Journal of General Virology (2009), **90**, 2166–2171 DOI 10.1099/vir.0.011585-0

Short Communication

Phylogenetic characterization of hantaviruses from wild rodents and hantavirus pulmonary syndrome cases in the state of Paraná (southern Brazil)

Sonia Mara Raboni,[1,2] Federico G. Hoffmann,[1] Renata C. Oliveira,[3] Bernardo R. Teixeira,[3] Cibele R. Bonvicino,[4] Vanessa Stella,[1] Suzana Carstensen,[1] Juliano Bordignon,[1] Paulo S. D'Andrea,[3] Elba R. S. Lemos[3] and Claudia Nunes Duarte dos Santos[1]

Correspondence
Claudia Nunes Duarte dos Santos
clsantos@tecpar.br

[1]Instituto Carlos Chagas, ICC/Fiocruz/PR, Brazil

[2]Universidade Federal do Paraná, Brazil

[3]Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, Brazil

[4]Instituto Nacional de Câncer, Rio de Janeiro, Brazil

Received 6 March 2009
Accepted 10 May 2009

Over 1100 cases of hantavirus pulmonary syndrome (HPS) have occurred in Brazil since 1993, but little is known about Brazilian hantaviruses, and many of their rodent hosts remain unknown. The Araucaria hantavirus (ARAUV) was described recently from HPS patients from Paraná, in southern Brazil, but its host could not be identified. In this study, rodents were captured from regions with high HPS prevalence to address this issue. ARAUV RNA was detected in three distantly related rodent species: *Oligoryzomys nigripes*, *Oxymycterus judex* and *Akodon montensis*. Furthermore, a specimen of *A. montensis* was infected with a Jaborá-like virus, implying that *A. montensis* can be infected by at least two different hantaviruses. The presence of the same hantavirus strain in three different rodent species and the co-circulation of two different strains in the same rodent species highlight the potential for genomic reassortment, which could have an impact on hantavirus transmission dynamics in nature and on human epidemiology.

Hantaviruses belong to the genus *Hantavirus* in the family *Bunyaviridae* and are found throughout most of the world. Similar to other members of the family, hantaviruses are enveloped viruses with an RNA genome comprised of three negative-sense, single-stranded segments. The large (L) RNA segment encodes an RNA-dependent RNA polymerase, the medium (M) segment encodes two envelope glycoproteins – Gn and Gc – processed from one precursor, and the small (S) segment encodes the nucleocapsid protein (N) (Plyusnin *et al.*, 1996).

Hantaviruses use small mammals as vectors, and in the wild, most of the different hantaviruses have been found to associate predominantly with a specific rodent species that acts as the host in a given geographical region (Plyusnin & Morzunov, 2001). Although sporadic spillover between rodents species has been suggested (Childs *et al.*, 1994; Delfraro *et al.*, 2008; Sousa *et al.*,. 2008), conclusive evidence that the studied viruses can establish productive infections in more than one rodent host species is still missing.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are FJ409556–FJ409561, FJ798975 and FJ798976.

Hantaviruses are transmitted to humans mainly through the inhalation of contaminated aerosols of rodent excreta (Lednicky, 2003; Schmaljohn & Patterson, 2001), but human-to-human transmission has also been described (Padula *et al.*, 1998). These viruses are associated with two clinical syndromes, haemorrhagic fever with renal syndrome, described in Europe and Asia with a mortality rate of 1–15 %, and hantavirus pulmonary syndrome (HPS), described in the Americas with a mortality rate ranging from 40 to 60 % (Lundkvist & Niklasson, 1994; Johnson *et al.*, 1999).

Despite the increasing incidence of HPS in Brazil (Elkhoury, 2005), little is known about the genetic diversity of its causative agents, Brazilian hantaviruses (Table 1), and identification of their rodent hosts remains incomplete. The south-western region of the state of Paraná (southern Brazil, 26° 07′ S 51° 31′ W) is one of the most heavily affected areas of Brazil, with a high number of HPS cases: 168 cases have been reported since 1993, 52 of which were fatal (Elkhoury, 2005). Two rodent species hosting hantavirus antibodies have been identified in the region: *Oligoryzomys nigripes* and *Akodon* sp. (Suzuki *et al.*, 2004). In addition, our group recently described the complete S

