Am. J. Trop. Med. Hyg., 89(3), 2013, pp. 489–494
doi:10.4269/ajtmh.12-0334

Hantavirus Fever without Pulmonary Syndrome in Panama

Blas Armien, Juan M. Pascale, Carlos Muñoz, Jamileth Mariñas, Heydy Núñez, Milagro Herrera, José Trujillo, Deyanira Sánchez, Yaxelis Mendoza, Brian Hjelle, and Frederick Koster*

Epidemiology and Microbiology, Gorgas Memorial Institute for Health Research, Panama City, Panama; Epidemiology, Ministry of Health, Panama City, Panama; Clinical Medicine, Social Security Health System, Panama City, Panama; Pulmonary Medicine, Santo Tomas Hospital, Panama City, Panama; Departments of Pathology, Biology, and Molecular Genetics and Microbiology, University of New Mexico Health Sciences Center, Albuquerque, New Mexico; Lovelace Respiratory Research Institute, Albuquerque, New Mexico

Abstract. In Panama, hantavirus pulmonary syndrome (HPS) is caused by Choclo virus, a species phylogenetically related to Andes and Maporal viruses. Up to 60% of the population has been positive for specific serum antibody in community-based surveys, but mortality is very uncommon. In four western Panama clinics, we tested individuals presenting with a severe febrile prodrome for acute hantavirus (HV) infection by immunoglobulin M enzyme-linked immunosorbent assay and reverse transcription polymerase chain reaction as well as clinically similar infections, such as dengue and leptospirosis. From 2006 to 2009, at least 21% of 117 patients diagnosed with HV infection had HV Fever (HF) with no evidence of pulmonary edema (no respiratory distress or radiographic lung infiltrates), and 44% of patients had very mild HPS (radiographic pulmonary edema but no respiratory insufficiency). HV infection caused by Choclo virus in Panama presents often as HF, which contrasts with HV in the Americas but is consistent with the high seroprevalence in endemic regions.

INTRODUCTION

Hantavirus (HV) infections were first described in the Americas during an outbreak in southwestern United States in 1993 and have since been found throughout North, South, and Central America.[1] The primary manifestation of HV infection is pulmonary edema; thus, HV pulmonary syndrome (HPS) or HV cardiopulmonary syndrome (HCPS) is often a dominant feature when severe pulmonary edema and cardiogenic shock are present. In Panama, Choclo virus was first described in 2000 during an outbreak in the agroecosystems of the Azuero Peninsula in western Panama.[2,3] Choclo virus is hosted by the fulvous rice rat (*Oligoryzomys fulvescens*)[2] and displays a peridomestic habitat preference.[4] Choclo virus is phylogenetically related to Maporal virus in Venezuela, Laguna Negra virus in Paraguay, and Andes virus in Argentina and Chile, and it seems to be responsible for almost all human infections in Panama.[4,5] Three other HVs are found in Panama, including two in the HPS-endemic region (Calabazo virus in *Zygodontomys brevicauda cherriei* and an unnamed virus in *Sigmodon hirsutus*) and one outside of the HPS-endemic region (a relative of Rio Segundo virus in *Reithrodontomys* species).[4,6]

A high prevalence of HV antibodies in serum, ranging from 12% to 45%, was noted in neighborhoods of HPS patients[3] and community-wide surveys[7,8] among individuals who had no history of hospitalization for respiratory insufficiency. A comparably high seroprevalence found in northern Argentina and Paraguay[9] and in Brazil[10–12] contrasts with low seroprevalence in Andes virus-endemic regions.[13,14] In four Panama communities, repeated seroprevalence surveys found that history-negative infections outnumbered hospitalized HPS in the same region by a ratio of 14:1.[8] These observations implied that a large fraction of Choclo virus infections were asymptomatic, did not develop pulmonary edema, or were mild HPS.

To identify mild as well as severe HV infections, we conducted active surveillance at four clinics in the endemic region of Panama for patients with febrile illnesses accompanied by prodromal symptoms typical of HV infection, including myalgias, headache, chills, and nausea. Diagnosis of HV infection was sought by three assays, and pulmonary involvement was assessed by symptoms, pulse oximetry, and chest radiography to identify graded severity of disease.

MATERIALS AND METHODS

Four communities located within HV-endemic agricultural ecosystems in Western Panama were selected for clinic-based patient recruitment. One community in Los Santos Province (Tonosí), two communities in Coclé Province (Aguadulce and Natá), and one community in Veraguas Province (Soná) had 24-hour clinics with onsite diagnostic capabilities for acute infections, an onsite immunoglobulin M (IgM) HV antibody assay, and previous experience in diagnosing at least 10 cases of HPS. Patient recruitment began in May of 2006 and ended in March of 2010. A total of 10,917 patients were seen for febrile illness in these four clinics, including 7,821 children under the age of 15 years.

