Hantavirus Pulmonary Syndrome, Central Plateau, Southeastern, and Southern Brazil

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Hantavirus pulmonary syndrome (HPS) is an increasing health problem in Brazil because of encroachment of sprawling urban, agricultural, and cattle-raising areas into habitats of subfamily Sigmodontinae rodents, which serve as hantavirus reservoirs. From 1993 through June 2007, a total of 884 cases of HPS were reported in Brazil (case-fatality rate 39%). To better understand this emerging disease, we collected 89 human serum samples and 68 rodent lung samples containing antibodies to hantavirus from a 2,500-km-wide area in Brazil. RNA was isolated from human samples and rodent lung tissues and subjected to reverse transcription–PCR. Partial sequences of nucleocapsid protein and glycoprotein genes from 22 human and 16 rodent sources indicated only Araraquara virus and Juquitiba virus lineages. The case-fatality rate of HPS was higher in the area with Araraquara virus. This virus, which may be the most virulent hantavirus in Brazil, was associated with areas that have had greater anthropogenic changes.

The genus Hantavirus of the family Bunyaviridae includes a large number of rodent-borne viruses (roboviruses) that are distributed worldwide. Hantaviruses are 80–120 nm in diameter and have an envelope that contains 3 single-stranded, negative-sense segments of RNA known as small (S), medium (M), and large (L). These segments are circular because of their 5′ and 3′ complementary termini and complex with a nucleocapsid (N) protein to form individual L, M, and S nucleocapsids (J). The S segment encodes an N nucleoprotein, the M segment encodes a glycoprotein precursor that is processed into Gn and Gc envelope glycoproteins, and the L segment encodes a viral RNA-dependent RNA polymerase. After the first description of hantavirus pulmonary syndrome (HPS) in the United States in 1993, dozens of hantavirus genotypes have been reported in North and South America. These genotypes have been associated with wild rodent species of the subfamily Sigmodontinae and are transmitted to humans mostly by contact with or through aerosols of excreta and secretions of infected rodents (2). Nevertheless, human-to-human transmission of hantavirus has been reported in Argentina and Chile (3).

In South America, hantaviruses have been reported in Argentina (Andes), Chile (Andes), Uruguay (Andes), Paraguay (Laguna Negra), Bolivia (Laguna Negra and Rio Mamoré), Venezuela (Caño Delgadito), and Brazil (Araraquara and Juquitiba) (4). Brazil is a large country (area 8,514,215 km²), having a predominantly tropical climate and high biodiversity that includes many important zoonoses. This biodiversity in natural ecosystems supports ≈450 of the 540 known species of Sigmodontinae rodents (5). Furthermore, a human population of ≈190 million is unevenly distributed, mostly in densely populated urban areas near the Atlantic coast of southeastern and northeastern Brazil (Brazilian Institute of Geography and Statistics, 2006, unpub. data).

HPS is an emerging health problem in Brazil because of the overlap of urban, agriculture, and cattle-raising areas with ecosystems containing several species of Sigmodonti-
nae rodents that are reservoirs of hantaviruses. From 1993 through June 2007, a total of 877 HPS cases were reported in Brazil (case-fatality rate 39%): 387 in southern Brazil, 264 in southeastern Brazil, 177 in midwestern Brazil, and 49 in northern and northeastern Brazil.

Five lineages of hantavirus have been associated with most documented HPS cases: Juquitiba virus (JUQV), Araquara virus (ARAV), Laguna Negra–like virus, Castelo dos Sonhos virus, and Anajatuba virus. HPS cases, especially those reported in northern and northeastern Brazil, were likely caused by other unknown hantaviruses (6–9; Brazilian Ministry of Health, 2007, unpub. data). Because of a lack of information about genetic diversity among hantaviruses in Brazil, we analyzed nucleotide sequences of hantaviruses infecting persons who contracted HPS and in hantaviruses in Brazil, we analyzed nucleotide sequences of hantaviruses infecting persons who contracted HPS and in Brazil (case-fatality rate 39%): 387 in southern Brazil, 264 in southeastern Brazil, 177 in midwestern Brazil, and 49 in northern and northeastern Brazil.

