Am. J. Trop. Med. Hyg., 70(6), 2004, pp. 682–687

HIGH SEROPREVALENCE OF HANTAVIRUS INFECTION ON THE AZUERO PENINSULA OF PANAMA

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Abstract. The first outbreak of hantavirus pulmonary syndrome (HPS) in Central America was documented on the Azuero peninsula of Panama in late 1999 and 2000. Reverse transcriptase–polymerase chain reaction evidence implicated only Choclo virus in symptomatic HPS with a mortality rate of 20%, although two rodent-borne hantaviruses (Choclo virus and Calabazo virus) were identified in the peridomestic habitat. Neighborhood serosurveys around case households found seroprevalence rates as high as 30%, the highest in the Americas except for western Paraguay. We report here population-based serosurveys for 1,346 adults and children in four communities, three on the Azuero peninsula and one in adjacent central Panama. Overall seroprevalence ranged from 33.2% in a population engaged in farming and fishing on Isla de Cañas, to 16.3% and 21.2% in two mainland agricultural communities, to 3.1% in central Panama, with a modest male predominance of 1.2:1. Nine percent of children 4–10 years old were seropositive, and seroprevalence increased with age in all communities, with highest levels of 52% in those 41–50 years old cohort on Isla de Cañas. Univariate analysis identified correlations between seroprevalence and multiple agricultural and animal husbandry activities. However, stepwise logistic regression models identified only raising animals (cows, pigs, goats, poultry) and fishing as significant independent variables. Human infection with hantavirus on the Azuero peninsula, either with Choclo virus or combined with Calabazo virus, is frequent but rarely results in hospitalization due to respiratory illnesses resembling HPS.

INTRODUCTION

Hantavirus infection was first described in the Americas in an outbreak in the Southwestern United States[1,2] ten years ago and subsequently, hantavirus infections with at least 15 different hantaviruses have been described in rodent reservoirs and in humans throughout the Americas.[3] The epidemiology of Sin Nombre and Andes viruses, the dominant strains in temperate regions, is characterized by a low incidence of symptomatic hantavirus cardiopulmonary syndrome (HCPS), a high ratio of symptomatic disease to asymptomatic infection, and a high case-fatality ratio between 42% and 58%.[4–8] One striking exception of this pattern of low seroprevalence is found in the Gran Chaco of western Paraguay where a lower case-fatality ratio of 11%,[9] primarily among European immigrants, is contrasted to increased seroprevalence rates of 40% among Amerindian populations.[10]

In January, 2000, an outbreak of hantavirus pulmonary syndrome (HPS) was detected in the Azuero peninsula of Panama, and a novel hantavirus, Choclo virus, was identified in a sigmodontine rodent vector (*Oligoryzomys fulvescens*) and in the blood of patients with typical HPS.[11] Among the first 25 hospitalized patients, the case-fatality ratio was 20%, and cardiogenic shock, a hallmark of severe Sin Nombre and Andes virus infections, was not documented. Preliminary serosurveys among the household and neighborhood contacts of hantavirus-seropositive patients residing on the Azuero peninsula identified seroprevalence rates of 6–30% in multiple villages (Bayard V and others, unpublished data).

The purpose of this study was to document the prevalence of antibody to hantavirus in human, population-based samples among four villages on or near the Azuero peninsula. The study sought to identify occupational, age-adjusted human activity, and peridomestic risk factors for infection, to initiate public health education prevention programs.

