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Confirmation of Choclo Virus as the Cause of Hantavirus Cardiopulmonary Syndrome and high serum antibody prevalence in Panama

Randin Nelson[1,*], **Raul Cañate**[2,*], **Juan Miguel Pascale**[2], **Jerry W. Dragoo**[3], **Blas Armien**[2], **Anibal G. Armien**[4], and **Frederick Koster**[1,5,†]

[1]Department of Internal Medicine, University of New Mexico Health Sciences Center, Albuquerque, NM

[2]The Gorgas Memorial Institute, Panama City, Panama

[3]Department of Biology, University of New Mexico

[4]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN

[5]Lovelace Respiratory Research Institute, Albuquerque, NM

Abstract

Choclo virus (CHOV) was described in sigmodontine rodents, *Oligoryzomys fulvescens*, and humans during an outbreak of hantavirus cardiopulmonary syndrome (HCPS) in 1999 to 2000 in western Panama. Although HCPS is rare, hantavirus-specific serum antibody prevalence among the general population is high suggesting that CHOV may cause many mild or asymptomatic infections. The goals of this study were to confirm the role of CHOV in HCPS and in the frequently detected serum antibody and to established the phylogenetic relationship with other New World hantaviruses. CHOV was cultured to facilitate the sequencing of the small (S) and medium (M) segments and to perform CHOV-specific serum neutralization antibody assays.

Sequences of the S and M segments found a close relationship to other *Oligoryzomys*-borne hantaviruses in the Americas, highly conserved terminal nucleotides, and no evidence for recombination events. The maximum likelihood and maximum parsimony analyses of complete M segment nucleotide sequences indicate a close relationship to Maporal and Laguna Negra viruses, found at the base of the South American clade. In a focus neutralization assay acute and convalescent sera from 6 Panamanian HCPS patients neutralized CHOV in dilutions from 1:200 to 1:6400. In a sample of antibody-positive adults without a history of HCPS, 9 of 10 sera neutralized CHOV in dilutions ranging from 1:100 to 1:6400. Although cross-neutralization with other sympatric hantaviruses not yet associated with human disease is possible, CHOV appears to be the causal agent for most of the mild or asymptomatic hantavirus infections, as well as HCPS, in Panama.

Keywords

hantavirus; phylogeny; neutralizing antibody; Bunyaviridae

[†]Address correspondence to: Frederick Koster, Lovelace Respiratory Research Institute, 2425, Ridgecrest Dr., Albuquerque, NM 87108, Phone: (505) 348-9614, Fax: (505) 348-8567, fkoster@LRRI.org.
[*]RN and RC contributed equally to this work.

INTRODUCTION

Hantavirus cardiopulmonary syndrome (HCPS) is a severe and often fatal infection of the lung and other tissues, caused by one of at least 16 hantaviruses (Family *Bunyaviridae*, Genus *Hantavirus*) distributed throughout most of the Americas [Schmaljohn and Hjelle, 1997] [Koster and Hjelle, 2004]. Hantaviruses are negative-strand RNA viruses containing three segments, a small (S), medium (M), and large (L) segments encoding nucleocapsid (N), glycoproteins GPC, and RNA-dependent RNA polymerase, respectively. Most hantaviruses in the Americas, including the Sin Nombre virus (SNV) in North America and the Andes virus (ANDV) in South America, are associated with mortality rates of 20 to 35% and are contrasted with low levels of seroprevalence in endemic regions, usually below 2%. One exception is the Laguna Negra virus (LNV) in the Gran Chaco of western Paraguay, where indigenous peoples uncommonly experience symptomatic infection and mortality yet seroprevalence to hantavirus infection exceeds 20% in many communities [Ferrer et al., 1998; Williams et al., 1997].