Informed written consent was obtained from all adult participants, and written assent was obtained from the parents of children. Consent forms were reviewed and approved by institutional ethics review boards at the University of New Mexico and the Gorgas Memorial Institute in Panama City along with the protocol review committee of the International Centers for Infectious Diseases Research program of the National Institute of Allergy and Infectious Diseases. All adults permanently residing in each community, free of known chronic infections, and presenting to the clinic with an acute febrile illness of more than 24-hours duration and symptoms suggesting HV prodrome were eligible for the study. Recruitment targeted patients with two or more prodrome symptoms (myalgia, headache, chills, nausea, and vomiting) and the absence of upper respiratory symptoms to avoid recruitment of the large numbers of influenza and other respiratory infections. Symptoms and physical examination were recorded on a standardized questionnaire form validated in a preliminary study at one site (Tonosí). Surveillance for all HPS was conducted through review of cases reported to the Ministry of Health. The diagnosis

* Address correspondence to Frederick Koster, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM 87108. E-mail: fkoster@lrri.org

TABLE 1
Definitions of four HV disease categories

Criteria	Definition of four HV disease categories			
	HF	Mild HPS group A	Mild HPS group B	Moderate to severe HPS
Febrile prodrome	+	+	+	+
Dyspnea*	Absent	Absent	Occasional	Marked
Chest X-ray	Normal	Abnormal	Abnormal	Abnormal
Hypoxemia	Absent	SaO2 > 90%	SaO2 > 85%	SaO2 < 85%
Oxygen therapy	None	None	Nasal cannula	CPAP/MV

CPAP/MV = continuous positive airway pressure/mechanical ventilation.
*Presence of tachypnea > 24/min and complaint of shortness of breath.

of HV infection required either IgM-positive serology by both of two assays or detection of Choclo virus RNA by reverse transcription polymerase chain reaction (RT-PCR) in serum.

Heparinized whole blood from arm venipuncture was separated by centrifugation, and plasma was stored at –20°C until analysis. Antibody to all known HVs indigenous to the Americas was cross-reacted to the N protein of Sin Nombre virus in binding assays. A strip immunoblot assay (SIA) for IgM antibody containing recombinant N protein of the 3H226 genotype of Sin Nombre virus was used as described; the criterion for positivity was a dark band to Sin Nombre N protein at a serum dilution of 1:200.[15] An enzyme immunoassay (EIA; Focus Diagnostics, Cypress, CA) used recombinant nucleocapsid protein from Sin Nombre virus.[14] Using the cutoff value established by the manufacturer, comparison with the Western blot test[13] and the EIA assay of the Centers for Disease Control[16] for 50 HPS subjects in Panama and a panel of healthy blood donor sera found a sensitivity of 100% and a specificity of 97%.

Genomic viral RNA was sought in acute blood from plasma, cell pellet, or both using a Choclo virus-specific RT-PCR assay with the following nested primers: HVF: 5′-AATGTCYTK GATGTKAACTC-3; HVR: 5′-KKCCAGGTGTWATYTCA TCA-3′ for first-round PCR (50°C for 30 minutes, 35 cycles at 94°C for 45 seconds and 57°C for 1 minute, 60 cycles at 72°C for 1 minute, and final extension for 5 minutes at 72°C) that amplifies a 317-bp fragment of the S gene. The second-round PCR amplifies a fragment of around 250 bp with HVCHF: 5′-AATGTCTTGGATGTGAACTC-3′; HVCHR: 5′-AGTGG GCATCGACACATAAA-3′ (94°C for 2 minutes, 35 cycles at 94°C for 45 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, and final extension for 5 minutes at 72°C). PCR products were electrophoresed on 2% agarose gels.

TABLE 3
Diagnostic assay results for all HV patients

Clinical diagnostic category	IgM+ (no PCR test)	PCR+/IgM–	IgM+/PCR+	IgM+/PCR–	Total HV
HF	11 (47.8)*	10 (62.5)	13 (28.9)	18 (54.5)	52 (44.4)
Mild HPS groups A + B†	9 (39.1)	4 (25.0)	26 (57.8)	13 (39.4)	52 (44.4)
Moderate to severe HPS	3 (13.0)	2‡ (12.5)	6 (13.3)	2§ (6.1)	13 (11.1)
Total	23	16	45	33	117

*n subjects (percent of that category among total with laboratory diagnostic result).
†Mild HPS combining both groups A and B (Table 1), because there were no differences between the two groups.
‡Serum or plasma sample not available for IgM assay.
§Sample for PCR degraded from one subject; sample obtained late in illness from another subject.

