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NOTE

SEROLOGICAL SURVEY OF HANTAVIRUS IN RODENTS FROM PRAIRIE DOG ECOSYSTEMS IN CHIHUAHUA, MEXICO

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Abstract—In northwestern Mexico, studies on hantavirus in rodent hosts are scarce. Our objective was to conduct serological tests to detect antibodies against hantavirus in rodents from the Janos–Nuevo Casas Grandes prairie dog complex (JCGC) in northwestern Mexico. In December 2007 and April 2008, we captured 149 rodents and tested for immunoglobulin G antibodies to New World hantaviruses. Three *Peromyscus maniculatus* from grassland habitats without prairie dog colonies were antibody positive. This is the first record of hantavirus prevalence in wild rodents from JCGC and from the state of Chihuahua. Further molecular analysis is needed to describe which hantavirus is circulating in this area.

Resumen—En el noroeste de México los estudios sobre hantavirus en roedores hospederos son escasos. Nuestro objetivo fue realizar un estudio serológico con el fin de detectar anticuerpos contra hantavirus en roedores del complejo de perros llaneros Janos–Nuevo Casas Grandes (CJCG), ubicado en el noroeste de México. En diciembre de 2007 y abril de 2008, capturamos 149 roedores y los analizamos para la presencia de anticuerpos IgG a hantavirus del Nuevo Mundo. Tres *Peromyscus maniculatus* de los pastizales sin colonias de perros llaneros fueron positivos a hantavirus. Este es el primer registro de la prevalencia de hantavirus en roedores silvestres en el CJCG y en el estado de Chihuahua. Estudios adicionales con análisis moleculares se deben realizar para conocer qué hantavirus se encuentra circulando en esta área.

Hantaviruses (family *Bunyaviridae,* genus *Hantavirus*) have emerged as an important rodent-borne zoonosis that is distributed globally (Jonsson et al., 2010). These viruses are the etiological agents of hemorrhagic fever with renal syndrome in Eurasia (Old Word hantaviruses) and hantavirus pulmonary syndrome (HPS) in the Americas (New World hantaviruses; Jonsson et al., 2010). The first human cases in the Americas were recognized in 1993 in the southwestern United States (Childs et al., 1994). Since then, cases of HPS have been reported in Central and South America (e.g., Hjelle et al., 1996; Fulhorst et al., 1997; Toro et al., 1998; Vincent et al., 2000). Currently, more than 40 genotypes of hantaviruses have been reported in the Americas; 20 of these are associated with diseases in humans (Hjelle and Torres-Pérez, 2010). Commonly, each unique virus type is maintained in nature by a single rodent host species in which it causes a chronic infection that may be largely asymptomatic (Jonsson et al., 2010). The transmission of hantavirus within host populations is believed to occur mainly through aggressive encounters between rodents (Mills et al., 1997; Klein et al., 2004).

Although there have been no human cases of HPS to date in Mexico (Vázquez-Pérez et al., 2012), four hantaviruses have been documented in rodents that occur in Mexico: El Moro Canyon virus, Playa de Oro virus, Limestone Canyon virus, and Sin Nombre virus (Hjelle et al., 1995; Chu et al., 2008; Milazzo et al., 2012),

with antibody prevalences ranging between 4% and 9% of samples tested. Additionally, three possible new hantaviruses were described recently: Carrizal virus, Huitzilac virus, and Montano virus (Kariwa et al., 2012). However, these strains may be members of Limestone Canyon virus (Montano virus) and El Moro Canyon virus or Rio Segundo virus (Carrizal virus and Huitzilac virus; Milazzo et al., 2012). Of these viruses, only Sin Nombre virus is known to cause HPS. Serological surveys of hantaviruses have been conducted in several areas of Mexico (e.g., Mantooth et al., 2001; Suzán et al., 2001; Castro-Arellano et al., 2009), though little is known about hantavirus prevalence in rodents from the northwestern portion of Mexico. Northwestern Mexico has environmental conditions similar to areas of the United States where HPS cases have occurred (Yates et al., 2002) and where rodents show molecular and serological evidence of hantavirus (e.g. Childs et al., 1994; Mills et al., 1998, 1999). The purpose of this study was to perform a serological survey on the Janos–Casas Grandes prairie dog complex (JCGC; Ceballos et al., 1993) in the previously unexamined state of Chihuahua, Mexico. We wanted to investigate hantavirus prevalence in wild rodents that inhabit this protected area that is part of one of the largest continuous black-tailed prairie dog (*Cynomys ludovicianus*) towns remaining in North America. The black-tailed prairie dog is considered a keystone species that can modify assemblages of small mammals (Ceballos et al., 1999; Cully et al., 2010); therefore, this species may influence rodent-borne zoonotic disease dynamics, which makes this study area an interesting place to investigate pathogens associated with wild rodents. Additionally, we chose this area because there are human settlements and farming activities within it that could increase human–wildlife contacts.

