Western North American Naturalist 73(3), © 2013, pp. 386–391

EVIDENCE OF HANTAVIRUS EXPOSURE IN RODENTS FROM NORTH TEXAS

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ABSTRACT.—Between 27 June 1997 and 16 May 1999, 423 rodents were collected from North Texas (Collin, Denton, and Grayson counties) by using Sherman live-traps (trapping success rate ~22%). Of the 423 rodents collected, 328 were tested for evidence of IgG antibodies to New World hantaviruses. Hantavirus antibodies were detected in 34 individuals (~10%). This is the first record of hantavirus antibody–positive rodents from the highly urbanized area of North Texas.

RESUMEN.—Entre el 27 de Junio 1997 y el 16 de Mayo 1999, 423 roedores fueron colectados usando trampas Sherman de capturas en vivo (éxito de captura ~22%) en el Norte de Texas (condados Collin, Denton, y Grayson). De los 423 roedores colectados, 328 roedores fueron puestos a prueba para evidenciar la presencia de anticuerpos IgG de Hantavirus del Nuevo Mundo. Los anticuerpos de Hantavirus fueron detectados en 34 individuos (~10%). Este es el primer registro de roedores con anticuerpo-positivo para Hantavirus de un área altamente urbanizada en el norte de Texas.

Members of the genus *Hantavirus* are rodent-borne viruses with cosmopolitan distributions (Jonsson et al. 2010). *Hantavirus* is the only genus within the family *Bunyaviridae* that does not utilize an arthropod vector; rather, hantaviruses are associated primarily with the rodent families Muridae (Old World) and Cricetidae (New World). Infection in rodent hosts is typically lifelong and thought to be asymptomatic (Mertz et al. 2006). The virus is shed in the saliva, urine, and feces of infected rodents. Both Old World and New World hantaviruses are known to infect humans either by inhalation of infectious aerosols or by direct contact of infectious materials with mucous membranes or broken skin (Vitek et al. 1996).

Six of the 17 North American hantavirus genotypes are associated with Hantavirus Pulmonary Syndrome (HPS; Mills et al. 2009). HPS commonly manifests with fever, headache, myalgia, and nausea (Duchin et al. 1994), followed by rapid onset of severe pulmonary illness (Zaki et al. 1995). HPS frequently concludes with the death of the patient (Nichol et al. 1993, Plyusnin et al. 1996). Between 1993 and 2009, 510 cases of HPS were reported in the United States (MacNeil et al. 2011). Of these cases, 498 (~98%) were reported in the western United States, and of these, 160 (~32%) resulted in death of the patient (MacNeil et al. 2011). *Sin Nombre virus* (SNV) is reported to be the cause of most HPS cases in the United States (Monroe et al. 1999). SNV is associated principally with *Peromyscus maniculatus* (Childs et al. 1994), which is a common rodent in rural areas. However, few studies have been conducted to determine hantavirus antibody prevalence in rodents from areas with high human population densities (Calisher et al. 2011).

In January 1997, a fatal case of HPS was reported in Hunt County, Texas (directly east of Collin County; Texas Department of Health 1997), located within the Dallas–Fort Worth Combined Statistical Area (CSA). Given the location of this case, and the paucity of active hantavirus sampling in urban and suburban areas, the purpose of this study was to determine prevalence of hantavirus antibody–positive rodent individuals in areas of high human population within the Dallas–Fort Worth CSA. Trapping efforts focused on the highly populated counties of Collin, Denton, and Grayson in North Texas (Fig. 1). These counties constitute a portion of the Dallas–Fort Worth CSA and have a collective population estimated at over 1.5 million people (U.S. Census Bureau 2010).