The other exception is Choclo virus (CHOV), the only virus ascribed to human disease in Panama [Bayard et al., 2004; Vincent et al., 2000]. CHOV was identified by RT-PCR from samples taken from the host *Oligoryzomys fulvescens*, a common peridomestic sigmodontine rodent in Panama. More than 140 cases of HCPS, including 28 fatalities, have been recorded in Panama since 2000. All hospitalized patients tested by RT-PCR and amplimer sequencing were shown to have CHOV infections (Pascale et al., unpublished data). HIgh serum antibody prevalence [Armien et al., 2004] may be due to infection exclusively with CHOV or may be due in part to infections with other hantaviruses. Hantaviruses in Panama not associated with human disease include Calabazo virus (CALV) [Hjelle et al., 1995; Salazar-Bravo et al., 2004; Vincent et al., 2000] and an unnamed hantavirus in *Sigmodon hirsutus* [Armien et al., 2009]. Extensive cross-reactivity to nucleoproteins of the American hantaviruses prevents the use of EIA or Western blot technologies when assigning an infecting strain [Hjelle et al., 1997]. Studies utilizing neutralizing antibody specificities, on the other hand, may permit tentative assignment [Chu et al., 1994]

This study was undertaken to culture CHOV, obtain a complete sequence of the viral S and M segments, identify phylogenetic relationships, and develop a focus neutralization assay in order to implicate CHOV in the high antibody prevalence among Panamanians.

METHODS

Viral Isolation

Virus was obtained from the spleen of a rodent, *O. fulvescens* (specimen voucher no. NK101588, UNM MSB 96073), captured on 6 March 2000 in Las Tablas, Los Santos Province, Panama. The virus is named for a cantina ‘El Choclo’ of interesting reputation in the neighborhood Barriada 8 Noviembre near Las Tablas. One-hundred mg of tissue was homogenized by a bead beater using 2.5-mm zirconium/silica beads in phosphate buffered saline (PBS) and diluted 1:50, 1:200, and 1:1000 in 1.0 ml complete Vero media (Eagle’s minimal essential medium [EMEM] containing 10% fetal bovine serum (FBS), gentamicin (50 μg/mL), and 20 mM glutamine). Vero E6 cells (Vero C1008, ATCC CRL 1586, passage 8) were grown to confluency in 25-cm^2 flasks in Vero complete media. Media was removed from the monolayer and the diluted homogenates were added, incubated on a slow plate rocker at room temperature for 2 h, then the tissue homogenate was removed. Fresh media with 2.5% FBS was added and the monolayers were incubated at 36°C in a 5% CO_2 atmosphere, with media changed twice weekly. Passage of the monolayer to fresh flasks after trypsinization (0.5% trypsin/5 mM EDTA) was accomplished after 4 weeks (first

passage) and thereafter every 2 to 2.5 weeks. All experiments involving infectious viruses were performed in a biosafety level 3 laboratory.

RT-PCR

A nested reverse transcriptase-polymerase chain reaction (RT-PCR) was used to detect viral RNA in culture supernatants from each passage. Typically 170-μL aliquots (approximately 2 μg total RNA) of supernatant media were extracted using the QIAampViral RNA kit (Qiagen Inc, Valencia, CA) according to the manufacturer's directions. RT-PCR was initiated using AMVrt and Amplitaq LD with the outer antisense primer for 1 h at 42°C. Subsequent reaction conditions were 94°C for 5 min, followed by 8 cycles consisting of 10 s at 94°C, 20 s at 50°C, and 60 s at 72°C, and finally by 28 cycles with the annealing temperature of 55°C. The outer primers at the 5′ end of the segment were 5′-ACTGCACGGCAAAAGCTTAAA-3′ (58F) and 5′-GGATATAAGCACCAATTGACCT-3′ (379R) producing a 320-bp amplimer. The inner pair was 5′-GGACCCGGATGAAGTTAACAA-3′ (102F) and 5′-AATTTTTGAGCTGCCACCAA-3′ (222R) producing a 120 bp amplimer. The products were visualized on agarose gel, purified and sequenced to confirm specificity to CHOV.