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Materials and Methods

Study Area

The 2,500-km-wide study area in Brazil included the Central Plateau in the midwestern region, the southeastern and southern regions, and the Central Plateau in Goiás State and the Federal District. The Central Plateau was originally a cerrado characterized by small trees and grasses adapted to climates with long dry periods. However, during the past 3 decades the Central Plateau has been modified by farming, creation of pastures, and extensive urbanization. The southeastern region is the most densely populated region of Brazil and includes the states of São Paulo and Minas Gerais, which have cerrado in western areas and neotropical Atlantic rain forest along the coast. The rain forest is an umbrella tropical forest on hillsides and has high precipitation caused by an orographic effect. The southern region has Araucaria forests at higher altitudes and neotropical Atlantic rain forest along the coast. These ecosystems sustain Sigmodontinae rodents and have been modified, segmented, and damaged by extensive sugar cane, soybean, and coffee farming; cattle raising; and rapid and poorly planned urbanization.

Collaborative Structure

Collection and serologic analysis of hantavirus samples from humans and rodents were performed at the Adolfo Lutz Institute in São Paulo and at the Virus Research Unit of the School of Medicine, University of São Paulo in Ribeirão Preto. Initial detection of hantavirus genomic RNA was conducted at the Virus Research Unit of the University of São Paulo. Samples positive for genomic RNA were sent to the Microbiology Department of the Institute of Biomedical Sciences at the University of São Paulo for further PCR amplification, DNA sequencing, and sequence analyses.

Human and Rodent Samples

Human serum samples obtained from HPS patients during 1998–2005 were analyzed by immunoglobulin (Ig) M capture ELISA by using antigens for Andes virus and ARAV. Rodents were captured alive by using traps in rural or sylvan environments at presumed sites of HPS cases during 2002–2005 as part of routine surveillance procedures conducted by the Adolfo Lutz Institute under the mandate of the Brazilian Ministry of Health. Sampling was reviewed and approved by appropriate ethical committees on human and animal research according to Brazilian Ministry of Health Resolutions 3747/95 and 196/96. Informed consent was obtained from all patients, and information was kept confidential by the Ministry of Health. Rodent collection in the field was authorized by the Brazilian Institute of Environment and Renewable Natural Resources. Samples containing hantavirus are under control of the Ministry of Health at classified sites according to United Nations Security Council Resolution 1540 and Brazilian Ministry of Science and Technology Resolution 10. Rodent blood samples were analyzed by IgG ELISA by using antigens for Sin Nombre virus and ARAV. Rodent lungs and human serum samples positive for hantavirus were used for RNA extraction.

Extraction of RNA

RNA was extracted from 300 μL of hemolyzed whole blood or serum samples from humans and from 300 μL of a suspension of macerated lung tissues from rodents. Samples were mixed with 1 mL of TRIzol LS Reagent (GIBCO/BRL, Gaithersburg, MD, USA) and 200 μL of chloroform–isoamyl alcohol (24:1), according to modifications described by Bowen et al. (10). Pellets were precipitated by centrifugation and resuspended in 10–20 μL of diethyl pyrocarbonate–treated water.

Reverse Transcription–PCR Primers, RNA Samples, and Reactions

Highly conserved regions of N and Gn genes of ARAV (GenBank accession nos. AF307325 and AF307327) were used to identify primers after aligning their nucleotide sequences to those of American hantaviruses. Two primer pairs, SAHN (amplifying 264 bp) and HANGn (amplifying 324 bp), were used (Table 1). The 2 sets of primers were used for diagnosis of hantavirus infection and DNA sequencing. Serum samples were considered positive for hantavirus if either N or Gn reverse transcription–PCR (RT-PCR) gave the expected amplicon size. Regions of hantavirus N and Gn genes were detected by RT-PCR in
RNA extracts of serum or hemolyzed whole blood from 19 HPS patients and RNA extracts of lung tissues from 15 rodents obtained during 2002–2005. Information on these samples is shown in Table 2.