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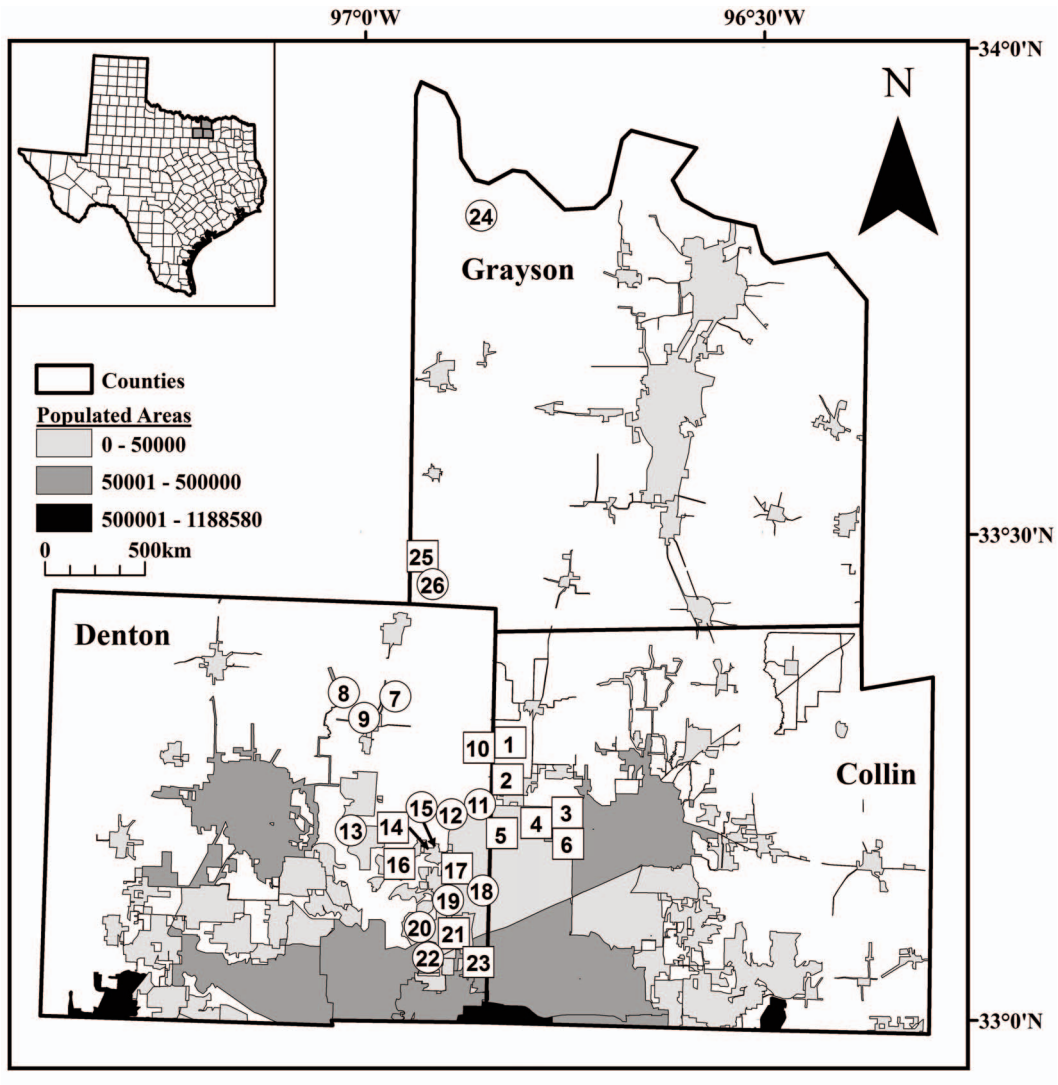

Fig. 1. Study area map of Collin, Denton, and Grayson counties within the Dallas–Fort Worth Combined Statistical Area. Populated areas are shaded according to population estimates of the recent U.S. Census (U.S. Census Bureau 2010). Circles designate collecting localities. Squares designate collecting localities with antibody-positive rodents. Numbers within circles and squares reference locality numbers in Table 1. Inset shows location of study area (gray-shaded area) within the state of Texas.

Twenty-six trapping localities (see Fig. 1, Table 1) were selected on the basis of preferred rodent habitat and visible evidence of rodent activity. Trapping localities included open fields and wooded areas within and between urban areas in the study region. Rodents were trapped with Sherman live-traps (H.B. Sherman Traps, Tallahassee, FL) baited with birdseed. Rodents were anesthetized and subsequently euthanized following guidelines set forth by the American Society of Mammalogists (Animal Care and Use Committee 1998). Blood was collected via capillary tube from the retrorbital capillary plexus of each animal. Sera were separated by centrifugation and frozen at –70 °C. All individuals collected were prepared as voucher specimens and deposited at the Sternberg Museum of Natural History (SMNH), Hays, Kansas.

