Genetic Diversity and Epidemiology of Hantaviruses in Argentina

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Phylogenetic analysis of a 292-nucleotide (nt) fragment of the hantavirus M genome segment from 36 rodent and 13 human samples from three known foci of hantavirus infection in Argentina was conducted. A 1654-nt fragment of the M genome segment was analyzed for 1 representative of 7 genetically distinct hantavirus lineages identified. Additionally, the nt sequence of the complete M genome segments of Lechiguanas, Oran, and Hu39694 hantavirus genotypes was determined. nt sequence comparisons reveal that 7 hantavirus lineages from Argentina differ from each other by 11.5%–21.8% and from Sin Nombre, Bayou, and Black Creek Canal viruses by 23.8%–26.5%. Phylogenetic analyses demonstrate that they form a unique, separate branch within the clade containing other New World sigmodontine-borne hantaviruses. Most *Oligoryzomys*-borne hantavirus genotypes clearly map together. The *Oligoryzomys*-borne genotypes Lechiguanas, Oran, and Andes appear to be associated with human disease. *Oligoryzomys longicaudatus* was identified as the likely rodent reservoir for Andes virus.

Worldwide, there is an increasing number of distinct hantavirus serotypes causing human diseases [1, 2]. At least 4 Old World serotypes have been linked to the human disease referred to as hemorrhagic fever with renal syndrome. Each of these hantaviruses is associated with a single rodent species as its primary natural reservoir: Hantaan with the striped field mouse *Apodemus agrarius,* Seoul with *Rattus norvegicus* and *Rattus rattus,* Dobrava with the yellow-necked field mouse *Apodemus flavicollis,* and Puumala with the bank vole *Clethrionomys glareolus* [3, 4].

Hantaviruses belong to the family Bunyaviridae. They contain a three-segmented, single-stranded, negative-sense RNA genome. The segments, known as large, medium, and small, encode the RNA-dependent RNA polymerase, the glycoproteins G1 and G2, and the nucleocapsid protein, respectively [5–9].

Since the recognition of hantavirus pulmonary syndrome (HPS) in the United States in 1993, 4 hantaviruses associated with this disease in North America have been characterized. Their natural reservoirs are the following indigenous New World rodents: the deer mouse *Peromyscus maniculatus* for Sin Nombre virus, the marsh rice rat *Oryzomys palustris* for Bayou virus, the cotton rat *Sigmodon hispidus* for Black Creek Canal virus, and the white-footed mouse *Peromyscus leucopus* for New York virus [10–14]. Other hantaviruses from North America that have not been associated with human disease include Prospect Hill and related viruses associated with the meadow vole *Microtus pennsylvanicus* and several other *Microtus* species [2, 15–17], El Moro Canyon, and Rio Segundo viruses discovered in the harvest mice *Reithrodontomys megalotis* and *Reithrodontomys mexicanus.* [18, 19].

Recently, serologic evidence of hantavirus infection with clinical manifestations of HPS has been reported from different countries in South America. In Brazil, 2 serologically confirmed fatal cases were reported from São Paulo State in 1995 [20]. Recently, a cluster of 23 cases resembling HPS occurred in western Paraguay. These were serologically confirmed as hantavirus infections. The sigmodontine rodent *Calomys laucha* was identified as a likely primary rodent reservoir for this virus [21].

In Argentina, serologic evidence of hantavirus infection in laboratory and wild rodents has been reported [22]. In 1995, there were 29 human HPS cases, which presented in clusters and were serologically confirmed as hantavirus infections [8, 23]. The Argentinian clusters of HPS cases occurred in three distinct geographic regions: the northwestern subtropical region of Oran, in Salta Province, close to the border with Bolivia; the central temperate region of the plains (pampas), in Buenos Aires and Santa Fe Provinces; and the southwestern, cold, forested region of El Bolson, Rio Negro Province, near the Andean range (Patagonia) (figure 1). During the spring of 1996, a severe outbreak, which affected 18 people, occurred in southwestern El Bolson [24]. Prior to this outbreak, a hantavirus referred to as Andes (AND) virus had been partially characterized from a fatal HPS case, which occurred in the same region in 1995 [25].

Serologic evidence of Sin Nombre–like hantavirus infection in Argentina has been reported in several rodents, including *Oligoryzomys flavescens* (rice rat), *Akodon azarae* (grass field mouse), and *Bolomys obscurus* (dark field mouse) in central

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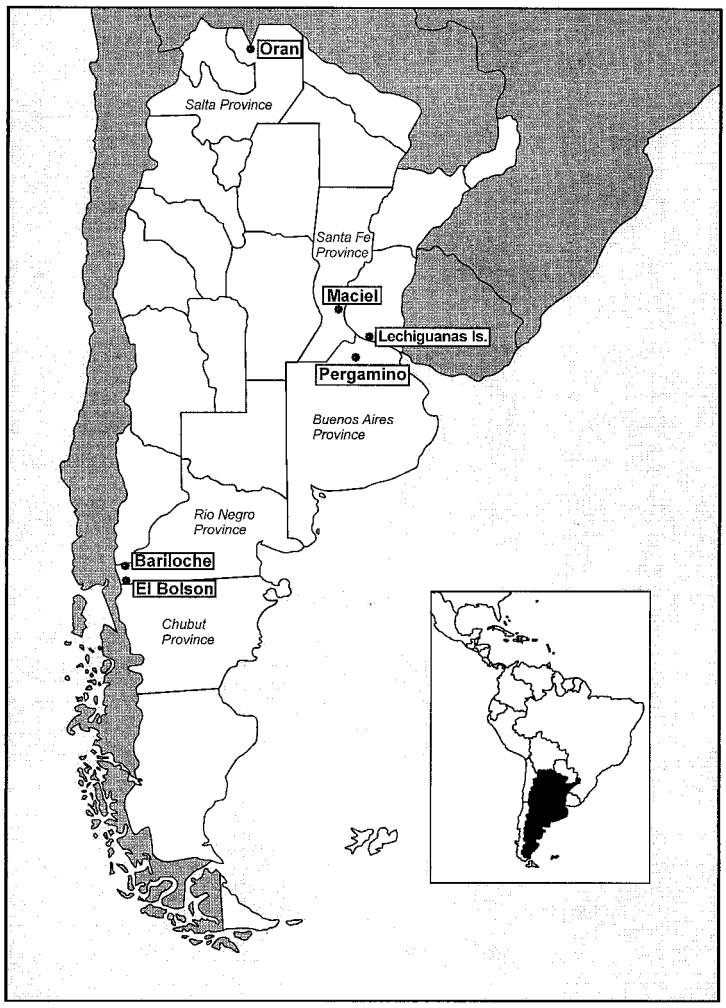

