Published in final edited form as:

Am J Trop Med Hyg. 2008 April ; 78(4): 669–674.

Choclo Virus Infection in the Syrian Golden Hamster

Eduardo J. Eyzaguirre,

University of Texas Medical Branch, Department of Pathology, 301 University Blvd., Galveston, TX 77555-0609, Telephone: 409-772-5548, Fax: 409-747-0060, E-mail: ejeyzagu@utmb.edu

Mary Louise Milazzo,

Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0609, Telephone: 409-747-2466, Fax: 409-747-2437, E-mail: mamilazz@utmb.edu

Frederick T. Koster, and

Lovelace Respiratory Research Institute, 2425 Ridgecrest Dr., SE Albuquerque, NM 87108, Telephone: 505-348-9614, Fax: 505-348-8567, E-mail: fkoster@lrri.org

Charles F. Fulhorst*

Department of Pathology, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0609, Telephone: 409-772-9713, Fax: 409-772-2437, E-mail: cfulhors@utmb.edu

Abstract

Andes virus and Choclo virus are agents of hantavirus pulmonary syndrome. Andes virus in hamsters almost always causes a disease that is pathologically indistinguishable from fatal hantavirus pulmonary syndrome. The purpose of this study was to assess the pathogenicity of Choclo virus in hamsters. None of 18 hamsters infected with Choclo virus exhibited any symptom of disease. No evidence of inflammation or edema was found in the lungs of the 10 animals killed on days 7, 9, 11, 13, and 16 post-inoculation or in the lungs of the 8 animals killed on day 28 post-inoculation; however, hantavirus antigen was present in large numbers of endothelial cells in the microvasculature of the lungs of the animals killed on days 7, 9, 11, and 13 post-inoculation. These results suggest that infection in the microvasculature of lung tissue alone does not result in the life-threatening pulmonary edema in hamsters infected with Andes virus.

INTRODUCTION

Hantavirus pulmonary syndrome (HPS) was first recognized as a distinct clinical entity in 1993 in the southwestern United States.¹ Subsequently, HPS was reported from 31 states in the contiguous United States (<http://www.cdc.gov/ncidod/diseases/hanta/hps/noframes/epislides/episl7.htm>), Canada, Panama, Brazil, Argentina, Bolivia, Paraguay, Chile, and Uruguay.^{2–8}

Seven members of the virus family *Bunyaviridae*, genus *Hantavirus* are known to cause HPS: Andes virus (ANDV), Bayou virus (BAYV), Black Creek Canal virus (BCCV), Choclo virus (CHOV), Laguna Negra virus (LANV), New York virus (NYV), and Sin Nombre virus (SNV).^{3,5,6,9–12} None of the 9 other hantaviruses native to the Americas, including Maporal virus (MAPV), has been associated with HPS or any other human disease.^{13–15}

Specific members of the rodent family Cricetidae are the principal hosts of the hantaviruses known to cause HPS. For example, the deer mouse (*Peromyscus maniculatus*) in the western United States is the principal host of SNV,¹⁶ the fulvous pygmy rice rat (*Oligoryzomys fulvescens*) in western Panama is the principal host of CHOV,³ the fulvous pygmy rice rat (*O. fulvescens*) in western Venezuela is the principal host of MAPV,¹⁵ and the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) in Argentina and Chile is the principal host of ANDV.^{17–20} It is assumed that humans usually become infected with hantaviruses by inhalation of aerosolized droplets of urine, saliva, or respiratory secretions from infected rodents or by inhalation of dust contaminated with infectious rodent secretions or excretions.

The severity of HPS ranges from mild febrile illness to fulminating, lethal pneumonitis.²¹ Virus genetics, inoculum dose, route of exposure, and human genetics likely affect the course and outcome of HPS.^{21,22}

Fatal HPS is characterized by rapid onset of severe respiratory distress, noncardiogenic pulmonary edema, cardiac depression, and shock.^{21,23,24} The most striking features at autopsy are large amounts of frothy fluid in the trachea, bronchi, and other airways, large volumes of pleural fluid, and congested, edematous lungs.^{23,25} Microscopic abnormalities in lung tissue include diffuse subacute interstitial pneumonitis, vascular congestion, and mild to moderate interstitial and alveolar edema.^{23,25} Immunohistochemical studies revealed hantavirus antigen in lung, heart, spleen, kidney, brain, and other tissues, with the most extensive staining within the endothelium of the microvasculature of lung tissue.²⁵

The results of a previous study indicated that ANDV strain Chile-9717869 in the Syrian golden hamster is highly lethal, regardless of inoculum dose, and that the disease in ANDV-infected hamsters is pathologically highly similar to fatal HPS.²⁶ The purpose of this study was to compare the pathogenicity of CHOV strain 588 to the pathogenicity of ANDV strain Chile-9717869 in the Syrian golden hamster.