This assay does not detect genomic RNA of either Calabazo or Rio Segundo viruses.

Evidence for five other acute infections that clinically mimic HV infection was sought in the clinic. Acute leptospirosis was identified by a specific IgM assay (PanBio, Brisbane, Queensland, Australia) and RT-PCR.[17] Acute malaria was sought by inspection of thick and thin smears of peripheral blood by an experienced technician. Acute mononucleosis was diagnosed by a titer > 128 in an IgM assay to Epstein–Barr virus (PanBio, Brisbane, Queensland, Australia) following the manufacturer's instructions. Influenza was sought by indirect immunofluorescent assay of nasal swab specimens. Dengue virus and *Rickettsia* acute infections were identified by measuring IgM titers by enzyme-linked immunosorbent assay (ELISA) and indirect flourescent antibody (IFA), respectively (Focus Diagnostics Cypress, CA).

A peripheral blood cell count, chest radiography, serum electrolytes with blood urea nitrogen (BUN) and creatinine, liver enzymes, and arterial blood gas analysis were performed at the discretion of the attending physician. All chest radiographs (61 HV+ and 13 HV– subjects) were read by a board-certified radiologist. Arterial blood gases were measured in 65 patients (Instrumentation Laboratory GEM Premier Model 3000). The clinical and laboratory data were used to define four categories of HV infection to distinguish mild HPS with and without hypoxemia or dyspnea (Table 1). Absence of radiographic evidence of pulmonary edema was denoted by the new category HV Fever (HF). Mild HPS included two groups: group A, with evidence of pulmonary edema by chest X-ray alone without respiratory symptoms or

TABLE 2
Demographic characteristics and outcome among four diagnostic categories of HV infection and non-HV disease

Demographic	HF	Mild HPS group A	Mild HPS group B	Moderate to severe HPS	HV-negative	Leptospirosis
N	52	25	27	13	27	6
Male (%)	55.8	52.0	59.3	61.5	63.0	83.3
Age (years) mean (SD); range	33.7 (16.3); 11–79	33.1 (17.5); 13–64	38 (14.0); 11–67	38 (20.5); 17–78	30.7 (14.7); 4–61	21 (13.5); 7–41
Clinic*	34/12/6	18/7/0	10/17/0	2/5/6	9/12/6	3/1/2
Hospitalized (%)	61.5	80.0	100.0	100.0	37.0	66.7
Days of hospitalization†	4.2, 1–9	5.4, 1–12	7.2, 1–18	9.3, 1–42	3.0, 1–8	3.0, 2–4
Days of symptoms†	7.6, 2–16	8.0, 1–25	11.4, 6–26	13.2, 2–46	6.2, 2–14	5.6, 3–7
Days of fever PTH†	4.1, 0–9	3.3, 0–13	4.1, 2–8	5.4, 2–15	3.6, 0–14	4.3, 3–7
Death (%)‡	0.0 (0)	0.0 (0)	0.0 (0)	53.8 (7)	0.0 (0)	16.7 (1)

HV-negative = negative for HV infection and leptospira infection; mild HPS group A, mild HV pulmonary syndrome without hypoxemia or dyspnea; PTH = prior to hospitalization.
*Distribution among clinics: Tonosí/Soná/Aguadulce and Natá.
†Days (mean, range); PTH before hospitalization.
‡Three patients died in the first 24 hours.

TABLE 4
Clinical characteristics among four diagnostic categories of HV disease

Symptoms	HF	Mild HPS group A	Mild HPS group B	Moderate to severe HPS	HV-negative	Leptospirosis
*N**	52	25	27	13	27	6
Weakness	84.6	80.0	85.2	84.6	66.7	100
Headache	88.5	76.0	88.9	75.0	85.2	100
Myalgia†	73.1	72.0	85.2	66.7	44.4	66.7
Cough‡	23.5	45.8	70.4	61.5	29.6	16.7
Nausea	44.2	40.0	51.9	23.1	33.3	50.0
Vomiting§	29.4	32.0	29.6	46.2	11.1	33.3
Diarrhea	11.5	8.0	25.9	16.7	11.1	16.7
Chills	9.6	4.0	14.8	23.1	11.1	0
Abdominal pain¶	3.8	4.0	3.7	38.5	7.4	0
Oliguria	0	0	0	15.3	0	0
Breath rate‖	16 [14–32] (34)	22 [19–36] (20)	26 [20–43] (27)	25 [20–40] (13)	21 [18–38] (16)	35 [20–60] (3)
% Sat O_2‖	98 [95–99] (12)	97 [95–99] (12)	94 [85–99] (24)	87 [50–90] (10)	98 [95–99] (5)	69 [45–94] (2)

