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Hantavirus Pulmonary Syndrome Outbreak in Argentina: Molecular Evidence for Person-to-Person Transmission of Andes Virus

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An increase of Hantavirus Pulmonary Syndrome (HPS) cases around a southwestern Argentina town and in persons living 1400 km away but in contact with those cases was detected during the spring of 1996. In order to evaluate person-to-person transmission we compared the homology of PCR-amplified viral sequences of 26 Argentine and Chilean cases. Sixteen of them were epidemiologically linked cases and had the same sequence (Epilink/96) in the S segment 3′ noncoding region and in the M segment partial G1 and G2 region (a total of 1075 nucleotides). Contrarily, two geographical and contemporary but nonepidemiologically related cases differed from Epilink/96 in the compared regions. No significant differences, such as glycosylation or hydrophilic pattern, were found between Epilink/96 and the other sequences. Nucleotide and deduced amino acid sequence homologies between samples from southern Argentina and Chile ranged from 90.9 to 100% and 96.4 to 100%, respectively. Phylogenetic analysis revealed that all the analyzed southwestern viruses belong to the Andes lineage. Although human infection principally occurs via inhalation of contaminated rodent excreta, our results with Andes virus show the first direct genetic evidence of person-to-person transmission of a hantavirus.

Key Words: Bunyaviridae; Hantavirus Pulmonary Syndrome; person-to-person transmission; Argentina; Chile.

INTRODUCTION

Hantaviruses, members of the family Bunyaviridae, are enveloped trisegmented, negative-strand RNA viruses. Hantaviruses are carried primarily by specific rodents or insectivore reservoirs and they are not spread by insects, birds, or humans. Several hantaviruses have been associated with zoonotic illness.

The three Old World hantaviruses, Hantaan (HTN), Seoul (SEO), and Puumala (PUU), are important causative agents of hemorrhagic fever with renal syndrome (HFRS) in China, Korea, Russia, and Europe (Plyusnin *et al.,* 1996). The mortality in HFRS ranges from 3–7% for HTN infection to 0.1–0.2% for PUU (Lee *et al.,* 1990; Mustonen *et al.,* 1994).

In 1993 another type of pathology was associated with hantaviruses infection in America. Hantavirus Pulmonary Syndrome (HPS) was characterized by febrile prodrome progressing to sudden onset of severe noncardiogenic pulmonary edema followed by bilateral pulmonary infiltrates and, in about 50% of cases, death (Elliott, 1994). This syndrome is caused by several hantaviruses, such as Sin Nombre virus (SN) (Nichol *et al.,* 1993; Duchin *et al.,* 1994) associated with *Peromyscus maniculatus* (Childs *et al.,* 1994), Black Creek Canal virus (BCC) associated with *Sigmodon hispidus* (Ravkov *et al.,* 1995), Bayou virus associated with *Orizomys palustris* (Morzunov *et al.,* 1995), and New York virus (NY) associated with *Peromyscus leucopus* (Hjelle *et al.,* 1995). The list of hantavirus is rapidly increasing and several more types are being characterized at the moment.

The determinants of hantavirus pathogenicity are poorly understood. For hantaviruses belonging to the same type, variants with palmitoylation differences (Isegawa *et al.,* 1994) or reassortant variants (Schmaljohn *et al.,* 1995; Plyusnin *et al.,* 1996) have been proposed to possess a higher virulence.

Human exposure to hantaviruses principally occurs through the respiratory tract route, via accidental inhalation of contaminated rodent urine, feces, or saliva. In the United States the epidemiology of HPS closely parallels the ecology of its rodent hosts, where the majority of HPS patients have had clearly identifiable peridomestic recreational or occupational exposure to rodents (Khan *et al.,* 1996; Nichol, 1996). Person-to-person transmission has not been previously demonstrated. An evaluation done between health care workers exposed to patients with confirmed HPS (Vitek *et al.,* 1996) and a recent review of HPS clusters associated with SN virus suggest that infection is rarely if ever transmitted from person to person (Wells *et al.,* 1997b). With regard to South America, serological evidence of human infection with hantavirus in Argentina, Bolivia, and Uruguay had already been assessed (Weissenbacher *et al.,* 1996; Parisi *et al.,* 1996), although the use of heterologous hantavirus antigens to

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identify antibodies may have underestimated the actual prevalence of infection. In the last few years HPS cases have been reported in Argentina (Levis *et al.,* 1995), Brasil (Iversson *et al.,* 1995), Chile (López *et al.,* 1997), Paraguay (Williams *et al.,* 1996), and Uruguay (unpublished data).

