Am. J. Trop. Med. Hyg., 72(1), 2005, pp. 54–58

HANTAVIRUS PULMONARY SYNDROME IN CENTRAL BOLIVIA: RELATIONSHIPS BETWEEN RESERVOIR HOSTS, HABITATS, AND VIRAL GENOTYPES

DARIN S. CARROLL, JAMES N. MILLS, JOEL M. MONTGOMERY, DANIEL G. BAUSCH, PATRICK J. BLAIR, JAMES P. BURANS, VIDAL FELICES, ALBERTO GIANELLA, NAOMI IIHOSHI, STUART T. NICHOL, JAMES G. OLSON, DUKE S. ROGERS, MILAGROS SALAZAR, AND THOMAS G. KSIAZEK

Division of Viral and Rickettsial Diseases, Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, Georgia; Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana; Naval Medical Research Center Detachment, U.S. Navy, Lima, Peru; National Center for Tropical Diseases, Santa Cruz, Bolivia; Department of Integrative Biology and M. L. Bean Life Science Museum, Brigham Young University, Provo, Utah

Abstract. In August 2002, two cases of hantavirus pulmonary syndrome (HPS) were confirmed in Mineros and Concepción, within the Santa Cruz Department of Bolivia. Extensive alteration of the native ecosystem, from dense forest to pasture or sugarcane, had occurred in both regions. An ecologic assessment of reservoir species associated with the human disease identified a single hantavirus antibody-positive *Oligoryzomys microtis* from Mineros and three hantavirus antibody-positive *Calomys callosus* from Concepción. In Mineros, the virus from the *O. microtis* was 90% similar to sequences published for Río Mamoré virus. Viral nucleotide sequences from two *C. callosus* were 87–88% similar to the sequence of Laguna Negra virus. The viral sequence from the *C. callosus* was 99% identical to viral sequences obtained from the HPS patient in this area, implicating *C. callosus* as the host and Laguna Negra virus as the agent responsible for the HPS case near Concepción.

INTRODUCTION

Sin Nombre virus, a New World hantavirus, was the causative agent for an outbreak of severe respiratory illness with a case fatality initially exceeding 75% of the cases in the southwestern United States in 1993.[1,2] Subsequent studies resulted in the discovery of approximately 30 hantaviruses throughout the Americas.[2–4] Although retrospective surveys found evidence of hantaviral infection in South America as early as 1985,[5,6] the first HPS-associated hantavirus for the region was discovered in 1993 in Brazil, and the first genetic characterization of a South American hantavirus was reported in 1996 following an outbreak of hantavirus pulmonary syndrome (HPS) in southwestern Argentina.[6–8] Since then, hundreds of human infections have been reported in Argentina, Brazil, Chile, Paraguay, and Uruguay, with mortality ranging from 30% to 70%.[6,9–11] The first hantavirus in Bolivia, Río Mamoré virus (RIOMV), was isolated from a small-eared pygmy rice rat (*Oligoryzomys microtis*) trapped in 1996.[12,13] This virus has not been associated with human disease. In 1997, Laguna Negra virus (LNV), hosted by the small vesper mouse (*Calomys laucha*), was identified in a patient in Santiago, Chile who had traveled extensively in Bolivia prior to the onset of illness.[14,15] In 2000, six HPS cases in southern Bolivia were linked to Bermejo virus, the first association of HPS with this hantavirus.[16]

In June and July 2002, two men living or working 100–250 km north of Santa Cruz, Bolivia (Figure 1) developed an illness compatible with HPS; one died. Laboratory testing (serology) in Bolivia showed that both patients were infected with a hantavirus. The Bolivian National Center for Tropical Diseases received reports of at least two additional suspected cases of HPS-like illness in the area. Herein, we report the results of an investigation to identify the virus and the rodent reservoir associated with HPS in Santa Cruz Department. We also describe the circumstances surrounding the transmission of hantaviruses to humans in this region, the prevalence of hantavirus infection in rodent populations in the disease endemic area, and the habitat associations of host species. A detailed description of the epidemiology of the human cases and results of a concurrent serosurvey conducted in the same region will be presented separately.

