

## Detection of the first incidence of *Akodon paranaensis* naturally infected with the Jabora virus strain (Hantavirus) in Brazil

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*We characterised hantaviruses circulating in different Akodon rodent species collected in midwestern Santa Catarina (SC), southern Brazil, where the Jabora hantavirus (JABV) strain was first identified in Akodon montensis. Genetic and phylogenetic analyses based on a partial S segment indicated that, in SC, Akodon paranaensis and A. montensis carried the same type of hantavirus. Additionally, we conducted the first genomic characterisation of the complete S segment from the Brazilian JABV strain. This is the first report of A. paranaensis infected with the JABV.*

Key words: phylogenetic analyses - rodents - hantavirus - Santa Catarina

Hantavirus pulmonary syndrome (HPS) is caused by the emerging rodent-borne viruses of the genus Hantavirus (Nichol et al. 1993). Since 1993, over 1,400 cases have been identified in 14 states in Brazil. Santa Catarina (SC) (southern Brazil, 27°10'S 51°44'W) is the second most affected Brazilian state, with a large number of cases (n = 226) (Brazilian Health Ministry Report on Hantavirus cases 1993-2011, unpublished data). Currently, there are five hantaviruses associated with HPS in Brazil: Juquitiba/Araucaria, Araraquara, Laguna Negra, Anajatuba and Castelo dos Sonhos; these are carried by *Oligoryzomys nigripes*, *Necromys lasiurus*, *Calomys* sp., *Oligoryzomys fornesi* and *Oligoryzomys utiaritensis*, respectively (Johnson et al. 1999, Suzuki et al. 2004, Raboni et al. 2005a, 2009a, Rosa et al. 2010, Travassos da Rosa et al. 2011). Two other hantaviruses, Rio Mearim and Jabora (JABV) have been identified in the rodent species *Holochilus sciureus* and *Akodon montensis*, respectively, but their roles in human disease have not been determined (Rosa et al. 2005, Oliveira et al. 2011).

Hantaviruses can co-circulate in the same locality and can be maintained side-by-side in different rodent species, as reported for Juquitiba and JABV related viruses and other Old World hantaviruses (Artois et al. 2007, Chu et al. 2009, Raboni et al. 2009b, Razzauti et al. 2009, Oliveira et al. 2011). In this study, we have identified JABV, which is associated with *A. montensis* and *Akodon paranaensis*, two related and sympatric rodent species in Midwestern SC (Jabora, 27°09'S 51°47'W). These two species of *Akodon* are widely distributed in

the central Southern Cone of South America and may be locally abundant in their preferred habitat (Musser & Carleton 2005). Additionally, we conducted the first genomic characterisation of the complete S segment from the JABV strain to better characterise the hantaviruses circulating in these two related rodent species.

RNA was extracted from lung tissue samples of five antibody-positive rodents from *A. paranaensis* (Akp8032, Akp8048) and *A. montensis* (Akm6266, Akm6943 and Akm9635) species, according to the manufacturer's instructions from the Trizol<sup>®</sup> Plus RNA Purification Kit. The rodent samples were analysed using the polymerase chain reaction with reverse transcription and nested reactions. For direct sequencing of overlapping amplicons, generic primer combinations (n = 8) were used for amplification and sequencing of the complete genomic S segment, including published (Raboni et al. 2005b, Oliveira et al. 2011) and unpublished (S Levis 2005, unpublished observations, A Guterres 2011, unpublished observations) oligonucleotide sequences. These sequences were designed based on the conserved regions of the S segment among South American hantaviruses (primers available on request). Amplicons of the expected size (approximately 1,800 bp) were recovered from two samples: one from *A. montensis* (Akm9635) and another from *A. paranaensis* (Akp8048). Sequence alignments were run in SeaView, using the MUSCLE algorithm. Obtained virus sequences (partial and complete) are accessible from GenBank (JN232078\_Akm9635, JN232080\_Akp8048 and JN232081\_Akp8032). Phylogenetic relationships were estimated using (i) a maximum likelihood (ML) phylogenetic inference method with 1,000 bootstrap replicates, implemented in PhyML 3 (Guindon & Gascuel 2003) under the GTR+G model of sequence evolution, which was chosen after hierarchically testing alternative models by computing likelihood ratios and (ii) a Bayesian Markov chain Monte Carlo method implemented in MrBayes v3.1.2 (Ronquist & Huelsenbeck 2003), using