Phylogenetic analysis of viruses from Argentina and Chile HPS cases showed that they were related to a newly recognized hantavirus referred to as Andes virus (López *et al.,* 1996, 1997).

An important increase of HPS cases, from 6 during 1995 to 20 in the spring of 1996, was detected 150 km around El Bolsón, including Bariloche and Esquel towns (southwestern Argentina). Two additional physicians, from Buenos Aires city, one with no history of being in the south region, were in contact with El Bolsón cases and got the disease in the same period. Epidemiological analysis suggested that the infections may have occurred via person-to-person transmission (Enria *et al.,* 1996; Wells *et al.,* 1997a; Yadón, personal communication).

This paper evaluates person-to-person transmission of Andes hantavirus by either nosocomial or house contact, using comparative analysis between different viral sequences of human cases that occurred during this outbreak (September 1996 to January 1997).

RESULTS

Comparative sequence analysis

Seropositive HPS human cases were examined for the presence of viral genetic material by nested and heminested RT–PCR. cDNA from all of them was successfully amplified from RNA extracted from autopsied tissues, serum, or blood samples. At least partial sequence comparisons were made among contact cases, temporally, geographically, and nongeographically clustered cases, and with previously characterized hantavirus cases.

Sequences of PCR products were obtained from both the M and S segments of viral RNA in 16 epidemiologically linked HPS human cases, in 9 from the same area and surroundings, including 3 already published and 1 case from Salta (Table 1). We examined G1a, the amino terminal region of G1, and a G2-encoding M segment fragment because they have been extensively used in hantavirus comparisons (Xiao *et al.,* 1994; Henderson *et al.,* 1995). We also analyzed the 3′ NCR region of the S segment, which had been shown to be useful for studying hantavirus evolution (Spiropoulou *et al.,* 1994; Plyusnin *et al.,* 1995). To lessen the probability of chance sequence identity between sequences, G1b, another reported variable region of the G1 gene (Johnson *et al.,* submitted for publication), was used.

Sequencing was performed on both DNA strands, giving consistent results. Since the sequences were obtained by directly sequencing the RT–PCR product, they represent the average sequence present, and the differences observed cannot be attributed to enzyme errors, which may occur during the amplification procedure. Moreover, multiple reiterations with the same sample but different preparations of RNA or other organs yield the same results (data not shown).

Percentage identities of both the nucleotide and the amino acid sequences of the M segment G1a, G1b, and G2 encoding regions (Table 2) and nucleotide sequences of the 3′ NCR region of the S segment (Table 3) were compared with previously published sequences of other hantaviruses.

Sixteen cases (I, A, B, P, L, Q, K, J, H, F, O, N, C, M, E, D), designated Epilink/96, had the same sequence in the M segment G1a, G1b, G2, and in 3′ NCR S fragment. The two El Bolsón human case sequences, G and T, belonging to contemporary but nonepidemiologically related patients, differed from Epilink/96. T differed in 1 of 167 nts in the G2 fragment and in 2 of 143 nts in the S fragment, and it was identical in G1a, whereas G differed in 8 of 167 nts in the G2 fragment and in 4 of 172 nts in the amino terminal portion of G1a. None of the viruses from southern Argentina and Chile studied differed from each other by more than 9.1% in either M or S analyzed fragments. ESQ-1/96 had identical sequences to Epilink/96 in G1b and G2, but a different sequence in G1a and also differed in another G2 fragment (nts 2053 to 2390), where two mutations were found, and in 3′ NCR region of the S segment. It was proposed that the collection time of specimens containing virus is a less important factor than the geographical origin of the strain (Plyusnin, *et al.,* 1995). Since ESQ-1/96 lived in Esquel but he visited El Bolsón in the summer of 1996, 30 days before the onset, this could explain the similarities between ESQ-1/96 and Epilink/96. In all viruses from southern Argentina and Chile the length and the insertion deletion patterns in the partial 3′ NCR S segment were conserved and the CTACCTCA motif was found three times without any variation.