MATERIALS AND METHODS

Ecologic investigation. From August 16–21, 2002, small mammals were collected from four localities in the Department of Santa Cruz, Bolivia. Three collection sites (Dinamarca, Universal Transverse Mercator coordinates (UTM) 20K 0457277 8113921; Las Empalizadas, 20K 0468038 8107777; and La Patria, 20K 0463980 8106647) were near the town of Mineros, approximately 100 km northeast of Santa Cruz de la Sierra (Figure 1). Four habitat types (forest, cultivated sugarcane fields, harvested sugarcane fields, and peridomestic areas) were studied at these sites. The fourth collection locality was near the town of Concepción, (20L0603157 8269311), approximately 250 km northeast of Santa Cruz (Figure 1). Disturbed forest and peridomestic environments were sampled at the fourth location.

Las Empalizadas was the abandoned sugarcane camp where a patient (fatal case) worked for eight days before his illness. Dinamarca was the relocation site for workers from Las Empalizadas after HPS was diagnosed there. La Patria was the closest permanent human settlement to the previously mentioned sugarcane camps. Concepción, the site of the non-fatal case, consisted of peridomestic habitats (adobe houses) and dense forests punctuated by areas cleared for cattle pasture. Processing of rodents followed standardized procedures.[17] Rodent carcasses were fixed in 10% formalin and preserved in 70% ethanol for permanent storage in the M. L. Bean Life Science Museum at Brigham Young University (BYU). Genbank accession numbers (AY439000-AY439002) were obtained for the cytochrome b (cyt *b*) sequences for representatives of the hantavirus antibody-positive rodents, and BYU voucher numbers were assigned for all individuals.

Rodent sequencing and identification. Total genomic DNA was extracted from liver tissue preserved in 95% ethanol using the DNeasy Tissue Kit (Qiagen, Valencia, CA). The primers in Table 1 were used to amplify and/or obtain the nucleotide sequence of the mitochondrial cyt *b* gene.[18–22]

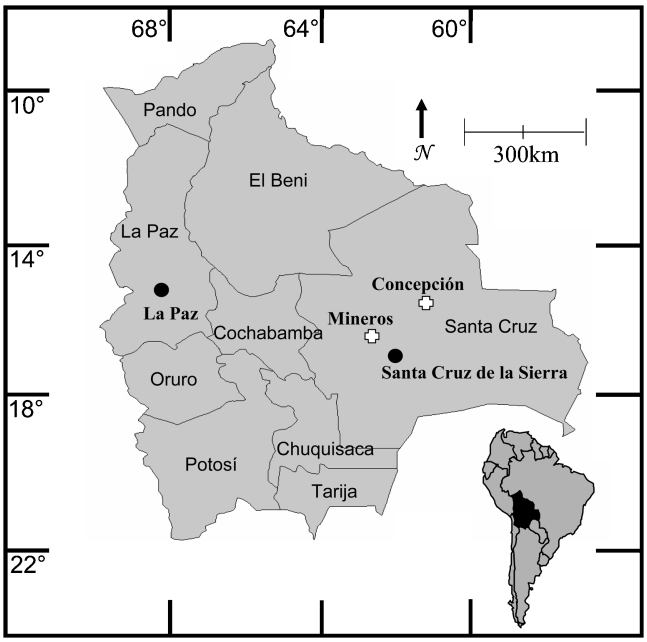

FIGURE 1. Map of Bolivia depicting areas of epidemiologic and ecologic/rodent reservoir investigations. The two areas investigated were Mineros and Concepción, as indicated by the white crosses.

The cyt *b* gene was amplified using a polymerase chain reaction (PCR) in a master mixture containing 1.0 μL of template DNA (approximate concentration estimated on a 2% agarose gel), 4 μL of dNTPs (1.25 mM), 2 μL of 10× *Taq* buffer, 0.5 μL of each primer (100 μM), 3 μL of $MgCl_2$ (25 mM), 14 μL of distilled water, and 0.25 μL of *Taq* polymerase (5 units/μL; Promega Corp., Madison, WI). The following thermal profiles were used for the majority of the PCRs: two minutes at 94°C, 39 cycles of one minute at 94°C, one minute at 45°C, and one minute at 72°C, plus five minutes at 72°C. The annealing temperature was increased from 45°C to 49°C for the three primers designed specifically for this study (Table 1). Sequences were determined using an ABI 570 Genetic Analyzer (Applied Biosystems, Inc., Foster City, CA) and edited and aligned using Sequencher version 4.1.1 (Gene Codes Corp., Ann Arbor, MI).