Focus and Neutralization Assays

Replicating virus was titered using a focus assay as published [Bharadwaj et al., 2000]. Vero E6 cells were seeded onto 48-well plates and incubated until confluent. Ten-fold dilutions (1:10 through $1:10^7$) of virus-containing culture supernatant were added to the monolayers in a 200-μL volume of viral culture medium (EMEM, HEPES buffer, 2.5% FBS, and 50 mg/mL gentamicin) and incubated for 2 h at 37°C. After adsorption, the supernatant was aspirated and 1 mL/well of viral overlay media (VCM and 1.2% methylcellulose) was added and incubated for 7 days. After 7 days the overlay media was removed; the monolayer was washed once with PBS. Ice cold methanol containing 0.5% H_2O_2 was added and incubated at room temperature for 30 min; fixative then was aspirated and PBS added for storage until immunoperoxidase assay.

For the immunoperoxidase assay, fixed cell monolayers were washed twice with PBS, and 200 μL/well of 1:1000-diluted rabbit anti-SN virus serum was incubated for 1 h at 37°C. After aspiration and washing twice with PBS, 200 μL of peroxidase-conjugated AffinPure Goat anti-rabbit IgG (H+L) (Pierce Immunochemicals, Rockford, IL) diluted 1:1000 in PBS was added and incubated for 1 h at 37°C. Following aspiration and washing, 200 μL of DAB/metal concentrate diluted with 1x peroxidase substrate buffer was added to the monolayers and incubated until brown foci appeared, usually 15 to 30 min. Foci/well were enumerated with a 20x inverted scope ocular.

Neutralizing antibody was measured in human serum using diluted test sera in the focus titration assay. Serum samples containing anti-hantavirus antibody were collected from family members and neighbors of HCPS patients in the community of San Jose, Los Santos Province from 2001 to 2003. Serum was diluted (1:20, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, and 1:12800 dilutions) was incubated for 2 h at 37°C with stock virus diluted to a concentration of 35 to 50 focus-forming units (FFU)/well prior to incubation on Vero cell monolayers. Neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that results in an 80% reduction in the number of foci compared to virus controls. Discrepant results were resolved by two additional focus neutralization assays. Stock CHOV used was E6 passage 3, with a titer of 4.3 $\log_{10}$ FFU/mL. Stock SNV and ANDV demonstrated approximately the same titer in the focus assay titration as was documented by the originating laboratory.

Viral Sequencing

To sequence complete S and M segments, nested primers were designed from consensus sequences of ANDV and LNV viruses in GenBank. To sequence the 5′ and 3′ termini of each segment, dephosphorylated terminal nucleotides were ligated with T4 ligase and appropriately sized amplimers were cloned into pCRII vector (Invitrogen, Carlsbad, CA). The resulting clones were sequenced by the dideoxy method. Sequences were acceptable only if forward and reverse sequencing results agreed. Multiple overlapping amplimers were sequenced to achieve an 80% duplication of the entire genome.

Phylogenetic Analysis

Sequences of the N protein gene of four strains of ANDV and one strain each of 20 other hantaviruses were compared to the sequence of the CHOV. The sequence of the GPC gene of the CHOV was compared to sequences of 4 strains of ANDV and 20 other hantaviruses (Table 1). The accession numbers of closely related viruses were Maporal virus (MAPV) (N, AY267347; GPC, AY363170) and LNV (N, AF005727; GPC, AF005728). Sequences were aligned using ClustalX [Thompson et al., 1994] followed by visual inspection using MacClade 4 [Maddison and Maddison, 2003]. The N protein and GPC gene sequences were 1302 (with stop codon) and 3465 characters in length, respectively.