Table 1. Brazilian hantaviruses and their putative reservoirs

Hantavirus	Putative reservoir	Place of detection (Brazil)	Reference
Juquitiba-like virus	*Oligoryzomys nigripes*	Paraná and Santa Catarina	Suzuki *et al.* (2004)
Araraquara virus	*Necromys lasiurus*	São Paulo and Minas Gerais	Suzuki *et al.* (2004)
Castelo dos Sonhos	Unknown	Pará	Figueiredo *et al.* (2009)
Anajatuba virus	*Oligoryzomys fornesi*	Maranhão	Rosa *et al.* (2005)
Rio Mearin virus	*Holochilus sciureus*	Maranhão	Rosa *et al.* (2005)
Laguna Negra-like virus	*Calomys laucha*	Mato Grosso	Figueiredo *et al.* (2009)
Jabora virus	*Akodon montensis*	Santa Catarina	Padula *et al.* (2007)

sequence of a hantavirus termed Araucaria (ARAUV) isolated from HPS patients from Paraná state, and demonstrated that it is closely related to the *O. nigripes*-associated strains ITA37 and ITA38 from neighbouring Paraguay (Raboni *et al.*, 2005). Unfortunately, we were not able to identify the rodent species that was locally associated with ARAUV at the time or to characterize the other genomic fragments of ARAUV. The latter is important to permit comparison with viruses for which only sequences of the M segment are known, and to be able to detect genomic reassortment among the segments. The current study focused on elucidating the local rodent host of ARAUV and characterizing the M segment of the genome from viruses isolated in rodents and in human patients.

To identify the rodent species acting as host to ARAUV, we sampled rodents in Paraná and searched for the presence of ARAUV-like RNA in seropositive rodents. Collecting efforts were maximized in areas with reported HPS cases. Fieldwork was conducted in November 2006 and rodents were captured by using Tomahawk ($40.6 \times 12.7 \times 12.7$ cm) and Sherman ($7.6 \times 9.5 \times 30.5$ cm) live traps set in six sites (in rural and natural environments) in areas of HPS infection. Capture sessions were carried out in a total of 16 linear transects with 20 capture stations on each at 10 m intervals, for five nights, for a total of 1426 trap-nights for the whole study. The most abundant species were *Akodon montensis* and *Akodon serrensis* and the overall capture success was 5 %. Six different genera and at least eight distinct species (seven rodent and one marsupial species) were recorded: *A. montensis* (31 specimens), *A. serrensis* (19), *Akodon paranaensis* (five), *O. nigripes* (seven), *Thaptomys nigrita* (three), *Oxymycterus* (*Oxy.*) *judex* (two), *Sooretamys angouya* (two) and two marsupials of the genus *Monodelphis*. Of the 69 captured rodent specimens from the subfamily Sigmodontinae, three had IgG antibodies against hantavirus (two specimens of *A. montensis* and one specimen of *Oxy. judex*).

All specimens were identified initially using external and cranial morphological analysis, and the identification of seropositive individuals was confirmed by karyological and molecular comparisons using the primers and conditions described by Delfraro *et al.* (2008). Seropositive animals were deposited as voucher specimens at the National Museum (MN), State of Rio de Janeiro; the remainder of the specimens will be deposited once their taxonomic status is resolved.

Blood and tissue samples were obtained following standard field biosafety procedures (Mills *et al.*, 1995) and stored in liquid nitrogen for further processing. All blood samples were screened by enzyme immunoassay for hantavirus antibodies using both ARAUV (Raboni *et al.*, 2007) and Andes virus (ANDV) (Padula *et al.*, 2000) antigens. Viral RNA was extracted from the lungs and kidneys of seropositive rodents using TRIzol (Gibco) and submitted to RT-PCR to detect and amplify fragments of the viral genome. Amplicons for a fragment of the viral S segment were obtained using the primers and conditions described by Raboni *et al.* (2005). To complement the genetic characterization, we sequenced a rodent-derived M segment from the specimen with the highest quality viral RNA: the specimen of *Oxy. judex*. In addition to studies about the local hosts of ARAUV, we also improved on the genomic characterization of the viral strain responsible for the HPS cases in the vicinity where rodents were captured. We sequenced the complete M segment from one of these HPS patients (HRP/02-72) and partially sequenced the M segment of two patients from the same area (HPR/02-71 and HPR/03-97). For the human-derived viruses, viral RNA was extracted from blood samples of HPS patients using a high pure viral RNA kit (Roche Applied Science). cDNA corresponding to the complete M segment was synthesized using a specific primer and an ImProm-II reverse transcription system (Promega), following the manufacturer's protocol. The resulting cDNA was subjected to PCR. Two fragments, of 1679 and 2019 bp, were generated and these covered the complete M segment. In all cases, fragments were sequenced with BigDye3 (Applied Biosystems) using PCR primers (sequences available on request). The resulting chromatographs were verified visually using vector NTI software (Invitrogen).