MATERIALS AND METHODS

Safety

All work with hamsters inoculated with CHOV or ANDV was done inside the Robert E. Shope, MD, Laboratory at the John Sealy Pavilion for Infectious Disease Research. The Shope Laboratory is the biosafety level 4 (BSL-4) facility located on the campus of the University of Texas Medical Branch, Galveston.

Viruses

Stocks of CHOV strain 588 and ANDV strain Chile-9717869 were prepared from cultures of Vero E6 cells inoculated with twice plaque-purified virus. Strain 588 originally was isolated from a fulvous pygmy rice rat (*O. fulvescens*) captured in 2001 in western Panama (R. Nelson, unpublished information) and strain Chile-9717869 originally was isolated from a long-tailed pygmy rice rat (*O. longicaudatus*) captured in 1997 in Chile.²⁷ The infectious titers of the stocks were measured in monolayers of Vero E6 cells grown in 24-well plastic cell culture plates (each well 1.33 cm²). Vero E6 cells harvested from the plates on day 12 post-inoculation (PI) were tested for hantavirus antigen, using an indirect fluorescent antibody test (IFAT) in which the primary antibody was a polyvalent anti-hantavirus hyperimmune mouse ascitic fluid (HMAF). (A preliminary experiment demonstrated that the IgG in this HMAF was highly reactive against strain 588 and strain Chile-9717869.) The titers of the CHOV and ANDV stocks were 5.9 log₁₀ median cell culture infectious doses (CCID₅₀)/0.1 mL and 5.8 log₁₀ CCID₅₀/0.1 mL, respectively.

Inoculation, husbandry, and sampling of hamsters

Twenty-five Syrian golden hamsters (9-week-old females) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN) and housed inside the Shope Laboratory for 6 days prior to inoculation with infectious virus or injection with sterile 0.01-M phosphate-buffered saline (PBS), pH 7.4. Four animals each were inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV, 14 animals each were inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV, 2 animals each were inoculated with PBS, and 5 animals each were inoculated with $2.0 \log_{10}$ CCID₅₀ of ANDV. The 4 hamsters inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV were to provide a basis for assessment of the strength of the $4.1 \log_{10}$ CCID₅₀ inoculum relative to the infectivity of CHOV in the Syrian golden hamster, the 2 animals inoculated with PBS were negative controls, and the 5 animals inoculated with ANDV were to provide an assurance that ANDV in the 18 animals inoculated with CHOV could cause a highly lethal HPS-like disease. Each animal was inoculated with 0.2 mL of virus suspension or injected with 0.2 mL of PBS at 1 site in the lumbar musculature, using a 27-ga $\times$ 5/8" hypodermic needle on a tuberculin syringe.

All animals were housed in microisolators maintained on a 112-cage ventilated rack (Laboratory Products, Inc., Seaford, DE). The animals inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV were housed in pairs, the animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV were housed in pairs, the negative control animals were housed together in a single microisolator, and the animals inoculated with ANDV were housed individually. Each animal was checked twice daily for symptoms of disease (e.g., lethargy, reluctance to move, inappetance, ruffled haircoat, tachypnea, and dyspnea). Strict barrier care was followed throughout the study to obviate virus transmission between animals in different microisolators.

The 18 animals inoculated with CHOV, 1 of the 5 animals inoculated with ANDV, and the 2 negative control animals were killed by intraperitoneal (IP) injection of a lethal dose (15 mg) of sodium pentobarbital. Two animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV were killed on days 7, 9, 11, 13, and 16 PI. The 4 other animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV, the 4 animals inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV, and the 2 negative control animals were killed on day 28 PI. Four animals inoculated with ANDV were found dead on day 10 or 11 PI. The fifth animal inoculated with ANDV was moribund on day 11. This animal was killed on day 11 PI by IP injection of 15 mg of sodium pentobarbital.