Phylogenetic analyses of DNA sequences were conducted using Maximum Parsimony (MP) and Maximum Likelihood (ML) in the software package PAUP* 4.0b10 [Swofford, 2002]. Fulhorst et al. (2004) excluded third base positions from their MP and ML analyses. Third base positions were included but only for transversion changes. Transversion changes continued to increase as genetic distance increased, suggesting that a phylogenetic signal may still be present for these changes at the third position, yet transition changes plateau, suggesting saturation of substitutions has occurred for transitions at the third base position. In the MP analysis all characters were weighted based on their rescaled consistency index (RCI). A heuristic search option with 100 replications of random addition of taxa and TBR branch swapping was used to generate parsimony trees. Bootstrap support [Felsenstein, 1985] for results of MP analyses were based on 1000 repetitions of resampling data using the heuristic search option, with 10 replications of random addition of taxa.

Modeltest (v 3.06) [Posada and Crandall, 1998] was used to calculate the appropriate model for ML analyses. The GTR + G + I (0.6787 and 0.3440 for G and I, respectively) was used to construct phylogenies for the S segment and the GTR + G (0.3439) model was used to construct the M segment phylogeny based on the results of the Modeltest analysis.

RESULTS

Isolation of the Virus

RT-PCR of serial supernatants from blind passages identified the appearance of detectable viral RNA from animal #588 by the fourth blind passage in both tissue inocula diluted 1:50 and 1:1000. Virus was first detected by focus titration assay from the third passage at <100 focus forming units (FFU)/mL, and the highest level of virus was found in the sixth passage with a titer of 5×10^4 FFU/mL. Viral RNA was extracted from sixth passage supernatants, or third passage of virus-positive supernatants, for subsequent genomic sequencing.

Nucleotide Sequence Analysis

The S segment of CHOV isolate 588 (Accession No. DQ285046) was 1516 nt in length, of which 1302 bases encode a predicted nucleocapsid protein 434 amino acids in length, beginning at nt 43. The S segment also contains a potential second ORF (open reading frame) beginning at nt 122, with a predicted protein of 63 amino acids in length. Terminal

nucleotides were conserved as seen in other hantaviruses [Chizhikov et al., 1995] with the 32- and 28-nt equivalent to the 5′ and 3′ ends, respectively, being identical to those of ANDV and LNV. The 20-nt differences between the CHOV and SNV in the S segment coding region included 16 nonsynonymous changes.

Comparison of the CHOV S segment sequences with those of other hantaviruses indicates the highest degree of identity with South American viruses (78.3 to 79.8% nt; 89.4 to 91.2% aa) (Table 2). Nucleotide similarity between CHOV and the North American viruses was close to that of the South American viruses and ranged from 75.9 to 79.3 % (83.2 to 89.2% aa). Comparison of deduced N protein amino acid sequences shows an 89.9% identity to MAPV, an 88.5% identity to SNV, and a 90% identity to ANDV.

Sequence Analysis of the M Segment

The complete M segment (Accession No. DQ285047) is 3465 nt in length, encoding a glycoprotein precursor of 1155 amino acids. Again, CHOV is more similar to the South American viruses (73.4 to 74.9% and 82.6 to 83.8% nt and aa, respectively) (Table 3). CHOV and North American viruses had nucleotide similarity ranging from 70.8 to 72.3% and amino acid similarity from 76.1 to 79.6%. Comparison of amino acid sequences shows that MAPV has an 83.6% identity to, a 79.6% identity to SNV, and a 83.7% identity to ANDV.

The tree topologies for the MP and ML analyses were similar. CHOV was basal to the South American viruses in the ML analyses for the S segment, whereas it formed a polytomy with MAPV and the remaining South American viruses in the MP analyses (Figure 1a and 1b). The relationship of CHOV to the South American viruses also was found in both the ML and MP analyses using the M segment (Figure 1c and 1d). However, the bootstrap support for this relationship was stronger than in the analysis of the S segment. The differences in tree topologies between this study and that of Fulhorst et al. (2004) can be explained by low bootstrap support of two nodes (compare Figure 1 to their Fig. 1 [p. 141]). Because bootstrap support is low in analyses from both studies, it is difficult to reject either hypothesis. In both studies the North American viruses are paraphyletic with respect to the South American viruses.

