Evidence of hantavirus exposure in rodents from north Texas

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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). *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 hantavirus antibody prevalence in rodents from areas with high human population densities (Calisher et al. 2011).

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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 retroorbital 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
conducted via enzyme-linked immunosorbent assays (ELISA), following standard protocols (Książek 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 reservoir 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

### 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.

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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.

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