Samples of oropharyngeal (OP) secretions, cardiac blood, urine, and lung were collected from each animal inoculated with CHOV, samples of cardiac blood and lung were collected from both negative control animals, and samples of cardiac blood and lung were collected from each animal inoculated with ANDV. The samples of OP secretions and urine were collected to assess whether secretions or excretions from CHOV-infected hamsters are potentially infectious to laboratorians. The OP secretions were collected on a sterile cotton swab wetted with 0.01-M PBS, pH 7.4, containing 10% v/v heat-inactivated (56°C for 30 min) fetal bovine serum (FBS). Urine was collected by cystocentesis. The samples of OP secretions, urine, and lung were stored at -80°C until tested for infectious hantavirus. The carcasses of the animals killed with sodium pentobarbital were fixed by immersion in 10% w/v neutral buffered formalin.

Antibody assay

The serum samples from the animals inoculated with CHOV and the serum samples from the negative control animals were tested for immunoglobulin G (IgG) against CHOV, using an IFAT described previously.²⁸ Serial 2-fold dilutions (from 1:20 through 1:640) of each sample were tested against acetone-fixed cell spots that contained Vero E6 cells infected with CHOV strain 588 mixed 1:1 with uninfected Vero E6 cells. Immunoglobulin G bound to antigen was revealed by using a goat anti-hamster IgG fluorescein isothiocyanate conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The titer of a positive sample was the reciprocal

of the highest dilution that produced a specific pattern of fluorescence in the cytoplasm of approximately 50% of the cells in duplicate cell spots.

Virus assay

The samples of OP secretions, urine, and lung from the hamsters inoculated with CHOV, the samples of lung from the animals inoculated with ANDV, and the samples of lung from the negative control animals were tested for infectious hantavirus by cultivation in monolayers of Vero E6 cells as described previously.²⁸ Cell spots prepared from the Vero E6 cell cultures were tested for hantavirus antigen, using an IFAT in which the primary antibody was the polyvalent anti-hantavirus HMAF described previously. Mouse IgG bound to cell-associated hantavirus antigen was revealed by using a goat anti-mouse IgG fluorescein isothiocyanate conjugate (Kirkegaard and Perry Laboratories).

Histopathology

Samples of brain, lung, thymus, heart, liver, spleen, and kidney were dissected from the formalin-fixed carcasses and embedded in paraffin. Thin (4 μ m) sections of each tissue were stained with hematoxylin and eosin and then examined with a light microscope at medium power ($\times 100$) and high power ($\times 400$). Perivascular and interstitial lymphocytic cellular infiltration were assessed in each tissue section at medium power ($\times 100$) and scored: 0, none; 1, mild (inflammatory infiltrate in $> 1\%$ but $< 25\%$ of the entire section); 2, moderate ($\geq 25\%$ but $< 50\%$); 3, severe ($\geq 50\%$).

Immunohistochemistry assay

Thin (4 μ m) sections of lung, thymus, and heart from the CHOV-inoculated animals killed on days 7, 9, 11, 13, and 16 PI, 3 of the hamsters inoculated with 4.1 log₁₀ CCID₅₀ of CHOV and killed on day 28 PI, and the animal inoculated with ANDV and killed on day 11 PI were tested for hantavirus antigen, using an immunohistochemistry assay described previously.²⁸ The tissue sections were deparaffinized in xylene and absolute ethanol, rehydrated in decreasing concentrations of absolute ethanol, and then treated with DAKO Target Retrieval Solution (DakoCytomation, Carpinteria, CA) at 90°C for 30 min. The primary antibody was immune serum from a rabbit inoculated 3 times with infectious ANDV strain Chile-9717869, with virus in complete Freund's adjuvant on day 0 and virus in incomplete Freund's adjuvant on day 30 and day 90 (P. E. Rollin, personal communication). The secondary antibody was a biotinylated goat anti-rabbit IgG (Vector Laboratories, Inc., Burlingame, CA). Goat antibody bound to rabbit IgG was detected by using the LSAB2 Streptavidin-Biotin System (DakoCytomation). The chromogen and counterstain were diaminobenzidine and hematoxylin, respectively. Nonspecific binding of rabbit IgG was minimized by treating the tissue sections with 10% normal goat serum (Vector Laboratories, Inc.) for 20 min before application of the rabbit immune serum, endogenous biotin was blocked by using the DAKO Biotin Blocking Kit (DakoCytomation) according to the manufacturer's instructions, and endogenous peroxidase activity was minimized by treating the tissue sections with 3% hydrogen peroxide for 10 min immediately before heat-induced antigen retrieval. All steps in the staining process were done in an Autostainer Universal Staining System (DakoCytomation). The negative controls included sections of lung from ANDV-infected hamsters stained with normal (nonimmune) rabbit serum and sections of lung from uninfected hamsters stained with the rabbit anti-ANDV immune serum. The prevalence of antigen-positive cells in each of 10 randomly selected high-power fields ($\times 400$) was scored: 1, $> 1\%$ but $< 25\%$; 2, $\geq 25\%$ but $< 50\%$; 3, $\geq 50\%$. The median of the scores for the 10 randomly selected high-power fields was used to compare the prevalence of antigen-positive cells in samples of tissues from different animals.