Neutralization of Choclo Virus by Human Sera

The presence of neutralizing antibody was detected by the focus reduction assay in sera from all 6 individuals with RT-PCR-confirmed CHOV infection and typical HCPS. Three adults with 3 to 5 days of symptomatic acute disease and who required intubation had lower serum titers (1:200 to 1:1600) than the 3 individuals with milder disease who did not require intubation and who were tested 30 to 60 days after hospitalization (Table 4). Among 10 individuals with IgG antibody, as detected by EIA and strip immunoblot, and who denied previous hospitalization for respiratory infection, 9 had significant neutralizing antibody to CHOV, with titers ranging from 1:100 to 1:6400. One individual in this group did not have a detectable CHOV-neutralizing antibody, as determined in four independent assays. Seronegative controls from Panama did not have neutralizing antibody to CHOV, and no subject from Panama had neutralizing antibody to either SNV or ANDV. Two patients with mild HCPS in New Mexico had neutralizing antibody to SNV isolate 777 but not to CHOV. The hantaviruses identified by RT-PCR in *Z. brevicauda* (CALV) and in *S. hirsutus* (unnamed virus) could not be grown in Vero cell culture despite multiple blind passages in two different laboratories, and therefore neutralizing antibody to these viruses is not reported.

DISCUSSION

The first goal of the study was to culture the virus and facilitate accurate identification of phylogenetic relationships. The complete genomic sequence of the S and M segments permitted the conclusion that CHOV is distinct from all other hantaviruses, according to established criteria [Elliott et al., 2000]. Even though CHOV and MAPV share the same host, *O. fulvescens*, these two strains are approximately 10% distinct at the amino acid level of the N protein. This distinction is likely due to independent evolution permitted by the extensive ecologic and physical separation between western Panama and western Venezuela. Rodents in the sigmodontine genus *Oligoryzomys* are natural hosts for at least 6 hantaviruses [Bharadwaj et al., 1997; Bohlman et al., 2002; Gonzalez Della Valle et al., 2002; Medina et al., 2009; Meissner et al., 2002; Powers et al., 1999]. The oryzomine rodent-borne viruses in the Argentina/Chile clade of hantaviruses show little amino acid diversity ([Bohlman et al., 2002; Medina et al., 2009], suggesting recent divergence. The greater diversity of CHOV from other oryzomine hantaviruses suggests its more remote divergence from the common ancestor.

The second goal of the study was to use the specificity of the neutralization assay to indicate whether the high serum antibody prevalence was due to previous CHOV infection. The focus neutralization assay of sera from CHOV-positive PCR-confirmed HCPS patients demonstrated the presence of neutralizing antibody titers of levels similar to other published reports [Bharadwaj et al., 2000]. As expected, there was no cross-neutralization between CHOV, ANDV, and SNV, but cross-neutralization studies with more closely related viruses, including CALV [Vincent et al., 2000] and MAPV, a hantavirus isolated from *O. fulvescens* in Venezuela [Fulhorst et al., 2004; Milazzo et al., 2002], is needed for definitive specificity.

In five communities in the Azuero peninsula investigators found antibody prevalence ranging from 3% to 45% [Armien et al., 2004]. Antibody was detected in samples from children as young as 4 years of age and increases steadily to highest prevalence in the 40- to 50-year-old cohort, suggesting that steady exposure and infection occurs throughout life in rural areas. From this cohort data it is estimated that among the 250,000 people of the Azuero peninsula there may be more than 10,000 individuals with serum anti-hantavirus antibody. Questionnaires indicated that none of the 275 antibody-positive individuals tested had given a history compatible with an HCPS-like illness, although histories of febrile respiratory illnesses not requiring hospitalization were common [Armien et al., 2004]. An ongoing clinical trial in four clinics during two years of observation has identified 110 individuals with fever and anti-hantavirus antibody detected by IgM-ELISA. CHOV RNA was detected in acute blood samples by RT-PCR, yet no progression to symptomatic pulmonary disease was found (B. Armien, unpublished data).

