, 2098–2101, 2114, 2115, 2364, 2365; and \textit{Sigmodon hispidus} CMC 1778, 2102, 2116. \textit{Liomys irroratus} was the most abundant species with 91 individuals (60.78\%) and was collected in all sites except one; \textit{B. musculus} was next with 24 individuals (15.68\%), then \textit{R. fulvescens} with 19 (12.41\%), followed by \textit{P. melanophrys} with 12 (7.84\%). The least abundant species were \textit{S. hispidus} and \textit{P. levispes}, for which only 3 (1.96\%) and 2 (1.3\%) specimens were collected, respectively. Only one of the 153 blood samples was positive for the hantavirus antigen used. The positive sample belonged to a \textit{L. irroratus} female collected at a site with 18–37 years of abandonment (Table 1).

Although only a single individual tested positive, our result suggests that hantaviruses are distributed in central Mexico and are present in rodents from southern Morelos. The possibility of an error in the diagnosis technique resulting in a false positive for heteromyids is quite unlikely, given that all samples were doubled-tested with strict controls and that other studies have reported the incidence of antibodies in this group of rodents (Mills et al. 1997, Alemán et al. 2006). Obtaining a higher number of seropositive individuals would be ideal for making a better estimation of hantavirus seroprevalence in this region of Morelos, but such an effort was beyond the main objective of this project. Notably, the single seropositive individual belongs to a heteromyid species, despite the high diversity and abundance of cricetid rodents from this assemblage, including 60 specimens of 5 species of cricetid rodents, some of which have been
Hantaviruses had been thought to be evolutionarily associated exclusively with the family Cricetidae, but recent studies have shown that some hantaviruses are also associated with insectivores (Arai et al. 2008); therefore, it is not appropriate to reject a priori the presence of hantaviruses in heteromyid rodents. The finding of hantaviral antibodies in *Liomys irroratus* represents one of the few reported cases of hantaviruses in heteromyid rodents. The presence of hantavirus antibodies in *Liomys* suggests that this finding be used as a basis for more extensive sampling and tests in the future.

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**LITERATURE CITED**


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