

Sympatry of 2 Hantavirus Strains, Paraguay, 2003–2007

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To explore geographic and host-taxonomic patterns of hantaviruses in Paraguay, we established sampling sites in the Mbaracayú Biosphere Reserve. We detected Jaborá virus and Itapúa37/Juquitiba–related virus in locations ≈20 m apart in different years, which suggested sympatry of 2 distinct hantaviruses.

Hantaviruses are rodent-borne viruses that may cause Hemorrhagic fever with renal syndrome or hantavirus pulmonary syndrome in humans, although some strains do not cause disease (1,2). In Paraguay in 1995, Laguna Negra virus carried by *Calomys laucha* (little laucha) caused an outbreak of hantavirus pulmonary syndrome in western Paraguay (3).

We have identified 4 additional strains in Paraguay: Alto Paraguay virus harbored by *Holochilus chacarius* (Chacoan marsh rat) in western Paraguay; and Ape Aime virus (AAIV) harbored by *Akodon montensis* (Montane akodont), Itapúa virus strain 37 (IPV37) harbored by *Oligoryzomys nigripes* (black-footed colilargo), and Bermejo virus strain Ñeembucu harbored by *O. chacoensis* (Chacoan colilargo) in eastern Paraguay (4,5). We have continued our surveillance of hantaviruses in the interior Atlantic forests within and near Reserva Natural del Bosque Mbaracayú (RNBM), a World Biosphere Reserve located within Departamento Canindeyú in eastern Paraguay (Figure 1).

The Study

We established and sampled 10 mark-recapture sites within and adjacent to RNBM during 2003–2007. Sampling of grids depended upon weather conditions, the purpose of the grid, and transitory human settlements. Each mark-recapture grid consisted of an 11 × 11 array of trap stations spaced 10 m apart, each of which had 1 standard live trap

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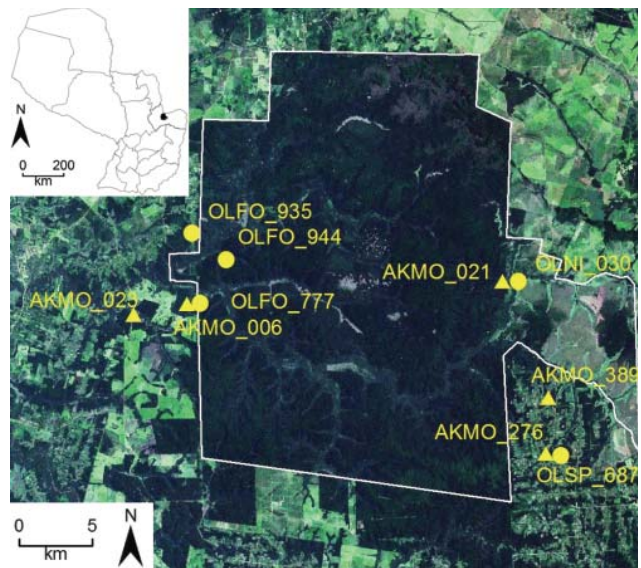


Figure 1. Satellite image of collection sites of hantavirus RNA-positive rodents, including selected Juquitiba virus (circles) and Jaborá virus (triangles) samples, Paraguay, 2003–2007. Inset shows location of study site in Paraguay. OLFO, *Oligoryzomys fornesi*; OLNI, *O. nigripes*; OLSP, *Oligoryzomys* sp.; AKMO, *Akodon montensis*.

(H.B. Sherman Traps, Tallahassee, FL, USA) placed on the ground, and another in branches or vines 2–3 m above ground to capture arboreal species. Grids were sampled for 8 nights, with at least 2 months between sampling sessions. Rodents captured in the mark-recapture grids were individually marked with passive integrated transponder tags, and ≈100 μL of blood was collected from the retroorbital sinus (once per trapping session). Animals were identified to species, age class, reproductive condition, sex, and weight and released.

Rodents were also collected in a series of traplines, each of which contained 50 traps placed ≈10 m apart. Animals collected in traplines were killed, standard collecting information was recorded, and liver, lung, heart, kidney, muscle tissues, and blood specimens were collected. All samples were snap-frozen in liquid nitrogen, transported to the Museum of Texas Tech University (TTU), and stored at –80°C. Standard voucher specimens were prepared from these animals, and have or will be deposited in the Museum of TTU or the Museo Nacional de Historia Natural del Paraguay. All field protocols followed American Society of Mammologists guidelines for the use of wild mammals in research (6), and were reviewed and approved by the TTU Animal Care and Use Committee.