If the majority of antibody positive individuals were infected with CHOV, as suggested by neutralization assays, and if the reported 140 cases of HCPS is a good estimation of total cases, the ratio of mild or asymptomatic CHOV infection to HCPS may be as high as 50:1 in Panama. LNV may also be associated with a high ratio of mild to severe infection, although strain-specific neutralization titers have not been published [Chu et al., 2006]. In regions where multiple hantaviruses co-circulate in rodent populations, such as Panama, Paraguay [Chu et al., 2006] and Brazil [Figueiredo et al., 2009; Raboni et al., 2009], neutralization assays studies can identify the dominant hantaviruses that infect humans. Since each rodent host has habitat preferences [Armien et al., 2009; Chu et al., 2003], the identification of hantavirus species causing human disease can have useful public health implications.

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Figure 1.

A. Maximum likelihood analysis of 1302 nt of the S segment of 25 hantaviruses. Tree based on the GTR + G (0.6787) + I(0.3440) model of nucleotide evolution. Numbers associated with node are Bayesian posterior probabilities. Model for Bayesian analyses is the same used for Maximum Likelihood (topologies were identical). B. Single most parsimonious tree based on 675 informative of 1302 total base pairs of the N protein. Characters were weighted based on a rescaled consistency index. Third base positions were analyzed using transversions only. Tree length = 587.794; RCI = 0.5148. Numbers above branches represent percentage of 1000 bootstrap replications supporting each node. Nodes with less than 50% support are collapsed. C. Maximum likelihood analysis of 3465 nt of the M segment of 16 hantaviruses. Tree based on the GTR + G(0.3439) model of nucleotide evolution. Numbers associated with node are Bayesian posterior probabilities. Model for Bayesian analyses is the same used for Maximum Likelihood (topologies were identical). D. Single most parsimonious tree based on 2077 informative of 3465 total base pairs of the GPC gene. Tree length = 6307; CI = 0.4635. Third base positions were analyzed using transversions only.

Numbers above branches represent percentage of 1000 bootstrap replications supporting each node. Nodes with less than 50% support are collapsed.

Table 1

Virus sequences used in this study. Accessions numbers are for N protein (top) and GPC gene (bottom of the pair – when present)

Virus	Genbank No.		General locality	Host
	S segment	M segment		
CHOV	DQ285046	DQ285047	Panama	*Oligoryzomys fulvescens*
ANDV	AF324902	AF324901	Argentina	*Homo sapiens*
ANDV	AF291702	AF291703	Chile	*Oligoryzomys longicaudatus*
ANDV	AF325966		Argentina	*Oligoryzomys chacoensis*
ANDV	AF482712		Argentina	*Homo sapiens*
BAYV	L36929	L36930	USA	*Homo sapiens*
BCCV	L39949	L39950	USA	*Sigmodon hispidus*
BMJV	AF482713		Argentina	*Oligoryzomys chacoensis*
ELMCV	U11427	U26828	USA	*Reithrodontomys megalotis*
HNTV	M14626	M14627	Korea	*Apodemus agrarius*
LECV	AF482714	AF028022	Argentina	*Oligoryzomys flavescens*
LNV	AF005727	AF005728	Paraguay	*Calomys laucha*
MACV	AF482716		Argentina	*Necromys benefactor*
MAPV	AY267347	AY363179	Venezuela	*Oligoryzomys fulvescens*
MONV	U32591		USA	*Peromyscus maniculatus*
MULEV	U54575		USA	*Sigmodon hispidus*
NYV	U09488	U36801	USA	*Peromyscus leucopus*
ORNV	AF482715	AF028024	Argentina	*Oligoryzomys longicaudatus*
PHV	M34011	X55129	USA	*Microtus pennsylvanicus*
PUUV	M32750	M29979	Russia	*Clethrionomys glareolus*
PRGV	AF482717		Argentina	*Akodon azarae*
RIOMV	U52136		Bolivia	*Oligoryzomys microtis*
RIOSV	U18100		Costa Rica	*Reithrodontomys mexicanus*
SEOV	M34881	M34882	Japan	*Rattus norvegicus*
SNV	L37904	L37903	USA	*Peromyscus maniculatus*