Blood samples were sent to the Centers for Disease Control and Prevention (Atlanta, GA) for testing of hantavirus antibodies. Tests were

TABLE 1. Summary of rodents tested for evidence of IgG antibodies reactant to SNV, from 26 localities in Collin, Denton, and Grayson counties within the Dallas–Fort Worth Combined Statistical Area. Locality numbers correspond to those numbers in Fig. 1. A total of 328 specimens were tested for hantavirus antibodies. Thirty-four specimens were antibody-positive. The numbers of antibody-positive rodents per species by locality is indicated within parentheses. Abbreviations are as follows: Loc. = locality number; Co. = county; COL = Collin County; DN = Denton County; GS = Grayson County; TN = number of trap-nights; Btay = *Baiomys taylori;* Mmus = *Mus musculus;* Opal = *Oryzomys palustris;* Pleu = *Peromyscus leucopus;* Pman = *Peromyscus maniculatus;* Rnor = *Rattus norvegicus;* Rful = *Reithrodontomys fulvescens;* and Shis = *Sigmodon hispidus.*

Loc.	Co.	Specific locality	TN	Btay	Mmus	Opal	Pleu	Pman	Rnor	Rful	Shis
1	COL	3.6 mi NW Prosper	50				3 (2)				1 (1)
2	COL	2.1 mi NW Propser	50				4 (2)	2			4
3	COL	5.5 mi NE Frisco	200	1			1	9 (1)			3 (3)
4	COL	3.2 mi SE Prosper	50					1			2 (1)
5	COL	10 mi W McKinney	100	1	1			11		3	14 (1)
6	COL	5.1 mi SE Prosper	50	1				2		1	4 (2)
7	DN	2.5 mi NE Aubrey	50								1
8	DN	1.9 mi NW Aubrey	50				2				
9	DN	1.6 mi NW Aubrey	50				1				
10	DN	4.2 mi SW Celina	50					3 (2)		2	2 (1)
11	DN	5 mi N Frisco	50					3		1	
12	DN	5 mi NW Frisco	50	1				10		3	
13	DN	10 mi NW The Colony	50								2
14	DN	1.8 mi E Little Elm	50	2		1	3				4 (1)
15	DN	1.9 mi E Little Elm	50	2			1	3		4	4
16	DN	1 mi E Little Elm	50	1			1	2		1	3 (3)
17	DN	5 mi N The Colony	100					2	2		2 (2)
18	DN	1.5 mi W Frisco	100								15
19	DN	3.4 mi N The Colony	50								1
20	DN	2 mi W The Colony	50							1	1
21	DN	The Colony	300	24	4		2 (2)	10 (1)		5	83 (4)
22	DN	2 mi SE The Colony	50				1				
23	DN	3 mi SE The Colony	50		1						19 (4)
24	GS	2 mi N Gordonville	100				1			1	4
25	GS	1 mi W Tioga	50	1				4 (1)			1
26	GS	1 mi S Tioga	50					7		1	3
Totals			1900	34	6	1	20 (6)	69 (5)	2	23	173 (23)

conducted via enzyme-linked immunosorbent assays (ELISA), following standard protocols (Ksiazek et al. 1995). ELISA methods tested for antibodies reactive to IgG antibodies of SNV. Because antibody presence is indicative of either current or past infection, such presence may or may not be associated with persistent viral shedding (Calisher et al. 2009). Due to the high cross-reactivity of New World hantavirus antibodies, this assay will detect but not distinguish between antibodies of a wide variety of hantaviruses associated with rodents (Mills et al. 1997).

A total of 423 rodents were trapped during 1900 trap-nights for an overall trap success of ~22%; however, quality blood sera samples were available for only 328 individuals. Thirty-four of the 328 specimens tested (~10%) were determined to have IgG antibodies reactive to SNV. Six *Peromyscus leucopus* (30%), 5 *P. maniculatus* (~7%), and 23 *Sigmodon hispidus* (~13%) were antibody positive (Table 1). This is the first documented occurrence of hantavirus antibodies in rodents from the highly urbanized area of North Texas.

