



4-5-2012

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

Elizabeth Arellano

Universidad Autónoma del Estado de Morelos, México, elisabet@buzon.uaem.mx

Iván Castro-Arellano

University of Connecticut, Storrs, CT, wnan@byu.edu

Gerardo Suzán

Universidad Nacional Autónoma de México, México

Francisco X. González-Cózatl

Universidad Autónoma del Estado de Morelos, México

Follow this and additional works at: <https://scholarsarchive.byu.edu/wnan>

Recommended Citation

Arellano, Elizabeth; Castro-Arellano, Iván; Suzán, Gerardo; and González-Cózatl, Francisco X. (2012) "Antibody seroprevalence to hantaviruses in rodents from Reserva de la Biosfera Sierra de Huatla, Morelos," *Western North American Naturalist*: Vol. 72 : No. 1 , Article 14. Available at: <https://scholarsarchive.byu.edu/wnan/vol72/iss1/14>

This Note is brought to you for free and open access by the Western North American Naturalist Publications at BYU ScholarsArchive. It has been accepted for inclusion in Western North American Naturalist by an authorized editor of BYU ScholarsArchive. For more information, please contact scholarsarchive@byu.edu, ellen_amatangelo@byu.edu.

ANTIBODY SEROPREVALENCE TO HANTAVIRUSES IN RODENTS FROM RESERVA DE LA BIOSFERA SIERRA DE HUAUTLA, MORELOS

Elizabeth Arellano^{1,6}, Iván Castro-Arellano^{2,5}, Gerardo Suzán³,
Francisco X. González-Cózat¹, and Ricardo Morales Jiménez⁴

ABSTRACT.—Hantavirus pulmonary syndrome (HPS) is a rare disease with a high mortality rate, caused by New World viral species of the genus *Hantavirus*. The presence of hantaviruses both north and south of Mexico suggests an extended presence through the country. Our objective was to conduct serologic tests to detect antibodies against hantaviruses in wild rodents of the tropical deciduous forest in Morelos, Mexico, and to report information about the distribution and prevalence of these viruses. Blood samples from 153 cricetid and heteromyid rodents were tested for immunoglobulin G (IgG) antibodies. One individual of *Liomys irroratus* was seropositive for a *Hantavirus* antigen. Our results suggest that hantaviruses occur in rodents from southern Morelos in central Mexico. Given the possibility of a false positive result, it is important that this finding be validated with further research.

RESUMEN.—El Síndrome Pulmonar por Hantavirus es una enfermedad rara con alta tasa de mortandad, causada por una especie viral del género *Hantavirus* del Nuevo Mundo. La presencia de hantavirus al norte y sur de México sugiere una presencia extendida a través del país. Nuestro objetivo fue llevar a cabo pruebas serológicas para detectar anticuerpos contra hantavirus en roedores del bosque tropical deciduo en Morelos, México y reportar información acerca de la distribución y prevalencia de estos virus. Muestras de sangre de 152 roedores cricétidos y heterómidos fueron puestas a prueba con anticuerpos inmunoglobulina G (IgG). Un individuo de *Liomys irroratus* fue seropositivo para un antígeno de *Hantavirus*. Nuestros resultados sugieren que los hantavirus ocurren en la parte central de México en roedores del sur de Morelos. Dada la posibilidad de un falso resultado positivo, es importante que estos resultados sean validados con estudios adicionales.

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-Solis et al. 2003, Milazzo et al. 2006).

Fourteen cricetid rodent species with antibody-positive individuals have been reported in Mexico: *Baiomys musculus*, *Habromys simulatus*, *Habromys* sp., *Oryzomys couesi*, *Peromyscus aztecus*, *P. beatae*, *P. hylocetes*, *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

¹Centro de Investigación en Biodiversidad y Conservación, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Cuernavaca, Morelos, C.P. 62209, México.

²Center for Environmental Sciences and Engineering, University of Connecticut, Storrs, CT 06269, USA.

³Departamento de Etología y Fauna Silvestre, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, Distrito Federal, C.P. 04510, México.

⁴Departamento de Enfermedades Emergentes, Instituto de Diagnóstico y Referencia Epidemiológicos, Secretaría de Salud, Prol. de Carpio 470, Distrito Federal, C.P. 11340, México.

⁵Present address: Department of Biology, Texas State University—San Marcos, San Marcos, TX 78666, USA.

