Molecular Epidemiology of Laguna Negra Virus, Mato Grosso State, Brazil

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We associated Laguna Negra virus with hantavirus pulmonary syndrome in Mato Grosso State, Brazil, and a previously unidentified potential host, the *Calomys callidus* rodent. Genetic testing revealed homologous sequencing in specimens from 20 humans and 8 mice. Further epidemiologic studies may lead to control of HPS in Mato Grosso State.

Hantavirus pulmonary syndrome (HPS) is a manifestation of an emerging zoonosis caused by New World viruses of the family *Bunyaviridae*, genus *Hantavirus*. Hantavirus is transmitted to humans by inhalation of aerosols of excreta from infected rodents of the subfamily *Sigmodontinae* (Rodentia, Cricetidae) (1,2). HPS was initially reported during an epidemic of severe respiratory disease that occurred in the southwestern United States in 1993 (1). HPS was subsequently identified in Brazil and other Latin American countries, which facilitated the recognition of new hantavirus species such as Laguna Negra virus (LNV), Andes virus, Choclo virus, Juquitiba virus, Araraquara virus, Castelo dos Sonhos virus, Anajatuba virus, as well as several other viruses detected in wild rodents which are not associated with HPS (3–9). Like particles of other bunyaviruses, hantavirus particles are spherical or pleomorphic and measure 80–120 nm in diameter; their genome comprises 3 RNA segments, and the small RNA fragment is used to characterize the nucleoprotein (N) gene and the hantavirus species (2).

During 1993–2009, a total of 1,246 cases of HPS were reported in Brazil; the state of Mato Grosso reported the fourth highest case count, diagnosed mainly in the municipalities of Tangarará da Serra and Campo Novo do Parecis. However, the circulating hantavirus species and its host remained unknown, and identification of these factors were the main objectives of this study.

**The Study**

Mato Grosso comprises 903,357.9 km² and has an estimated population of 2,803,274 inhabitants living in 141 municipalities. Nineteen municipalities have reported cases of HPS, mainly near Brazil’s BR-364 highway, located between the north and southwestern sections of the state. The climate is equatorial subhumid, with an annual rainfall of 1,700 mm, and temperature range 24°–40°C; the landscape consists of savannah (*Cerrado*) and pre-Amazon rainforest. The economic activities are agricultural production and ecologic tourism.

HPS was diagnosed in 24 persons who were IgM positive for LNV during 2001–2006 in the municipalities of Barra do Bugres (n = 1), Campo Novo do Parecis (n = 13), Diamantino (n = 3), Nova Olimpia (n = 1), Santo Afonso (n = 1), São José do Rio Claro (n = 1), and Tangarará da Serra (n = 4) (Figure 1). Detailed information of patient

![Figure 1. State of Mato Grosso, Brazil, indicating municipalities where hanta pulmonary syndrome cases occurred.](http://dx.doi.org/10.3201/eid1806.110948)
samples submitted for nucleotide sequencing is provided in the Table.

During a 2001 ecological–epidemiologic study conducted in the municipalities of Tangará da Serra and Campo Novo do Parecis, researchers obtained blood and viscera samples from wild rodents (10). The researchers followed Brazilian Institute for the Environment and Renewable Natural Resources guidelines for the capture and handling of rodents and using biosafety level 3 protocols. The samples were tested for hantavirus; animals with positive test results were identified taxonomically by morphometry and molecular analysis of mitochondrial DNA (cytochrome b gene) (10,11).

For hantavirus detection, we conducted reverse transcription PCR to synthesize complementary DNA with generic hantavirus primers as described (1,2). We obtained N gene partial nucleotide sequences by using the Sanger method with the same primers (3,4,6,9). At least 3 amplicons per sample were sequenced in both directions to improve coverage and confidence for results. The obtained sequences were aligned with other hantavirus sequences available at the GenBank database (www.ncbi.nlm.nih.gov) with ClustalW software in BioEdit version 7.1.3 (www.mbio.ncsu.edu/BioEdit/bioedit.html). We implemented the maximum-likelihood and Bayesian methods by using PHYML (www.atgc-montpellier.fr/phyml/versions.php) and Mr. Bayes version 3.2 (http://mrbayes.scs.fsu.edu) software, respectively, for phylogenetic reconstructions. We used Modeltest version 3.7 (http://gel.ahabs.wisc.edu/mauve) to determine the best nucleotide substitution model. We analyzed 2 million replicates, with the sample fixed at every 1,000 trees generated, and used TRACER (www.evolve.zoo.ox.ac.uk) to determine whether the Bayesian analysis reached appropriate convergence (3,6,9,12).

We obtained amplicons from 20 of the 24 samples from persons with HPS and partial sequence of the N gene (~434 bp) from 16 of the 24 samples from patients who were symptomatic at the time of sampling. During the ecologic study, 126 rodents were captured: 68 (53.9%) commensal synanthropic species, 49 (38.8%) wild rodents [Calomys callidus (n = 46), Proechimys sp. (n = 1), and Necromys lasiurus (n = 2)], and 9 (7.1%) unidentified species. IgG was detected in 8 (17.4%) C. callidus rodents (2 captured in Campo Novo do Parecis, 6 in Tangará da Serra). Amplicons were produced in lung/heart samples from 7 of the 8 IgG-positive rodents; 3 of those were selected for nucleotide sequencing of the N gene (Table).