RESULTS

None of the 18 animals inoculated with CHOV and neither of the negative control animals exhibited any symptom of disease. In contrast, 4 of the 5 animals inoculated with ANDV were found dead on day 10 ($N = 1$) or day 11 ($N = 3$) PI, and the fifth animal inoculated with ANDV was reluctant to move on day 10 PI and moribund on day 11 PI.

The body cavities and internal organs of the 18 animals inoculated with CHOV and the 2 negative control animals were unremarkable at necropsy. Note that the lungs of these 20 animals were neither congested nor edematous. The gross abnormalities in each of the 5 animals inoculated with ANDV were limited to the thoracic cavity and included large amounts of frothy tracheal fluid, reddened lungs that failed to collapse when the thoracic cavity was opened, and large volumes (2.1 to 6.0 mL) of clear, straw-colored pleural fluid.

Antibody (IgG) against CHOV was found in all 4 animals inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV. Thus, the $4.1 \log_{10}$ CCID₅₀ inoculum contained at least 100 median infectious doses (ID₅₀) of CHOV strain 588.

Antibody (IgG) against CHOV was found in 11 (78.5%) of the 14 animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV (Table 1). The antibody titers in the antibody-positive animals ranged from 40 through 640: 40 in 1 of the animals killed on day 9 PI, 160 in 1 of the animals killed on day 11 PI, ≥ 640 in the other animal killed on day 11 PI, and ≥ 640 in the 8 animals killed on days 13, 16, and 28 PI. Neither of the negative control animals was antibody-positive against CHOV.

Infectious hantavirus was isolated from the lungs of the 10 animals inoculated with CHOV and killed on days 7, 9, 11, 13, and 16 PI, 2 of the 4 animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV and killed on day 28 PI, 1 of the 4 animals inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV and killed on day 28 PI, and each of the 5 animals inoculated with ANDV. Infectious hantavirus also was isolated from the samples of OP secretions or urine from 6 of the 18 animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV (Table 1) and from the samples of urine from 2 of the 4 animals inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV.

The samples of brain, lung, thymus, heart, liver, spleen, and kidney from the 18 animals inoculated with CHOV and the samples of brain, lung, thymus, heart, liver, spleen, and kidney from the negative control animals were microscopically unremarkable. Note that there was no inflammatory cellular infiltrate or edema in the samples of lung from the CHOV-infected animals (Figure 1A), regardless of inoculum dose. In contrast, microscopic examination of the sample of lung from the animal inoculated with ANDV and killed on day 11 PI revealed mild, focal subacute interstitial pneumonitis, mild perivascular lymphocytic inflammatory infiltrate, interstitial edema, and focal alveolar edema (Figure 1B).

Hantavirus antigen was detected in endothelial cells in the microvasculature of the lung of each animal killed on or before day 13 PI and 1 of 3 animals inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV and killed on day 28 PI (Table 1). Hantavirus antigen also was detected in endothelial cells in the microvasculature of the hearts of animals killed on days 9, 11, 13, and 28 PI, endothelial cells in the microvasculature of the thymuses of animals killed on days 7, 9, 11, 13, and 28 PI, small numbers of macrophages in the lungs of the 4 animals killed on days 9 and 11 PI, and endothelial cells in the microvasculature of the lung of the animal inoculated with ANDV and killed on day 11 PI. The most intensive staining in the CHOV-infected animals was found in endothelial cells in the microvasculature of lungs of the 4 animals killed on days 9 and 11 PI (Figure 1C). Note that the prevalence of antigen-positive cells in the samples of lung from the 4 animals inoculated with CHOV and killed on days 9 and 11 PI was similar to

the prevalence of antigen-positive cells in the sample of lung from the hamster inoculated with ANDV and killed on day 11 PI (Figure 1D).

DISCUSSION

The results of a previous study indicated that ANDV strain Chile-9717869 in the adult Syrian golden hamster is highly lethal, regardless of inoculum dose.²⁶ The morphological abnormalities in the ANDV-infected Syrian golden hamsters in that study and in this study included frothy tracheal fluid, congested, edematous lungs, large volumes of pleural fluid, and (microscopically) subacute interstitial pneumonitis, interstitial edema, and alveolar edema.