*All subjects had fever.
†Myalgia less frequent in non-HV infection ($P = 0.01$).
‡Cough frequency increasing with severity of HPS ($P = 0.0003$).
§Vomiting tended to be less frequent in non-HV infection ($P = 0.06$).
¶Abdominal pain increasing with severity of HPS ($P = 0.002$).
‖Breath rate and % Sat O_2: mean [range] (*N* for data available).

hypoxemia, and group B, with compatible pulmonary infiltrate, and respiratory insufficiency (respiratory rate > 24/min) or hypoxemia by pulse oximetry.

Data were transferred from field collection forms to a database (Microsoft Access 2007) for statistical analyses using Epi-Info Software (version 3.5.3; Centers for Disease Control and Prevention, Atlanta, GA) and TIBCO Spotfire S+ 8.1 for Windows. Differences in clinical characteristics between groups were analyzed by non-parametric tests of proportion (Fisher–Freeman–Halton Exact Test) and Wilcoxon rank sum tests and reported if the difference was significant at $P < 0.05$.

RESULTS

A total of 150 subjects were recruited into the study, including 16 children between the ages of 5 and 15 years (Table 2). The use of both the IgM ELISA and PCR assays identified 117 subjects with presumed HV infection, including 11 of the children recruited (Table 3). The IgM assay was positive in 86% (101) of subjects with presumed HV infection. Subjects without pulmonary edema (HF) had less frequent positive IgM (81%) than those subjects with pulmonary edema (91%). Among 16 presumed infections without IgM antibody, 2 infections were not tested for IgM, and 14 infections with positive RT-PCR assays had either HF (10) or mild pulmonary edema without hypoxemia or dyspnea (4). Of 33 subjects with positive IgM and negative PCR, 18 subjects had HF, and 13 subjects had mild HPS; the remaining two subjects with moderate/severe HPS had inadequate samples for PCR test. IgG immunoblot serology was performed on some acute sera, although lack of follow-up samples prevented documenting seroconversion in the IgG serotype. Of 22 IgG-positives, 19 were IgM-positive; the three negatives presumably represented remote infection and were not counted as HV infection. Among 30 IgM-positive acute sera tested, 19 were IgG-positive, and 11 were IgG-negative, presumably because of early infection.

Six subjects were found to have acute leptospirosis infection, whereas no subjects had malaria, dengue, influenza, rickettsial infection, or infectious mononucleosis (Table 2). Temporally, 68% of the HV infections detected in these four clinics were diagnosed during the 2007–2008 interval, when HPS diagnoses were elevated above usual levels in the national surveillance system (data not shown).

Symptoms recorded at first clinical presentation did not display dramatic differences between the four categories of HV infection (Table 4). Weakness, myalgia, and headache were equally common among all HV severity groups, whereas cough ($P < 0.0003$, Fisher–Freeman–Halton Exact Test) and abdominal pain ($P < 0.002$) significantly increased in frequency with increasing severity of illness. Cough was reported in patients with no evidence of pulmonary edema. HV-negative patients had significantly less myalgia ($P < 0.01$) and tended to have less vomiting ($P = 0.06$), but otherwise, they were clinically indistinguishable from the HV-positive patients.

By definition, all HF patients with recorded chest films ($N = 25$) had normal X-rays, and all HPS patients had evidence of pulmonary infiltrates consistent with edema (Table 5). It is important to note that, of 48 radiographed patients with mild HPS,

TABLE 5
Chest radiographic findings at initial clinic visit

		HV diseases					
Chest X-ray findings	*N*	HF	Mild HPS group A*	Mild HPS group B	Moderate to severe HPS	HF-negative % (*n*)	Leptopira-positive % (*n*)
Bilateral infiltrates	51 (34.0)	–	56.0 (14)	85.2 (23)	100 (13)	–	16.7 (1)
Parahilar infiltrates	12 (8.0)	–	32.0 (8)	11.1 (3)	–	3.7 (1)	–
Normal chest X-ray	36 (24)	48.1 (25)	–	–	–	29.6 (8)	50.0 (3)
No information in the record†	51 (34.0)	51.9 (27)	12.0 (3)	3.7 (1)	–	66.7 (18)	33.3 (2)
Total	150	52	25	27	13	27	6

*Percent of diagnostic category with chest X-ray finding (*N*).
†Twenty-four (47.1%) patients were ambulatory, and twenty-seven (52.9%) patients were hospitalized.