⁶E-mail: elisabet@uaem.mx

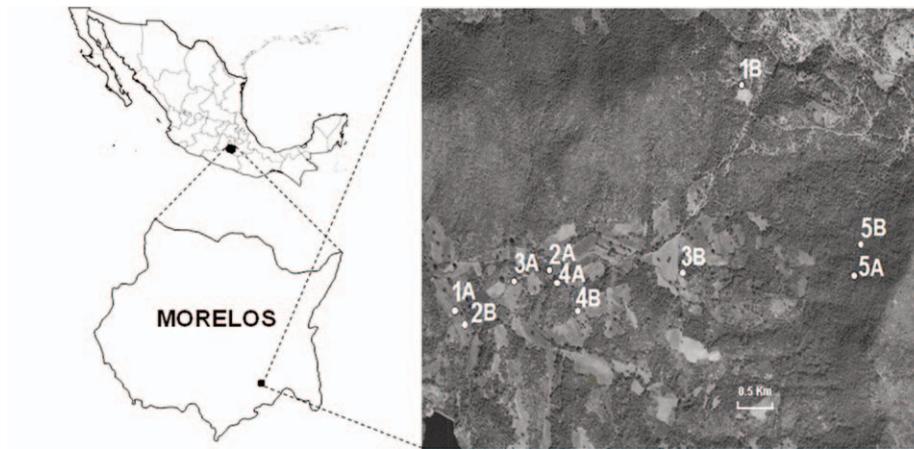


Fig. 1. Location of collecting sites at Reserva de la Biosfera Sierra de Huautla, Morelos, Mexico. Site codes correspond to time of abandonment shown in Table 1. Distances between sites ranged from 0.1 to 1.5 km.

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-Solís 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* (Musser and Carleton 2005) and that several peromyscine-borne hantaviruses are known from North America, many of these rodent 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 Biosfera 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 cropland. 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 30×50 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 G (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 1X on 0.01 M (PBS) buffer solution at pH 7.4, then diluted 1:25 on Blocking Buffer (PBS 1X 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 nucleocapsid recombinant antigen and a control recombinant antigen. A conjugate mixture of IgG anti-*Rattus norvegicus* and anti-*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: *Liomys irroratus* CMC 1713–1745, 1757, 2073–2093, 2103–2106, 2324–2357; *Baiomys musculus* CMC 1746–1756, 2094, 2107–2112, 2358–2363; *Peromyscus levipes* CMC 1758, 2095; *P. melanophrys* CMC 1759–1766, 2096–2097, 2113, 2363; *Reithrodontomys fulvescens* CMC 1767–1777, 2098–2101, 2114, 2115, 2364, 2365; and *Sigmodon hispidus* CMC 1778, 2102, 2116. *Liomys irroratus* was the most abundant species with 91 individuals (60.78%) and was collected in all sites except one; *B. musculus* was next with 24 individuals (15.68%), then *R. fulvescens* with 19 (12.41%), followed by *P. melanophrys* with 12 (7.84%). The least abundant species were *S. hispidus* and *P. levipes*, 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 *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

TABLE 1. Number of individuals collected at each site. Letters A and B represent replicates of each site. An asterisk (*) indicates the identification and location of the specimen positive for *Hantavirus*. Site codes correspond to those in Figure 1.

Time since abandonment	0–4 years		5–8 years		9–17 years		18–37 years		No abandon	
	1A	1B	2A	2B	3A	3B	4A	4B	5A	5B
<i>Liomys irroratus</i> (n = 93)	22	0	5	7	6	10	12*	11	7	13
<i>Baiomys musculus</i> (n = 24)	11	0	0	6	0	0	0	3	0	4
<i>Peromyscus levipes</i> (n = 2)	0	1	0	0	0	0	0	0	0	1
<i>Peromyscus melanophrys</i> (n = 12)	0	2	1	0	3	2	1	1	1	1
<i>Reithrodontomys fulvescens</i> (n = 19)	9	1	1	4	0	1	0	2	1	0
<i>Sigmodon hispidus</i> (n = 3)	3	0	0	0	0	0	0	0	0	0
Total (n = 153)	45	4	7	17	9	13	13	17	9	19