None of the 18 hamsters infected with CHOV in this study exhibited any symptom of disease. Together, the asymptomatic infections and the absence of gross and histological abnormalities in the 4 hamsters inoculated with $2.1 \log_{10}$ CCID₅₀ of CHOV and 14 hamsters inoculated with $4.1 \log_{10}$ CCID₅₀ of CHOV strongly suggest that CHOV strain 588 in the adult Syrian golden hamster is not pathogenic, regardless of inoculum dose.

The pathophysiology that underlies the evolution of the life-threatening pulmonary edema in HPS is not well understood. However, the absence of necrosis in the lungs of the majority of fatal HPS cases in combination with the marked similarity between serum and the protein-rich fluid in the lungs of HPS cases suggest that the edema in the interstitium of lung tissue and alveoli is a consequence of increased capillary permeability (i.e., fluid leakage from the pulmonary microvasculature).^{23,24}

The endothelial cell of the microvasculature in lung tissue is a major target of SNV infection in humans.²⁵ The results of an *in vitro* study indicated that SNV alone has no measurable effect on the permeability of monolayers of human lung microvascular endothelial cells.²⁹ Thus, the occurrence of the capillary leak in HPS likely is dependent upon interactions between endothelial cells and other cells or soluble factors in infected lung tissue.³⁰

The results of a study of autopsy samples from fatal HPS cases suggested that activation of lymphocytes, monocytes, and macrophages and production of tumor necrosis factor- α (TNF- α), interleukin (IL)-2, interferon- γ (IFN- γ), and other vasoactive cytokines in lung and spleen play a significant role in the pathogenesis of the capillary leak in HPS.³¹ An *in vitro* study done thereafter demonstrated that human SNV-specific cytotoxic T-cells can increase the permeability of monolayers of SNV-infected human endothelial cells.³²

The MAPV prototype strain HV 97021050 in the Syrian golden hamster can cause a lethal disease that is pathologically similar to the HPS-like disease caused by ANDV strain Chile-9717869 in the Syrian golden hamster.^{26,28} The endothelial cell in the microvasculature in lung tissue is a major target of MAPV infection and ANDV infection in the Syrian golden hamster and, importantly, subacute interstitial pneumonitis is a key feature of MAPV infection and ANDV infection in the Syrian golden hamster.^{26,28,33}

The results of this study indicate that the endothelial cell in the microvasculature in lung tissue is a major target of CHOV infection in the Syrian golden hamster. The absence of inflammation and edema in the lungs of the CHOV-infected hamsters suggests that the cellular inflammatory response in lung tissue plays a key role in the development of the pulmonary edema in the HPS-like disease in ANDV-infected hamsters and MAPV-infected hamsters. Studies to assess the role of hantavirus-specific cytotoxic T cells, macrophages, and vasoactive cytokines in ANDV- or MAPV-infected hamsters may provide insight into the pathogenesis of the life-threatening pulmonary edema in HPS.

The 5 hamsters inoculated with ANDV and 18 hamsters inoculated with CHOV in this study were similar in age, identical in gender, from the same genetic stock, inoculated in the same manner, housed in the same facility, and cared for by a single laboratorian. Thus, the occurrence of HPS-like disease only in the ANDV-infected hamsters likely is a consequence of genetic differences between ANDV strain Chile-9717869 and CHOV strain 588. A specific objective of research currently supported by the National Institutes of Health is to identify the genetic elements of the hantavirus genome that are causally associated with severe HPS-like disease in the Syrian golden hamster.

The results of previous studies indicated that SNV in the Syrian golden hamster is highly infectious but apathogenic, regardless of the inoculum dose.^{26,33} The failure of SNV to cause HPS-like disease in the Syrian golden hamster was attributed to the inability of the virus to efficiently disseminate from the intramuscular injection site and subsequently infect large numbers of endothelial cells in lung and other solid tissues.³³ Thus, the reason that SNV was apathogenic in the Syrian golden hamsters in previous studies appears to be different from the reason that CHOV was apathogenic in the Syrian golden hamsters in this study.