A total of 1,150 small mammals from ≥20 species were captured, including 13 sigmodontine rodent species

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(1,140 animals) (online Technical Appendix, available from www.cdc.gov/EID/content/15/12/1977-Techapp.pdf). The dominant rodent species in Mbaracayú were *A. montensis* (55.7%), *Necromys lasiurus* (hairy-tailed akodont) (10.8%), *C. callosus* (big laucha) (6.5%), and *O. fornesi* (Fornes' colilargo) (6.3%).

Antibodies to hantavirus antigens were detected in blood specimens by using an indirect immunofluorescent antibody assay and irradiation-sterilized slides of Vero E6 cells infected with Andes virus as described (4). Seven species were antibody positive: *A. montensis*, *N. lasiurus*, *O. fornesi*, *O. nigripes*, *Oligoryzomys* sp., *Oryzomys megacephalus* (Azara's broad-headed *Oryzomys* sp.), and *Oxymycterus delator* (Paraguayan hocicudo). Antibodies to hantavirus antigens were 3× more abundant in blood samples from males than females (online Technical Appendix).

Total RNA was extracted from antibody-positive blood clots from mark-recapture samples or lung tissue from killed animals. Nested reverse transcription-PCR was performed to amplify a 371-nt small (S) hantavirus RNA segment (4). Hantavirus RNA was detected in 23 *A. montensis*, 5 *O. fornesi*, 1 *O. nigripes*, and 1 *Oligoryzomys* sp. Of these animals, all but 2 *A. montensis* were males, which indicated that male rodents play the primary role in maintenance and transmission of hantavirus.

A representative sample (15 *A. montensis*, 3 *O. fornesi*, 1 *O. nigripes*, and 1 *Oligoryzomys* sp.) were selected for additional PCR, sequencing, and phylogenetic analysis. PCR-amplified cDNAs of a 1,014-nt amino terminus region of the S segment were purified by agarose gel electrophoresis and cloned into pCRII (Invitrogen, Carlsbad, CA, USA) (4,5). M13 forward and reverse primers were used for sequencing. For sequence comparison and phylogenetic analysis, sequences of representative New and Old World hantaviruses were obtained from GenBank. Phylogeny reconstruction was conducted by using Modeltest version 3.6 analysis (<http://darwin.uvigo.es/software/modeltest.html>), maximum-likelihood estimation, and Bayesian inference (Figure 2).

Bayesian analysis based on the 1,014-nt sequence showed that all sequences from *A. montensis* formed a strongly supported clade, which included AAIV-related hantaviruses from Itapúa Department, Jaborá virus (JABV) from southern Brazil, and strains from RNBM in Paraguay (Figure 2, clade C1). Phylogenetic analyses identified 3 subclades representing virus sequences from animals in the RNBM, Itapúa, and southern Brazil. This type of geographic clustering is similar to Sin Nombre-related viruses in deer mice in North America (7). All S segment sequences from *A. montensis* were closely related, with nucleotide sequence differences between RNBM strains and AAIV and JABV of 4% and 12%, respectively, and derived amino acid differences of 0% or 1%, respectively (Table 1).