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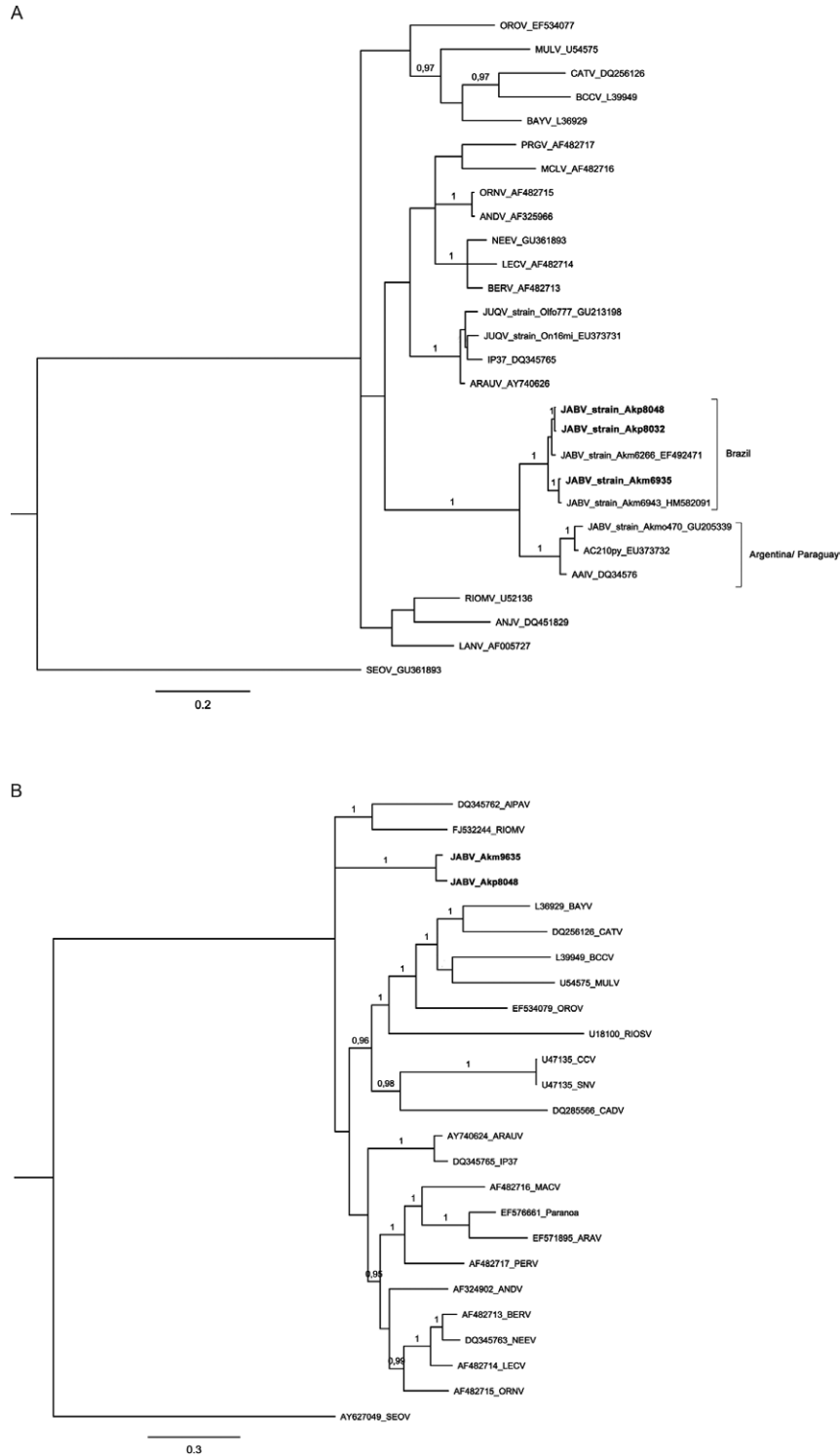