TABLE 6
Laboratory findings at first clinic visit for all diagnostic categories

	Laboratory results *n*, mean (range)					
	HF	Mild HPS group A	Mild HPS group B	Moderate to severe HPS	HV-negative	Leptospirosis
Hb (g/dL)	52, 13.8 (10.6–16.6)	25, 13.5 (9.7–15.2)	27, 14.7 (11.7–17.7)	13, 14.0 (8.3–19.5)	27, 13.7 (9.4–17.3)	6, 12.9 (10.5–14.49)
Hematocrit (%)	52, 40.4 (33.9–48.0)	25, 39.6 (26.9–47.1)	27, 43.0 (33.0–51.5)	13, 41.6 (27.6–55.7)	27, 40.2 (26.0–49.0)	6, 37.8 (28.4–43.0)
Leukocytes ($\times 1{,}000/mm^3$)	52, 5,740 (1,600–18,200)	25, 5,092 (2,600–8,840)	27, 9,304 (2,280–57,900)	13, 8,426 (3,120–15,900)	27, 7,142 (2,200–13,600)	6, 6,968 (3,380–12,300)
Neutrophils (%)	51, 70.0 (36.5–94.0)	25, 75.0 (55.7–91.0)	27, 75.6 (54.9–92.5)	13, 74.6 (42.0–91.5)	27, 75.4 (31.9–94.0)	6, 74.0 (54.0–86.0)
Lymphocytes (%)	51, 21.4 (4.0–55.0)	25, 17.7 (7.0–32.0)	26, 16.6 (7.0–48.0)	13, 15.8 (5.1–41.0)	27, 17.0 (5.0–47.0)	6, 18.3 (7.0–38.0)
Platelets* ($\times 1{,}000/mm^3$)	52, 137 (20–352)	25, 156 (70–357)	27, 97 (34–214)	13, 81 (26–155)	27, 181 (91–343)	6, 178 (123–249)
Creatinine (mg/dL)	25, 0.99 (0.45–2.79)	13, 0.94 (0.50–1.7)	24, 1.00 (0.50–1.86)	13, 1.14 (0.7–2.97)	8, 0.86 (0.40–1.62)	2, 1.20 (1.09–1.3)

Hb = hemoglobin; HV-negative = negative for HV and leptospira infections; mild HPS group A = HV pulmonary syndrome without hypoxemia or dyspnea.
*Platelet counts were significantly lower ($P < 0.001$, Wilcoxon rank sum test) in HV+ subjects with age > 11 years (mean; SD; range = 125.0; 63.8; 20–357) compared with HV− subjects (175; 54.9; 91–289).

22 patients (group A) reported no dyspnea or hypoxemia. Among 13 non-HV subjects with chest radiographs, 85% were normal, and only 15% displayed bilateral infiltrates.

Among 23 clinical pathology assays, only the platelet counts were significantly lower in HV+ subjects compared with HV− subjects (Table 6). HV-infected subjects tended to have lower pO2 tension in arterial blood (67 versus 84) than HV-negative subjects, but serum hepatic enzyme levels and all other assay results were not different (data not shown). Among HV-infected subjects, only the platelet count tended to be decreased with increasing disease severity, but the ranges were broadly overlapping.

DISCUSSION

Sin Nombre infections without pulmonary edema have been described only rarely in North America.[18,19] The large number of HV infections in this study without pulmonary edema or with mild pulmonary edema in the absence of respiratory insufficiency led us to define two new categories of HV illness. The novel designation HF is intended to be a clinical parallel with dengue fever uncomplicated by pulmonary edema and hemorrhage. The division of mild HPS into two groups is intended to emphasize the frequency of radiographic pulmonary edema in the absence of respiratory insufficiency, and it suggests that HPS is likely underdiagnosed in Panama. The ratio of total HV infections to moderately severe HPS of 9:1 in this study is similar to the ratio of annual seroconversions to hospitalized HPS of 14:1 calculated in these communities.[8]

The IgM ELISA and RT-PCR assays were not concordant in all subjects. In hospitalized subjects with HPS in Panama, consistent concordance between the two assays has been shown (Pascale JM, unpublished observations). Some of the negative RT-PCR assays may indicate sample collection after rapid viral clearance in a mild infection. As noted in one Andes virus infection,[20] the absence of IgM-positive assays in the presence of positive PCR assays may represent very early infection. The lack of follow-up samples also prevents unequivocable diagnosis in those subjects without IgG serology performed. Even if only subjects with both assays positive are considered, however, the broad spectrum of clinical HV disease and frequency of mild infection are apparent in Panama.