To confirm the field identifications of the hantavirus antibody-positive rodents, we obtained reference sequences from GenBank for representative genera of sigmodontine rodents and compared sequence data from the cyt *b* gene of the three voucher specimens analyzed in this study. Missing data were coded as ?. Phylogenetic analyses, including maximum parsimony (MP) and neighbor-joining (NJ), were performed using PAUP*.[23] For the maximum parsimony analysis all characters were weighted equally, and we performed a heuristic search with 100 replicates of random taxon addition with tree bisection-reconnection branch swapping. Confidence in nodes was assessed using the bootstrap procedure with 1,000 resampling replicates. Bootstrap values ≥ 70% were considered well-supported.[22,24,25]

Extraction of viral RNA, reverse transcriptase–PCR (RT-PCR), and sequencing. Tissues were frozen on dry ice in the field and stored at −70°C until processed. To extract RNA, 50–100 mg of rodent lung or spleen tissue was homogenized with 1 mL of TRIzol (Invitrogen Life Technologies, Frederick, MD). Total RNA was prepared as previously described,[26] and the RT-PCR was conducted using an Access RT-PCR kit (Promega Corp.) following the manufacturer's instructions. Briefly, the RT products were amplified using primers (Table 1) specific for the S segment of the hantavirus genome, and hemi-nested reactions were generated using 1 μL of first-round product.[14,26] Purified PCR amplicons were sequenced directly with the Big Dye Sequencing kit (Applied Biosystems, Inc.) and analyzed on an ABI 377 Genetic Analyzer (Applied Biosystems, Inc.). Nucleotide sequences were aligned and analyzed as described in the rodent cyt *b* analyses.

TABLE 1
Primer sequences used to amplify viral (S-segment) and rodent mitochondrial (cytochrome *b*) fragments

Virus primers	
Sense strand SS143C-N	5′-TGG A(C/G)C CIG ATG AIG TTA ACA C-3′
Anti-sense SS743R-N	5′-TCI ATC CA(G/A) TC(C/T) TTI ACA AA-3′
Sense strand SS283C-N	5′-CCA ACA GGG ITT GA(A/G) CC(T/A) GAT GA-3′
Anti-sense PPT716R	5′-AAI CCI ATI ACI CCC AT-3′
Rodent cytochrome *b* primers	
L14724[19]	5′-CGA AGC TTG ATA TGA AAA ACC ATC GTT G-3′
L14648[21]	5′-TGA ATY TGA GGR GGC TTC TCA GTA-3′
700L[18]	5′-CCC CAG CAC ATA TTA AAC CAG AAT G-3′
F1[20]	5′-TGA GGA CAR ATA TCH TTY TGR GG-3′
H15915[19]	5′-AAC TGC AGT CAT CTC CGG TTT ACA AGA C-3′
H15149[19]	5′-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A-3′
CBH3[22]	5′-GGC AAA TAG GAA RTA TCA TTC-3′
L738-*Calomys*	5′-CCC AGA TGT GCT CGG AGA CCC-3′
H738-*Calomys*	5′-GGG TCT CCG AGC ACA TCT GGG-3′
H1105-*Calomys*	5′-GGA TAT CAT TCT CAA TTA TGC TGG C-3′

RESULTS

Ecologic investigation and rodent identification. Rodent trapping results for each site are listed in Table 2. Relative densities in each habitat and trap site are provided as trap success (numbers of captures per 100 trap nights, where trap nights is the number of traps set multiplied by the number of nights set). It is likely that some of the currently recognized hantavirus rodent host species are composites of cryptic species that are difficult to identify morphologically.[18] Therefore, field identifications of the hantavirus antibody-positive rodents were confirmed through phylogenetic analysis of nucleotide sequence data. The entire cyt *b* gene (1,143 basepairs) was sequenced for BYU 19014 (identified in the field as *O. microtis*) from the Dinamarca site. This sequence was compared with GenBank cyt *b* sequences for 18 samples of *Oligoryzomys* representing eight species. Both NJ and MP analy-

TABLE 2
Sites and habitats of rodent collections in Santa Cruz Province, Bolivia, August 2002*

Mineros; Dinamarca August 16–17, 2002

General habitat	T	A sp	B sp	Cc	Or sp	Olm	O(i)	Hs	TC	TS
Peridomestic	99	0	0	0	0	2	1	0	3	3.0%
Disturbed forest	198	8	8	1	2	13 (1/7.7%)	4	1	37	18.7%
Cultivated sugarcane	40	0	0	1	0	1	0	0	2	5.0%
Total	337	8	8	2	2	16	5	1	42	12.46%