reported as potential reservoirs of hantaviruses. 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 for a hantavirus in a heteromyid rodent, and it is the first one in the genus *Liomys*. Antibodies for hantaviruses have been found previously in *Perognathus fasciatus* and *P. parvus* in the southwestern United States (Mills et al. 1997) and in *Heteromys* in Colombia (Alemán et al. 2006). Although it is clear that understanding the determinants for hantavirus presence in heteromyids requires molecular, phylogeographic, and ecological studies of this group of organisms, the presence of hantaviruses in heteromyids may be explained by a spillover infection caused by the contact of individuals of this family with infected cricetid rodents. Experimental evidence (Suzán et al. 2009) and mechanistic models (Peixoto and Abramson 2006) have indicated that the rodent assembly structure is important in determining levels of hantavirus prevalence. At our study site, *Peromyscus* species, which are the most probable reservoirs of hantaviruses, are not abundant, such that it would be necessary to perform extensive sampling to detect very low levels of prevalence. High levels of prevalence (23%) have been reported for *P. levipes* in Tamaulipas (Castro-Arellano et al. 2009), but at that site *P. levipes* is the most abundant species of the rodent assembly (Castro-Arellano 2005). At RBSH, *L. irroratus* is the dominant rodent species, and the probability of an individual from this species encountering an

infected cricetid rodent is higher. However, even though our sampling did not find cricetid rodents that were seropositive for hantaviruses in RBSH, it is highly probable that these viruses are present in this region of Morelos.

Given the low level of prevalence observed, there is a possibility that our finding represents a false positive result of the ELISA methodology. This finding should be validated with other methodologies, such as PCR. We do not have the tissues required to do such validation. We suggest that this finding be used as a basis for more extensive sampling and tests in the future.

We thank Armando Pérez Barrios, Rachel Mercado Vallejo, Gabriela Mendoza Nakano, Dayana Mayares Salvador, and Alfredo Pacheco for helping with fieldwork. Partial financial support for this study was provided by a grant to FXGC (PROMEP/Fondo de Consolidación 2007).

LITERATURE CITED

- ALEMÁN, A., H. IGUARÁN, H. PUERTA, C. CANTILLO, J. MILLS, W. ARIZ, AND S. MATTAR. 2006. Primera evidencia serológica de infección por *Hantavirus* en roedores, en Colombia. *Revista de Salud Pública* 8:1–12.
- ARAI, S., S.N. BENNETT, L. SUMIBCAY, J.A. COOK, J. SONG, A. HOPE, C. PARMENTER, V.R. NERURKAR, T.L. YATES, AND R. YANAGIHARA. 2008. Short report: phylogenetically distinct hantaviruses in the masked shrew (*Sorex cinereus*) and dusky shrew (*Sorex monticolus*) in the United States. *American Journal of Tropical Medicine and Hygiene* 78:348–351.
- CASTRO-ARELLANO, I. 2005. Ecological patterns of the small mammal communities at El Cielo Biosphere Reserve, Tamaulipas, México. Doctoral dissertation, Texas A&M University, College Station, TX. 151 pp.
- CASTRO-ARELLANO, I., G. SUZÁN, R. FLORES-LEÓN, R. MORALES-JIMÉNEZ, AND T.E. LACHER JR. 2009. Survey for antibody to hantaviruses in Tamaulipas, México. *Journal of Wildlife Diseases* 45:207–212.
- CEBALLOS, G., AND G. OLIVA. 2005. Los mamíferos silvestres de México. CONABIO – Fondo de Cultura Económica, México D.F. 988 pp.