Table 2

Nucleotide differences (below diagonal) and amino acid differences (above diagonal) between pairs of hantaviruses for the S segment

	ANDV1	ANDV2	ANDV3	ANDV4	BMJV	LECV	ORNV	MACV	PRGV	MAPV	CHOV	RIOM	LNV	ELMC	RIOS	SNV	MONV	NYV	MULE	BAYV	BCCV	SEOV	HTNV	PUUV	PHV
ANDV1	0	0	15	1	14	15	14	26	21	36	41	38	41	71	76	59	52	52	59	49	57	157	151	123	114
ANDV2	74	0	15	1	14	15	14	26	21	36	41	38	41	71	76	59	52	52	59	49	57	157	151	123	114
ANDV3	209	210	0	16	6	7	1	24	18	40	43	41	43	72	78	56	53	53	62	47	58	162	151	124	115
AND4	79	9	213	0	15	16	15	26	22	35	41	39	42	72	77	60	53	52	60	50	56	157	151	123	115
BMJV	203	205	163	209	0	1	5	24	18	37	38	41	43	71	75	53	50	52	62	47	58	161	149	123	113
LECV	197	202	166	202	110	0	6	25	19	36	39	40	44	72	74	54	51	53	63	48	59	161	149	123	112
ORNV	207	206	8	209	164	165	0	23	17	39	42	40	42	71	77	55	52	52	61	46	57	161	150	123	114
MACV	241	241	227	240	231	228	227	0	17	42	46	45	50	70	77	62	57	54	60	52	57	159	155	124	115
PRGV	237	237	216	239	231	216	216	218	0	40	44	43	44	71	79	59	53	52	59	48	59	159	152	127	114
MAPV	257	254	260	252	279	262	256	285	265	0	44	36	45	67	69	59	57	55	59	50	57	155	151	121	111
CHOV	267	279	263	283	274	274	263	281	275	281	0	47	51	68	73	50	47	47	65	49	62	165	155	123	108
RIOM	258	245	244	246	259	251	244	276	264	234	280	0	29	72	77	65	58	56	58	50	56	161	156	124	116
LNV	267	266	272	269	266	259	267	284	276	281	270	226	0	74	81	62	58	54	61	54	61	163	156	124	117
ELMC	300	301	285	303	292	294	283	313	309	301	314	297	321	0	38	64	65	65	73	65	70	163	164	127	116
RIOS	328	326	322	328	321	320	317	329	323	308	320	324	316	268	0	70	73	71	80	69	76	167	164	123	119
SNV	305	300	294	305	288	288	292	285	305	314	304	303	301	297	311	0	27	27	72	54	67	167	162	129	114
MONV	276	281	283	282	289	291	279	307	291	294	294	295	315	298	324	217	0	16	68	48	58	166	159	128	109
NYV	294	284	295	283	302	292	293	285	294	289	299	292	299	294	332	213	198	0	68	51	60	162	158	128	110
MULE	293	307	301	307	299	291	303	307	310	296	310	290	305	312	351	314	314	304	0	30	41	168	160	124	115
BAYV	296	295	274	297	293	292	274	304	297	279	305	286	285	303	322	294	292	286	248	0	33	162	155	121	107
BCCV	301	313	301	313	273	277	301	296	307	285	301	291	294	317	342	317	311	311	251	248	0	162	156	126	112
SEOV	480	480	476	477	478	475	474	476	487	487	490	503	489	491	506	494	504	484	508	512	481	0	75	161	160
HTNV	469	475	473	475	479	481	472	468	476	465	484	478	476	486	509	484	491	497	482	461	474	343	0	166	163
PUUV	409	421	427	427	427	424	427	418	442	418	433	424	416	417	416	417	432	425	415	425	408	475	510	0	86
PHV	395	391	400	398	399	402	398	415	418	390	394	405	396	405	414	396	395	394	408	398	403	484	486	347	0

