

## Serosurvey of Wild Rodents for Hantaviruses in Panama, 2000–2002

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**ABSTRACT:** Five hundred fifty-six samples representing 24 species of small mammals (two species of marsupials and 22 rodents) were collected in Panama between February 2000 and July 2002. The samples were examined for antibodies to hantaviruses by means of enzyme-linked immunosorbent assay or immunoblot assays. The serologic results indicated that several rodent species might act as hantaviral reservoirs in Panama: Costa Rican pygmy rice rat (*Oligoryzomys fulvescens costaricensis*), four positive of 72 tested (5.6%); Cherrie's cane rat (*Zygodontomys brevicauda cherriei*), five of 108 (4.6%); Mexican deer mouse (*Peromyscus mexicanus*), one of 22 (5%); Mexican harvest mouse (*Reithrodontomys mexicanus*), one of seven (14%); Chiriquí harvest mouse (*Reithrodontomys creper*), one of two (50%); and Sumichrast's harvest mouse (*Reithrodontomys sumichrasti*), three of four (75%). Hantavirus infection in *Peromyscus mexicanus* and the three species of *Reithrodontomys* was caused by Rio Segundo hantavirus, a species of virus not previously reported from Panama. At least three hantaviruses, therefore, are known to infect populations of wild rodents in the country. However, given the total number of animals tested, the role of these rodent species in the epidemiology and epizootiology of hantavirus infections remains unclear.

**Key words:** Hantavirus, Heteromyidae, Muridae, Panama, seroprevalence, Sigmodontinae.

Hantavirus pulmonary syndrome (HPS), first described in the southwestern United States in 1993 (Nichol et al. 1993), is a distinctive but uncommon viral pneumonitis with a mortality rate of about 38%. Hantavirus pulmonary syndrome occurs throughout most of the Americas. Since its description in 1993, more than 350 cases in North America and close to 1,000 cases in South America have been documented (CDC, 2003). Hantavirus pulmonary syndrome is caused by many American han-

taviruses (Family *Bunyaviridae*)—small, tripartite, negative-strand RNA viruses. Sin Nombre virus (SNV) is the most common pathogenic hantavirus through most of North America, with rare infections caused by New York virus in the northeastern United States and Bayou virus and Black Creek Canal virus in the southeastern United States. In the Patagonian region of Argentina and Chile, Andes virus causes a severe form of HPS that can include renal involvement; person-to-person transmission of Andes virus has been documented.

Most other regions of South America have also had sporadic cases of HPS in areas including central and northwestern Argentina, southern Bolivia, Amazonian Peru, the Gran Chaco region of Paraguay, central and southeastern Brazil, and Uruguay. Rodents with antibodies to hantaviruses have been captured in Peru, Venezuela, Mexico, and Costa Rica, although HPS has not been documented in these countries (e.g., Hjelle et al., 1994; Suzán et al., 2001).

Each hantavirus species is generally carried by one of several rodent species belonging to the murid subfamilies Sigmodontinae or Arvicolinae; a single instance of one hantavirus, Thottapalayam, from an Asiatic house shrew (*Suncus murinus*) has occurred in India (Zeller et al., 1989; Hjelle and Yates, 2001). Transmission of SNV to humans is believed to occur by inhalation of aerosolized excreta or saliva from infected rodents but this has not been confirmed in rodent models (Botten et al., 2002); a potential role for ectoparasites in