The largest open reading frame of the 3417 nt M segment from patient HRP/02-72 encoded a glycoprotein precursor from nt 52 to 3468. The ARAUV Gn glycoprotein extended from aa 1 to 651 (nt 52–2004), including the conserved putative cleavage WAASA motif (aa 647–651), and the Gc glycoprotein extended from aa 652 to 1139 (nt 2005–3466). Five putative *N*-linked glycosylation sites at residues 138, 350, 402, 524 and 930 were predicted for ARAUV glycoprotein precursor as well as the three main *O*-

glycosylation clusters at residues 96, 306 and 582 (Tischler *et al.*, 2003).

In our study, we found two specimens of *A. montensis* (10027 and 10028) and one of *Oxy. judex* (10056) that were positive by enzyme immunoassay for ARAUV and ANDV hantavirus IgG antibodies and viral RNA, indicating that they were infected with hantavirus. The seropositive rodents were collected on different days in the same trapping period and on different transects of capture. The voucher numbers and capture coordinates of infected rodents were: MN 71600 (*A. montensis* 10027), which was captured in a mixed forest of pines and bamboos (26° 33′ 12.0″ S 51° 23′ 46.1″ W); MN 71601 (*A. montensis* 10028), which was captured in an area of forest regrowth (26° 24′ 36.9″ S 051° 23′ 43.3″ W); and MN 71602 (*Oxy. judex* 10056), which was captured in an area of primary forest with large trees (26° 28′ 49.4″ S 051° 19′ 17.8″ W). Nucleic acid extractions and PCR assays of seropositive rodents were performed in three independent laboratories with different unopened aliquots, and identical results were observed in all cases, thus confirming the findings.

To characterize the genetic diversity of the viral strains isolated in the state of Paraná and assess their phylogenetic relationships, we compared the obtained sequences to a reference panel that covered most hantavirus diversity from South America. Reference sequences were downloaded from public databases for the two segments used in this study (M and S). Bayesian estimations of phylogenies were conducted in MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003), running four simultaneous chains for 2×10^6 generations, sampling trees every 1000 generations and using default priors. We used a general time-reversible model of nucleotide substitution (Rodriguez *et al.*, 1990) in which rate variation followed a discrete gamma distribution. We assessed convergence by measuring the standard deviation of the split frequency among parallel chains. Chains were considered to have converged once the mean split frequency was lower than 0.01. We summarized the results with a majority-rule consensus of 1500 trees collected after convergence was reached; trees collected before chains reached convergence were discarded. Maximum-likelihood searches were conducted in Treefinder (October 2008 version; Jobb *et al.*, 2004), selecting the best-fitting model of nucleotide substitution using the Bayesian Information Criterion model selection routine in Treefinder. We evaluated support for the nodes with 1000 bootstrap pseudoreplicates.

In all sequence comparisons of the viral S segment, we included the sequence from a specimen of *O. nigripes* (10091) trapped in another region of Paraná state, as S segment sequences from the *O. nigripes*-associated ARAUV reported by Suzuki *et al.* (2004) do not overlap with the fragment used in this study. The viral strains isolated from *Oxy. judex* (10056), *O. nigripes* (10091) and *A. montensis* (10028) were all very similar (99 % sequence identity in pairwise comparisons), and phylogenies based on the partial S segment sequences (Fig. 1) indicated that they were all closely related to the other ARAUV sequences previously characterized from HPS patients (Raboni *et al.*, 2005) and to some recently reported Juquitiba virus (JUQV)-like S segment sequences (Delfraro *et al.*, 2008). This result shows the epidemiological link between ARAUV from HPS patients and ARAUV found in the rodents, which was missing in our previous report (Raboni *et al.*, 2007).