Phylogenetic analysis

In order to study phylogenetic relationships, sequences of the amino terminal region of the G1 fragment (nts 88–259) and the G2 fragment (nts 2780–2946) of the M segment, which were available for all cases, were analyzed.

Parsimony trees constructed from G1 + G2 (Fig. 1) illustrated that Argentine and Chilean sequences clustered together with the Andes AH1 virus. The results were supported by a very high bootstrap value of 99%. The case from northern Argentine SAL-1/96 is closely related to viruses in the more divergent cluster. Argentine and Chilean sequence cluster is related to the Paraguayan Laguna Negra (LN) virus. The South American sequences showed the highest degree of similarity with that of BAY and BCC virus. Epilink/96 was closer to ESQ-1/96 and T sequences than to the rest. Moreover,

TABLE 1

HPS Cases Compared in This Study

Case	Residence	Relationship and type of contact with previous cases up to 45 days before onset	Date of onset	Date of death
I[a]	El Bolsón	NKC.	9-22-96	9-26-96
A[a]	El Bolsón	Doctor of I.	10-12-96	10-20-96
B[a]	El Bolsón	I's mother.	10-13-96	10-19-96
P	Bariloche	Visiting Bariloche Private Hospital at the same time El Bolson cases were hospitalized.	10-13-96	10-22-96
L	El Bolsón	Housekeeper of I and B.	10-21-96	10-25-96
Q[a]	Bariloche	Spouse of P. Staying at Bariloche Private Hospital giving birth when El Bolson cases were being transferred.	10-22-96	11-2-96
K	El Bolsón	I's friend. Visited him when hospitalized.	10-23-96	
J	Buenos Aires[b]	I's brother-in-law. Stayed at B's home during her funeral. Traveled with L and H in a car.	10-31-96	
H	Buenos Aires[b]	I's sister, spouse of J. Stayed at B's home during her funeral. Traveled with L and J in a car.	11-4-96	
F	El Bolsón	Doctor of I, B, and G; daily contact with A.	11-7-96	
O	Bariloche	Visiting a non-HPS case patient at Bariloche Private Hospital.	11-7-96	11-13-96
N	Bariloche	Bariloche Private Hospital receptionist when El Bolsón cases were hospitalized. Friendly relation with P and Q.	11-8-96	11-17-96
C	El Bolsón	Spouse of A, transferred to Buenos Aires Hospital.	11-8-96	
M	Buenos Aires	J's and H's daughter. Traveled by car with L, J, and H (No history of visiting the area).	11-28-96	
E	Buenos Aires[b]	Friend of C, also a doctor. Took care of her daily at Buenos Aires Hospital.	11-28-96	12-16-96
D	Buenos Aires	Doctor of C at Buenos Aires Hospital.	12-5-96	
AH1[a]	El Bolsón	AH1's father (not included in the study).	4-22-95	4-29-95
ESQ-1/96	Esquel[b]	NKC.	2-9-96	2-14-96
CH-1/96	Chile	NKC.	2-23-96	2-28-96
G	El Bolsón	NKC.	9-23-96	
T	El Bolsón	NKC.	10-4-96	
U (ESQ-2/96)	Esquel	NKC.	11-20-96	11-22-96
SAL-1/96	Orán	NKC.	12-6-97	
ESQ-3/96	Esquel	NKC.	12-19-96	12-24-96
CH-2/96	Chile	NKC.	12-19-96	12-26-96
CH-3/96	Chile	NKC.	1-7-97	1-20-97

Note. NKC, no known contacts with HPS cases. Cases shown in bold letters correspond to Epilink/96.

[a] Living with another HPS case. Housemate case groups: (AH1 and his father); (I, his mother B, and their housekeeper L); (P and his spouse Q), (A and his spouse C); (M and their parents J and H).