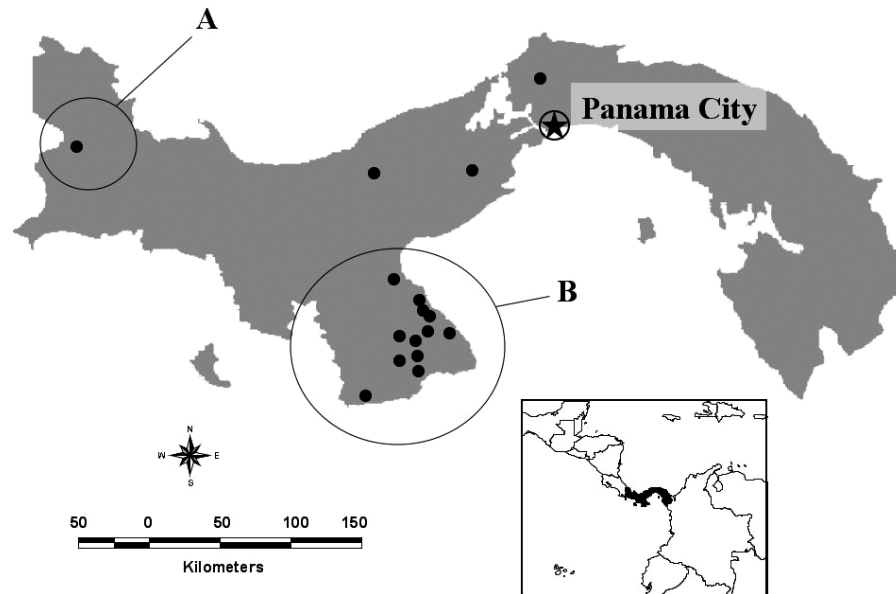


FIGURE 1. Map of the sampling localities in Panama. (A) Area in the country where wild populations of rodents have been found infected with Rio Segundo virus. (B) Península del Azuero, the region of endemicism of hantavirus pulmonary syndrome (HPS) in Panama, where the only hantaviruses found to date are Choclo and Calabazo.

transmission dynamics has been suggested as well (Houck et al., 2001).

In December 1999, cases identified as HPS were recorded from the Azuero peninsula of Panama (Fig. 1). Within 5 wk of the initial case, 12 additional putative cases were reported. As of this writing, almost 40 cases of HPS have been identified in Panama, with a case fatality rate of 22%. After the original outbreak investigation, it was shown that the Costa Rican pigmy rice rat (*Oligoryzomys fulvescens costaricensis*) harbored a novel hantavirus (Choclo virus) linked to human disease, whereas a second, also novel, hantavirus (Calabazo virus), found in Cherrie's cane rat (*Zygodontomys brevicauda cherriei*) to date has not been linked to cases of HPS. Results of the laboratory phase of the investigation have been published elsewhere (Vincent et al., 2000).

This study was undertaken to identify species and populations of small wild mammals that might be of importance in the epidemiology of HPS in Panama, as well as in the maintenance of the enzootic

cycle of hantaviruses in that country. We concentrated on several localities throughout central and western Panama, attempting to sample several types of vegetation and different levels of human intervention; thus, our sampling included areas covered with tropical rain forest, cloud forest, and tropical dry forest, etc. We conducted intensive trapping in and around the area of endemicism of HPS—the District of La Tablas (Península del Azuero), where human-induced biological disturbance and numerous crop types, including corn, watermelon, bean, coffee, and sugar cane, characterize the region (Fig. 1).

The overarching climate regime of the Península del Azuero is characterized by extreme seasonality, with rainfall covering the region between May and December and a dry season from January to April. However, subsumed within this regime is a strong gradient of precipitation, with the northeast portion of the peninsula receiving the least amount of precipitation and the southwest receiving the most; highland areas of the peninsula receive more pre-

precipitation regardless of their geographic location on the peninsula. Dry deciduous forest occurs primarily in the drier eastern portion, with evergreen forest types predominating in the higher regions and the southwest. Mangrove vegetation locally lines discrete portions of the coast. Patches of evergreen forest remain in the Cerro Hoya highlands in the southwest of the peninsula.