The genomes of hantaviruses consist of 3 RNA segments, designated small (S), medium (M), and large (L).¹³ These segments encode the nucleocapsid (N) protein, glycoprotein precursor (GPC), and RNA-dependent RNA polymerase, respectively. Analyses of N protein gene sequences and GPC gene sequences in a recent study indicated that MAPV is phylogenetically closely related to ANDV and that CHOV is phylogenetically more closely related to ANDV and MAPV than to SNV.¹⁴ Conceptually, the ability to efficiently disseminate from a peripheral inoculation site to lung in the Syrian golden hamster may have emerged after the separation of the SNV and CHOV-ANDV-MAPV lineages. Further, the capacity to elicit a strong cellular immune response in lung tissue in the Syrian golden hamster may have emerged after the separation of the CHOV and ANDV-MAPV lineages.

In this study, CHOV was isolated from urine samples collected on day 28 PI from 2 of the 4 animals inoculated with 2.1 log₁₀ CCID₅₀ and from urine samples collected on day 28 PI from 2 of 4 animals inoculated with 4.1 log₁₀ CCID₅₀. Thus, Syrian golden hamsters may be infectious to humans long after they become infected with CHOV.

Acknowledgments

Pierre E. Rollin (Centers for Disease Control and Prevention, Atlanta, GA) kindly provided the rabbit immune serum used in the immunohistochemistry assay. Robert B. Tesh (World Reference Center for Emerging Viruses and Arboviruses, University of Texas Medical Branch, Galveston) provided the polyvalent anti-hantavirus HMAF used in the indirect fluorescent antibody tests. Miguel A. Grimaldo (University of Texas Medical Branch, Galveston) provided logistical support for the work done inside the Shope Laboratory. Cristina Cassetti (National Institutes of Health) facilitated administration of the financial support for this study.

Financial support: This study was financially supported by National Institutes of Health Grants AI-63235 ("Animal models of hantavirus cardiopulmonary syndrome") and AI-67947 ("Viral determinants of hantavirus pulmonary disease in the hamster").

REFERENCES

- Centers for Disease Control and Prevention (CDC). Outbreak of acute illness—southwestern United States, 1993. *MMWR Morb Mortal Wkly Rep* 1993;42:421–424. [PubMed: 8502213]
- Stephen C, Johnson M, Bell A. First reported cases of hantavirus pulmonary syndrome in Canada. *Can Commun Dis Rep* 1994;20:121–125. [PubMed: 7920363]
- Vincent MJ, Quiroz E, Gracia F, Sanchez AJ, Ksiazek TG, Kitsutani PT, Ruedas LA, Tinnin DS, Caceres L, Garcia A, Rollin PE, Mills JN, Peters CJ, Nichol ST. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology* 2000;277:14–19. [PubMed: 11062031]