Mineros; Las Empalizadas August 2002

General habitat	T	A sp	Cc	TC	TS
Cleared sugarcane	97	1	16	17	17.5%
Cultivated sugarcane	80	5	4	9	11.3%
Total	117	6	20	26	14.69%

Mineros; La Patria August 17–18, 2002

General habitat	T	M(m)	A sp	Cc	Or sp	Olm	TC	TS
Peridomestic	226	0	1	1	0	0	2	0.9%
Disturbed forest	200	1	0	13	1	2	17	8.5%
Total	426	1	1	14	1	2	19	4.46%

Concepción; August 21–22, 2002

General habitat	TS	A sp	Cc	Cf	Or sp	Olm	O(i)	M(m)	TC	TS
Peridomestic	110	0	6	0	5	6	0	0	17	15.5%
Disturbed forest	480	38	37 (3/8.1%)	1	28	27	5	1	137	28.5%
Total	590	38	43	1	33	33	5	1	154	26.10%

* T = number of traps set; A sp = *Akodon* species; B sp = *Bolomys* species; Cc = *Calomys callosus*; Or sp = *Oryzomys* species; Olm = *Oligoryzomys microtis*; O(i) = *Oxymycterus inca*; Hs = *Holochilus sciureus*; TC = total captures; TS = percent trap success; M(m) = *Marmosa murina* (field indentification); Cf = *Calomys fecundus.* Numbers in parentheses are the number of hantavirus-positive individuals/hantavirus antibody prevalence for that species in that habitat.

ses associated BYU 19014 with *O. microtis* (GenBank assession no. U58381). The Bootstrap value for this node was 100%. Cytochrome *b* sequences were obtained for two samples identified in the field as *Calomys callosus* (BYU 19015 and 19016, 1,130 and 1,143 basepairs, respectively). These sequences were compared with those of 61 samples of *Calomys* available from GenBank representing nine species. Both NJ and MP analyses associated BYU 19015 and 19016 with samples identified as *C. callosus* with 95% nodal support.

Viral RT-PCR and DNA sequencing. The nucleotide sequence generated from the antibody-positive *O. microtis* captured in Mineros was 90% similar to those of RIOMV (GenBank accession no. U52137-39). Sequencing of PCR products from two *C. callosus* captured in Concepción identified a virus that was 87–88% similar to published sequences for LNV. In addition, the topology produced by the NJ analysis shows that the viral RNA amplicons from *C. callosus* and *O. microtis* each form a monophyletic cluster with sequences obtained from LNV and RIOMV when compared with sequences from all other hantaviruses known from this region (Figure 2).

DISCUSSION

Viral RNA sequencing demonstrated that one case patient was infected with an LNV-like virus, which implicates a member of the genus *Calomys* as the reservoir. In addition, the comparison of the viral sequences from the case patient and the *C. callosus* captured in the same area (> 99% identity) links the rodent and human viruses. This is congruent with the recent collection of hantavirus antibody-positive *C. callosus* in northern Argentina.[27] *Calomys laucha* is recognized as the primary rodent reservoir for LNV.[14] It is not common for a New World hantavirus to be hosted by multiple species of rodents. Spillover is unlikely since the Concepción study site is outside of the recognized range of *C. laucha*, and no individuals of this species were collected at this location. It is possible that LNV infected the common ancestor to both *C. laucha* and *C. callosus* and has remained associated with both species with minimal genetic divergence.

The identification of *O. microtis* as the host of the only hantavirus detected in the Mineros region at the location of the human case is consistent with the previous recognition of this rodent as the host of RIOMV.[12] Unfortunately, no sample was available from the fatal human case for comparison with the rodent virus sequence. Given the phylogenetic similarity of the rodent virus amplicon to that of RIOMV, the geographic proximity of the captured RIOMV PCR-positive rodent to the site where the patient became ill, and the lack of detection of any other hantaviruses in the vicinity of the human case, it appears possible that this variant of RIOMV may be the pathogen responsible for the HPS case in this region. Río Mamoré virus has not been associated with human disease; however, another South American hantavirus (Bermejo virus) was shown to be associated with human disease several