The results of this study are based on rodent trapping conducted between 29 February 2000 and 30 July 2002. Rodent trapping was intermittent throughout the period of study, with most of the animals collected between May and July 2001. Trapping was conducted with Sherman (H. B. Sherman Traps, Inc., Tallahassee, Florida, USA) and Tomahawk (Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) traps baited with a combination of rolled oats, birdseed, molasses, and tuna; vanilla extract also was added sporadically to the bait.

In areas with relatively pristine vegetation (e.g., Bugaba District), up to three grids of 10×10 m (100 traps) were established, whereas in highly disturbed habitats found in and around human habitations of the peninsula, traps were set at approximately 10-m intervals in linear transects in a complex matrix of riparian vegetation, residential, and agricultural areas. The number of trap stations in these disturbed habitats was not standardized. In all cases, all traps were set for a minimum of three consecutive nights.

Mammals were handled and sampled according to the recommendations of Mills et al. (1995). Briefly, blood was obtained from retro-orbital sinus by heparinized capillary tubes, and then the animals were euthanized with an overdose of inhalant anesthesia (methoxyflurane, Pitman-Moore, Mundelein, Illinois, USA). The following data were recorded: species; sex; age; mass; reproductive condition; length of body, tail, hind foot, and ear; and presence and nature of external wounds. Blood and samples of spleen, liver, kidneys,

heart, and lungs were collected into separate labeled cryovials with clean sterilized instruments for each animal. All biologic samples were placed immediately into liquid nitrogen. After processing, each carcass was placed either directly into 80% ethanol or into 10% formalin for 3 days, followed by immersion in 70% ethanol for long-term preservation. All animals are deposited in the Museum of Southwestern Biology (University of New Mexico, Albuquerque, New Mexico, USA) or the Gorgas Memorial Institute (Panama City, Rep. of Panama).

The first 120 animals were initially screened by enzyme-linked immunosorbent assay (ELISA) with SNV antigen and confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) for some individuals (Vincent et al., 2000). Animals collected after March 2000 were tested by a strip immunoblot assay for detection of antibodies to SNV nucleocapsid (N) antigen (Yamada et al., 1995; Hjelle et al., 1997). Reverse transcriptase PCR was used to confirm the identity of the hantavirus infecting rodents that tested positive.

During the trapping period covered by this report, a total of 556 individuals of 22 rodent and two marsupial species from 20 localities in 10 districts were collected in Panama. Most of the localities sampled (73%) were located within the Península del Azuero, where the majority of Panama's cases of HPS have been identified (Fig. 1).

Rodent species diversity had an ecologic component. Most of the diversity was found (14 species) in pristine protected forests, whereas only six species (*Liomys adspersus*, *Z. brevicauda*, *Sigmodon hispidus*, *O. fulvescens*, *Rattus rattus*, and *Mus musculus*) were found in the more disturbed habitats of the peninsula.

In general, the Panamanian endemic species *L. adspersus* was the most common rodent species captured (21% of all rodents), followed by *Z. brevicauda* (19%), *S. hispidus* (15%), and *O. fulvescens* (13%), with other species captured less

TABLE 1. Antibody prevalence (number positive/number tested) for hantaviruses in mammals collected from February 2000 to January 2002 in Panama.

District <sup>a</sup>	Species <sup>b</sup>							Total
	Zbrevic	Ofulves	Pmexic	Rmexic	Rsumic	Rcreper	Others <sup>c</sup>	
Bugaba	NT <sup>d</sup>	NT	1/22	1/7	3/4	1/2	0/15	6/50
Las Tablas	4/63	3/35	NT	NT	NT	NT	0/141	7/239
Parita	0/3	1/9	NT	NT	NT	NT	0/9	1/21
Pocri	1/18	0/9	NT	NT	NT	NT	0/9	1/36
Others <sup>e</sup>	0/24	0/19	NT	NT	NT	NT	0/167	0/210
Total	5/108	4/72	1/22	1/7	3/4	1/2	0/341	15/556

<sup>a</sup> District Bugaba is identified by circle A in Figure 1, Districts Las Tablas, Parita, and Pocri are encircled by B in Figure 1.