- CHU, Y.K., R. OWEN, C. SANCHEZ-HERNANDEZ, M.L. ROMERO-ALMARAZ, AND C.B. JONSSON. 2007. Genetic characterization and phylogeny of a hantavirus from Western México. *Virus Research* 131:180–188.
- FELDMAN, H., A. SANCHEZ, S. MORZUNOV, C.F. SPIROPOULOU, P.E. ROLLIN, AND T.G. KSIAZEK. 1993. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Research* 30: 351–367.
- GONZÁLEZ-CÓZATL, F.X., D.S. ROGERS, AND E. ARELLANO. 2009. Diversidad críptica en la CNMA: descubriendo nuevas especies de roedores mexicanos. Pages 55–64 in F.A. Cervantes, J. Vargas-Cuenca, and Y. Hortelano-Moncada, editors, 60 años de la Colección Nacional de Mamíferos del Instituto de Biología UNAM.
- HJELLE, B., B. ANDERSON, N. TORRES-MARTINEZ, W. SONG, W.L. GANNON, AND T.L. YATES. 1995. Prevalence and geographic genetic variation of hantaviruses of New World harvest mice (*Reithrodontomys*): identification of a divergent genotype from a Costa Rican *Reithrodontomys mexicanus*. *Virology* 207:452–459.
- MANTOOTH, S.J., M.L. MILAZZO, R.D. BRADLEY, C.L. HICE, G. CEBALLOS, AND R.B. TESH. 2001. Geographical distribution of rodent-associated hantaviruses in Texas. *Journal of Vector Ecology* 26:7–14.
- MILAZZO, M.L., M.N. CAJIMAT, J.D. HANSON, R.D. BRADLEY, M. QUINTANAV, AND C. SHERMAN. 2006. Catacamas virus, a hantaviral species naturally associated with *Oryzomys couesi* in Honduras. *American Journal of Tropical Medicine and Hygiene* 75:1003–1010.
- MILLS, J.N., J.E. CHILDS, T.G. KSIAZEK, C.J. PETERS, AND W.N. VELLECA. 1995a. Methods for trapping and sampling small mammals for virologic testing. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA.
- MILLS, J.N., T.G. KSIAZEK, B.A. ELLIS, P.E. ROLLIN, S.T. NICHOL, T.L. YATES, W.L. GANNON, C.E. LEVY, D.M. ENGELTHALER, T. DAVIS, ET AL. 1997. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. *American Journal of Tropical Medicine and Hygiene* 56:273–284.
- MILLS, J.N., T.L. YATES, J.E. CHILDS, R.R. PARMETER, T.G. KSIAZEK, P.E. ROLLIN, AND C.J. PETERS. 1995b. Guidelines for working with rodents potentially infected with *Hantavirus*. *Journal of Mammalogy* 76: 716–722.
- MONROE, M.C., S.P. MORZUNOV, AND A.M. JOHNSON. 1999. Genetic diversity and distribution of *Peromyscus*-borne hantaviruses in North America. *Emerging Infectious Diseases* 5:75–86.
- MUSSER, G.G., AND M.D. CARLETON. 2005. Superfamily Muroidea. Pages 894–1522 in D.E. Wilson and D.M. Reeder, editors, *Mammal species of the world: a taxonomic and geographic reference*. 3rd edition. Johns Hopkins University Press, Baltimore, MD. 142 pp.
- PATZ, J.A., P. DASZAK, G.M. TABOR, A.A. AGUIRRE, M. PEARL, J. EPSTEIN, N.D. WOLFE, A.M. KILPATRICK, J. FOFOPOULOS, D. MOLYNEUX, AND D.J. BRADLEY. 2004. Unhealthy landscapes: policy recommendations on land use change and infectious disease emergence. *Environmental Health Perspectives* 112:1092–1098.
- PEIXOTO, I.D., AND G. ABRAMSON. 2006. The effect of biodiversity on the hantavirus epizootic. *Ecology* 87: 873–879.
- RAMOS, C. 2008. Los hantavirus causantes de la fiebre hemorrágica con síndrome renal y del síndrome pulmonar. *Salud Pública de México* 50:334–340.
- SCHMALJOHN, C., AND B. HJELLE. 1997. Hantaviruses: a global disease problem. *Emerging Infectious Diseases* 3:95–104.
- SUZÁN, G., A. ARMIEN, J.N. MILLS, E. MARCE, AND G. CEBALLOS. 2008. Epidemiological considerations of rodent community composition in fragmented landscapes in Panama. *Journal of Mammalogy* 89:684–690.
- SUZÁN, G., G. CEBALLOS, J. MILLS, T.G. KSIAZEK, AND T.L. YATES. 2001. Serologic evidence of hantavirus infection in sigmodontine rodents in Mexico. *Journal of Wildlife Diseases* 37:391–393.
- SUZÁN, G., E. MARCE, J.T. GIERMAKOWSKI, J.N. MILLS, AND G. CEBALLOS. 2009. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PLoS ONE* 4:e5461.
- VADO-SOLÍS, I., C. PÉREZ-OSORIO, H. LARA-LARA, H.A. RUIZ-PIÑA, M. CÁRDENAS-MARRUFOV, AND M.L. MILAZZO. 2003. Evidencia serológica de infección por hantavirus en población humana del estado de Yucatán, México. *Revista Biomédica* 14:221–225.
- VINCENT, M.J., E. QUIROZ, F. GRACIA, A.J. SANCHEZ, T.G. KSIAZEK, P.T. KITSUTANI, L.A. RUEDAS, D.S. TINNIN, L. CACERES, A. GARCIA, ET AL. 2000. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology* 277:14–19.
- YATES, T.L., J.N. MILLS, AND C.A. PARMENTER. 2002. The ecology and evolutionary history of an emergent disease: hantavirus pulmonary syndrome. *BioScience* 52:989–998.

Received 26 August 2010
Accepted 15 November 2011