4. da Silva MV, Vasconcelos MJ, Hidalgo NT, Veiga AP, Canzian M, Marotto PC, de Lima VC. Hantavirus pulmonary syndrome. Report of the first three cases in Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 1997;39:231–234. [PubMed: 9640788]
5. López N, Padula P, Rossi C, Lázaro ME, Franze-Fernández MT. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology* 1996;220:223–226. [PubMed: 8659118]
6. Johnson AM, Bowen MD, Ksiazek TG, Williams RJ, Bryan RT, Mills JN, Peters CJ, Nichol ST. Laguna Negra virus associated with HPS in western Paraguay and Bolivia. *Virology* 1997;238:115–127. [PubMed: 9375015]
7. Centers for Disease Control and Prevention (CDC). Hantavirus pulmonary syndrome—Chile, 1997. *MMWR Morb Mortal Wkly Rep* 1997;46:949–951. [PubMed: 9338456]
8. Padula PJ, Colavecchia SB, Martínez VP, González Della Valle MO, Edelstein A, Miguel SD, Russi J, Riquelme JM, Colucci N, Almiron M, Rabinovich RD. Genetic diversity, distribution, and serological features of hantavirus infection in five countries in South America. *J Clin Microbiol* 2000;38:3029–3035. [PubMed: 10921972]
9. Morzunov SP, Feldmann H, Spiropoulou CF, Semenova VA, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. *J Virol* 1995;69:1980–1983. [PubMed: 7853545]
10. Khan AS, Gaviria M, Rollin PE, Hlady WG, Ksiazek TG, Armstrong LR, Greenman R, Ravkov E, Kolber M, Anapol H, Sfakianaki ED, Nichol ST, Peters CJ, Khabbaz RF. Hantavirus pulmonary syndrome in Florida: association with the newly identified Black Creek Canal virus. *Am J Med* 1996;100:46–48. [PubMed: 8579086]
11. Hjelle B, Lee SW, Song W, Torrez-Martinez N, Song JW, Yanagihara R, Gavrilovskaya I, Mackow ER. Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse, *Peromyscus leucopus*: genetic characterization of the M genome of New York virus. *J Virol* 1995;69:8137–8141. [PubMed: 7494337]
12. Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 1993;262:914–917. [PubMed: 8235615]
13. Nichol ST.; Beaty, BJ.; Elliott, RM.; Goldbach, R.; Plyusnin, A.; Schmaljohn, CS.; Tesh, RB. Family *Bunyaviridae*. In: Fauquet, CM.; Mayo, MA.; Maniloff, J.; Desselberger, U.; Ball, LA., editors. *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press; 2005. p. 695–716.
14. Milazzo ML, Cajimat MNB, Hanson JD, Bradley RD, Quintana M, Sherman C, Velasquez RT, Fulhorst CF. *Catacamas virus*, a hantaviral species naturally associated with *Oryzomys couesi* (Coues' oryzomys) in Honduras. *Am J Trop Med Hyg* 2006;75:1003–1010. [PubMed: 17124003]
15. Fulhorst CF, Cajimat MNB, Utrera A, Milazzo ML, Duno GM. Maporal virus, a hantavirus associated with the fulvous pygmy rice rat (*Oligoryzomys fulvescens*) in western Venezuela. *Virus Res* 2004;104:139–144. [PubMed: 15246651]
16. Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, Gage KL, Rollin PE, Sarisky J, Ensore RE, Frey JK, Peters CJ, Nichol ST. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *J Infect Dis* 1994;169:1271–1280. [PubMed: 8195603]
17. Calderon G, Pini N, Bolpe J, Levis S, Mills J, Segura E, Guthmann N, Cantoni G, Becker J, Fonollat A, Ripoll C, Bortman M, Benedetti R, Enria D. Hantavirus reservoir hosts associated with peridomestic habitats in Argentina. *Emerg Infect Dis* 1999;5:792–797. [PubMed: 10603213]
18. Cantoni G, Padula P, Calderon G, Mills J, Herrero E, Sandoval P, Martinez V, Pini N, Larrieu E. Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina. *Trop Med Int Health* 2001;6:811–816. [PubMed: 11679129]
19. Levis S, Morzunov SP, Rowe JE, Enria D, Pini N, Calderon G, Sabattini M, St Jeor SC. Genetic diversity and epidemiology of hantaviruses in Argentina. *J Infect Dis* 1998;177:529–538. [PubMed: 9498428]

20. Torres-Perez F, Navarrete-Droguett J, Aldunate R, Yates TL, Mertz GJ, Vial PA, Ferres M, Marquet PA, Palma RE. Peridomestic small mammals associated with confirmed cases of human hantavirus disease in southcentral Chile. *Am J Trop Med Hyg* 2004;70:305–309. [PubMed: 15031522]
21. Koster, FT.; Levy, H. Hantavirus cardiopulmonary syndrome: a new twist to an established pathogen. In: Fong, IW.; Alibek, K., editors. *New and Evolving Infections of the 21st Century*. New York: Springer-Verlag; 2006. p. 151-170.
22. Vapalahti O, Lundkvist A, Vaheri A. Human immune response, host genetics, and severity of disease. *Curr Top Microbiol Immunol* 2001;256:153–169. [PubMed: 11217403]
23. Nolte KB, Feddersen RM, Foucar K, Zaki SR, Koster FT, Madar D, Merlin TL, McFeeley PJ, Umland ET, Zumwalt RE. Hantavirus pulmonary syndrome in the United States: a pathological description of a disease caused by a new agent. *Hum Pathol* 1995;26:110–120. [PubMed: 7821907]
24. Hallin GW, Simpson SQ, Crowell RE, James DS, Koster FT, Mertz GJ, Levy H. Cardiopulmonary manifestations of hantavirus pulmonary syndrome. *Crit Care Med* 1996;24:252–258. [PubMed: 8605797]
25. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, Rollin PE, Ksiazek TG, Nichol TS, Mahy BWJ, Peters CJ. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. *Am J Pathol* 1995;146:552–579. [PubMed: 7887439]
26. Hooper JW, Larsen T, Custer DM, Schmaljohn CS. A lethal disease model for hantavirus pulmonary syndrome. *Virology* 2001;289:6–14. [PubMed: 11601912]
27. Meissner JD, Rowe JE, Borucki MK, St Jeor SC. Complete nucleotide sequence of a Chilean hantavirus. *Virus Res* 2002;89:131–143. [PubMed: 12367756]
28. Milazzo ML, Eyzaguirre EJ, Molina CP, Fulhorst CF. Maporal viral infection in the Syrian golden hamster: a model of hantavirus pulmonary syndrome. *J Infect Dis* 2002;186:1390–1395. [PubMed: 12404153]
29. Sundstrom JB, McMullan LK, Spiropoulou CF, Hooper WC, Ansari AA, Peters CJ, Rollin PE. Hantavirus infection induces the expression of RANTES and IP-10 without causing increased permeability in human lung microvascular endothelial cells. *J Virol* 2001;75:6070–6085. [PubMed: 11390609]
30. Terajima M, Hayasaka D, Maeda K, Ennis FA. Immunopathogenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: do CD8+ T cells trigger capillary leakage in viral hemorrhagic fevers? *Immunol Lett* 2007;113:117–120. [PubMed: 17897725]
31. Mori M, Rothman AL, Kurane I, Montoya JM, Nolte KB, Norman JE, Waite DC, Koster FT, Ennis FA. High levels of cytokine-producing cells in the lung tissues of patients with fatal hantavirus pulmonary syndrome. *J Infect Dis* 1999;179:295–302. [PubMed: 9878011]
32. Hayasaka D, Maeda K, Ennis FA, Terajima M. Increased permeability of human endothelial cell line EA.hy926 induced by hantavirus-specific cytotoxic T lymphocytes. *Virus Res* 2007;123:120–127. [PubMed: 16979772]
33. Wahl-Jensen V, Chapman J, Asher L, Fisher R, Zimmerman M, Larsen T, Hooper JW. Temporal analysis of Andes virus and Sin Nombre virus infection of Syrian hamsters. *J Virol* 2007;81:7449–7462. [PubMed: 17475651]