Recruitment into the study was biased in the sense that clinic personnel focused on infections that were similar to HPS prodrome, including dengue, malaria, and leptospirosis. Febrile illnesses with only upper respiratory symptoms were specifically not recruited, and therefore, HV incidence relative to other viral infections cannot be derived. The high public profile of HV infections in Panama and the accumulated skill in recognizing HPS infection among diagnosticians have enhanced the recognition of infections that mimic HV infection, including leptospirosis. This study suggests, however, that symptoms and laboratory analyses offer minimal distinctions between HV and leptospirosis, requiring specific assays for the latter treatable infection.[21,22] The lack of consistent thrombocytopenia in mild Choclo virus infection is similar to other clinical mimics, such as dengue, malaria, and leptospirosis, but it is distinct from the almost universal thrombocytopenia found in the more severe Sin Nombre virus infection.[23]

The high frequency of HV fever and mild HPS in Choclo virus infection contrasted with the high mortality in Andes virus

infection may reflect the highly specific interaction between HV species and the host integrin receptors.[24] Andes virus is uniformly lethal in hamsters,[25,26] and viral glycoprotein–integrin interaction is highly specific.[27] However, in hamsters infected with Choclo virus, robust viral replication in the lung is not accompanied by pulmonary inflammation or mortality.[28] Examination of the molecular specificity of this viral protein–integrin interaction may provide clues to the ameliorated pathogenesis in most Choclo virus infections.

Previous antibody prevalence surveys conducted in Panama to identify populations and locations at risk have found sustained increases in incidence of seroconversions compared with hospitalized HPS.[8] Similar seroprevalence rates in Paraguay[9] and Brazil[10–12] may also be consistent with frequent mild disease that does not require hospitalization but may pose a significant economic burden. The identification of all HV infections will improve environmental risk assessments, public health intervention planning, and HV vaccine field evaluations. Increasing seroprevalence in stable populations[8] suggests an increasing incidence of HV infections in Panama. The changing agroeconomy in Panama emphasizing rice monoculture, with increased rodent food supply and reduced rodent diversity,[29,30] may be increasing the risk of human HV infection.[4]

Received May 22, 2012. Accepted for publication May 21, 2013.

Published online July 8, 2013.

Acknowledgments: The authors thank the International Centers for Infectious Diseases Research (ICIDR) program of the National Institutes of Health, the Ministry of Health and Social Security, the University of New Mexico, the Gorgas Memorial Institute of Studies of Health, the Panamanian Institute of Livestock and Agricultural Research, and the National Environment Authority for their support. We also thank people from the communities, several state organizations, and the human survey team of the Ministry of Health and Social Security.

Financial support: This study was supported by an Opportunity Pool Award and supplement from the International Centers for Infectious Diseases Research program of the National Institutes of Health (U19-AI 45452); funds from Instituto Conmemorativo Gorgas de Estudios de la Salud, Hantavirus Research Project 04-90-0075-8; the Ministry of Health, Panama; and Secretaria Nacional de Ciencia y Tecnología, Innovation and Technology Program ftd06-089.

Disclaimer: Members of the Hantavirus Research Group were Ariosto Hernandez, Ayvar Hernandez, Alexander Cardenas, and Orlando Rivas (Tonosí Hospital); A. Arjona (Joaquín Pablo Franco Sayas Hospital); Ana Montenegro, Ovidio Mendoza, and Mario Ávila (Ministry of Health); Yamizel Zaldivar, Jonnatan Montenegro, Ricardo Cumbrera, Carlos Justo, Dianik Moreno, and Demetrio Serracin (Gorgas Memorial Institute); Joel Nuñez, Milagro de Guerra, Aida Romero, Florencio Rujano, Mario Aquino, and Katy Morales (Ezequiel Abadía Hospital); Fernando Rivera, Susana Hesse, and María Peña (Luis Fábrega Hospital); Yanibeth Guevara, Javier Reyes, and Marukel Salamin (Regional Rafael Estevez Hospital); Rolando Reyna and Fernando Gracia (Santo Tomás Hospital); Anibal G. Armien (University of Minnesota); and Samuel González (El Vigía Hospital).