Fig. 1: phylogenetic trees of hantavirus based on a Bayesian analysis of genetic distances generated from comparisons of a partial sequences of the S segment (700 nt) (A) and complete sequences of the S segment (B). The numerical value at the node indicates the posterior probability that supported the interior branch. GenBank accessions are indicated and sequences obtained in this study are shown in bold. Hantavirus strain abbreviations: AAV: Ape Aime virus from Paraguay; AC210py: virus from Argentina; ALPA: Alto Paraguay virus from Paraguay; ANAJV: Anajatuba virus from Brazil; ANDV: Andes virus, strain Nort from Argentina; ARAUV: Araucaria virus from Brazil; ARAV: Araraquara virus from Brazil; BAYV: Bayou virus from the United States (USA); BCCV: Black Creek Canal virus from USA; BERV: Bermejo virus from Argentina; NEEV: Bermejo virus, strain Ñembucú from Paraguay; CADV: Cano Delgado virus from Venezuela; CATV: Catacamas virus from Honduras; IP37: Itapúa virus, strain 37 from Paraguay; Jabora: JAB virus from Brazil and Paraguay; JUQV: Juquitiba virus from Brazil; LANV: Laguna Negra virus from Paraguay; LECV: Lechiguanas virus from Argentina; MULV: Muleshoe virus from USA; MACV/MCLV: Maciel virus from Argentina; NYV: New York virus from USA; ORNV: Oran virus from Argentina; OROV: Playa de Oro virus from the Mexico; Paranoa virus from Brazil; PRGV: Pergamino virus from Argentina; RIOMV: Rio Mamoré virus from Bolivia; RIOS: Rio Segundo virus from Costa Rica; SEOV: Seoul virus from China.

the GTR+G model of nucleotide substitution. Two simultaneous runs of four chains each were run for 1 million generations and sample frequency = every 100th generation; a consensus tree (burn-in of 25%) was constructed from the remaining trees. Posterior probabilities above 0.95 and bootstrap values above 70% at the nodes were accepted as significant. We also assessed the phylogenetic relationships between JABV and other hantaviruses with a partial section of the S segment sequence (700 nucleotides due to the large number of partial sequences available).

Phylogenetic trees calculated by ML (not shown) and Bayesian methods, based on partial and complete sequences, indicated similar topology at the relevant nodes (Fig. 1). Phylogenetic analyses of these sequences (partial and complete) indicated that all hantaviruses carried by *A. montensis* and *A. paranaensis* form a distinct and monophyletic lineage. ML and Bayesian analyses based on partial S segment indicated that the sequences circulating in *A. paranaensis* are closely related to *A. montensis* viruses from Brazil, Argentine and Paraguay (JABV/AC210py/AAIV). Although *A. montensis* sequences are not monophyletic, *A. paranaensis* (Akp8032 and Akp8048) sequences are grouped with significant support and are tightly associated with the *A. montensis* virus strain (Fig. 1). The JABV-like virus clade could be divided into two well-supported subclades: one composed of Paraguayan and Argentinean viruses and the other composed of Brazilian viruses (Akp and Akm samples). The ML tree that

was constructed using the amino acid sequences of the S segment indicated highly similar bootstrap values and a branching pattern obtained from the nucleotide sequence phylogenetic analysis (not shown).

In pairwise comparisons of a nucleotide sequence, calculated using MEGA 5 (Tamura et al. 2011), the genetic distance among JABV strains from Brazilian *A. montensis* and *A. paranaensis* ranged from 0.1-3.4% and amino acid derived differences ranged from 0-1.3%. This suggests that spillover infection of JABV-related viruses is actively occurring among Akodontini rodent species in southern Brazil. Furthermore, the nucleotide differences between JABV strains from Brazil and JABV/AAIV-related viruses from Paraguay and Argentina (Ac210py) ranged from 11.5-14.7% (Table).

All five *Akodon* specimens were karyotyped to confirm morphologic identification. Phylogenetic reconstructions of three rodent specimens (Akm6943, Akp8048, Akp8032), based on the mitochondrial DNA cytochrome b gene (Smith & Patton 1993), were also obtained. These reconstructions were used to confirm species identification and to estimate phylogenetic relationship of the hantavirus-positive specimens, using the same phylogeny model of evolution and parameters described above for hantaviruses. All rodent specimens collected were fixed in 10% formalin or prepared as skin and skull and placed as voucher specimens in the mammal collection of the National Museum of the Federal University of Rio de Janeiro.