As seen in previous studies (Mills et al. 1998, Mantooth et al. 2001), *P. leucopus* and *P. maniculatus* both tested positive for hantavirus antibodies. *Peromyscus maniculatus* is the primary reservoir of SNV and *Monongahela virus* (MGLV). SNV is the leading cause of HPS (Monroe et al. 1999), and MGLV has also been associated with HPS (Song et al. 1996). *Peromyscus leucopus* is both a known reservior for *New York virus* (NYV; Hjelle et al. 1995) and a host to *Blue River virus* (BRV; Morzunov et al. 1998). Although NYV has been associated with HPS, BRV has not. The third antibody-positive rodent species in this study was *S. hispidus,* a known reservoir for *Black Creek Canal virus* (BCCV; Rollin et al. 1995) and host to *Muleshoe virus* (MSV; Rawlings et al. 1996). Because of the ability of all antibody-positive rodent species to harbor

multiple hantavirus species, amplification and sequencing of the viral genome would be necessary to confirm the specific identification of the virus.

None of the 5 remaining species collected in this study (*Baiomys taylori*, *Mus musculus*, *Oryzomys palustris*, *Rattus norvegicus*, and *Reithrodontomys fulvescens*) tested positive for IgG antibodies to hantavirus, though these species have been reported to be antibody positive in previous studies (LeDuc et al. 1986, Childs et al. 1994, McIntyre et al. 2005). Two of these species, *O. palustris* and *R. norvegicus*, are reservoirs for hantaviruses with known human health implications in North America (Lee et al. 1982, LeDuc et al. 1986, Hjelle et al. 1996, Ksiazek et al. 1997). It is not clear whether the failure to identify antibody-positive rodents of these species was a result of small sample size (Table 1) or the "dilution effect" (Ostfeld and Keesing 2000). The "dilution effect" is a phenomenon in which increased rodent biodiversity is correlated negatively with virus antibody prevalence within rodent communities. Previous studies have determined increased species diversity to have a negative effect on both abundance of reservoir hosts and their infection prevalence with hantavirus (Dizney and Ruedas 2009, Suzán et al. 2009). Localities where at least one of these species was collected had an average species richness of ~3.6 species (range 2–6 species) per locality (Table 1); however, more stringent trapping protocols would be necessary to accurately estimate measures of biodiversity.

Of the 2 reservoir species with human health implications in North America, *O. palustris* is the principal reservoir for *Bayou virus* (BAYV; Hjelle et al. 1996, Ksiazek et al. 1997), which is thought to be the second leading cause of HPS in the United States (Morzunov et al. 1995). The distribution of *O. palustris* includes the majority of the southeastern United States, encompassing numerous metropolitan areas (Wilson and Reeder 2005); however, due to the non-peridomestic behavior of *O. palustris*, the risk of human infection is relatively low (McIntyre et al. 2005). The second species, *R. norvegicus*, is associated with *Seoul virus* (SEOV; Lee et al. 1982, LeDuc et al. 1986), which has been linked to Hemorrhagic Fever with Renal Syndrome (HFRS; LeDuc et al. 1982). *Rattus norvegicus* is an invasive, commensal species that occurs commonly in urban areas (Hall 1981). LeDuc et al. (1986) screened 1616 *R. norvegicus* from all continents, excluding Antarctica, and 341 individuals (~21%) tested positive for hantavirus antibodies. The city of Baltimore, Maryland, had the highest prevalence, with 108 of 170 screened individuals (~64%) testing positive. In addition, Baltimore recently had a domestically acquired case of HFRS due to SEOV (Woods et al. 2009). Given the human health implications of these case studies, the results presented herein warrant further rodent sampling for hantavirus screening in the Dallas–Fort Worth CSA, as well as other urbanized areas.

We thank Curtis Schmidt at the Sternberg Museum for help accessing records of cataloged specimens. We also thank the Centers for Disease Control and Prevention for funding and performing ELISA assays. Comments from Robert Bradley and 2 anonymous reviewers improved the manuscript. Nicté Ordóñez-Garza provided the Spanish abstract translation.

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Received 13 June 2012
Accepted 29 May 2013