Reverse Transcription

RT reactions were prepared in a final volume of 22 μL by mixing 5 μL of extracted RNA, 0.113 mmol/L deoxyribonucleoside triphosphates (dNTPs), 0.68 μmol/L of either SAHN-C or HANGn-C primer, and 4.4 μL of 5× RT buffer (250 mmol/L Tris-HCl, pH 8.3, 15 mmol/L MgCl₂, 50 mmol/L dithiothreitol). Reaction mixtures were heated at 95°C for 3 min to linearize RNA and cooled to 4°C. A total of 10 U RNase inhibitor (Pharmacia, Piscataway, NJ, USA) and 10 U of Moloney murine leukemia virus reverse transcriptase (Pharmacia) were added to each sample, and

Table 1. Primers used for reverse transcription–PCR of hantaviruses, Brazil, 1998–2007

| Gene/primer | Sequence (5′→3′) | Nucleotide annealing site |
|-------------|-----------------|--------------------------|
| N/SAHN-C    | CAAAACGATTGATCACAACGGG | 213–236 of hantavirus small RNA segment |
| N/SAHN-S    | GTGAGACATCCTAGGAAAGTAT | 454–477 of hantavirus small RNA segment |
| G1/HANGn-C  | GGGGAGTAATGGCCTGAAAC | 1301–1320 of hantavirus medium RNA segment |
| G1/HANGn-S  | ACATTACGAGTTGCCATGCGG | 1602–1625 of hantavirus medium RNA segment |

N, nucleocapsid; G1, glycoprotein 1.

Table 2. Geographic origin of human and rodent sources of hantviruses, Brazil, 1999–2005