Authors' addresses: Blas Armien, Epidemiology, Gorgas Memorial Institute for Health Research, Panama City, Panama, E-mail: barmien@gorgas.gob.pa. Juan M. Pascale and Yaxelis Mendoza, Microbiology, Gorgas Memorial Institute for Health Research, Panama City, Panama, E-mails: jmpascal@yahoo.com and ymendoza@gorgas.gob.pa. Carlos Muñoz, Jamileth Mariñas, and Deyanira Sánchez, Epidemiology, Ministry of Health, Panama City, Panama, E-mails: cbmunozg@cableonda.net, drajamilethm@gmail.com, and deyaluly@hotmail.com. Heydy Núñez and Milagro Herrera, Clinical Medicine, Social Security Health System, Panama City, Panama, E-mails: hmnm07@hotmail.com and dramherrera@yahoo.es. José Trujillo, Pulmonary Medicine, Santo Tomas Hospital, Panama City, Panama, E-mail: jmtmrt14@cableonda.net. Brian Hjelle, Department of Pathology, University of New Mexico Health Sciences Center, Albuquerque, NM, E-mail: bhjelle@salud.unm.edu. Frederick Koster, Lovelace Respiratory Research Institute, Albuquerque, NM, E-mail: fkoster@lrri.org.

REFERENCES

1. Jonsson CB, Figueiredo JL, Vapalahti O, 2010. A global perspective on hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev 23:* 412–441.
2. 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, 2000. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology 277:* 14–19.
3. Bayard V, Kitsutani PT, Barria EO, Ruedas LA, Tinnin DS, Munoz C, deMosca IB, Guerrero G, Kant R, Garcia A, Caceres L, Gracia F, Quiroz E, de Castillo Z, Armien B, Libel M, Mills JN, Khan AS, Nichol ST, Rollin PE, Ksiazek TG, Peters CJ, 2004. Outbreak of hantavirus pulmonary syndrome, Los Santos, Panama, 1999–2000. *Emerg Infect Dis 10:* 1635–1642.
4. Armien AG, Armien B, Koster FT, Pascale JM, Avila M, Gonzalez P, de la Cruz M, Zaldivar Y, Mendoza Y, Gracia F, Hjelle B, Lee SJ, Yates TL, Salazar-Bravo J, 2009. Hantavirus infection and habitat associations among rodent populations in agroecosystems of Panama: implications for human disease risk. *Am J Trop Med Hyg 81:* 59–66.
5. Nelson R, Canate R, Pascale JM, Dragoo JW, Armien B, Armien AG, Koster F, 2010. Confirmation of Choclo virus as the cause of hantavirus cardiopulmonary syndrome and high serum antibody prevalence in Panama. *J Med Virol 70:* 2289–2295.
6. Salazar-Bravo J, Armien B, Suzan G, Armien A, Ruedas LA, Avila M, Zaldivar Y, Pascale JM, Gracia F, Yates TL, 2004. Serosurvey of wild rodents for hantaviruses in Panama, 2000–2002. *J Wildl Dis 40:* 103–109.
7. Armien B, Pascale JM, Bayard V, Munoz C, Mosca I, Guerrero G, Armien A, Quiroz E, Castillo Z, Zaldivar Y, Gracia F, Hjelle B, Koster F, 2004. High seroprevalence of hantavirus infection on the Azuero peninsula of Panama. *Am J Trop Med Hyg 70:* 682–687.
8. Armien B, Pascale JM, Munoz C, Lee S-J, Choi KL, Avila M, Broce C, Armien AG, Gracia F, Hjelle B, Koster F, 2011. Incidence rate for hantavirus infections without pulmonary syndrome, Panama. *Emerg Infect Dis 17:* 1936–1939.
9. Ferrer JF, Jonsson CB, Esteban E, Galligan D, Basombrio MA, Peralta-Ramos M, Bharadwaj M, Torrez-Martinez N, Callahan J, Segovia A, Hjelle B, 1998. High prevalence of hantavirus infection in Indian communities of the Paraguayan and Argentinean Gran Chaco. *Am J Trop Med Hyg 59:* 438–444.
10. Campos GM, Moro de Sousa RL, Badra SJ, Pane C, Gomes UA, Figueiredo LT, 2003. Serological survey of hantavirus in Jardinopolis County, Brazil. *J Med Virol 71:* 417–422.
11. Mendes WS, da Silva AAM, Aragao LFC, Aragao NJL, Raposo M de L, Elkhoury MR, Suzuky A, Ferreira IB, de Sousa LT, Pannuti CS, 2004. Hantavirus infection in Anajatuba, Maranhao, Brazil. *Emerg Infect Dis 10:* 1496–1498.
12. Raboni SM, Delfraro A, de Borba L, Teixeira BR, Stella V, de Araujo MR, Carstensen S, Rubio G, Maron A, Lemos ER, D'Andrea PS, Duarte dos Santos CN, 2012. Hantavirus infection prevalence in wild rodents and human anti-hantavirus serological profiles from different geographic areas of South Brazil. *Am J Trop Med Hyg 87:* 371–378.
13. Levis S, Rowe JE, Morzunov S, Enria DA, St. Joan S, 1997. New hantaviruses causing hantavirus pulmonary syndrome in Central Argentina [letter]. *Lancet 349:* 998–999.
14. Castillo HC, Sanhueza HL, Tager FM, Munoz NS, Ossa AG, Vial CP, 2002. Seroprevalence of antibodies against hantavirus in 10 communities of the IX region of Chile where hantavirus cardiopulmonary syndrome cases were reported. *Rev Med Chil 130:* 1–10.
15. Hjelle B, Jenison S, Torrez-Martinez N, Herring B, Quan S, Polito A, Pichuantes S, Yamada T, Morris C, Eigh F, Lee HW, Artsob H, Dinello R, 1997. Rapid and specific detection of Sin Nombre virus antibodies in patients with hantavirus pulmonary syndrome