Figure 1. Map of Argentina showing provinces and major locations where samples were collected from rodents and persons who had had hantavirus pulmonary syndrome.

Argentina and in *Oligoryzomys longicaudatus* (long-tailed rice rat), *Oligoryzomys chacoensis* (no common name), and *Akodon varius* (raton variado) in northwestern Argentina [26]. These rodent species belong to the same subfamily, Sigmodontinae, family Muridae, as other New World rodents that serve as primary reservoirs for HPS-associated hantaviruses in North America. The first partially genetically characterized South American hantavirus, Rio Mamore virus, although not associated so far with human disease, has been recovered from a sigmodontine rodent, *Oligoryzomys microtis,* from Bolivia [27].

Our laboratory recently reported (in a study that included the phylogenetic analysis of a 147-nucleotide [nt] fragment of the virus M genome segment from human HPS case samples and rodents [*O. flavescens, A. azarae* and *B. obscurus*] from a limited geographic area of central Argentina [28]) the existence of 4 novel hantavirus genotypes: Lechiguanas (LEC; harbored by *O. flavescens*), Pergamino (PRG; harbored by *A. azarae*), Maciel (MAC; harbored by *B. obscurus*), and Hu39694, with LEC and Hu39694 also being associated with human disease.

The current studies were done to gain a better understanding of the geographic distribution, genetic diversity, and epidemiology of South American hantaviruses. From central Argentina, a larger number of samples from HPS cases and seropositive rodents from the same areas were analyzed. To clarify the phylogenetic relationship of these newly recognized virus lineages to previously characterized hantaviruses, a 1654-nt fragment of the M genome segment was sequenced for 5 hantavirus genotypes from central Argentina, and the nt sequence of the complete virus M genome segment was determined for LEC and Hu39694. These sequences were analyzed and compared with previously characterized hantaviruses.

This report also describes the polymerase chain reaction (PCR) amplification, sequencing, and phylogenetic analysis of the complete M genome segment of a new hantavirus genotype, Oran (ORN), from the northwestern location of Oran, which was recovered from a human with HPS and 2 seropositive rodents. The identification of the likely primary reservoir for AND virus from El Bolson is also reported.

Materials and Methods

Patient and rodent samples. Human whole blood, blood clot, or autopsy tissue samples of lung, liver, and kidney (or all

3 samples) were obtained from 2 HPS cases from Oran (Salta Province, northwestern Argentina; figure 1), from 16 HPS cases from Pergamino, Lechiguanas Islands, Salto Island, San Nicolas, and San Pedro (Buenos Aires Province), and from Acebal and San Lorenzo (Santa Fe Province) in central Argentina. A blood sample from an HPS patient residing in Pergamino but with an uncertain site of exposure was also examined. All patients included in this study acquired infection during 1987–1996, and hantavirus infection was confirmed by IgM and IgG ELISAs reactive to Sin Nombre virus. Whole blood, obtained during the acute period of the disease and postmortem tissue samples were stored at −80°C.

Rodent lung and kidney tissue samples were obtained from sacrificed animals. The animals had been trapped in 1992–1996 for hantavirus and arenavirus studies at the same locations where the HPS cases occurred. The samples were from Oran, in Salta Province (2 *O. longicaudatus,* 1 *O. chacoensis,* and 1 *A. varius*); from the central Argentina localities of Pergamino, Lechiguanas Islands, Zarate, San Pedro, and San Nicolas in Buenos Aires Province, and Alcorta, Maximo Paz, Jaun Bautista Molina, Uranga, Maciel, and Oliveros in Santa Fe Province (20 *O. flavescens,* 35 *A. azarae,* 7 *B. obscurus,* and 1 *Holochilus brasiliensis*); and from the southwestern Argentina localities of El Bolson and Bariloche in Rio Negro Province and Lake Puelo in Chubut Province (17 *O. longicaudatus,* 1 *Abrotrix longipilis*). All rodent samples studied were seropositive for hantavirus by ELISA.

Total RNA extraction. Total RNA was extracted from whole blood, blood clot, and autopsy samples of human and rodent tissues as previously described [29]. In brief, ~100 mg of tissue or 50 μL of whole blood was mixed with 300 μL of cell lysis solution containing guanidine thiocyanate, extracted with phenol-chloroform, and purified with RNA matrix beads (RNaid PLUS kit; Bio 101, La Jolla, CA). RNA was eluted from the matrix by use of RNase-free water and at −80°C.