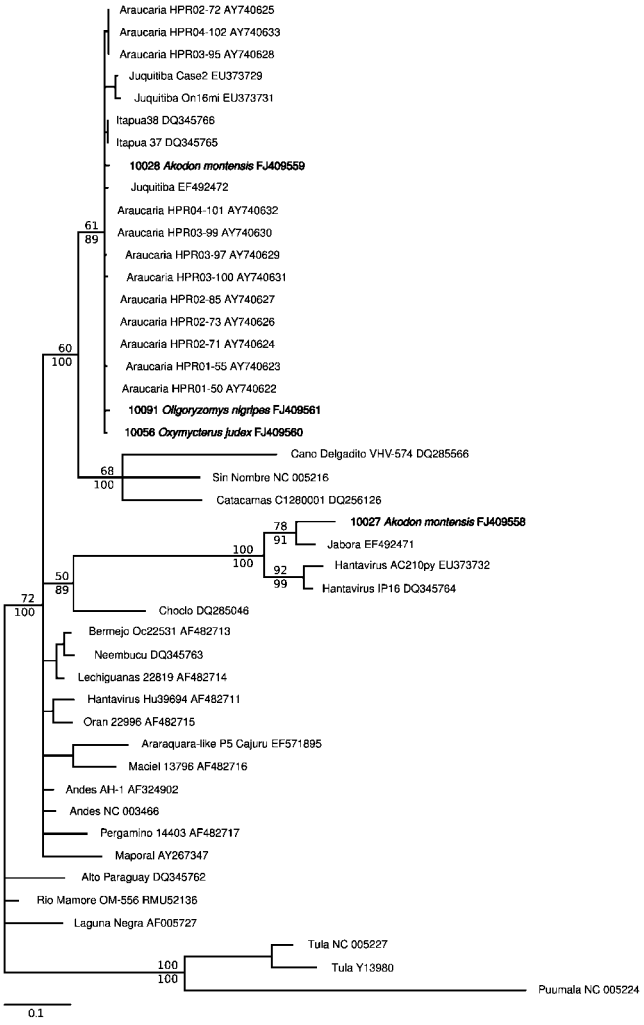

Fig. 1. Maximum-likelihood tree based on a 312 nt alignment of the S segment, depicting phylogenetic relationships among hantavirus sequences from Paraná, southern Brazil, and a reference panel of sequences obtained from GenBank. Samples in bold correspond to sequences added in this study from the state of Paraná, Brazil. Support for relevant nodes is provided as maximum-likelihood bootstrap support values (above) and Bayesian posterior probabilities (below). Nodes with support below 50 % were collapsed. Phylogenetic resolution other than grouping strains from Paraná with other strains is limited due to the limited amount of sequence data available in public databases. Bar, 0.1 nucleotide substitutions per site.

During our routine epidemiological assessments in the same region of rodent capture, we found a relatively high level of human hantavirus seroprevalence in individuals who had never reported HPS symptoms (Raboni *et al.*, 2007). We found that an *A. montensis* specimen (10027) in this area was infected with a hantavirus genotype that was 25 % different from ARAUV at the nucleotide level in the fragment of the S segment analysed. The virus from specimen 10027 was most closely related to Jaborá virus (JABV; ~ 90 % sequence identity), isolated in Santa Catarina state (southern border of Paraná state; GenBank accession no. EF495338), and was also phylogenetically close to a strain from Paraguay, on the border to the west of Paraná (Chu *et al.*, 2003). In both reports, the identified rodent species was *A. montensis*. Until now, these viruses have not been related to human disease. The co-circulation of two hantaviruses exhibiting differences in virulence could explain the high seroprevalence (8.4 %) observed in individuals without disease history in this region (Raboni *et al.*, 2007).

The complete coding region of the M segment from the human sample HPS/02-72 showed 73 % identity with Laguna Negra virus and 49.5 % with Seoul virus at the RNA level, and 88 and 58 % identity, respectively, at the amino acid level. Phylogenies based on the complete HPS-derived viral M segment showed that ARAUV grouped within the South American hantaviruses (Fig. 2a), and distance comparisons of the complete and partial M segment sequences of ARAUV with the reference sequences revealed that ARAUV was very similar to the partial JUQV-like sequences from *O. nigripes* trapped in this same region (Suzuki *et al.*, 2004), corroborating the data we obtained previously using the complete viral S segments from the same HPS patients (Raboni *et al.*, 2005). A more detailed analysis that included partial sequences derived from two additional human patients (HPR/02-71 and HPR/03-97) and a rodent (*Oxy. judex* 10056) provided further evidence of the strong phylogenetic affinities and high sequence similarities between the JUQV-like sequence reported by Suzuki *et al.* (2004) and the ARAUV-like virus isolated from rodents and humans from Paraná (Fig. 2b). Taken together, our analyses of S and M segments demonstrated that the sequences of ARAUV isolates were very similar to JUQV isolates reported by others (Delfraro *et al.*, 2008; Suzuki *et al.*, 2004), and, due to historical precedence, we propose that ARAUV should be named JUQ-like virus.