by a strip immunoblot assay suitable for field diagnosis. *J Clin Microbiol 35:* 600–608.
16. Feldmann H, Sanchez A, Morzunov S, Spiropoulou CF, Rollin PE, Ksiazek TG, Peters CJ, Nichol ST, 1993. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Res 30:* 351–367.
17. Cardona MN, Moros RM, Lopez EA, Perez JL, Hernandez RC, 2008. Diagnostico de leptospirosis mediante la PCR en pacientes con sindrome febril icterohemorragico. *Rev Soc Venez Microbiol 28:* 24–30.
18. Zavasky DM, Hjelle B, Peterson MC, Denton RW, Reimer L, 1999. Acute infection with Sin Nombre hantavirus without pulmonary complications. *Clin Infect Dis 29:* 664–666.
19. Kitsutani PT, Denton RW, Fritz CL, Murray RA, Todd RL, Pape WJ, Wyatt Frampton J, Young JC, Khan AS, Peters CJ, Ksiazek TG, 1999. Acute Sin Nombre hantavirus infection without pulmonary syndrome, United States. *Emerg Infect Dis 5:* 701–705.
20. Galeno H, Mora J, Villagra E, Fernandez J, Hernandez J, Mertz GJ, Ramirez E, 2002. First human isolate of hantavirus (Andes virus) in the Americas. *Emerg Infect Dis 8:* 657–661.
21. Bruce MG, Sanders EJ, Leake JA, Zaidel O, Bragg SL, Aye T, Shutt KA, Deseda CC, Rigau-Perez JG, Tappero JW, Perkins BA, Spiegel RA, Ashford DA, 2005. Leptospirosis among patients presenting with dengue-like illness in Puerto Rico. *Acta Trop 96:* 36–46.
22. Ellis T, Imrie A, Katz AR, Effler PV, 2008. Underrecognition of leptospirosis during a dengue fever outbreak in Hawaii, 2001–2002. *Vector Borne Zoonotic Dis 8:* 541–547.
23. Koster F, Foucar K, Hjelle B, Scott A, Chong Y-Y, Larson R, McCabe M, 2011. Rapid presumptive diagnosis of hantavirus cardiopulmonary syndrome by peripheral blood smear review. *Am J Clin Pathol 116:* 665–672.
24. Gavrilovskaya IN, Shepley M, Shaw R, Ginsberg MH, Mackow ER, 1998. Beta3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure. *Proc Natl Acad Sci USA 95:* 7074–7079.
25. Hooper JW, Larsen T, Custer DM, Schmaljohn CS, 2001. A lethal disease model for hantavirus pulmonary syndrome. *Virology 289:* 6–14.
26. Campen MJ, Milazzo ML, Fulhorst CF, Obot Akata CJ, Koster F, 2006. Characterization of shock in a hamster model of hantavirus infection. *Virology 356:* 45–49.
27. Matthys VS, Gorbunova EE, Gavrilovskaya IN, Mackow ER, 2010. Andes virus recognition of human and Syrian hamster beta3-integrins is determined by an L33P substitution in the PSI domain. *J Virol 84:* 352–360.
28. Eyraguirre EJ, Milazzo ML, Koster FT, Fulhorst CF, 2008. Choclo virus infection in the Syrian golden hamster. *Am J Trop Med Hyg 78:* 669–674.
29. Suzan G, Marce E, Giermakowski JT, Armien B, Pascale J, Mills J, Ceballos G, Gomez A, Aguirre AA, Salazar-Brazo J, Armien A, Parmenter R, Yates T, 2008. The effect of habitat fragmentation and species diversity loss on hantavirus prevalence in Panama. *Ann N Y Acad Sci 1149:* 80–83.
30. Suzan G, Marce E, Giermakowski JT, Mills JN, Ceballos G, Ostfeld RS, Armien B, Pascale JM, Yates TL, 2009. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. *PLoS One 4:* e5461.