Nested reverse transcription (RT)–PCR and sequence analysis. Amplification products were obtained by nested RT-PCR assays performed as described previously [10]. DNA was visualized by 1% agarose gel electrophoresis, and bands of the correct predicted size were excised from the gel and purified using a GeneClean kit (Bio 101). The nt sequence was determined by a manual or automatic dyedeoxy cycle sequencing technique (Applied Biosystems, Foster City, CA) as previously described [10].

Oligonucleotide primer design. Generic primers were designed on the basis of the conserved regions of sequences among hantaviruses, including Prospect Hill virus strain PH-1, Bayou virus, Black Creek Canal virus, El Moro Canyon virus, and Sin Nombre virus strains NMh10 (New Mexico h10) and CC107 (Convict Creek 107). Primers +2653 and −3687 (first round; numbers correspond to nt positions of aligned sequences mentioned above), and +2750 and −3398 (second round) were used to amplify a 649-nt fragment of the M genome segment of the G2 glycoprotein–encoding region from several human whole blood and rodent tissue samples from central Argentina. On the basis of the specific sequence obtained from 1 human HPS case and 1 *O. flavescens,* a nested set of specific primers was designed to optimize the routine diagnostic RT-PCR assay. These included the first-round primers +2765 and −3348 and the second-round primers +2781 and −3221, which amplify a 441-nt fragment (table 1). The routine diagnostic RT-PCR of *A. azarae* lung tissue samples was carried out using the same three primers, but the second-round reverse primer was replaced with a specific new primer, −3133. This primer was designed on the basis of the 441-nt fragment sequence information, and it generated a 353-nt DNA fragment.

To extend sequencing of the hantavirus M segments, additional primers were designed. As sequence information from each virus became available, specific primers were designed to fill gaps in the sequences. The exact terminal sequences for the M genome segments were determined by ligation of the individual RNA segment termini, followed by RT-PCR amplification and sequencing through the ligation site [30].

Sequences were compared and aligned using the GAP, PILEUP and LINEUP programs of the GCG software package (Genetic Computer Group, Madison, WI).

Phylogenetic analysis. Phylogenetic analyses of the nt sequences of different hantavirus strains were done using both the maximum parsimony method (PAUP, version 3.1.1) [31] and the distance-based neighbor-joining method (MEGA, version 1.02) [32]. For maximum parsimony analyses, phylogenetic trees were obtained by the heuristic search method, using a 4:1 weighting of transversions over transitions [29]. For neighbor-joining, the Kimura 2-parameter algorithm, which includes estimation of differences in the rate of transitional and transversional substitutions, was used. Bootstrap confidence limits were obtained by 1000 heuristic or Kimura 2-parameter search repetitions. The following previously published hantavirus sequences were included in the analysis: Hantaan strain 76118 [33], Seoul strain SR11 [34], Prospect Hill strain PH-1 [9], Tula [35], Puumala strains CG 1820 [36] and Sotkamo [37], Bayou [12], Black Creek Canal [38], El Moro Canyon [18], New York RI1 and 1 strains [39], and Sin Nombre strains NMh10 and CC107 [29, 40].

Table 1. Primers for nested reverse transcription–polymerase chain reaction (RT-PCR) of Argentinian hantaviruses. Primer numbers correspond to base numbers of M segment sequence.

First-round RT-PCR	+2765 5′-CTGTATGTGAGTACCAAG-3′	−3348 5′-CTGTCCAGATTTAGTGTTCCA-3′
Second-round PCR	+2781 5′-AGGTAACACTATATCTGG-3′	−3221 5′-TCAGAAGAGCAGTCAGTGTCATG-3′
Second-round PCR (for *Akodon azarae* samples)	+2781	−3133 5′-CTAGCAAGGTTTGCAACTGT-3′