<sup>b</sup> Abbreviations: Zbrevic=*Zygodontomys brevicauda*; Ofulves=*Oligoryzomys fulvescens*; Pmexic=*Peromyscus mexicanus*; Rmexic=*Reithrodontomys mexicanus*; Rsumic=*Reithrodontomys sumichrasti*; Rcreper=*Reithrodontomys creper*. All seropositive rodents were confirmed via reverse transcriptase polymerase chain reaction.

<sup>c</sup> Includes two didelphid marsupials (five *Marmosa mexicana* and five *M. robinsoni*), two dasyproctid rodents (*Dasyprocta punctata*), seven echimyid rodents (*Proechimys semispinosus*), 126 heteromyid rodents (eight *Heteromys desmarestianus* and 118 *Liomys adspersus*). Other murid rodents were negative and included (in increasing order): one *Tylomys watsoni*, two *Nyctomys sumichrasti*, two *Oryzomys albigularis*, two *Oryzomys alfari*, three *Oryzomys couesi*, four *Oryzomys bolivaris*, four *Melanomys caliginosus*, seven *Scotinomys xerampelinus*, 12 *Rattus rattus*, 20 *Oryzomys talamancae*, 54 *Mus musculus*, and 85 *Sigmodon hispidus*.

<sup>d</sup> None tested.

<sup>e</sup> Other districts where rodents were tested but yielded no antibody positive samples included (sample size in parenthesis): Capira (6), Chagres (10), Guarare (62), Montijo (78), Ola (18), and Tonosi (36).

frequently (Table 1). Murid rodents were the most common group of species captured, representing over 75% of the sample.

All animals were tested for antibodies and reactivity to SNV antigen; however, only murid rodents were found to have antibodies to the SNV antigen used. Infection rates were 2.7% of all species and 3.7% of murid rodents. The highest hantaviral antibody prevalence was found among *Reithrodontomys sumichrasti*, three of four (75%); *Reithrodontomys creper*, one of two (50%); and *Reithrodontomys mexicanus*, one of seven (14%). The most consistently seropositive species were *O. fulvescens*, four of 72 (6%), and *Z. brevicauda*, five of 108 (2.7%), all from the Península del Azuero, where the majority of Panamanian cases of HPS have emerged.

Of the 15 rodents that tested positive, 14 (94%) were male and 11 (69%) were adults. All positive *O. fulvescens* and *Z. brevicauda* were adult males. Among all positive rodents, 37.5% (all males) had wounds present. The higher prevalence in animals of larger size (i.e., adults) and

among males is consistent with previous reports for other rodents with antibody to SNV, in which it has been hypothesized that the mode of transmission might include a number of active or passive interactions between individuals, including biting and scratching and mating activities (e.g., Mills et al., 1999).

Our results thus far suggest that, in Panama, only rodents of the family Muridae are involved in circulation of hantaviruses. This agrees with data from elsewhere in the Americas, where both murid and heteromyid rodents are present. Mills et al. (1997) did not find any heteromyid species with antibodies to SNV, although both virus and rodents alike are highly abundant and diverse in the American Southwest. This also agrees with data presented by Hjelle and Yates (2001), suggesting a long coevolutionary relationship between hantaviruses and murid rodents.

The localities sampled in Panama can be divided into two geographic areas in terms of the hantavirus-infected rodents: the western region of the country (Bugaba District) and the eastern region of the Península del Azuero. Reverse transcrip-

tase PCR analysis confirms a strong geographic component to hantaviral infections of rodents in Panama. All rodents with antibody were from anthropogenically disturbed habitats in the Península del Azuero and were infected with either Calabazo or Choclo viruses, whereas rodents from Bugaba were infected with Rio Segundo virus (Fig. 1).