Fieldwork was conducted on JCGC, which is located within the grasslands and scrublands of northwestern Chihuahua (around 30°50′N, 108°25′W) approximately 50 km south of the Mexico–United States border (Ceballos et al., 1999). The study area is part of the Biosphere Reserve of Janos, a priority area for conservation in Mexico (List et al., 2010). The study consisted of two sampling sites in each of the following habitat types (totaling six sampling sites): (1) grasslands without prairie dogs colonies, (2) grasslands with active prairie dogs colonies, and (3) mesquite (*Prosopis*) scrublands. The grasslands consisted of grasses and annual herbs such as *Bouteloa*, *Aristida hamulosa*, and *Fouqueria splendens*. In each sampling site we established a 7×7 grid consisting of 49 Sherman traps ($8 \times 8 \times 23$ cm; H. B. Sherman traps, Tallahassee, FL) with traps set at 10-m intervals (3,600 m^2) and baited with a mixture of rolled oats, peanut butter, and vanilla extract. Each grid was considered independent and separated by at least 300 m (Ceballos et al., 1999). We sampled each sampling site during three consecutive nights over two sampling periods (December 2007 and April 2008). Once captured, we identified, weighed, sexed, and ear-tagged the animals. We collected blood from the retro-orbital sinus (~0.1 mL) using capillary tubes and transferred the blood to Nobuto blood filter strips (Cole-Parmer, Vernon Hills, IL). After handling, we released animals at their sites of capture. Procedures for trapping and handling rodents met the guidelines approved by the American Society of Mammalogists (Sikes et al., 2011) and were approved by the animal care committee of the Universidad Nacional Autónoma de México and by the Secretaría de Medio Ambiente y Recursos Naturales, Mexico (license no. FAUT-0250).

At each habitat type we recorded the number of individuals captured, species richness (*S*), and the Shannon diversity index (*H*; Krebs, 1989). We performed the serological tests at the Department of Pathology, University of Texas Medical Branch at Galveston. Serum samples were tested for immunoglobulin G reactive against Caño Delgadito virus strain VHV-574, using an enzyme-linked immunosorbent assay described previously (Fulhorst et al., 1997). Caño Delgadito virus is highly cross-reactive with North American hantaviruses such as Sin Nombre virus and Black Creek Canal virus (Fulhorst et al., 1997). This strain has been used in other serological surveys of hantavirus in North America (e.g., Mantooth et al., 2001; Milazzo et al., 2012). Moreover, Caño Delgadito antibody-positive rodents have reported El Moro Canyon virus, Limestone Canyon virus, or Sin Nombre virus infections (by deoxyribonucleic acid sequencing, not virus isolation; Milazzo et al., 2012). We used a lysate of Vero E6 cells infected with strain VHV-574 as test antigen. The control antigen was a lysate of uninfected Vero E6 cells. Serial fourfold dilutions (from 1:80 through 1:5,120) of each blood sample were tested against the test antigen and the control antigen. We used a mixture of immunoglobulin G anti-*Rattus norvegicus* peroxidase conjugate and anti-*Peromyscus leucopus* peroxidase conjugate in conjunction with the 2,2′-Azino-di-(3-ethylbenzthiazoline-6-sulfonate (ABTS) microwell peroxidase substrate system (Kirkegaard and Perry Laboratories, Gaithersburg, MD) to detect linked immunoglobulins. Optical densities (OD) at 405 nm (reference = 490 nm) were measured with a Dynex MRX II microplate reader (Dynatech Industries, Inc., McLean, VA). The adjusted OD (AOD) of a blood–antigen reaction was the OD of the well coated with the test antigen minus the OD of the well coated with the control antigen. We considered a serum sample as positive if the AOD at 1:80 was $\geq$0.200, the AOD at 1:320 was $\geq$0.200, and the sum of the AODs for the series of fourfold dilutions (from 1:80 through 1:5,120) was $\geq$0.750. The titer of a positive sample was the reciprocal of the highest dilution for which the AOD was $\geq$0.200.