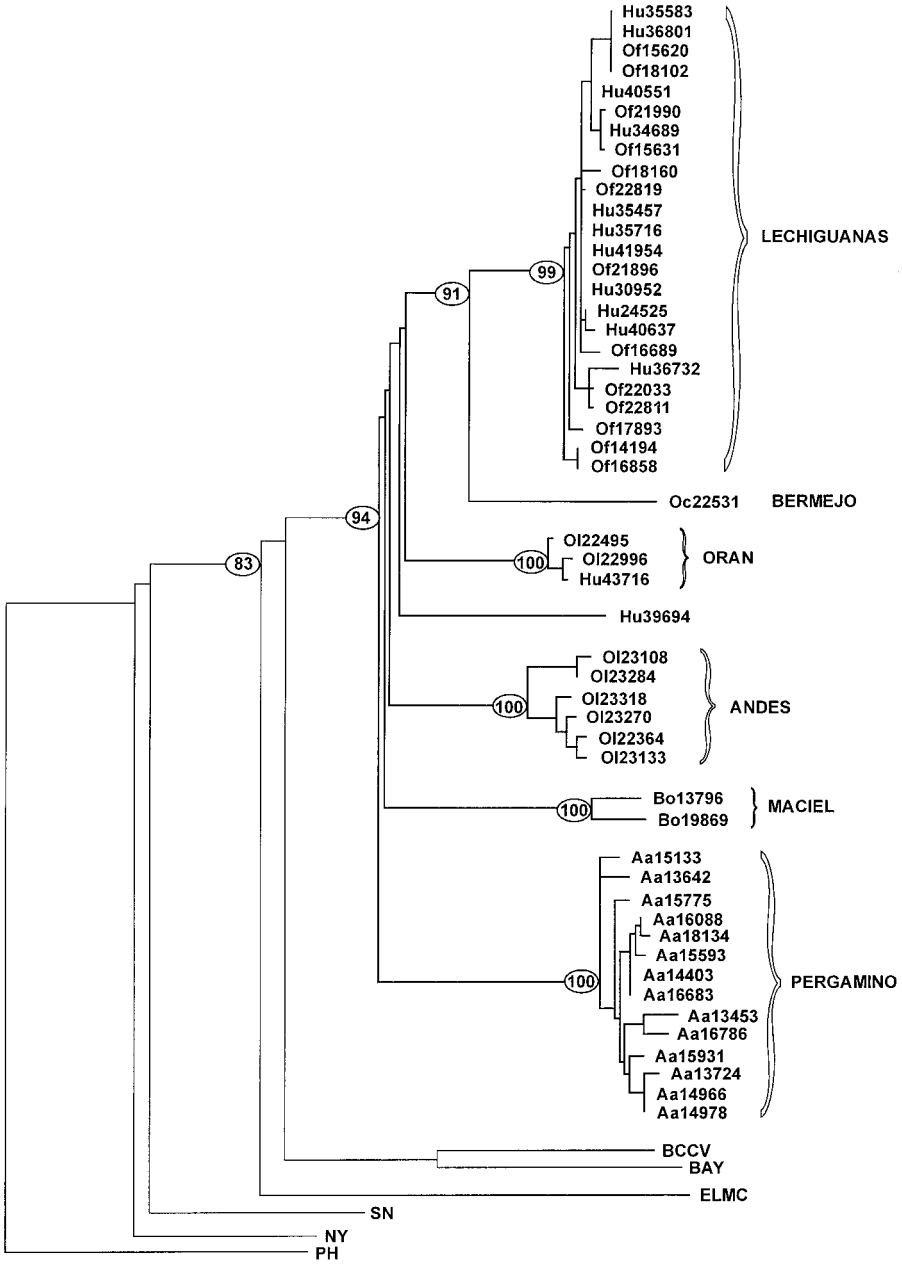

Figure 2. Phylogenetic analysis of 292-nucleotide fragment of M genome segment (bases 2815–3106) with neighbor-joining method, using Kimura 2-parameter algorithm. For major branches, bootstrap confidence limits (from 1000 repetitions) are indicated at node. Major nodes with bootstrap limit of <75%, have no no. indicated. Prefixes to South American sample numbers: Hu = human, Of = *Oligoryzomys flavescens,* Oc = *Oligoryzomys chacoensis,* Ol = *O. longicaudatus,* Bo = *Bolomys obscurus,* Aa = *Akodon azarae.* Viruses: BCCV = Black Creek Canal, BAY = Bayou, ELMC = El Moro Canyon, SN = Sin Nombre, NY = New York, PH = Prospect Hill.

Results

Phylogenetic relationship between newly recognized South American hantaviruses. From a total of 19 HPS cases and 85 rodent samples tested, 13 and 36 hantavirus sequences were amplified, respectively. Phylogenetic analysis (figure 2) of nt sequence differences in a 292-nt fragment of the G2 glycoprotein–encoding region of the virus M genome segment (bases corresponding to LEC 2815–3106) revealed a significant genetic diversity between the samples from the three distant geographic regions, as well as within each geographic region studied. The analysis also indicated that most of these hantavirus genotypes are genetically different from other previously characterized hantaviruses. Both neighbor-joining (figure 2) and maximum parsimony (data not shown) phylogenetic analyses of the 292-nt fragment confirmed the existence of 7 phylogenetically distinct hantavirus lineages (LEC, Bermejo [BMJ], ORN, Hu39694, AND, MAC, and PRG); bootstrap analysis and a standard error test provided good support for these nodes. One group of these hantavirus sequences was closely related to the previously known AND hantavirus, on the basis of high sequence similarity in an overlapping 150-nt fragment to the published AND M segment sequence. However, the genetic sequences of the 6 other groups did not match any published

hantavirus sequences. Of these 6 genotypes, LEC and BMJ appear to be closely related to each other.

The 7 hantavirus genotypes are described as follows: (1) LEC genotype: Comparison of the nt sequences recovered from 11 HPS cases and 13 virus sequences from *O. flavescens* from 9 different locations in central Argentina (up to 200 km apart) demonstrated a direct genetic link between hantaviruses associated with human HPS cases and with *O. flavescens* infection (96.6% nt identity). The genotype diverged 11.5%–17.7% at the nt level from the northwestern virus genotypes, 17.7%–21.3% from other central and southwestern genotypes, and 23.8%–26.1% from Black Creek Canal, Bayou, and Sin Nombre (table 2) [28]; (2) BMJ genotype: This genotype, which was recovered from *O. chacoensis* from Oran, is closely related to the LEC genotype (11.5% nt divergence). The genotype diverged 17.8%–21.8% from other Argentinian hantaviruses; (3) ORN genotype: Comparison of hantavirus sequences from 2 *O. longicaudatus* and 1 HPS case from northwestern Oran showed a direct genetic link between them (98.6% nt identity). The ORN genotype differed 15.4%–21.5% from other Argentinian hantavirus genotypes; (4) Hu39694 genotype [28]: The hantavirus sequence from the HPS case who was residing in Pergamino but who was uncertain about the site of exposure diverged 16.4%–20.7% from the other genotypes. This virus genotype will be referred to as Hu39694 until its geographic distribution and reservoir can be determined; (5) AND genotype: Virus sequences from 6 *O. longicaudatus* from the southwestern locations of El Bolson and Lake Puelo showed 94.2% nt sequence identity with AND virus, a previously characterized hantavirus from a fatal human HPS case in the same area [25]. This virus diverged 19.3%–21.8% at the nt level from the other Argentinian genotypes; (6) MAC genotype [28]: This distinct hantavirus genotype was identified from 2 *B. obscurus* from Maciel and Oliveros, the northern-most locations studied in central Argentina. The nt sequence for MAC diverged 20.4%–21.8% from the other Argentinian genotypes; (7) PRG genotype [28]: Virus nt sequences from 14 *A. azarae* from 10 locations in central Argentina were related to but distinct from (20.4%–21.8% divergence) the other Argentinian hantaviruses.

nt and amino acid sequence differences between the 7 hantavirus genotypes described above, based on the nt and amino acid sequence of a 1654-nt fragment of the M genome segment, are shown in table 2.