METHODS

Study sites. Four communities were selected to survey in 2001 due to the previous identification of symptomatic patients in each village, and to the high seroprevalence observed in household and neighborhood contacts of seropositive patients (Figure 1). San Jose and Pocri (Los Santos province) are communities engaged in agricultural activities and cattle farming located in lowland scrub vegetation. The villages are located 10 and 30 minutes from the provincial capital and regional medical center and are served on weekdays by a local health center. Of the 307 households participating in the study, 85% had tile or cement floors and 99% had metal or tile roofs. All but one household used the continuously available municipal water supply, and all households disposed of solid waste by municipal collection and by burning or burying locally. Jaquito (Cocle province) is an agricultural community in central Panama located in a lowland scrub ecologic zone. Employment is primarily in the sugar cane processing industry. All houses had metal or tile roofs and dirt floors were rare. Animal husbandry adjacent to the home was uncommon (12%) in Jaquito. Otherwise characteristics of the peridomestic environment were similar among the three mainland communities, with adjacent pasture in 32–58%, pig barns in 2–12%, chicken coops in 45–62%, food storage in 12–36%, uncovered refuse in 14–36%, and abandoned vehicles and machinery in 5–8%.

Isla de Cañas (Los Santos province) is a small island surrounded by mangrove forest on the Pacific coast 50 km south of San Jose, with an impoverished farming community of 327 residents engaged in watermelon culture, and subsistence rice, corn, and cattle farming. Access to the mainland is limited except during high tide, the provincial capital is approximately two hours away, and there is no staffed health center. Of the 79 households included in the study, 68% had an

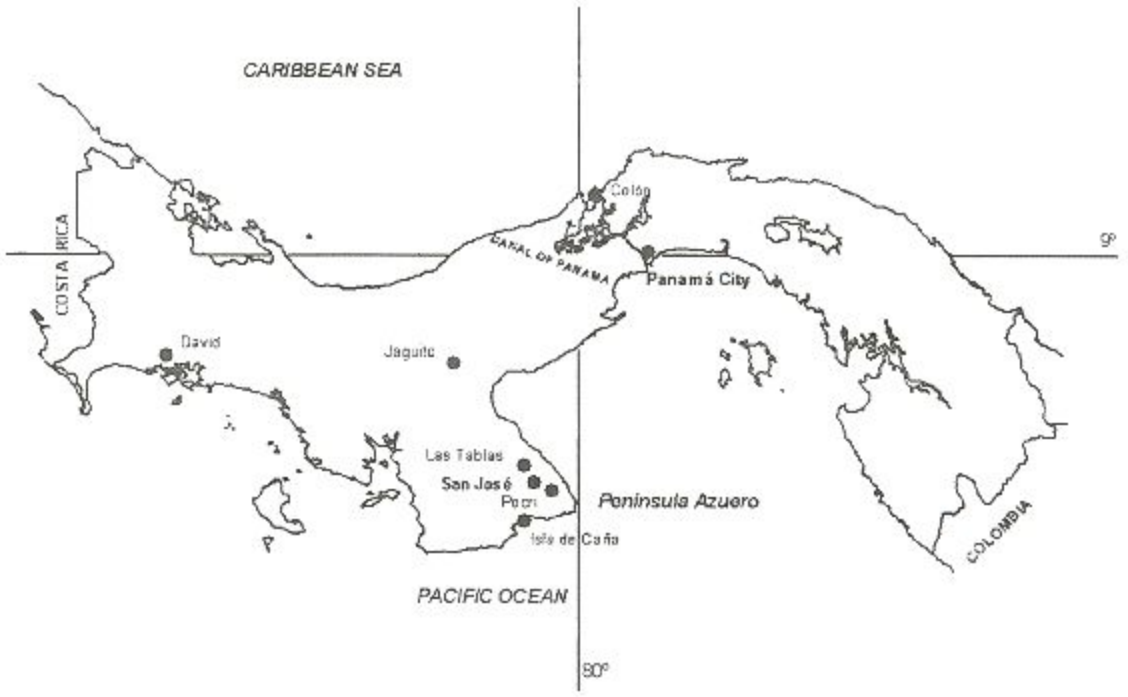

FIGURE 1. Map of Panama showing the location of the Azuero peninsula and the study communities of Jaquito, San Jose, Pocri, and Isla de Cañas.

earthen floor, 45% had wood or stick walls, and 49% had a palm-thatched roof. Since there was no municipal garbage collection, refuse was burned or buried locally. Water was delivered to 82% of the households by intermittently available municipal distribution, and 18% used private wells. The peridomestic environment was characterized by adjacent pasture in 58%, pig barns in 5%, chicken coops in 56%, food storage in 38%, uncovered refuse in 35%, and abandoned vehicles or machinery in 8%.