[b] Visitors to El Bolsón.

both trees constructed with G1 and G2 gave similar topologies. Chilean sequences did not form a definite cluster; however, travel records of these cases were incomplete.

Although the amino terminal region of N protein was previously utilized for phylogenetic analysis (Hjelle *et al.,* 1994), few sequence differences among different cases were found in this region (data not shown).

Encoded protein features

Despite relative nucleotide sequence diversity among G1 and G2 fragments of South American viruses, deduced amino acid sequences were highly conserved, indicating a strong evolutionary pressure to maintain the sequence integrity. This fact may indicate that the reservoir for the virus is the same species. Averages of all pair comparisons among viral sequences from southern Argentina and Chile revealed that nearly 88% of the nucleotide differences were in the third codon position and most corresponded to G-A or C-T transitions.

No amino acid differences were found between all samples in the G1a region. In G1b, Epilink/96 and the closely related ESQ-1/96 differed in one amino acid from the other sequences from southern Argentina and Chile (I instead of T at residue 642).

In G2 Epilink/96, ESQ-1/96, and case T had an A instead of a T at residue 939 (Fig. 2). AH-1/96 and G differed from the other sequences in one amino acid, a V instead of I at residue 914, and SAL-1/96 differed from the other sequences from southern Argentina and Chile in two amino acids. No significant differences, such as glycosylation signals, hydrophilicity patterns, or evidence

TABLE 2

Nucleotide and Amino Acid Sequence Homologies between Partial M Segments of Southern Argentine, Chilean, and Two Characterized North American HPS Viruses

M fragment (% identity)	BCC			BAY			CH-1/96			CH-2/96			CH-3/96			ESQ-1/96			U (ESQ-2/96)			ESQ-3/96			AH-1			Epilink/96			T[a]		G[a]	
	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a	G1b	G2	G1a*	G2	G1a*	G2
BCC	—	—	—	74	80.9	79.0	64.6	70.4	74.3	62.4	73.9	73.7	63.2	71.2	73.1	64.0	72.0	74.3	63.2	74.3	73.7	63.2	74.3	74.9	64.0	73.5	75.5	64.2	72.0	74.3	61.6	73.7	62.2	75.5
BAY	80.2	91.8	87.3	—	—	—	61.4	72.4	70.7	61.8	75.1	70.1	61.8	73.2	71.3	61.2	75.5	70.7	61.6	75.5	70.1	61.6	75.5	72.5	62.4	75.5	72.5	61.2	75.5	70.7	61.6	70.1	61.1	72.5
CH-1/96	56.8	82.4	76.4	52.7	82.4	76.4	—	—	—	92.9	93.0	94.6	93.1	93.8	92.8	93.5	93.4	94.6	92.1	94.2	94.6	92.7	94.2	94.6	92.5	94.6	93.4	93.3	93.4	94.6	92.4	85.6	91.3	93.4
CH-2/96	56.2	82.4	76.4	52.1	82.4	76.4	99.4	100	100	—	—	—	96.1	94.2	95.8	95.9	93.8	97.6	96.7	95.3	100	97.2	95.3	97.6	95.3	95.7	95.2	95.7	93.8	97.6	95.9	94.0	97.1	95.2
CH-3/96	56.2	82.4	76.4	52.1	82.4	76.4	99.4	100	100	100	100	100	—	—	—	96.3	94.9	95.8	95.7	94.9	95.8	95.5	94.9	94.6	97.6	96.9	97.0	96.1	94.9	95.8	97.7	97.0	98.8	97.0
ESQ-1/96	56.2	81.2	74.6	52.1	81.2	74.6	99.4	98.8	98.2	100	98.8	98.2	100	98.8	98.2	—	—	—	95.9	94.6	97.6	96.1	94.6	96.4	96.3	95.7	95.2	99.8	100	100	100	95.2	98	95.2
U (ESQ-2/96)	56.2	82.4	76.4	52.1	82.4	76.4	99.4	100	100	100	100	100	100	100	100	100	98.8	98.2	—	—	—	99.2	100	97.6	95.3	96.5	95.2	95.7	94.6	97.6	94.8	99.4	95.9	95.2
ESQ-3/96	56.2	82.4	76.4	52.1	82.4	76.4	99.4	100	100	100	100	100	100	100	100	100	98.8	98.2	100	100	100	—	—	—	95.1	96.5	94.0	95.9	94.6	96.4	95.9	97.0	95.9	94.0
AH-1	56.2	82.4	78.2	52.1	82.4	78.2	99.4	100	98.2	100	100	98.2	100	100	98.2	100	100	96.4	100	100	98.2	100	100	98.2	—	—	—	—	96.1	95.7	95.2	98.3	95.8	99.4	100
Epilink/96	56.2	81.2	74.6	52.1	81.2	74.6	99.4	98.8	98.2	100	98.8	98.2	100	98.8	98.2	100	100	100	100	98.8	98.2	100	98.8	98.2	100	98.8	96.4	—	—	—	100	94.6	98	95.2
T	56.1[b]	NA	74.6	50.9[b]	NA	74.6	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	100	100[b]	NA	98.2	100[b]	NA	96.4	100[b]	NA	100	—	—	97.7	94.6
G	56.1[b]	NA	78.2	50.9[b]	NA	78.2	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	96.4	100[b]	NA	98.2	100[b]	NA	98.2	100[b]	NA	100	100[b]	NA	96.4	100	99.4	—	—