Phylogenetic relationship of South American hantaviruses to other hantaviruses. The phylogenetic relationship of newly recognized South American virus genotypes to previously characterized hantaviruses was determined on the basis of the nt sequence differences among the 1654-nt fragments of the M segment (figure 3) and among the complete virus M segments (for LEC, ORN, and Hu39694; data not shown). Both maximum parsimony and neighbor-joining phylogenetic analyses of the 1654-nt fragment of the M segment (positions 1456–3109 of the LEC sequence) were performed. The neighbor-joining tree and the single maximum parsimony tree obtained displayed very similar topology (figure 3A, B). Although divergent from each other, all 6 newly described hantavirus genotypes together with AND virus form a unique, well-supported clade on the phylogenetic tree. This new clade diverges at the same node as 3 other groups of Sigmodontinae-borne New World hantaviruses: *Orizomys* or *Sigmodon* species–borne viruses (Bayou and Black Creek Canal), *Peromyscus* species–borne viruses (Sin Nombre and New York), and *Reithrodontomys* species–borne viruses (El Moro Canyon). Although bootstrap support for Argentinian, Sin Nombre/New York, and Bayou/Black Creek Canal clades is strong (100%, 98%, and 96%, respectively), both maximum parsimony and neighbor-joining analyses, as well as phylogenetic analysis of the entire M segments, were unable to resolve unambiguously the phylo-

Table 2. Comparison of the nucleotide (above dashes) and amino acid (below dashes) sequences from a 1654-nucleotide fragment of the M segment of 7 Argentinian hantaviruses (LEC, BMJ, ORN, AND, Hu39694, PGM, and MAC) with other known hantaviruses.

Virus	LEC	BMJ	ORN	AND	Hu39694	PRG	MAC	SN	BAY	BCC	TUL	PUU	SEO	HTN
LEC	—	88.5	82.3	79.7	82.3	80.0	78.7	76.2	73.9	73.9	70.9	69.6	63.6	62.1
BMJ	98.4	—	82.2	79.3	81.9	79.6	78.2	74.7	73.5	75.2	70.0	68.5	64.0	62.5
ORN	95.4	97.1	—	80.0	83.6	78.5	79.3	74.9	74.6	74.9	71.1	68.3	63.3	61.2
AND	95.4	94.5	95.3	—	80.7	78.2	79.6	75.1	75.3	75.2	70.3	68.9	63.3	62.2
Hu39694	95.5	97.3	94.1	94.7	—	79.3	79.4	75.0	74.0	73.6	69.2	68.6	63.1	61.4
PRG	93.5	92.9	94.0	92.0	93.5	—	78.2	74.8	73.5	74.2	70.6	70.0	64.1	63.2
MAC	91.6	91.1	91.7	90.6	91.3	92.6	—	74.3	73.5	74.1	69.6	68.7	62.7	62.2
SN	86.0	84.8	84.9	85.6	85.5	84.6	83.8	—	75.3	75.0	71.8	69.0	62.2	61.2
BAY	84.6	83.5	84.8	84.6	84.8	83.7	82.9	87.1	—	79.6	69.9	66.9	63.9	63.4
BCC	83.8	83.3	83.8	83.1	83.7	82.9	82.4	85.1	91.3	—	70.9	68.1	63.4	61.3
TUL	75.5	74.9	75.8	75.5	75.5	76.5	74.5	77.3	75.8	75.6	—	74.3	62.4	61.6
PUU	73.7	73.5	73.3	73.5	73.3	74.0	73.0	73.3	71.1	71.5	81.5	—	62.1	61.0
SEO	62.2	61.8	61.6	61.6	62.2	61.5	60.7	59.1	61.2	60.0	59.5	59.5	—	75.4
HTN	62.0	61.6	61.5	61.6	61.8	62.4	61.4	59.8	61.3	59.8	59.5	59.3	82.6	—

NOTE. Virus strains: LEC = Lechiguanas, BMJ = Bermejo, ORN = Oran, AND = Andes, Hu = human, PRG = Pergamino, MAC = Maciel, SN = Sin Nombre, BAY = Bayou, BCC = Black Creek Canal, TUL = Tula, PUU = Puumala, SEO = Seoul, HTN = Hantaan.