| Composite taxon | City       | State        | Region        | Amplicon† |
|-----------------|------------|--------------|---------------|-----------|
| PR_DF_Hsp_19    | Paranoá    | Distrito Federal | Central Plateau | N         |
| SS_DF_Nlas_13   | São Sebastião | Distrito Federal | Central Plateau | Gn        |
| SS_DF_Nlas_10   | São Sebastião | Distrito Federal | Central Plateau | Gn, N     |
| SS_DF_Nlas_11   | São Sebastião | Distrito Federal | Central Plateau | Gn, N     |
| SS_DF_Nlas_12   | São Sebastião | Distrito Federal | Central Plateau | Gn, N     |
| CO_GO_Hsp_20    | Goiás      | Distrito Federal | Central Plateau | Gn         |
| AR_SP_Hsp_21    | Araxá      | Minas Gerais | Central Plateau | Gn, N     |
| SG_MG_Nlas_8    | São Gotardo | Minas Gerais | Central Plateau | Gn, N     |
| SG_MG_Nlas_9    | São Gotardo | Minas Gerais | Central Plateau | Gn, N     |
| AG_SP_Ost_1     | Aguai      | São Paulo    | Southeast     | Gn         |
| BA_SP_Hsp_2     | Bataia     | São Paulo    | Southeast     | Gn, N     |
| BA_SP_Hsp_1     | Bataia     | São Paulo    | Southeast     | Gn, N     |
| CJ_SP_Hsp_3     | Cajurú     | São Paulo    | Southeast     | Gn, N     |
| CJ_SP_Hsp_4     | Cajurú     | São Paulo    | Southeast     | N          |
| CC_SP_Hsp_5     | Cassia dos Coqueiros | São Paulo     | Southeast | Gn, N     |
| CC_SP_Hsp_6     | Cassia dos Coqueiros | São Paulo     | Southeast | Gn, N     |
| CC_SP_Nlas_2    | Cassia dos Coqueiros | São Paulo | Southeast | Gn, N     |
| CC_SP_Nlas_3    | Cassia dos Coqueiros | São Paulo | Southeast | Gn, N     |
| CC_SP_Nlas_4    | Cassia dos Coqueiros | São Paulo | Southeast | Gn, N     |
| CC_SP_Nlas_1    | Cassia dos Coqueiros | São Paulo | Southeast | Gn, N     |
| CC_SP_Nlas_5    | Cassia dos Coqueiros | São Paulo | Southeast | Gn, N     |
| CV_SP_Hsp_7     | Cravinhos  | São Paulo    | Southeast     | Gn, N     |
| GU_SP_Hsp_9     | Guariba     | São Paulo    | Southeast     | N          |
| GU_SP_Hsp_8     | Guaraí-SP   | São Paulo    | Southeast     | Gn, N     |
| IB_SP_Nlas_6    | Ibaté       | São Paulo    | Southeast     | Gn         |
| IB_SP_Nlas_7    | Ibaté       | São Paulo    | Southeast     | Gn, N     |
| JD_SP_Hsp_10    | Jardinópolis | São Paulo | Southeast | Gn, N     |
| JU_SP_Hsp_11    | Juá         | São Paulo    | Southeast     | Gn, N     |
| PO_SP_Hsp_12    | Pontal      | São Paulo    | Southeast     | Gn         |
| RB_SP_Hsp_13    | Ribeirão Bonito | São Paulo     | Southeast | Gn, N     |
| RP_SP_Ako_1     | Ribeirão Preto | São Paulo     | Southeast | Gn, N     |
| SC_SP_Hsp_14    | São Carlos   | São Paulo    | Southeast     | Gn, N     |
| SE_SP_Hsp_15    | Sertãozinho | São Paulo    | Southeast     | Gn, N     |
| ST_SP_Hsp_17    | Sertãozinho | São Paulo    | Southeast     | Gn, N     |
| ST_SP_Hsp_16    | Sertãozinho | São Paulo    | Southeast     | Gn, N     |
| TB_SP_Hsp_18    | Taubaté     | São Paulo    | Southeast     | Gn, N     |
| SE_SC_Oti_01    | Seara       | Santa Catarina | South | Gn         |
| CX_RS_Hsp_22    | AY740623    | Rio Grande do Sul | South | Gn, N     |

The first 2 letters indicate the city, the next 2 the state, and the next 3 the animal source (Hsp, human; Nlas, Necromys lasiurus; Ost, oligoryzomys stramineus; Ako, Akodon sp.; Oti, O. nigrivisus). Numbers indicate sample number.

†N, nucleocapsid; Gn, glycoprotein.
samples were incubated for 2 h at 37°C. The cDNA was used as a template in the subsequent PCR or stored at –20°C.

**PCR**

PCRs were conducted in 50-μL volumes containing 0.3 μM of SAHN or HANGn primer pairs, 0.05 mmol/L dNTPs, 3 μL RT products, and 5 μL 10× buffer (100 mmol/L Tris-HCl, pH 8.5, 500 mmol/L KCl). Mixtures were heated to 80°C, 1U of thermostable Taq DNA polymerase (Pharmacia) was added, and mixtures were subjected to 35 cycles at 95°C for 30 s, 55°C for 45 s, and 72°C for 240 s. Samples were then subjected to an elongation step at 70°C for 5 min to promote DNA strand extension. Eight microliters of amplified DNA was subjected to electrophoresis on 1.7% agarose gels in Tris-acetate-EDTA buffer and stained with ethidium bromide. Amplicons were visualized by using a 312-nm UV transilluminator, and their sizes were determined by comparison with a 100-bp DNA ladder.

**Nucleotide Sequencing**

Direct nucleotide sequencing of N (261 bp) and Gn (324 bp) PCR products was performed by using the Big Dye Termination Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) with 25 cycles at 95°C, 55°C for 45 s, and 72°C for 180 s. Products were analyzed in ABI PRISM 3100 and ABI 377 sequencers (Applied Biosystems). Sequences obtained were deposited in GenBank (accession nos. EU170162–EU170239).