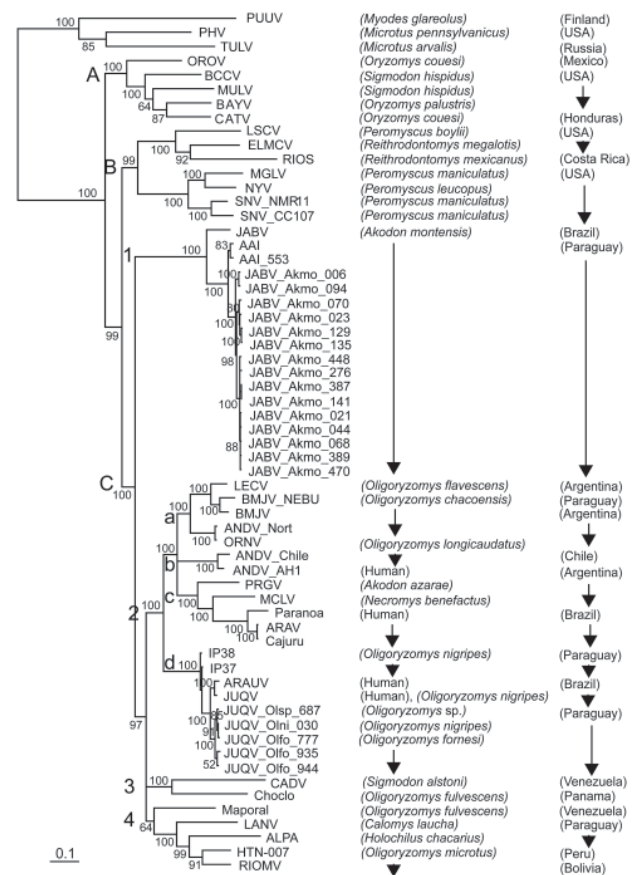


Figure 2. Phylogenetic tree based on Bayesian analysis of the small (S) segment of American hantaviruses, Paraguay, 2003–2007. The tree is based on 1,014 nucleotides of partial S segment of North and South American hantaviruses. Clades (upper case letters), subclades (numbers), and groups (lower case letters) are indicated on the left. Numerical values at the nodes indicate posterior probabilities that supported each interior branch. Scale bar indicates mean number of nucleotide substitutions per site. Alignment and editing of nucleotide sequences were conducted by using Vector NTI version 10.3.1 (Invitrogen, Carlsbad, CA, USA). Sample identifier numbers are the same as in Figure 1. Complete sequencing and abbreviation information is available online at www.cdc.gov/EID/content/15/12/1977-F2.htm.

In contrast, all virus sequences from *O. fornesi*, *O. nigripes*, and *Oryzomys* sp. at RNBM formed a strongly supported clade with viruses related to Jucuitiba virus (JUQV) from Brazil and Itapúa virus strain 37, which was originally detected in *O. nigripes* from Itapúa Department in eastern Paraguay (Figure 2, clade C2d). Nucleotide sequence differences between JUQV strains from RNBM were 0%–2%. Nucleotide sequence differences between JUQV strains from RNBM and Itapúa virus strain 37 or JUQV (Brazil) were 5% or 4%, respectively, and derived amino acid differences were 0% (Table 1). This clade is phylogenetically

Table 1. Nucleotide and amino acid sequence similarities of small gene segments among hantaviruses identified in Paraguay and nearby countries, 2003–2007*

Virus	JABV				BMJV-NEBU			JUQV		PRGV	ARAV	
	LANV	RIOMV	ALPA	Akmo_006	AAI	ANDV	IPV37	Oifo_777				
LANV	–	83	78	75	75	76	79	80	80	80	78	78
RIOMV	93	–	81	77	78	77	80	80	79	79	78	79
ALPA	92	96	–	77	77	78	78	78	78	77	77	78
JABV	85	89	88	–	88	89	76	77	78	77	78	77
JABV Akmo_006	86	88	88	99	–	96	76	75	77	76	76	76
AAIV	88	90	90	99	99	–	77	76	77	77	77	76
ANDV	90	90	89	86	86	88	–	83	82	81	82	82
BMJV-NEBU	89	90	88	86	85	88	98	–	84	83	84	81
IPV37	90	90	89	86	86	88	96	95	–	95	95	81
JUQV_Oifo_777	89	88	87	86	86	87	96	94	100	–	96	80
JUQV	90	90	89	86	85	88	96	95	100	100	–	81
PRGV	90	90	89	85	84	87	96	95	93	92	93	–
ARAV	91	91	90	90	89	90	96	94	94	94	94	–

*Values above the diagonal are percentage nucleotide sequence similarities, and values below the diagonal are percentage amino acid sequence similarities. LANV, Laguna Negra virus; RIOMV, Rio Mamore virus; ALPA, Alto Paraguay virus; JABV, Jaborá virus; AAIV, Ape Aime virus; ANDV, Andes virus; BMJV-NEBU, Bermejo virus from Ñeembucú; IPV37, Itapúa virus strain 37; JUQV, Juquitiba virus; PRGV, Pergamino virus; ARAV, Araraquara virus.

distinct from viruses that form the *Akodon montensis* clade at RNBM and more closely related to Andes (clade C2b) and Bermejo-Ñeembucú (clade C2a) viruses. This finding suggests that spillover infection of JUQV-related viruses is actively occurring among oryzomyine rodent species at RNBM, as reported for other hantaviruses in oryzomyines (8) and other rodent hosts of Old World hantaviruses (9,10). Additional data are needed to determine the primary oryzomyine reservoir of JUQV and to better understand mechanisms by which spillover occurs.

In addition to spillover infection of JUQV among oryzomyine rodents, we identified 2 virus strains (JUQV and JABV) in close proximity (collected ≈ 20 m apart on the same grid in the same sampling session) on 2 occasions, in sites separated by ≈ 30 km (Figure 1; Table 2). Thus, these 2 distinct hantaviruses appear to be maintaining a sympatric status across a considerable expanse of landscape, rather than reflecting a temporary or localized phenomenon. We use the term sympatric to underscore that these viruses are in the same community and are near enough (their rodent reservoirs) to interact.