Note. Values above dashes are nucleotide sequence homologies and those below dashes are amino acid sequence homologies. NA, not available. G1a, G1b, and G2 regions, GenBank Accession numbers, and references of the sequences used here are described under Materials and Methods.

[a] G1b was not done.

[b] Only nts 88–259 were analyzed.

for reassortment, were found between Epilink/96 and the other cases in the regions sequenced.

DISCUSSION

Although all hantavirus variants circulating in the southern Argentine–Chilean region were genetically similar, it is remarkable that all 16 cases (Epilink/96) that had the same M segment (partial G1 and G2) and 3′ noncoding S segment sequences were obviously epidemiologically linked; each patient was in close contact (household, health caring, marital contact, and/or traveling together within a car) with one or more members of this group, as shown in Table 1 and previously reported (Enria *et al.,* 1996; Wells *et al.,* 1997a). These cases include patients from El Bolsón, Bariloche, and Buenos Aires. The last 2 cases were 130 and 1400 km from El Bolsón, respectively, where the index case (I) occurred. The detection of the same sequence in different people might be explained by infection with a genetically identical hantavirus. However, the more geographically distant the cases, the more unlikely it is that a unique source of infection or same variant within the rodent local populations occurred. Alternatively, infection could happen through person-to-person transmission.

Buenos Aires cases (E and D) were a friend and a doctor, respectively, of C. E was in the endemic area 50 days before the onset of disease, but D had never been there. Bariloche cases N, O, P, and Q occurred in people visiting or working at the Private Hospital to which many of the El Bolsón patients were transferred. All of the sequences were the Epilink/96 group. Case M had never visited El Bolsón, but traveled by car for 20 h with a symptomatic infected person (L) and stayed with her infected parents (J and H). No other HPS cases were detected in Buenos Aires or Bariloche during that time. Finding the same sequence in the patients and their contacts in the hospital and the lack of other HPS cases in these cities strongly suggests human-to-human transmission of the virus. Case M must also have been infected in a similar manner.

Knowledge of virus genotypes in the rodent populations would have provided more insight into potential routes of transmission. However, rodent trapping success rates near El Bolsón have been very low and no rodents were captured inside houses of the patients (Yadón, personal communication). Infected rodents captured in Lago Puelo, 15 km from El Bolsón, have yielded different sequences from the Epilink/96 cases (unpublished results). Two El Bolsón human case sequences belonging to contemporary but epidemiologically unrelated patients (T and G) differed from the Epilink/96 sequences in 3 of 482 nts, and 12 of 339 nts, respectively, suggesting the existence of a different source of infection.