**Phylogenetic Analysis**

Nucleotide sequences of N and Gn genes were aligned with those of orthologs from other South American hantaviruses (Table 3) by using ClustalX version 8 software (11). Alignments were edited by using SeAl version 2.0a11 software (http://tree.bio.ed.ac.uk/software/seal). Preliminary analysis of N and Gn amplicons and references (Table 2) (concatenated in a 573-member dataset that included 58 taxa for which N and Gn are available) or individual genes by using the RDP3 program (12) indicated no intergene recombination or reassortment that would be detected by recombination at the border between N and Gn genes in the 573-bp concatamer. This finding confirmed our previous results (13).

To improve the phylogenetic signal, we used the 573-bp contamer and inferred the maximum clade credibility (MCC) tree for 58 South American hantaviruses by using BEAST version 1.4.8 software (14) and the Bayesian Skyline model under exact conditions as described (13). The MCC tree was sampled from 20 million trees after Markov chain Monte Carlo algorithm sampling was stable after a preburning stage of 30 million chains. Seven of 38 taxa had neither N nor Gn sequences (Table 2), but this absence did not preclude use of tree-based Bayesian methods and maximum likelihood methods. These methods account for missing nucleotides as undefined character-states during phylogenetic reconstruction and do not use global pairwise distances. Statistical support for clustering of newly iso-

### Table 3. Reference hantavirus gene sequences

| Virus         | Country of origin | Glycoprotein sequences | Nucleocapsid sequences |
|---------------|-------------------|------------------------|------------------------|
| Laguna Negra  | Paraguay          | AF005728               | AF005727               |
| Lechiguana    | Argentina         | AF028022               | AF482714               |
| Oran          | Argentina         | AF028024               | AF482715               |
| Pergamino     | Argentina         | A028028                | AF482717               |
| Araraquara Lutz | Brazil        | AY970821               | None                   |
| Araraquara Johnson | Brazil    | AF307327               | AF307325               |
| Andes Chile   | Chile             | AY228238               | AY228237               |
| Andes AH-1    | Argentina         | AF324901               | AF324902               |
| Choclo Panamá | Panamá            | DQ285047               | DQ285046               |
| Cano Delgadito | Paraguay         | DQ284451               | DQ285566               |
| Juquitiba     | Brazil            | AY963900               | EF446280               |
| Rio Mamoré    | Bolivia           | A7953445               | U13455                 |
| Araucária HR0271 | Brazil        | None                   | A740624                |
| Araucária HR0150 | Brazil        | None                   | A740622                |
| Araucária HR4101 | Brazil        | None                   | A740632                |
| Araucária HR0273 | Brazil        | None                   | A740626                |
| Araucária HR0272 | Brazil        | None                   | A740625                |
| Araucária HR4102 | Brazil        | None                   | A740633                |
| Araucária HR0399 | Brazil        | None                   | A740630                |
| Araucária HR0395 | Brazil        | None                   | A740628                |
| Araucária HR0397 | Brazil        | None                   | A740629                |
| Araucária HR3100 | Brazil        | None                   | A740631                |
| Araucária HR0285 | Brazil        | None                   | A740627                |
| Araucária HR0155 | Brazil        | None                   | A740623                |
lated viruses was evaluated by running 500 nonparametric bootstraps with GARLI version 0.95 (15) and PhyML version 2.4.5 software (16). We also conducted a 5% jackknife resampling analysis of 1,000 neighbor-joining trees by using maximum likelihood distance with PAUP version 4b10 software (17). The best evolutionary model was estimated with GARLI software (i.e., general time reversible + Γ + I model).