This study provides additional evidence that the scenario of hantavirus transmission in South America could be more complex than previously thought. We found highly similar hantaviruses (JUQ-like virus) occurring in three distantly related rodent species (*O. nigripes*, *A. montensis* and *Oxy. judex*) in the same location, in agreement with the identification of a JUQ-like virus in two different species in Uruguay: *O. nigripes* and *Oxymycterus nasutus* (Delfraro *et al.*, 2008). This could be due to an incidental infection of *A. montensis* and *Oxy. judex* with a JUQ-like virus from *O. nigripes* (the presumed reservoir for JUQ-like virus).

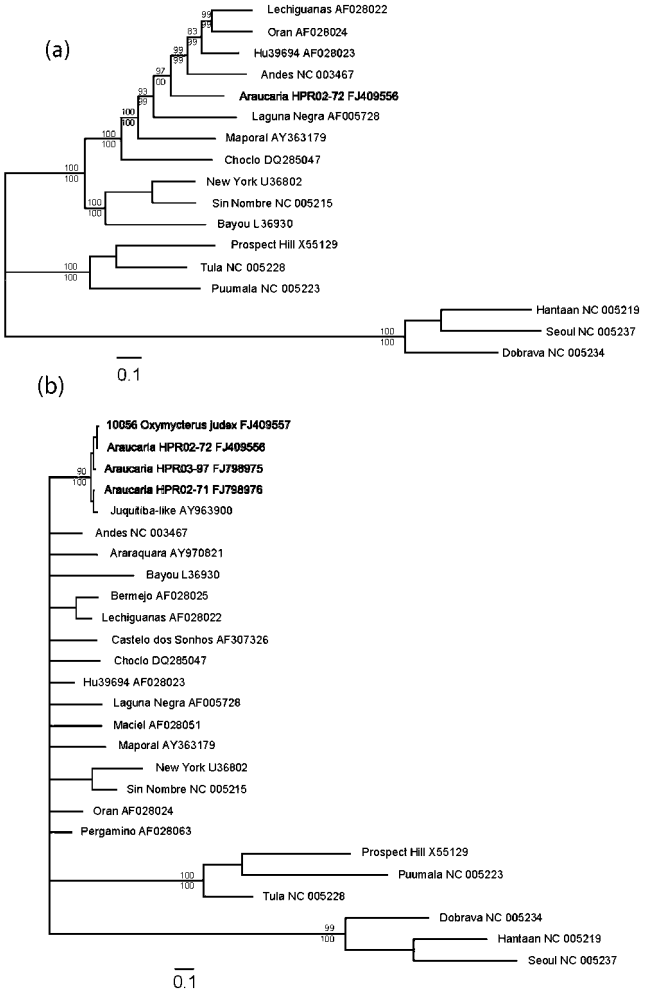

Fig. 2. Maximum-likelihood trees depicting phylogenetic relationships between the ARAUV complete segment M sequence (a) and based on a 500 nt alignment of the M segment, depicting phylogenetic relationships among hantavirus sequences from Paraná, southern Brazil (b), compared with a reference panel of sequences obtained from GenBank. Samples in bold correspond to sequences from this study from the state of Paraná (HPR indicates sequences that are from human patients). Support for relevant nodes is provided as maximum-likelihood bootstrap support values (above) and Bayesian posterior probabilities (below). Nodes with support below 50 % were collapsed. Bars, 0.1 nucleotide substitutions per site.

Alternatively, it could imply that host switching is more common than previously believed. It is worth noting that none of the *O. nigripes* specimens trapped in the area of HPS was positive for hantavirus antibodies. In fact, JUQ-like RNA in the HPS area was obtained from *A. montensis* and *Oxy. judex* specimens, suggesting that chronic infection in the two species is possible. We can only speculate that these two rodents act as primary reservoir hosts (able to maintain and transmit the virus for long periods), as there are few data from *in vivo* studies of South

American species. The possibility of human infection through contact with these infected rodents is real, and therefore further investigation of this issue is needed. The fact that *Oxy. judex* could act as a hantavirus reservoir deserves consideration, as this species has broad geographical distribution in areas of HPS transmission in the south of Brazil (Hoffmann *et al.*, 2002).