All strains recovered from human and C. callidus rodent specimens were related and formed a monophyletic cluster with the LNV (GenBank accession no. AF005727), with a mean genetic divergence of 4.8%. These strains were included in subclade II, which comprises Anajatuba, Laguna Negra Virus, Brazil

### Table. Characteristics of human patients and Calomys callidus rodents with positive serologic results for hantavirus and partial nucleotide sequence of gene N, Mato Grosso, Brazil*

| ID no. | Age, y/sex | Sample | Date of sample collection | Outcome | GenBank accession no. | Municipality |
|-------|------------|--------|---------------------------|---------|-----------------------|-------------|
| Humans |            |        |                           |         |                       |             |
| H 678213 | 38/M      | Serum  | 2005 Mar 10               | Cure    | JQ775513              | Barra do Bugres (15°42’1"S; 57°10’52"W) |
| H 650736 | 33/M      | Serum  | 2001 Dec 19               | Death   | JQ775504              | Campo Novo do Parecis (13°40’31"S; 57°53’31"W) |
| H 660462 | 23/F      | Serum  | 2002 Jul 8                | Cure    | JQ775506              | Campo Novo do Parecis |
| H 671696 | 20/F      | Serum  | 2003 Aug 23               | Death   | JQ775505              | Campo Novo do Parecis |
| H 682807 | 20/M      | Serum  | 2004 Aug 22               | Death   | JQ775507              | Campo Novo do Parecis |
| H 695689 | 27/F      | Serum  | 2005 Aug 22               | Cure    | JQ775508              | Campo Novo do Parecis |
| H 696558 | 22/M      | Blood  | 2005                      | Cure    | JQ775512              | Campo Novo do Parecis |
| H 711891 | 42/M      | Serum  | 2006 Aug 17               | Death   | JQ775503              | Campo Novo do Parecis |
| H 678484 | 42/M      | Serum  | 2002 May 14               | Cure    | JQ775517              | Diamantino (14°24’31"S; 56°26’46"W) |
| H 706738 | 13/F      | Serum  | 2006 May 22               | Cure    | JQ775516              | Campo Novo do Parecis |
| H 712518 | 33/M      | Serum  | 2006 Aug 29               | Cure    | JQ775518              | Campo Novo do Parecis |
| H 653486 | 13/F      | Serum  | 2002 Feb 22               | Cure    | JQ775514              | Campo Novo do Parecis |
| H 695325 | 24/M      | Serum  | 2005 Nov 11               | Cure    | JQ775515              | Campo Novo do Parecis |
| H 713175 | 17/M      | Serum  | 2006 Sep 26               | Cure    | JQ775509              | Campo Novo do Parecis |
| H 651686 | 31/M      | Serum  | 2002 Jan 22               | Cure    | JQ775511              | Campo Novo do Parecis |
| H 710031 | 7/F       | Serum  | 2006 Jul 18               | Death   | JQ775510              | Campo Novo do Parecis |
| Rodents |            |        |                           |         |                       |             |
| AN 650204 | NA    | Lung   | NA                        | NA      | JQ775500              | Campo Novo do Parecis |
| AN 650228 | NA    | Lung   | NA                        | NA      | JQ775502              | Campo Novo do Parecis |
| AN 649993 | NA    | Heart  | NA                        | NA      | JQ775501              | Campo Novo do Parecis |

*Source: Secretaria de Vigilância Epidemiológica do Estado do Mato Grosso and Instituto Evandro Chagas, Secretaria de Vigilância em Saúde), and Ministério da Saúde. NA, data not available.

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Rio Mamore, Rio Mearim, and Alto Paraguay viruses (Figure 2). The genetic distance between strains recovered from rodents and humans was 5.5%, whereas the genetic distance between the human strains was 6.8%. Analysis of homology showed no difference between the partial amino acid sequences of human and rodent strains and LNV (100% homology). The homology of nucleotide sequences between the LNV strains was 89.9%–93.4% (online Appendix Table, wwwnc.cdc.gov/EID/article/18/6/11-0948-TA1.htm). Most changes were silent mutations in the nucleotide sequences, indicated by the genetic divergence between LNV strains (Δdiv = 0.2%–9.8%).

LNV was initially confirmed in 1997 by serologic testing of a patient with HPS who died. The patient lived in Santiago, Chile, but was probably infected in Santa Cruz, Bolivia (13). In 1999, molecular analysis of the small N gene and medium Gn and Gc gene segments of the hantavirus genome in samples from HPS patients from Bolivia, western Paraguay, and Chile facilitated the genetic characterization of LNV and its association with the small vespertine mouse *Calomys laucha*, which is considered the primary host of LNV. Subsequent studies in Argentina have also demonstrated the circulation of LNV in patients with HPS and in the large vespertine mouse *Calomys callosus* (4,9,13–15).

**Conclusions**

Our phylogenetic analysis of partial sequences of the N gene showed LNV as the cause of HPS, and the possible association of the organism with *C. callidus* rodents in western Brazil. These findings highlight the intense circulation of LNV in Matto Grosso municipalities located near the BR-364 highway. The vegetation and the equatorial climate of the area provide an excellent microenvironment for the maintenance of *C. callidus* rodents, as do areas in Bolivia, Paraguay, and northern Argentina, where HPS caused by LNV has been reported (4,9,13–15).

The high nucleotide and amino acid homology between strains recovered from humans and the *C. callidus* rodent in Matto Grosso and the LNV prototype detected in Paraguay and Argentina suggest that LNV was transmitted by the rodent host *C. callidus* and led to the HPS cases that occurred in the vicinity of the highway BR-364 in southwestern Matto Grosso. No correlation was observed between the human LNV strains and year, geographic distribution, or between the severity of disease and the genetic diversity of LNV found in Brazil. The genetic data obtained in this study provide a better understanding of the molecular characterization of LNV and its association with HPS in southwestern Matto Grosso. Finally, on the basis of the phylogenetic analysis, the rodent species *C. callidus* is suggested as a potential reservoir for LNV. Further analyses of complete genome data are needed to confirm this result and to assess whether the *C. callidus* rodent is the sole carrier of LNV in Matto Grosso.

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