Case-Fatality Rate of HPS in Brazil

The Central Plateau and southeastern region of Brazil are covered mostly by cerrado, where Necromys lasiurus, the reservoir of ARAV, is enzootic. The HPS case-fatality rate for this area was compared with the HPS case-fatality rate for the southern region, which is characterized by Atlantic rain forest and Araucaria subtropical forest environments. In these environments, Oligoryzomys nigripes, a reservoir of JUQV, is enzootic. HPS case-fatality rates for the Central Plateau and southeastern region were compared with HPS case-fatality rates for hantaviruses in other regions of Brazil. These comparisons were made by using the Fisher exact test at a 5% significance level (Brazilian Ministry of Health, 2007, unpub. data).

Results

Composite Phylogenetic Tree of N and Gn Genes

We studied 89 human samples and 68 rodent samples that had positive serologic results; N and Gn genes from 22 human and 16 rodent sources were obtained. The N/Gn composite tree (online Appendix Figure, available from www.cdc.gov/EID/content/15/4/561-appF.htm) indicates that human and rodent samples were associated with ARAV or JUQV reference strains with posterior probabilities >0.9. As expected, ARAV samples from rodents and human cases in São Paulo State, the Central Plateau, and Minas Gerais State (on the border of the plateau) were mixed in the tree because human cases are almost always derived from rodents (online Appendix Figure). Nevertheless, highly supported clusters of ARAV suggest that viruses may show some geographic partitioning (e.g., clusters of isolates from N. lasiurus in São Sebastião in the Central Plateau differ from clusters from São Paulo in the southeastern region). Furthermore, 13 of 14 ARAV samples obtained from rodents were from N. lasiurus and 1 was from Akodon spp. All rodent and human ARAV samples were from the Central Plateau (cerrado) or southeastern region (tropical rain forests). Distribution of ARAV extends 1,000 km across the dry northern cerrado region and includes western São Paulo State to the boundary of the Atlantic rain forest in the southeastern region.

ARAV are more related to Pergamino virus than to JUQV (online Appendix Figure). Pergamono virus infects A. azarae, which inhabit Argentina, and JUQV infects Oligoryzomys spp., which inhabit southern Araucaria and eastern Atlantic forests. Two rodent samples nested with JUQV (high posterior probability 0.93) 100% of the time during jackknife resampling and 86% of the time during 500 nonparametric bootstrap iterations (online Appendix Figure). These samples were identified as AG_SP_Ost_1 isolated from O. stramineus in Aguai, São Paulo, and SE_SC_Oni_1 isolated from O. nigripis in Seara, Santa Catari-na State located in southern Brazil near the northern border with Argentina (Table 2). These data indicate that these 2 samples were divergent lineages of JUQV infecting Oligoryzomys spp. Detection of AG_SP_Ost_1 in Aguai, São Paulo, was expected because Itapuá JUQV is also found in the adjacent Atlantic rain forest. Two additional samples, SG_MG_Nlas_8 from N. lasiurus in São Gotardo, Minas Gerais State (Central Plateau), and CX_RS_Hsp_22 from a human case in Caxias do Sul, Rio Grande do Sul (southern region), appeared to have some distant association with ARAV, JUQV, and Oran hantavirus, which are associated with O. longicaudatus from subtropical Oran Department in Argentina. This association was indicated by the low support of nodes connecting these 3 virus lineages to SG_MG_Nlas_8 and CX_RS_Hsp_22. Although more data are needed to better understand these associations, the most parsimonious explanation is that a hantavirus associated with Necromys spp./Akodon spp. that originated in the southern region may have been the source of the lineage that led to Oligoryzomys spp.-associated JUQV, which has lineages across the Araucária pine forests and along the rain forests into southeastern Brazil.