We found two distantly related viruses (JUQ-like virus and JABV) infecting the same rodent host species: *A. montensis*. Previously, JABV (GenBank accession no. EF492471) RNA was isolated from *A. montensis* rodents in southern Brazil, and Chu *et al.* (2006) found RNA from a hantavirus similar to JABV (strain IP16, GenBank accession no. DQ345764) in *A. montensis* specimens from Paraguay. More recently, an IP16-related hantavirus, strain AC210py (GenBank accession no. EU373732), was identified from a specimen of *Akodon cursor* from Paraguay (Padula *et al.*, 2007). So far, no *Akodon*-borne hantavirus has been reported to be associated with HPS cases in South America (Padula *et al.*, 2007), but precise determination of the true reservoir of JAB-like viruses is needed if we are to elucidate the transmission cycle and role of these viruses in nature. Infection of the same species by distantly related hantaviruses could be interpreted as incidental, but the epidemiological relevance of this infection is not clear. However, the co-circulation of two distinct hantavirus genotypes with the associated potential for genomic reassortment could have an impact on hantavirus transmission dynamics in nature, and thus on human epidemiology.

Acknowledgements

We are indebted to Gisélia Rubio and Edson dos Santos from Departamento de Vigilância, SESA-PR, Edilsom Semczuk from Centro de Controle de Zoonoses of Foz do Iguaçu for helping with rodent trapping logistics. We are also grateful to Drs Adriana Delfraro and Juan Arbiza from Universidad de la Republica, Montevideo, Uruguay, for confirming viral sequencing data and Dr Christian Probst for helpful discussions. We thank Paulo Arauco for technical assistance in sequencing. The authors thank CNPq, CNPq/Prosul, Fiocruz, Fundação Araucária, CYTED/RIVE and Fundo Paraná for financial support. C. N. D. S., P. S. D. and C. R. B. are CNPq fellowship recipients.

References

Childs, J. E., Ksiazek, T. G., Spiropoulou, C. F., Krebs, J. W., Morzunov, S., Maupin, G. O., Gage, K. L., Rollin, P. E., Sarisky, J. & other authors (1994). Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *J Infect Dis* **169**, 1271–1280.

Chu, Y. K., Owen, R. D., Gonzalez, L. M. & Jonsson, C. B. (2003). The complex ecology of hantaviruses in Paraguay. *Am J Trop Med Hyg* **69**, 263–268.

Chu, Y. K., Milligan, B., Owen, R. D., Goodin, D. G. & Jonsson, C. B. (2006). Phylogenetic and geographical relationships of hantavirus strains in eastern and western Paraguay. *Am J Trop Med Hyg* **75**, 1127–1134.

Delfraro, A., Tomé, L., D'Elía, G., Clara, M., Achával, F., Russi, J. C. & Rodonz, J. R. (2008). Juquitiba-like hantavirus from 2 nonrelated rodent species, Uruguay. *Emerg Infect Dis* **14**, 1447–1451.

Elkhoury, M. R. (2005). Aspectos epidemiológicos da infeccão e patogenicidade por hantavirus no Brasil. Boletim Epidemiológico Eletrônico. Ministério da Saúde/Secretaria de Vigilância em Saúde 5, 1–5. http://www.saude.gov.br/svs (in Portuguese).

Figueiredo, L. T. M., Moreli, M. L., de Souza, L. M., Borges, A. A., Figueiredo, G. G., Machado, A. M., Bisordi, I., Nagasse-Sugahara, T. K., Suzuki, A. & other authors (2009). Hantavirus pulmonary syndrome, Central Plateau, Southeastern, and Southern Brazil. *Emerg Infect Dis* **15**, 561–567.

Hoffmann, F. G., Lessa, E. P. & Smith, M. F. (2002). Systematics of *Oxymycterus* with description of a new species from Uruguay. *J Mammal* **83**, 408–420.

Jobb, G., von Haeseler, A. & Strimmer, K. (2004). TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol Biol* **4** doi:10.1186/1471-2148-1184-1118

Johnson, A. M., de Souza, L. T. M., Ferreira, I. B., Pereira, L. E., Ksiazek, T. G., Rollim, P. E., Peters, J. C. & Nichol, S. T. (1999). Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. *J Med Virol* **59**, 527–535.