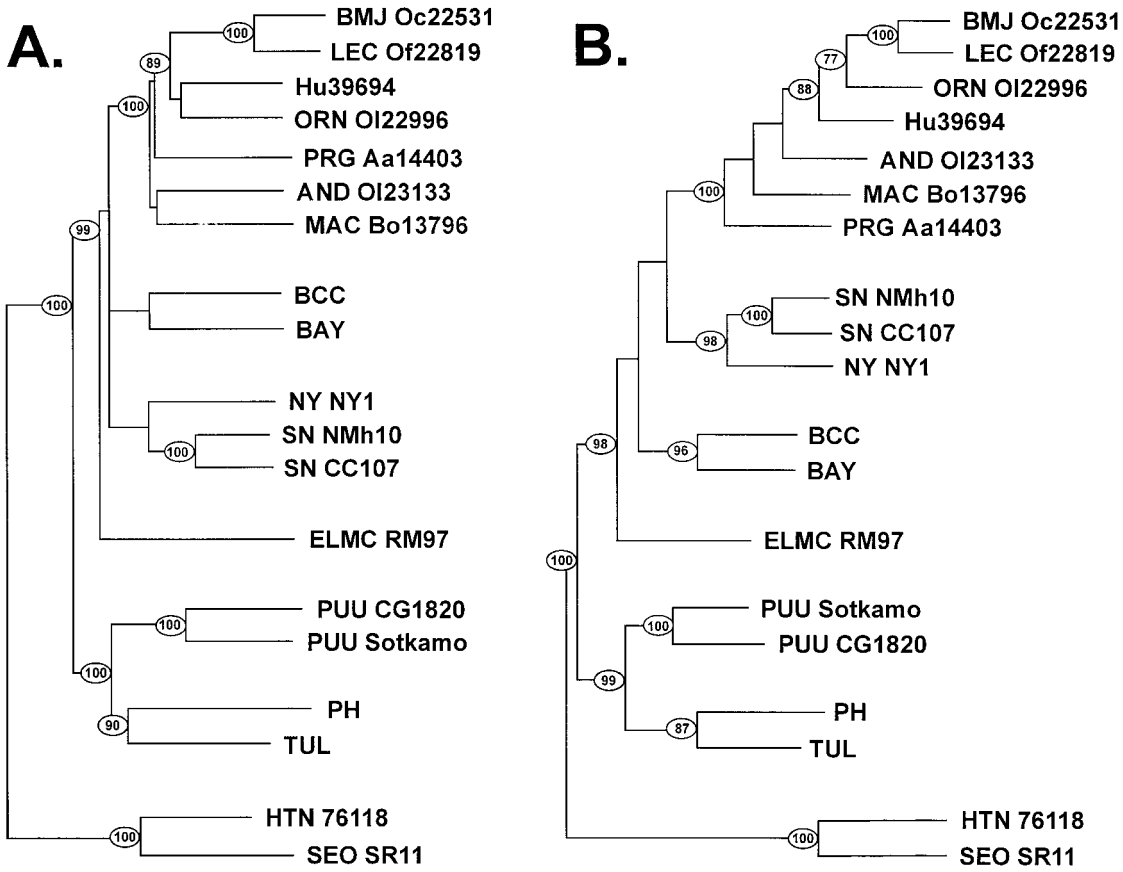

Figure 3. Phylogenetic analysis of 1654-nucleotide fragment (bases 1456–3109) of M genome segment. For major branches, bootstrap confidence limits (from 1000 repetitions) are indicated at node. Major nodes with bootstrap limit of <75%, have no no. indicated. **A**, Analysis with neighbor-joining method, using Kimura 2-parameter algorithm. All bootstrap nos. listed also represent nodes with SE value >95%. **B**, Analysis using maximum parsimony, heuristic search method with weighting of 4:1 for transversions over transitions. Viruses: BMJ = Bermejo, LEC = Lechiguanas, ORN = Oran, PRG = Pergamino, AND = Andes, MAC = Maciel, BCC = Black Creek Canal, BAY = Bayou, NY = New York, SN = Sin Nombre, ELMC = El Moro Canyon, PUU = Puumala, PH = Prospect Hill, TUL = Tula, HTN = Hantaan, SEO = Seoul.

genetic relationship between El Moro Canyon virus and the other members of this branch (figure 3A, B).

In the new clade of Argentinian hantaviruses, 2 genotypes, *Oligoryzomys*-borne LEC and BMJ, are closely related to each other, and together with Hu39694 and *Oligoryzomys*-borne ORN genotypes, they form a well-supported subclade (88% bootstrap support). Of interest, *Oligoryzomys*-borne AND virus appears to be positioned outside this group.

The neighbor-joining and maximum parsimony phylogenetic analyses of the complete virus M segments, which included those of the LEC, Hu39694, and ORN genotypes, did not reveal any changes in the position of the clade containing these *Oligoryzomys*-borne lineages (data not shown).

Genetic characterization of LEC, ORN, and Hu39694 M genome segments. The complete sequences of LEC, ORN, and Hu39694 M genome segments were determined to be 3653, 3646, and 3654 nt in length. These numbers include: three, three, and four 3′ end nt for LEC, ORN, and Hu39694, which, due to the natural length heterogeneity of the 3′ viral RNA ends in RNA preparations [41], were not resolved by directly sequencing PCR products of ligated M segment termini but were inferred from the hantavirus consensus sequence [42]. The glycoprotein precursor coding frame was found from position 53–3469 for LEC, ORN, and Hu39694 lineages and would be predicted to encode a glycoprotein precursor 1138 amino acids in length.

The nt identities among LEC, ORN, and Hu39694 range from 82.3% to 83.6%, and the deduced amino sequence identity is 94.1%–95.5%. These virus sequences appear to be most similar to the previously characterized Bayou, Black Creek Canal, and Sin Nombre (strains NMh10 and CC107) viruses at both the nt and protein levels (table 2). Alignment and comparisons of the predicted LEC, ORN, and Hu39694 virus glycoprotein precursor amino acid sequences with the deduced glycoprotein precursor sequences of previously characterized hantaviruses revealed conservation of the potential *N*-glycosyl-

ation sites (positions 138, 351, and 402 in G1 and position 931 in G2), the 57 cysteine residues, and the potential transmembrane regions of G1 and G2 proteins [9].