Conclusions

Coexistence of hantaviruses in 2 rodent species at mark-recapture sites has been observed (11–13). Serologic analyses in these studies would not have differentiated whether distinct strains of hantaviruses were co-circulating or active spillover infection was occurring among sympatric rodents at collection sites. Recently, Raboni et al. reported JUQV circulating in 3 sympatric rodent species in southern Brazil and 2 distinct hantaviruses (Jabora and JUQV) in 1 rodent species (*A. montensis*) (14). We have not detected JUQV in *A. montensis* in Paraguay. To address host-jumping of hantaviruses among sympatric rodent species in RNBM and other regions in South America, future longitudinal studies are warranted. Such studies are critical to understanding evolutionary adaptation of hantaviruses in rodents in South America, the ability of these viruses to adapt to new rodent reservoirs, and their emergence and maintenance in the environment.

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Table 2. Incidence of sympatry of 2 hantaviruses and their presumed reservoirs, Paraguay, 2003–2007*

ID no.	Species	Collection date	Collection site	Virus antibody	Virus RNA
JAB_Akmo_006	<i>Akodon montensis</i>	2005 Sep 15–18	Mark-recapture	–	–
		2005 Nov 12, 15, 17		+	+
		2006 Feb 27–Mar 6		+	+
JUQV_Oifo_777	<i>Oligoryzomys fornesi</i>	2005 Feb 14–16		–	–
		2005 Sep 12		+	+
JAB_Akmo_276	<i>A. montensis</i>	2007 Jun 12	Trapline	+	+
JUQV_Oisp_687	<i>Oligoryzomys</i> sp.	2006 Aug 18		+	+
JAB_Akmo_021	<i>Akodon montensis</i>	2003 Sep 12	Trapline	+	+
JUQV_Oini_030	<i>O. nigripes</i>	2003 Sep 13		+	+

*ID, identification; JAB, Jaborá virus; JUQV, Juquitiba virus.

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Dr Chu is a research scientist at Southern Research Institute. His research interests include the ecology and evolution of hantaviruses.

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Sympatry of 2 Hantavirus Strains, Paraguay, 2003–2007

Technical Appendix

Table. Small mammals collected at Mbaracayú Reserve, Paraguay, 2003–2007*

Species	No. collected			No. positive for antibodies against hantavirus antigens			No. positive for hantavirus RNA		
	M	F	Total (%)	M	F	Total	M	F	Total
Akodon montensis *	319	315	641 (55.7)	50	15	65	21	2	23
Calomys callosus *	41	21	63 (5.5)	0	0	0	0	0	0
Calomys tener	1	1	2 (0.2)	0	0	0	0	0	0
Calomys sp.	5	2	7 (0.6)	0	0	0	0	0	0
<i>Clyomys laticeps</i>	1	0	1 (0.1)	0	0	0	0	0	0
<i>Dasyopus novemcinctus</i>	0	0	1 (0.1)	0	0	0	0	0	0
<i>Dasyopus septemcinctus</i>	0	1	1 (0.1)	0	0	0	0	0	0
<i>Gracilinanus sp.</i>	1	0	1 (0.1)	0	0	0	0	0	0
Holochilus chacarius	1	0	1 (0.1)	0	0	0	0	0	0
<i>Monodelphis domestica</i>	2	2	4 (0.3)	0	0	0	0	0	0
<i>Mus musculus</i>	0	1	1 (0.1)	0	0	0	0	0	0
Necromys lasiurus	74	50	124 (10.8)	1	2	3	0	0	0
Oligoryzomys fornesi *	41	30	72 (6.3)	4	0	5	4	0	5
Oligoryzomys nigripes *	27	15	44 (3.8)	1	0	1	1	0	1
Oligoryzomys sp.	5	10	15 (1.3)	1	0	1	1	0	1
Oryzomys angouya	14	7	30 (2.6)	0	0	0	0	0	0
Oryzomys buccinatus	0	1	1 (0.1)	0	0	0	0	0	0
Oryzomys megacephalus	33	18	51 (4.4)	1	0	1	0	0	0
Oryzomys sp.	5	13	18 (1.6)	0	0	0	0	0	0
Oxymycterus delator *	25	31	57 (5.0)	0	1	1	0	0	0
Oxymycterus misionalis	3	2	5 (0.4)	0	0	0	0	0	0
<i>Rattus rattus</i>	1	0	1 (0.1)	0	0	0	0	0	0
Scapteromys aquaticus	2	2	4 (0.3)	0	0	0	0	0	0
Unknown	2	3	5 (0.4)	0	0	0	0	0	0
Total	603	525	1,150 (100)	58	18	77	27	2	30

*Species in **boldface** are sigmodontine rodents (family Cricetidae, subfamily Sigmodontinae). Species followed by an asterisk included ≥ 1 animals of undetermined sex. Thus, the total is greater than males plus females.