Lednicky, J. A. (2003). Hantaviruses: a short review. *Arch Pathol Lab Med* **127**, 30–35.

Lundkvist, A. & Niklasson, B. (1994). Haemorrhagic fever with renal syndrome and other hantavirus infections. *Rev Med Virol* **4**, 177–184.

Mills, J. N., Childs, J. E., Ksiazek, T. G., Peters, C. J. & Velleca, W. M. (1995). *Methods for Trapping and Sampling Small Mammals for Virologic Testing.* U.S. Department of Health & Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Padula, P. J., Edelstein, A., Miguel, S. D. L., Lopez, N. M., Rossi, C. M. & Rabinovich, R. D. (1998). Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* **241**, 323–330.

Padula, P. J., Rossi, C. M., Della Valle, M. O., Martínez, P. V., Colavecchia, S. B., Edelstein, A., Miguel, S. D., Rabinovich, R. D. & Segura, E. L. (2000). Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. *J Med Microbiol* **49**, 149–155.

Padula, P., Martinez, V. P., Bellomo, C., Maidana, S., San Juan, J., Tagliaferri, P., Bargardi, S., Vazquez, C., Colucci, N. & other authors (2007). Pathogenic hantaviruses, northeastern Argentina and eastern Paraguay. *Emerg Infect Dis* **13**, 1211–1214.

Plyusnin, A. & Morzunov, S. P. (2001). Virus evolution and genetic diversity of hantaviruses and their rodent hosts. *Curr Top Microbiol Immunol* **256**, 47–75.

Plyusnin, A., Vapalahti, O. & Vaheri, A. (1996). Hantaviruses: genome, structure, expression and evolution. *J Gen Virol* **77**, 2677–2687.

Raboni, S. M., Probst, C. M., Bordignon, J., Zeferino, A. & Duarte dos Santos, C. N. (2005). Hantaviruses in Central South America: phylogenetic analysis of the S segment from HPS cases in Paraná, Brazil. *J Med Virol* **76**, 553–562.

Raboni, S. M., Levis, S., Rosa, E. S. T., Bisordi, I., Delfraro, A., Lemos, E., Correia, D. C. & Duarte dos Santos, C. N. (2007). Hantavirus infection in Brazil: development and evaluation of an enzyme immunoassay and immunoblotting based on N recombinant protein. *Diagn Microbiol Infect Dis* **58**, 89–97.

Rodriguez, F., Oliver, J. L., Marin, A. & Medina, J. R. (1990). The general stochastic model of nucleotide substitution. *J Theor Biol* **142**, 485–501.

Ronquist, F. & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Rosa, E. S., Mills, J. N., Padula, P. J., Elkhoury, M. R., Ksiazek, T. G., Mendes, W. S., Santos, E. D., Araújo, G. C., Martinez, V. P. & other authors (2005). Newly recognized hantaviruses associated with hantavirus pulmonary syndrome in northern Brazil: partial genetic characterization of viruses and serologic implication of likely reservoirs. *Vector Borne Zoonotic Dis* **5**, 11–19.

Schmaljohn, C. S. & Patterson, J. L. (2001). *Bunyaviridae*: the viruses and their replication. In *Fields Virology*, 4th edn, pp. 1293–1309. Edited by D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott Williams & Wilkins.

Sousa, R. L. M., Moreli, M. L., Borges, A. A., Campos, G. M., Livonesi, M. C., Figueiredo, L. T. M. & Pinto, A. A. (2008). Natural host relationships and genetic diversity of rodent-associated hantaviruses in southeastern Brazil. *Intervirology* **51**, 299–310.

Suzuki, A., Bisordi, I., Levis, S., Garcia, J., Pereira, L. E., Souza, R. P., Sugahara, T. K., Pini, N., Enria, D. & Souza, L. T. (2004). Identifying rodent hantavirus reservoirs, Brazil. *Emerg Infect Dis* **10**, 2127–2134.

Tischler, N. D., Fernández, J., Müller, I., Martínez, R., Galeno, H., Villagra, E., Mora, J., Ramírez, E., Rosemblatt, M. & Valenzuela, P. D. (2003). Complete sequence of the genome of the human isolate of Andes virus CHI-7913: comparative sequence and protein structure analysis. *Biol Res* **36**, 201–210.