The major conserved type-specific linear epitope, which is reported to be located at aa 58–88 in Sin Nombre virus G1 protein [43], contains 12, 12, and 14 amino acid substitutions in the G1 of LEC, Hu39694, and ORN viruses, respectively, compared with the Sin Nombre virus protein sequence, and 16, 16, and 17 amino acid substitutions, respectively, compared with the Bayou sequence. This makes it potentially useful for differential serodiagnostics of this new group of hantaviruses. The corresponding protein sequences of LEC and Hu39694 are identical to each other and differ at four amino acid positions from the ORN virus genotype. Whether these substitutions lead to a change in the immunologic specificity of this epitope remains to be determined.

Discussion

Since the identification of Sin Nombre virus as a causative agent of HPS in 1993, the public has become increasingly aware of the extensive presence in New World sigmodontine rodents of numerous hantaviruses that are yet to be identified and that are potentially pathogenic for humans (reviewed in [1, 2]). Original scientific efforts were focused primarily on the investigation of genetic diversity and pathogenic potential of North American hantaviruses and their association with specific rodent hosts [10–14]. However, considering the narrow host range of hantaviruses and the high genetic diversity of sigmodontine rodents in South America [44], a larger number and a greater genetic diversity of hantaviruses might be found on this subcontinent.

Epidemiologic [24, 45] and serologic [23, 26, 46, 47] data obtained during the last decade indicate the presence of hantaviruses in indigenous South American rodents and the existence of HPS-like human disease in several South American countries. Recently, there has been partial genetic characterization of several new, genetically distinct hantaviruses: a partial S genome sequence of Rio Mamore virus amplified from *O. microtis* from Bolivia [27], the partial S and M genome sequences of AND virus recovered from an autopsy sample of a person with HPS in southern Argentina [25], and 4 genetically distinct hantavirus genotypes (LEC, PRG, MAC, and Hu39694) in central Argentina that were identified in a recent report from our laboratory [28] of the phylogenetic analysis of a 147-nt region of the virus M genome segment.

Serologic surveys of rodents and diagnosis of human HPS cases during the last 3 years by the National Institute of Human Viral Diseases (Pergamino) resulted in the identification of three major foci of hantavirus infections in Argentina and indicated the presence of hantaviruses in several common sigmodontine rodent species [26, 46, 47].

Genetic analysis of the virus M genome segment from 3 geographic regions of Argentina, which are separated by >4000 km, revealed a high genetic diversity of hantavirus sequences between and within these regions. The phylogenetic analysis of a 292-nt fragment of the M genome segment indicated that at least 7 genetically distinct hantavirus genotypes circulate in Argentina. Comparisons of the nt sequences of a 1654-nt fragment of the M genome segments determined for 1 representative of each of these 7 virus genotypes (LEC, BMJ, ORN, Hu39694, MAC, PRG, and AND) indicated an nt difference of 11.5%–21.8% between different genotypes within this group and of at least 23.8% from the corresponding M genome segment region of Bayou, Black Creek Canal, and Sin Nombre (the most closely related of the North American hantaviruses; table 2). The highest genetic diversity was observed in a limited geographic region of central Argentina, where 4 distinct genotypes were recovered from 3 different rodent species and human autopsy samples, possibly because of the more extensive sampling from this region (80/104 total samples).

The phylogenetic analysis of the nt sequences of the 1654-nt fragment shows a branching order similar to that of the tree obtained with the 292-nt fragment (figures 2, 3). All the Argentinian hantaviruses form a new, distinct, well-supported branch, which diverges from the same ancestral node as 3 other groups of New World Sigmodontinae-borne hantaviruses: *Peromyscus*-borne Sin Nombre–like viruses, *Oryzomys*- or *Sigmodon*-borne Bayou and Black Creek Canal viruses, and *Reithrodontomys*-borne El Moro Canyon virus. Phylogenetic analyses of the 1654-nt M genome fragment, as well as that of the entire M genome segments (available for LEC, Hu39694, and ORN), consistently fail to resolve the phylogenetic relationship between the 4 major groups of New World Sigmodontinae-borne hantaviruses. Considering the lengths of sequences and number of taxa analyzed, this lack of resolution may not be due to insufficient data but may reflect the rapid radiation of New World hantaviruses from a common ancestor in a historically short period of time.

Within the clade of newly recognized South American hantaviruses, LEC and BMJ virus genotypes appear to be more closely related (11.5% nt sequence differences), and together with Hu39694 and ORN genotypes they form a well-supported subclade (figure 3A, B). This subclade includes *Oligoryzomys*-borne hantavirus genotypes from both northwestern (BMJ, associated with *O. chacoensis,* and ORN, associated with *O. longicaudatus*) and central (LEC, associated with *O. flavescens*) Argentina. This is consistent with the hypothesis that cospeciation of hantaviruses with their specific rodent hosts is a predominant pattern in the evolution of these viruses (reviewed in [1, 2, 48]). Hu39694 virus genotype was recovered from the blood of a person with HPS who was residing in central Argentina but who was uncertain of the site of exposure. However, considering this patient's travel history and possible rodent exposure before the onset of disease, he may have acquired infection somewhere in Buenos Aires or Santa Fe Provinces, in central Argentina. The natural rodent host for this hantavirus genotype has not yet been identified. However, on

the basis of the position of this genotype on the phylogenetic tree, it will possibly be another *Oligoryzomys* species from central Argentina.