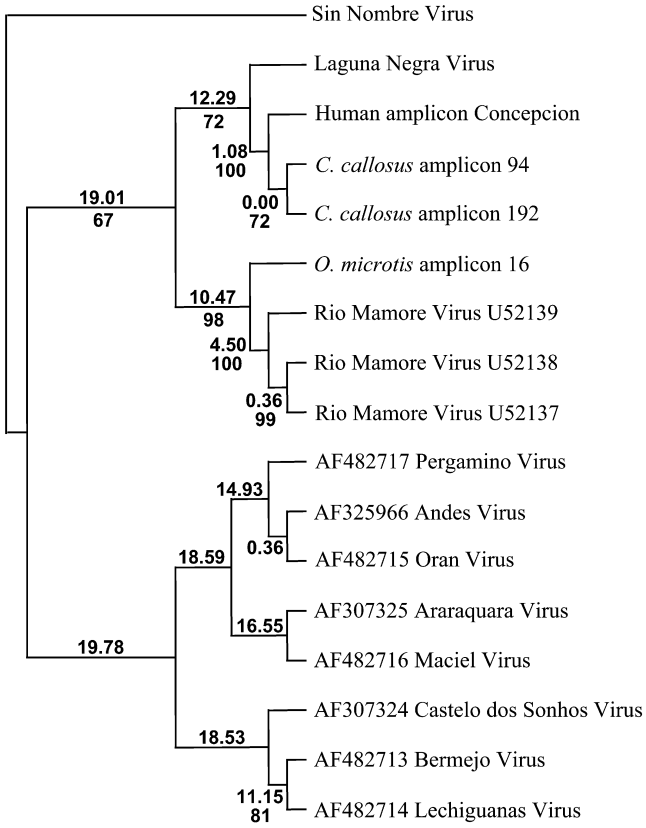

FIGURE 2. Neighbor-joining tree using 288 basepairs of the N gene from the S segment, depicting the phylogenetic relationship of hantaviruses isolated from central Bolivia compared with Genbank nucleotide sequences from previously identified South American hantavirus species. Sin Nombre virus was used as the outgroup taxon. The values with decimals represent the average absolute genetic divergence of the taxa united by the indicated node. The whole number values are bootstrap values (1,000 replicates) taken from a maximum parsimony tree. Nodes without bootstrap values were collapsed in the parsimony analysis.

years after the host and virus were described.[16] Alternatively, trap success, and therefore the number of rodents tested, were much lower in Mineros than Concepción. It is possible that another hantavirus circulating in the area is responsible for human disease, but was undetected by our sampling. Continued human and rodent surveillance are needed to positively identify the virus and host responsible for HPS near Mineros.

The potential for intermittent HPS cases in these regions likely will continue due to the presence of housing that is highly permeable to rodents, and the constant availability of food, water, and shelter for wild rodents in the vicinity of human habitations. These conditions could contribute to disease outbreaks should environmental circumstances lead to a sudden increase in reservoir population density, increased transmission of the virus within the rodent population, or higher levels of virus infection and shedding by the host population.

These data were collected during a case investigation to identify high-risk areas for transmission of hantaviruses to humans. Thus, all sites sampled were somewhat disturbed. Changes in environmental conditions can contribute to an increase in populations of some hantavirus reservoir species and, in turn, an increased risk of human disease.[28,29] In this study, the HPS cases occurred in areas of extensive anthropogenic perturbation. Dinamarca was at the boundary of natural forest being cleared to cultivate sugarcane. The study site near Concepción was adjacent to slash and burn deforestation. These disturbances decrease species diversity and simplify local ecosystems, including the rodent assemblages.[30,31] *Calomys* and *Oligoryzomys* are murid genera that contain species that adapt well to disturbance. In this sense, the situation in Santa Cruz Department is similar to that associated with a recent HPS outbreak in Panama and suggests that environmental perturbations create conditions that favor hantavirus reservoir species and result in increased risk for HPS.[32] In Santa Cruz Department, long-term sampling of these rodent populations could be used to test the hypothesized relationship between decreasing biodiversity and increasing numbers of hantavirus-infected rodents. These studies should include the sampling of disturbed and undisturbed habitats with comparisons of the species composition and prevalence of hantavirus infection in host populations.

Received November 24, 2003. Accepted for publication January 22, 2004.

Acknowledgments: We are grateful for technical help provided by Rosa Rios and Tania Garron (Bolivian National Center for Tropical Diseases) and Roger Castillos (Naval Medical Research Center Detachment, Lima, Peru), as well as administrative support from Gloria Talledo and Carolina Guevara (Naval Medical Research Center Detachment, Lima, Peru).