Our results indicate that two subsets of viruses can be identified in terms of their abilities to infect multiple species. We found at least four rodent species that were infected by Rio Segundo virus, whereas Choclo virus and Calabazo viruses each infected a single species of rodent. Whether this is because of the rodent-virus dynamics, the mode of transmission, or the geographic and ecologic setting in which these viruses occur is unknown at this time. It is further possible that hantavirus from rodents of the genus *Reithrodontomys* is more amenable to host switching than other hantaviruses. For example, Limestone Canyon virus, from brush mouse (*Peromyscus boylii*) in Arizona (USA), clearly is a *Reithrodontomys* hantavirus on the basis of sequence data (Sanchez et al., 2001). These significant lacunae in our knowledge base strongly argue in favor of the continuation of long-term studies on the ecology and epidemiology of these and related hantaviruses on a regional scale.

Vincent et al. (2000) observed an inverse relationship between rodent abundance and prevalence of infection by the virus that causes HPS; specifically, the most abundant murid rodent (*Z. brevicauda*) did not harbor the virus responsible for the HPS outbreak of 2000. Our data tentatively support this observation: the third most abundant rodent species in our survey (*O. fulvescens*) is the rodent reservoir of Choclo virus, the only virus known to cause HPS in Panama.

Interestingly, these two species of rodents (*Z. brevicauda* and *O. fulvescens*) occur sympatrically, and in many cases syntopically (same microhabitat associa-

tion), in all Districts sampled to date. On the foregoing basis, we consider three factors as potentially significant in transmission of hantaviruses from rodents to humans and the consequent initiation of onset of HPS infection: 1) ecologic differences are subtle between *O. fulvescens costaricensis* and *Z. brevicauda*, 2) these two rodent species exhibit different behavioral patterns with regard to entering human habitations, 3) a combination (in varying degrees) of factors 1 and 2 might act in the maintenance and transmission of the virus.

These are all testable hypotheses that require detailed studies of microhabitat preferences, individual activity patterns, etc. In turn, testing these hypotheses could prove to have important implications for ecologic or other measures of rodent control. Moreover, they could prove to be working hypotheses for other rodent-virus systems in which more than one rodent species is involved in the cycle of infection. For example, evidence is ample that at least two species of rodents (*Abrothrix olivaceus* and *Abrothrix longipilis*), in addition to the apparent primary host, *Oligoryzomys longicaudatus*, maintain high hantavirus seroprevalence in some regions in Chile (Toro et al., 1998).

At least two hantaviruses are known from harvest mice (*Reithrodontomys* spp.): Rio Segundo virus (*R. mexicanus*) and El Moro Canyon virus (*R. megalotis*; Schmaljohn and Hjelle, 1997). Rio Segundo was originally described in animals from central Costa Rica (Hjelle et al., 1995); thus, it is not surprising that we now report it from northern Panama, an area adjacent to the Costa Rican border. What is significant, however, is that more than one species of harvest mouse and at least one deer mouse (*Peromyscus mexicanus*) appeared to be infected with (or to circulate) Rio Segundo virus and that the highest infection rate occurred in *R. sumichrasti* rather than in *R. mexicanus*. All animals infected with Rio Segundo virus were collected sympatrically, suggesting a potential case

of spillover from one species to others. A similar pattern also was found by Mills et al. (1997) in sympatric assemblages of small mammal species for the southwestern United States, although the alternative hypothesis of a hantavirus with plastic host tolerance cannot be discounted at this time. Much remains to be learned about the ecology of infection in rodent-hantavirus systems in the New World, in general, and in tropical areas, in particular. Our data tentatively suggest that hantaviruses from *Reithrodontomys* spp. display greater tolerance to host switching than do hantaviruses found in *Peromyscus* spp. Careful study (ecologic, epidemiologic, and molecular) is required to test this hypothesis versus its equally untested alternative that these hantaviruses are circulating in multiple species because of spillover from a primary host.

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