Amino acid and nt sequence differences among this subclade of Argentinian hantaviruses are up to 5.9% and 16.4%, respectively (table 2). These differences are as great as those observed among North American Sin Nombre–like hantaviruses, which, although closely related to each other, are associated with distinct species or subspecies of closely related *Peromyscus* rodents [10, 11, 39, 49] and have been described by others as distinct virus species [39, 49]. Further studies of South American hantaviruses and their rodent hosts are needed to determine whether LEC, BMJ, ORN, and Hu39694 genotypes are taxonomically distinct entities (i.e., separate virus species) or divergent strains of a single novel virus. The phylogenetic relationship between this *Oligoryzomys*-borne subclade and other Argentinian hantaviruses and among PRG, MAC, and AND virus genotypes is not resolved by the current phylogenetic analyses.

Of interest, the previously recognized, highly pathogenic AND virus was recovered in southwestern Argentina from the same rodent species *(O. longicaudatus)* as ORN virus in the northwestern region of the country, but it is positioned on the phylogenetic tree outside the clade containing other *Oligoryzomys*-borne virus genotypes. Considering that the two locations are separated by ~4000 km, a possible explanation could be that biogeographic factors have played a role in constituting the AND virus genotype. *O. longicaudatus* is known to inhabit the Andes mountain range from northern Salta and Jujuy Provinces to southern Tierra del Fuego Province [44]. The original exploration of such a wide geographic range may have involved extensive migrations over a long period of time, and this alone may be enough to explain the divergence of AND virus from its northwestern counterpart.

A similar situation exists in Europe, where Puumala virus has undergone cospeciation with different populations of *Clethrionomys glareolus,* which are widely distributed from France to the Ural mountains, and from Fennoscandia to the Balkans, resulting in the same range of nt sequence divergence among geographically distant virus strains [1, 36, 50–52]. However, since the ORN genotype was recovered from only 2 *O. longicaudatus,* the possibility of circulation of this virus genotype in another rodent species and spillover into *O. longicaudatus* cannot be completely excluded. Another attractive possibility is that rodents morphologically identified as *O. longicaudatus* are in fact 2 different sibling species in the north and south of the country. Experiments investigating intraspecific phylogenetic relationships of *O. longicaudatus* are ongoing.

Comparisons of nt and corresponding amino acid differences among *Oligoryzomys*-borne hantavirus genotypes and other newly recognized Argentinian hantavirus lineages associated with non-*Oligoryzomys* rodent species (PRG and MAC) show that while the range of nt divergence within the *Oligoryzomys*-borne group of hantavirus genotypes (up to 19.3%) and among the entire group of Argentinian hantaviruses (up to 21.8%) is similar, the corresponding range of amino acid sequence divergence is different (up to 5.9% and up to 9.4%, respectively; table 2). The higher conservation of the surface glycoprotein amino acid sequences among geographically distant and phylogenetically distinct *Oligoryzomys*-borne hantavirus

Table 3. Rodent species from South America, associated hantavirus strains, and hantavirus pulmonary syndrome (HPS) in humans.

Rodent species*	Location	Virus strain	Disease	Mortality[†]
Oligoryzomys flavescens (rice rat)	Central Argentina	LEC	HPS	26%
Oligoryzomys chacoensis (no common name)	Northwestern Argentina	BMJ	None described	
Oligoryzomys longicaudatus (long-tailed mouse)	Northwestern and southwestern Argentina	ORN AND	HPS HPS	33% 58%
Oligoryzomys microtis[‡] (pygmy rice rat)	Bolivia	RM	None described	
Akodon azarae (grass field mouse)	Central Argentina	PRG	None described	
Akodon varius (raton variado)	Northwestern Argentina	None found	None described	
Bolomys obscurus (dark field mouse)	Central Argentina	MAC	None described	
Holochilus brasiliensis (South American water rat)	Central Argentina	None found	None described	
Abrotrix longipilis (long hairy mouse)	Southwestern Argentina	None found	None described	
Calomys laucha[§] (vesper mouse)	Paraguay	LN	HPS	?

NOTE. Virus strains: LEC = Lechiguanas, BMJ = Bermejo, ORN = Oran, AND = Andes, RM = Rio Mamore, PRG = Pergamino, MAC = Maciel, LN = Laguna Negra.

* No. of rodents of each species tested is given in Materials and Methods section. All rodents tested by polymerase chain reaction were positive for hantavirus antibody by ELISA.

[†] D. Enria, unpublished data.

[‡] [28].

[§] [21].

genotypes may reflect an original evolutionary adaptation to the internal genetic environment of *Oligoryzomys* rodents. This would suggest that both biogeographic factors and cospeciation with specific rodent hosts have been involved in the evolution of these hantavirus lineages. Unfortunately, the nt sequence of Rio Mamore virus recovered from *O. microtis* from Bolivia [27] is not available from GenBank for comparison.

Most of the *Oligoryzomys*-borne hantavirus genotypes described in this report, including LEC from central Argentina, ORN from northwestern Argentina, and AND virus from southwestern Argentina, have the ability to cause HPS with a high mortality in humans (table 3). The pathogenicity of the BMJ genotype has not been evaluated since it has not been linked to any human disease. However, on the basis of the known pathogenic potential of its closest relatives, it is likely that this genotype may be capable of causing severe HPS in humans.

MAC and PRG hantavirus genotypes were discovered in the non-*Oligoryzomys* rodents *(B. obscurus* and *A. azarae)* and are likely to represent true novel virus species. These are not currently linked to human disease. Whether this reflects lower pathogenicity of these 2 viruses or differences in the rodent behavior, resulting in reduced contact with humans, remains to be determined.

Viral sequences described in this paper can be obtained from GenBank. Accession numbers are AF028029–AF028063 for the 292-nt fragments of hantavirus M segment, AF028025–AF028028 for the 1654-nt fragments of the M genome segment, and AF028022–AF028024 for the complete M genome segments of LEC, ORN, and Hu39694 viruses.

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