Financial support: This work was supported in part by The Department of Defense Global Emerging Infectious Surveillance Program and Brigham Young University.

Disclaimer: The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of Defense, the Department of the Navy, or the U.S. Government.

Authors' addresses: Darin S. Carroll, James N. Mills, Joel M. Montgomery, Stuart T. Nichol, and Thomas G. Ksiazek, Special Pathogens Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Mailstop A-26, 1600 Clifton Road NE, Atlanta, GA 30333, E-mail: dcarroll@cdc.gov. Daniel G. Bausch, Tulane School of Public Health and Tropical Medicine, SL-17, J. B. Johnston Building, Room 511, 1430 Tulane Avenue, New Orleans, LA 70112. Patrick J. Blair, James P. Burans, Vidal Felices, James G. Olson, and Milagros Salazar, U.S. Navy, Medical Research Detachment Lima, Peru, Unit Number 3800, American Embassy, APO AA 34031-3800. Alberto Gianella and Naomi Iihosi, Centro Nacional de Enfermedades Tropicales, Bolivian National Center for Tropical Diseases, 2do. Anillo esq. Av. Centenario, Casilla 2974, Santa Cruz de la Sierra, Bolivia. Duke S. Rogers, 401 WIDB, Department of Integrative Biology and M. L. Bean Life Science Museum, Brigham Young University, Provo, UT 84602-5181.

REFERENCES

1. Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, Rollin PE, Nichol S, Umland ET, the Hantavirus Study Group, 1994. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. *N Engl J Med 330:* 949–955.
2. Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs JE, Zaki S, Peters CJ, 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science 262:* 914–917.
3. Schmaljohn CS, Hjelle B, 1997. Hantaviruses: A global disease problem. *Emerg Infect Dis 3:* 95–104.
4. Peters CJ, 1998. Hantavirus pulmonary syndrome in the Ameri-