The Epilink/96 sequence regions (1075 nts) did not change during continuous person-to-person transmis-

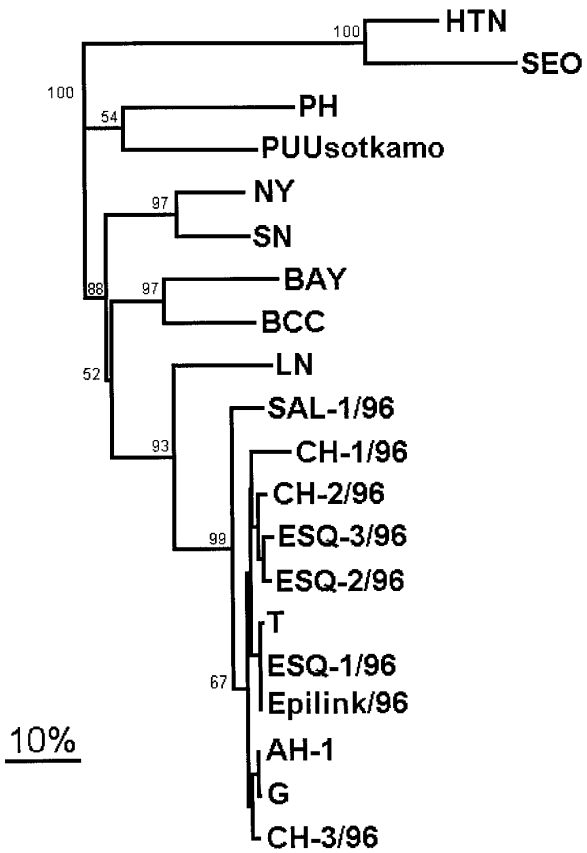

FIG. 1. Phylogenetic relationship of southwestern American and other hantaviruses based on nucleotide sequence differences in two M segment PCR fragments (nts 88 to 259 and nts 2780 to 2946). Sequences were analyzed by the maximum parsimony method. The percentage of bootstrap supporting each node is indicated at selected branch points and was obtained from 500 replicates. Horizontal distances indicate approximate nucleotide sequence percentage differences between virus variants. Vertical distances are for graphic representation only.

sion. A recent report (Hjelle *et al.,* 1996) suggests that in order to identify the precise place of infection a perfect matching of nucleotide sequences between humans and rodents is required (about 500 nts were used). A similar criteria could be required to demonstrate person-to-person transmission. Person-to-person transmission of hantaviruses has never been reported. However, a family case was recently detected in southern Chile where both parents died and their two children and one brother-in-law contracted the disease (CDC, 1997). Another family cluster was detected in Brazil (Zaparoli *et al.,* 1995). Clusters of SN infections have also been reported. In one of them, two of four infected adults living in the same house died (Duchin *et al.,* 1994). In another, a 4-year-old child and his mother were infected and the mother died 12 days after child's initial symptoms (Armstrong *et al.,* 1995). In all cases infected persons could have been exposed to rodent contact (Wells *et al.,* l997b). In contrast to SN cases, contact with rodents was not evident in the Argentine outbreak. Differences between Andes and SN virus outbreaks might be attributable to different biological properties and transmission potentials of the two viruses.

It is of interest that only one lineage has been found in 25 samples from Chile and Argentina, despite the large geographic distance (2200 km). In this regard infected *Oligoryzomis longicaudatus*, a potential reservoir of Andes virus, were found in both the El Bolsón and the Salta regions (Levis *et al.,* 1996).

The direct genetic evidence presented here strongly supports person-to-person transmission and confirms the results of epidemiological investigations (Enria *et al.,* 1996; Wells *et al.,* 1997a; Yadón *et al.,* personal communication). The data suggest the possible existence of three- and four-person transmission chains, assuming that only one rodent to human transmission event occurred.