FIGURE 1.

High-power ($\times 400$) images of (A and C) the lung of a hamster infected with Choclo virus and (B and D) the lung of a hamster infected with Andes virus. Both animals were killed on day 11 post-inoculation. (A) Lung of the hamster infected with Choclo virus and stained with hematoxylin and eosin. Note the absence of inflammation and edema. (B) Lung of the hamster infected with Andes virus and stained with hematoxylin and eosin. Note the subacute interstitial pneumonitis and diffuse alveolar edema. (C) Lung of the hamster infected with Choclo virus and stained for hantavirus antigen. Virus antigen is diffusely distributed within endothelial cells. (D) Lung of the hamster infected with Andes virus and stained for hantavirus antigen. Note that the distribution of antigen is similar to the distribution of antigen in the lung of the animal infected with Choclo virus. The primary antibody and the chromogen used in the immunohistochemistry assay were a rabbit anti-Andes virus immune serum and diaminobenzidine, respectively. This figure appears in color at www.ajtmh.org.

TABLE 1
Results of laboratory tests on hamsters inoculated with 4.1 log₁₀ median cell culture infectious doses (CCID₅₀) of Choclo virus strain 588

	Days post-inoculation (PI)						Total
	7	9	11	13	16	28	
Virus recovered from [*]							
Throat swab	0/2	1/2	1/2	2/2	0/2	0/4	4/14
Lung	2/2	2/2	2/2	2/2	2/2	2/4	12/14
Urine	0/2	1/2	0/2	0/2	0/2	2/4	3/14
Antibody status [†]	0/2	1/2	2/2	2/2	2/2	4/4	11/14
Hantaviral antigen in [‡]							
Lung	2/2 (2)	2/2 (3)	2/2 (2,3)	2/2 (1,2)	0/1	1/3 (2)	9/12
Heart	0/2	2/2 (1)	1/1 (1)	2/2 (1)	0/1	1/3 (1)	6/11
Thymus	1/2 (1)	2/2 (1)	2/2 (1)	2/2 (1)	0/2	2/2 (1)	9/12

* Number positive/number tested

[†] The antibody (IgG) titers were measured by an indirect fluorescent antibody test and ranged from less than 20 (negative) through 640. The titer in the antibody-positive animal killed on day 9 post-inoculation (PI) was 40, the antibody titer in 1 of the animals killed on day 11 PI was 160, and the antibody titers in the 9 other antibody-positive animals were $\geq$ 640.

[‡] The number(s) in parentheses are the median scores for the prevalence of antigen-positive cells in 10 randomly selected high-power ($\times$ 400) microscope fields in sections of lung, heart, or thymus from the antigen-positive animals (see Materials and Methods).