Virulence of Hantaviruses Determined by Case-Fatality Rate

The Central Plateau and southeastern and southern regions contain >80% of the HPS cases reported in Brazil in 2007. However, the case-fatality rate of HPS was distinct in these and other regions. These rates were 44.5% (149 deaths in 335 reported cases) in areas with ARAV in the Central Plateau and the southeastern region and 35.4% (192 deaths in 542 cases) in areas with ARAV in the remaining southern regions. This difference was statistically significant (p = 0.011, by Fisher exact test, and χ² 3.0978, df 1, p = 0.0051, by Fisher exact test, χ² 4.8293, df 1, p = 0.045) (Brazilian Ministry of Health, 2007, unpub. data). Although some geographic overlap of ARAV and JUQV occurs, these results suggest that ARAV strains may have higher virulence than JUQV or other hantaviruses in Brazil.
Discussion

The MCC composite tree (online Appendix Figure) shows that all 38 samples from HPS patients and from rodents captured near human cases were related to ARAV or JUQV groups. Moreover, ARAV lineages obtained in the Central Plateau or southeastern region grouped in a robust monophyletic group independent of human or rodent origin. This finding reinforces the probability of zoonotic origin of human cases from a rodent-borne zoonotic pool and supports the view that closely related hantavirus lineages associated with distinct rodent species may be experiencing cross-species transmission (spillover) (13, 18).

ARAV appears to have dispersed from the cerrado origin. This bust monophyletic group independent of human or rodent species was found in the Central Plateau or southeastern region grouped in a rodent-borne zoonotic pool. Moreover, ARAV lineages obtained in rodents captured near human cases were related to ARAV (13, 18). Detection of ARAV lineages in the Central Plateau shows that all 38 samples from HPS patients and from rodents associated with distinct rodent species may be experiencing cross-species transmission (spillover) (13, 18).

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Rodent behavior is a factor in transmission of hantaviruses to humans, and *N. lasiurus* is an opportunistic and aggressive rodent species that is gradually being encroached upon in environments experiencing anthropogenic change in the southeastern region and the Central Plateau of Brazil. Conversely, *O. nigripes* has adapted to the Atlantic and Araucaria pine forests and has been found in lineal natural habitats bordering cultivated areas (5, 7, 19). Detection of ARAV-Pergamino and JUQV with high support but may be the most divergent lineages so far detected. Levels of support in the MCC tree indicated that SG_MG_Nlas_8 and CX_RS_Hsp_22 would always branch from poorly defined nodes in the tree in a basal position relative to ARAV-Pergamino, JUQV, and Oran lineages. These results suggest that SG_MG_Nlas_8 and CX_RS_Hsp_22 may represent distinct lineages linked to Argentinean Oran and Pergamino hantavirus lineages from which ARAV and JUQV originated.

Rooting of the tree and lineage associations suggest that ARAV and JUQV may have originated from a *Necromys* spp./Akodon spp.–associated hantavirus from the southern part of South America. This theory supports our finding of 1 *N. lasiurus* infected by ARAV (SG_MG_Nlas_9) and another *N. lasiurus* infected by a highly divergent lineage (SG_MG_Nlas_8) that outgroups with ARAV and JUQV. Both rodents were collected in São Gotardo in Minas Gerais State, at the edge of the central plains. The role of distinct rodent species as potential reservoirs and sources of human infection in Brazil and South America needs to be better understood. However, our data corroborate reports showing that *N. lasiurus* is a reservoir of ARAV, that *O. nigripes* is a reservoir of JUQV, and that rodents transmit hantaviruses to humans (6–8).
exposed to the hantavirus but did not have severe clinical symptoms. Further study is needed to determine which factors influence the severity of disease manifestation in humans caused by infections with hantviruses, specifically with ARAV or JUQV (6–8).

We have identified the viruses circulating in our study area as ARAV and JUQV. Based on geographic distribution of these viruses and the assumption that no other unknown lineage is causing disease in humans, we suggest that ARAV may be responsible for >50% of HPS cases reported in Brazil. ARAV was associated with areas experiencing greater anthropogenic changes and disorganized human population growth than other more stable areas. ARAV may be the most virulent hantavirus in Brazil.

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