Human infection with hantaviruses as well as other zoonoses is an accidental, dead-end event, which typically is irrelevant for the maintenance of virus in nature. There are a few notable exceptions, such as urban yel-

TABLE 3

Nucleotide Sequence Homologies between Partial S Segments of Southern Argentine, Chilean, and Two Characterized American HPS Viruses

S fragment* (% identity)	BCC	BAY	CH-1/96	CH-2/96	CH-3/96	ESQ-1/96	U (ESQ-2/96)	ESQ-3/96	AH-1	Epilink/96	T
BCC	—	74.8	76.2	73.4	75.5	75.5	76.2	75.5	76.2	76.2	75.5
BAY		—	79	81.1	77.6	79.7	82.5	81.8	79	83.2	79.7
CH-1/96			—	93	97.9	99.3	97.9	97.2	100	97.9	99.3
CH-2/96				—	90.9	93.7	92.3	91.6	93	93.7	93.7
CH-3/96					—	97.2	95.8	95.1	97.9	95.8	97.2
ESQ-1/96						—	98.6	97.9	99.3	98.6	100
U (ESQ-2/96)							—	99.3	97.9	98.6	98.6
ESQ-3/96								—	97.2	97.9	97.9
AH-1									—	97.9	99.3
Epilink/96										—	98.6

Note. S fragment, GenBank accession numbers, and references of the sequences used here are indicated under Materials and Methods.

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                911                                                   965
Epilink/96      GNTISGYKRMMATKDSFQSFNLTEPHITANKLEWIDPDGNTRDHVNLVLNRDVSF
T               ..................................................
G               ...V.....................T.........................
AH-1/96         ...V.....................T.........................
ESQ-1/96        ..................................................
ESQ-2/96        .........................T.........................
ESQ-3/96        .........................T.........................
CH-1/96         .........................T.........................
CH-2/96         .........................T.........................
CH-3/96         .........................T.........................
SAl-1/96        ....................D....T..................I.......
LN              .......Q.L............D....SS.................I.......
BAY             ...V..F..L..........VS.A...T.S...V...N.IK..I.......I..
BCC             ...V..F..L..........VS.V...TT....S...S.IK..I..I.......
SN              ...V..FQ.....R.......V......S.R.......SSIK..I.M........
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FIG. 2. Predicted amino acid sequence of the G2 region of Epilink/96 aligned with analogous sequences of hantavirus from the Americas (numbered relative to SN virus). A dot indicates identity with respect to Epilink/96 strain. A conserved potential N-glycosylation site is bold and underlined.

low fever and dengue. Interpersonal transmission of Lassa and Machupo viruses is possible, but not other arenaviruses (Peters *et al.,* 1996). The finding of person-to-person transmission related to a particular variant of hantavirus should not be surprising; viruses may differ biologically.

In the southern Argentina outbreak, the epidemic index case occurred early in September at El Bolsón and the data suggest that the virus spread by person-to-person transmission. The mechanisms promoting this remarkable mode of transmission remain to be determined.

Although all Andes-like sequences are similar, minor changes among viruses are sometimes responsible for major distinguishing biological properties. It is not known whether the major route of infection occurred via parenteral or person-to-person, via fomites, infectious droplets, or sexual transmission. Transmission could be influenced by the disease stage, coinfection with other pathogens, virus load level, and type of virus. Factors that contribute to human susceptibility to hantavirus infection remain to be identified.

For southern Argentina and Chile, emphasis must be placed on reduction of the nosocomial spread of HPS through the strict implementation of universal biosafety precautions and reducing exposure of health personnel to Andes virus.

On the basis of information presented herein, PCR and sequencing proved to be powerful tools for establishing both the uniqueness of Epilink/96 viruses and their similarities to others in the entire region. This technique could be a useful tool in future prospective analyses of the modes of transmission of Andes virus during an outbreak and in monitoring the effectiveness of infection control measures in halting the spread of nosocomially and community transmitted HPS infection. Investigation of the virulence properties of the virus will be possible with the isolation of Andes virus and comparison of the complete sequences of M and S segments of transmissible and nontransmissible variants.

Andes virus, a new recognized HPS virus, possesses the novel feature of being the first hantavirus in the world associated with a severe, predominantly pulmonary illness transmitted person to person.