Table 3

Nucleotide differences (below diagonal) and amino acid differences (above diagonal) between pairs of hantaviruses for the M segment

	ANDV1	ANDV2	ORNV	LECV	MAPV	LNV	CHOV	SNV	NYV	BAYV	BCCV	ELMC	SEOV	HTNV	PHV	PUUV
ANDV1	0	9	85	72	168	142	188	238	257	262	267	291	512	506	476	524
ANDV2	184	0	81	71	166	146	187	242	259	267	272	295	511	507	478	524
ORNV	711	708	0	51	174	150	194	246	256	269	267	299	507	505	486	533
LECV	713	718	629	0	161	146	187	238	252	269	266	290	505	497	483	527
MAPV	835	841	842	827	0	205	189	257	267	278	283	302	521	500	490	534
LNV	820	832	807	812	876	0	201	257	267	269	268	288	512	508	485	535
CHOV	870	874	879	895	889	922	0	236	258	245	260	276	522	512	484	532
SNV	953	960	968	947	970	970	961	0	58	212	222	226	521	505	462	512
NYV	965	974	986	999	965	955	987	647	0	234	237	240	523	508	471	512
BAYV	971	971	998	995	970	1003	964	911	947	0	130	259	509	503	477	526
BCCV	959	961	995	1007	988	965	975	893	915	752	0	264	517	510	488	527
ELMC	1044	1050	1035	1057	1061	1019	1013	939	960	986	963	0	509	487	475	522
SEOV	1418	1410	1410	1413	1454	1413	1421	1414	1410	1371	1355	1358	0	258	594	621
HTNV	1400	1402	1432	1417	1392	1400	1407	1401	1376	1374	1382	1377	937	0	587	619
PHV	1375	1385	1397	1392	1365	1360	1396	1327	1325	1375	1378	1333	1545	1512	0	314
PUUV	1452	1471	1487	1469	1462	1454	1477	1422	1452	1463	1451	1454	1600	1590	1061	0

Table 4

Neutralization titers of Panamanian sera to CHOV isolate 588

Clinical Group	Pt #	Clin. Severity	Neut. CHOV	Neut. SNV	Neut. ANDV
Acute HCPS	15355	fatal	1:400	<1:20	<1:20
(day 5–8 of sx[a])	16617	moderate	1:200	<1:20	<1:20
	15467	moderate	1:1600	<1:20	<1:20
Convalescent HCPS	18483	mild	1:3200	<1:20	<1:20
(day 15–20 of sx[a])	15586	mild	1:6400	<1:20	<1:20
	17644	mild	1:3200	<1:20	<1:20
Seropositive/no resp hx[b]	384	No resp hx	1:100	<1:20	<1:20
	390	No resp hx	1:1600	<1:20	<1:20
	401	No resp hx	1:200	<1:20	<1:20
	407	No resp hx	1:400	<1:20	<1:20
	409	No resp hx	1:400	<1:20	<1:20
	412	No resp hx	1:1600	<1:20	<1:20
	703	No resp hx	1:6400	<1:20	<1:20
	705	No resp hx	<1:20	<1:20	<1:20
	717	No resp hx	1:1600	<1:20	<1:20
	733	No resp hx	1:3200	<1:20	<1:20
Seronegative/neg hx	7 sera	No resp hx	<1:20	<1:20	<1:20
Acute HCPS, USA	NM 1	Mild	<1:20	1:800	<1:20
	NM 2	mild	<1:20	1:1600	<1:20

[a] interval between onset of symptoms and diagnosis of HCPS.

[b] seropositive and no history of acute respiratory infection requiring hospitalization.