We captured a total of 149 rodents during 1,764 trap-nights, representing 11 species, eight genera, and two families (Heteromyidae and Cricetidae; Table 1). There was a marked difference in trapping success (number of

TABLE 1—Rodents tested for hantavirus antibodies in grassland with prairie dog colonies (GLPD), grassland without prairie dog colonies (GL), and mesquite scrublands (MS) in the Janos–Nuevo Casas Grandes prairie dog complex, in Chihuahua, Mexico. Number of positive individuals is in parentheses.

	Habitat type					
	GLPD		GL		MS	
Rodent species	December	April	December	April	December	April
Baiomys taylori	—	—	25	1	—	—
Dipodomys merriami	—	1	2	—	18	8
Dipodomys ordii	—	—	—	—	1	2
Dipodomys spectabilis	—	—	3	2	1	1
Neotoma albigula	—	—	2	—	7	—
Onychomys leucogaster	9	—	6	—	7	—
Peromyscus leucopus	—	—	—	—	1	—
Peromyscus maniculatus	—	—	9 (3)	—	13	4
Perognathus flavus	4	1	5	—	3	3
Reithrodontomys megalotis	—	—	—	—	0	1
Sigmodon hispidus	—	—	—	—	5	4
Overall prevalence (%)	0	0	5.77	0	0	0

individuals captured/trapping effort) between sampling periods (0.14 individuals/trap-night in December 2007 and 0.03 individuals/trap-night in April 2008; Table 1). Four rodent species captured in the study area have been previously identified as potential hantavirus reservoir species: *Peromyscus maniculatus*, *P. leucopus*, *Reithrodontomys megalotis*, and *Sigmodon hispidus* (Hjelle and Torres-Pérez, 2010). Of these, *P. maniculatus* was the most abundant species, with 26 individuals captured (17.45%; Table 1). Rodents were mainly captured in the mesquite scrublands (53%, $S = 10$, $H = 1.9$), followed by grasslands (37%, $S = 7$; $H = 1.57$) and prairie dog grasslands (10%, $S = 3$; $H = 0.85$). None of the potential hantavirus-reservoir hosts was captured in prairie dog grasslands (Table 1). Three *P. maniculatus* (~11%), all captured in grasslands and in the first sampling period, were antibody positive (Table 1). No other species tested positive. This is the first documented occurrence of hantavirus antibodies in rodents from JCGC, as well as from the state of Chihuahua.

Peromyscus maniculatus is the main reservoir of Sin Nombre virus, which is the leading cause of HPS in the United States (Monroe et al., 1999). Sin Nombre virus has been reported in Mexico, specifically in *P. maniculatus* (in the states of Nuevo León, San Luis Potosí, and Veracruz), *Peromyscus eremicus* (in Nuevo León state), *P. leucopus* (in Tamaulipas state), and *R. megalotis* (in Zacatecas state; Hjelle et al., 1995; Milazzo et al., 2012). Because of high cross-reactivity of the serological analyses conducted in this study, it is not possible to determine which hantavirus is circulating in the study area. To address this, further molecular analyses are needed.

The black-tailed prairie dog is a keystone species from the grasslands of North America that can modulate the assemblages of small mammals within their colonies (Ceballos et al., 1999; Cully et al., 2010). Additionally, hantavirus dynamics in rodent hosts may be influenced by abundance, diversity, and composition structure of small mammal assemblages (Clay et al., 2009; Suzán et al, 2009; Carver et al., 2011). Our findings showed that only *P. maniculatus* inhabiting grasslands without prairie dogs were antibody positive. Although our sample size is too small to claim that habitat type influences hantavirus prevalence, the fact that *P. maniculatus* and other potential hantavirus reservoir hosts are absent or rare in grasslands with prairie dog colonies in the JCGC (Cruzado, 2008; Rubio, unpub. data) suggests that prairie dog colonies may buffer hantavirus transmission. However, to address this topic, as well as temporal dynamics of hantavirus and rodent populations and communities, a more extensive study should be conducted. Recognition of hantavirus distribution and reservoir host ecology in Mexico will enhance prevention and control of emerging hantavirus diseases.

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