MATERIALS AND METHODS

Study population

Virus sequences amplified from 16 HPS patients with known epidemiological relationships were examined together with another 6 nonlinked cases and 3 previously described cases from Argentina and Chile. For comparative purposes 1 case from Oran, Salta, northern Argentina, was analyzed. Epidemiological contact data of these cases are depicted in Table 1.

Total RNA extraction, RT–PCR amplification, and sequencing

RNA was extracted from 200 mg of autopsy tissues in 8 HPS cases (I, B, P, L, ESQ-2/96, ESQ-3/96, CH-2/96, CH-3/96) or 200 μl whole blood or blood clots from 11 cases (A, Q, K, J, F, C, H, M, E, D, SAL-1/96). Samples were homogenized in 600 μl of acid guanidine thiocyanate solution, followed by phenol–chloroform extraction and ethanol precipitation (Chomczynski and Sacchi, 1987). For cases T, G, O, and M, only serum was available, so after phenol–chloroform extraction RNA was purified with an RNA matrix (RNaid kit, Bio101, La Jolla, CA). Amplification of viral RNA was done by nested or heminested RT–PCR reaction with a GeneAmp reagent kit (Perkin–Elmer Cetus). Synthesized DNA products were separated on agarose gels, gel-purified, and directly sequenced by the dideoxy cycle sequencing technique (*fmol* DNA Sequencing System, Promega).

Phylogenetic and sequence difference analyses

The primers used for amplification of the different fragments in S and M (G1a and G2) segments were as described (López *et al.,* 1996, 1997). Two additional primers were used: for the 3′ noncoding region fragment (3′ NCR) of the S segment +5′AGTATGTTAAGGCCTATAGGT 3′ (positions 1674–1694), numbered in the antigenome sense of Andes virus, and for G1a region of the M segment −5′CTTGGGTGAAACTCTTCTGG 3′ (positions 273–292), numbered relative to SN virus. For amplification of the G1b region of the M segment, primers SM 1687C, SM 1723C, ASM 2016R, and SM 2255R (Johnson *et al.,* 1997) were kindly provided by Dr. S. Nichol.

For nucleotide and amino acid sequence analysis two regions of the G1 encoding region of the M segment, designated G1a (nts 88 to 595), and G1b (nts 1736 to 1992); one region of the G2 encoding region of the M segment, designated G2 (nts 2780 to 2946), numbered in the antigenome sense sequence relative to SN virus were amplified. An additional 3′ NCR of the S segment (nts 1696 to 1838), numbered in the antigenome sense sequence relative to Andes virus, was used. In selected cases, virus was subjected to amplification of other regions specified under Results. Analysis of nucleotide and deduced amino acid sequence differences among hantavirus G1a, G1b, G2, and S amplimers were performed using NALIGN and PALIGN programs of the PCGENE 6.8 software from Intelligenetics Inc. (Mountain View CA).

Maximum parsimony analysis of viruses was carried out using the PHYLIP package version 3.57c (Felsenstein, 1993). Published sequences used for the S segment comparisons were BCC virus L39949 (Ravkov *et al.,* 1995), BAY virus L36929 (Morzunov *et al.,* 1995), AH-1 AF004660, CH-1/96: AF005947, ESQ-1/96, AF005948 (López *et al.,* 1997).

For M segment comparison, the following sequences were used: HTN virus strain 76-118, M 14627 (Schmaljohn *et al.,* 1987), Seoul virus strain Sr-11, M 34882 (Arikawa *et al.,* 1990); PUU virus strain Sotkamo, X61034 (Vapalahti *et al.,* 1992); PH virus strain PH-1, X55129 (Parrington *et al.,* 1991); NY virus strain RI-1, U36801 (Hjelle *et al.,* 1995); SN virus strain NMH10, L25783 (Spiropoulou *et al.,* 1994); BAY virus L36930 (Morzunov *et al.,* 1995); BCC virus, L39950 (Ravkov *et al.,* 1995); Laguna Negra virus AF005728 (Johnson *et al.,* submitted for publication); AH-1 AF004659 (López *et al.,* 1997), U51040 (López *et al.,* 1996); CH-1/96 AF005943, AF005949; ESQ-1/96 AF005944, AF005950, AF005952 (López *et al.,* 1997).

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