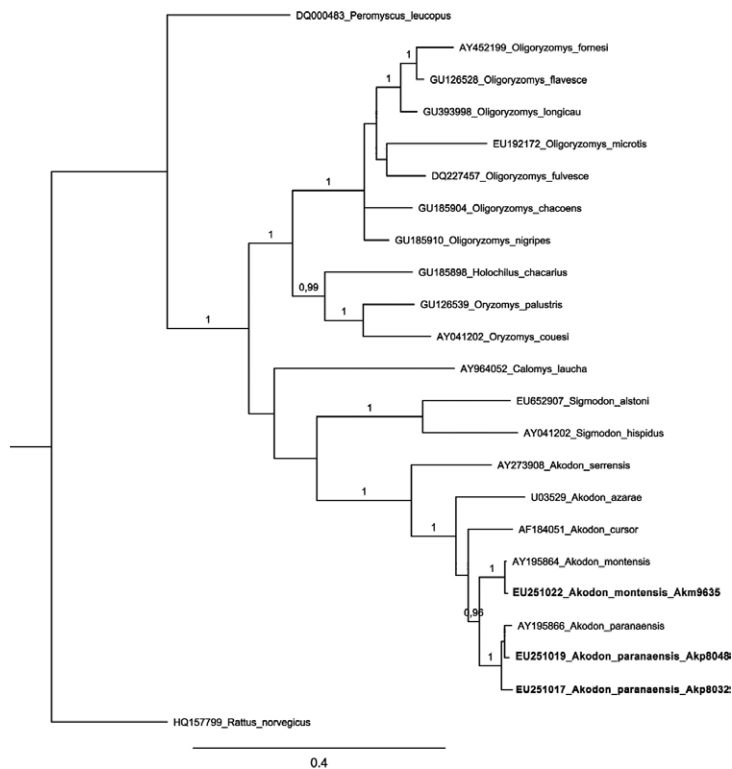


Fig. 2: phylogenetic tree of hantavirus rodent hosts based on partial sequences of mitochondrial cytochrome b gene. The numerical value at the node indicates the posterior probability that supported the interior branch. GenBank accessions are indicated and sequences obtained in this study are shown in bold.

TABLE  
Estimates of evolutionary divergence between Akodontini hantavirus strains of S sequences

Comparison	Pairwise distances <sup>a</sup> (%)
Between Pergamino virus and Jabora (JABV)-like virus clade <sup>b</sup>	26.2-28.3
Between Argentinean (Ac210py) and Paraguayan (AAIV; Akmo470) virus strains <sup>b</sup>	1.9-4.2
Between Brazilian JABV subclade and Argentinean_Paraguayan virus subclade <sup>b</sup>	11.5-14.7
Within Brazilian JABV subclade <sup>b</sup>	0.1-3.4
Between Akp8048 and Akp9635 <sup>c</sup>	3.2

*a*: range of pairwise distances calculated by the Kimura two-parameter (Kimura 1980) using MEGA 5; *b*: comparison of genetic distances between partial sequences; *c*: comparison of genetic distances between complete sequences.

Karyologic analyses confirmed that three *Akodon* specimens belong to *A. montensis* (2n = 24, FNa = 42) and the other two specimens to *A. paranaensis* (2n = 44, FNa = 44). The Bayesian and ML (not shown) trees indicated similar topologies for Akodontini (Fig. 2). These analyses grouped the haplotypes of *Akodon* sequenced specimens (Akm6943, Akp8048, Akp8032) with the GenBank sequence of *A. montensis* and *A. paranaensis*.

Genetic and phylogenetic analyses based on S partial and complete segments indicated that, in SC, *A. paranaensis* and *A. montensis* carried the same type of hantavirus. According to some ecological mathematics models, the presence of multiple hosts increases the possibility of disease emergence (McCormack & Allen 2007). In this study, JABV was identified for the first time in *A. paranaensis*. The phylogenies obtained from S segments indicate that the *A. paranaensis* strain is monophyletic and related to the virus circulating in the sympatric *A. montensis*. Spillover of JAB-like virus from its real host to other sympatric rodent species cannot be excluded and, therefore, further investigation of this issue is needed. Studies utilising phylogenetic methods to generate and compare evolutionary scenarios of hantaviruses and their rodent hosts are critical to better understanding the evolution of hantaviruses, especially in South America. Additionally, a longitudinal study and new rodent collection expeditions in different areas are needed to elucidate whether *A. paranaensis* rodents are true reservoirs or only sporadic hosts.

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