cas. Scheld WM, Craig WA, Hughes JM, eds. *Emerging Infections*. Second edition. Washington, DC: American Society for Microbiology Press, 17–63.
5. Weissenbacher MC, Cura E, Segura EL, Hortal M, Baek LJ, Chu YK, Lee HW, 1996. Serological evidence of human hantavirus infection in Argentina, Bolivia and Uruguay. *Medicina (B Aires) 56:* 17–22.
6. Johnson AM, de Souza LTM, Ferreira IB, Pereira LE, Ksiazek TG, Rollin PE, Peters CJ, Nichol ST, 1999. Genetic investigation of novel hantaviruses causing fatal HPS in Brazil. *J Med Virol 59:* 527–535.
7. Lopez N, Padula P, Rossi C, Lazaro ME, Franze-Fernandez MT, 1996. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology 220:* 223–226.
8. Nichol ST, Ksiazek TG, Rollin PE, Peters CJ, 1996. Hantavirus pulmonary syndrome and newly described hantaviruses in the United States. Elliot RM, ed. *The Bunyaviridae*. New York: Plenum Press, 269–280.
9. Levis S, Morzunov SP, Rowe JE, Enria D, Pini N, Calderón G, Sabattini M, St. Jeor SC, 1998. Genetic diversity and epidemiology of hantaviruses in Argentina. *J Infect Dis 177:* 529–538.
10. Toro J, Vega JD, Khan AS, Mills JN, Padula P, Terry W, Yadon Z, Valderrama R, Ellis BA, Pavletic C, Cerda R, Zaki S, Wun-Ju S, Meyer R, Tapia M, Mansilla C, Baro M, Vergara JA, Concha M, Calderón G, Enria D, Peters CJ, Ksiazek TG, 1998. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. *Emerg Infect Dis 4:* 687–694.
11. Padula PJ, Rossi CM, Della Valle MO, Martinez PV, Colavecchia SB, Edelstein A, Miguel SD, Rabinovich RD, Segura EL, 2000. Development and evaluation of a solid-phase enzyme immunoassay bases on Andes hantavirus recombinant nucleoprotein. *J Med Microbiol 49:* 149–155.
12. Bharadwaj M, Botten J, Torrez-Martinez N, Hjelle B, 1997. Rio Mamore virus: genetic characterization of a newly recognized hantavirus of the Pygmy rice rat, *Oligoryzomys microtis*, from Bolivia. *Am J Trop Med Hyg 57:* 368–374.
13. Hjelle B, Torrez-Martinez N, Koster FT, 1996. Hantavirus pulmonary syndrome-related virus from Bolivia (letter). *Lancet 347:* 57.
14. Johnson AM, Bowen MD, Ksiazek TG, Williams RJ, Bryan RT, Mills JN, Peters CJ, Nichol ST, 1997. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. *Virology 238:* 115–127.
15. Espinoza R, Vial P, Noriego LM, Johnson A, Nichol ST, Rollin PE, Wells R, Zaki S, Reynolds E, Ksiazek TG, 1998. Hantavirus pulmonary syndrome in a Chilean patient with recent travel in Bolivia. *Emerg Infect Dis 4:* 93–95.
16. Padula P, Della Valle MG, Alai MG, Cortada P, Villagra M, Gianella A, 2002. Andes virus and first case report of Bermejo virus causing fatal pulmonary syndrome. *Emerg Infect Dis 8:* 437–439.
17. Mills JN, Childs JE, Ksiazek TG, Peters CJ, Velleca WM, 1995. *Methods for Trapping and Sampling Small Mammals for Virologic Testing.* Atlanta: U. S. Department of Health and Human Services.
18. Peppers LL, Carroll DS, Bradley RD, 2002. Molecular systematics of the genus *Sigmodon* (Rodentia:Muridae): evidence from the mitochondrial cytochrome-b gene. *J Mamm 83:* 396–407.
19. Irwin DM, Kocher TD, Wilson AC, 1991. Evolution of the cytochrome b gene in mammals. *J Mol Evol 32:* 128–144.
20. Whiting AS, Bauer AM, Sites JW Jr, 2003. Phylogenetic relationships and limb loss in sub-Saharan African scincines (Squamata: Scincidae). *Mol Phylogenet Evol 29:* 582–598.
21. Martin Y, Gerlach G, Schlotterer C, Meyer A, 1999. Molecular phylogeny of European muroid rodents based on complete cytochrome *b* sequences. *Mol Phylogenet Evol 16:* 37–47.
22. Palumbi SR, 1996. Nucleic acids I: the polymerase chain reaction. Hillis DM, Moritz C, Mable BK, eds. *Molecular Systematics*. Second edition. Sunderland, MA: Sinauer Associates, 205–247.
23. Swofford DL, 2001. *PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods).* Version 4. Sunderland, MA: Sinauer Associates.
24. Felsenstein J, 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39:* 783–791.
25. Hillis DMBJ, 2003. An empircal test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst Biol 42:* 182–192.
26. Blair PJ, Riley JL, Levine BL, Lee KP, Craighead N, Francomano T, Perfetto SJ, Gray GS, Carreno BM, June CH, 1998. CTLA-4 ligation delivers a unique signal to resting human CD4 T cells that inhibits interleukin-2 secretion but allows Bc1-X(L) induction. *J Immunol 160:* 12–15.
27. Pini N, Levis S, Calderón G, Ramirez J, Bravo D, Lozano E, Ripoll C, St. Jeor S, Ksiazek TG, Barquez RM, Enria D, 2003. Hantavirus infection in humans and rodents, northwestern Argentina. *Emerg Infect Dis 9:* 1070–1076.
28. Plyusnin A, Morzunov SP, 2001. Virus evolution and genetic diversity of hantaviruses and their rodent hosts. *Cur Top Microbiol Immunol 256:* 47–75.
29. Glass GE, Yates TL, Fine JB, Shields TM, Kendall JB, Hope AG, Parmenter CA, Peters CJ, Ksiazek TG, Li CS, Patz JA, Mills JN, 2002. Satellite imagery characterizes local animal reservoir populations of Sin Nombre virus in the southwestern United States. *Proc Natl Acad Sci USA 99:* 16817–16822.
30. Ostfeld RS, Keesing F, 2000. The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Can J Zool 78:* 2061–2078.
31. Vandermeer J, 1997. Biodiversity loss in and around agroecosystems. Grifo F, Rosenthal J, eds. *Biodiversity and Human Health*. Washington, DC: Island Press, 111–127.
32. Vincent MJ, Quiroz E, Gracias F, Sanchez A, Ksiazek T, Kitzutani P, Ruedas LA, Tinnin D, Caceres L, Garcia A, Rollin PE, Mills JN, Peters CJ, Nichol ST, 2000. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology 277:* 14–19.