Peridomestic trapping in all four locales has identified both rodents known to be natural hosts for hantaviruses in Panama, the Costa Rican pigmy rice rat *Oligoryzomys fulvescens costaricensis* harboring the Choclo virus, and the Cherrie's cane rat *Zygodontomys brevicauda cherriei* harboring the Calabazo virus.[11,12] Longitudinal studies in each locale have identified spatial and temporal differences in the abundance and relative prevalence of these rodent species and their seroprevalence,[13] and meaningful analysis of complex relationships will require further data.

Study participants. Informed written consent was obtained from all adult participants and from parents or legal guardians of minors. Consent and assent forms were reviewed and approved by institutional ethics review boards at the University of New Mexico and the Gorgas Memorial Institute in Panama City, and by the protocol review committee of the International Centers for Infectious Diseases Research program of the National Institute of Allergy and Infectious Diseases. All adults and children more than two years of age who were permanently residing in each community according to the year 2000 national census were eligible for the study. The percent of the community consenting for study was 82% in Pocri, 86% in San Jose, and 68% in Isla de Cañas. The reasons for non-inclusion were absence during the week of the survey, attending school on the mainland (Isla de Cañas), and fear of phlebotomy. Every other house (50%) in Jaquito on each block was approached for participation and cooperation was 100%. After informed consent/assent was obtained, a questionnaire was administered and venous blood was collected for serology. The ethnic origins of individuals from all populations were comparable, a mixture of European, African-American, and Amerindian intermarriage in a region originally populated by Amerindians before the Spanish conquest 500 years ago.[14]

Serology. Heparinized whole blood from arm venipuncture was separated by centrifugation and plasma for serology was stored at −20°C until analysis. Antibody to all known hantaviruses in the Americas cross-react to the N protein of Sin Nombre virus in binding assays.[15] A strip immunoblot assay (SIA) for IgG antibody containing recombinant N protein of the 3H226 genotype of Sin Nombre virus was used as described.[15] An enzyme immunoassay (EIA) used recombinant nucleocapsid protein from Sin Nombre virus.[16] All sera were tested by both assays and the discordance of the EIA and SIA in this seroprevalence study was 3% (Pascale J, unpublished data). For the purposes of this study, the criterion for seropositivity was a positive reaction in both assays.

Data analysis. Descriptive statistics were calculated for community, age cohort, sex, and occupation groups (Epi-Info, Centers for Disease Control and Prevention, Atlanta, GA). Univariate analysis used the Spearman rank correlation coefficient and the chi-square statistic to calculate the odds ratio and 95% confidence limits comparing seropositivity with demographic characteristics and bivariate risk factor descriptors derived from the questionnaire. Odds ratios are presented as crude ratios and ratios adjusted for age and sex. Significant independent variables were identified by stepwise logistic regression models (SPSS, Chicago, IL) using all significant variables derived from univariate analysis.

RESULTS

Seroprevalence. Although end point titrations of sera for IgG reactivity to N antigen were not performed, the intensity of the strip immunoblot of seropositive sera was indistinguishable from that of sera from documented HPS patients (Figure 2). Optical density of positive reactions in the EIA was also comparable between seropositive individuals in the community and sera from patients in the acute stages of HPS. Only one subject among the 1,346 studied had had a previous diagnosis of HPS, which was confirmed by a reverse transcriptase–polymerase chain reaction (RT-PCR) and IgM-specific serology. Among 12 participants with a history of hospitalization, none had a discharge diagnosis consistent with HPS, excepting this one case, and none was seropositive. Among 66 questionnaire respondents describing a previous respiratory illness resembling mild HPS but not requiring hospitalization, 12 (18%) had antibody to hantavirus, which was not different from those denying a previous history.

Subjects on Isla de Cañas were younger (mean ± SD = 30.1 ± 10.0 years, range = 2–90) than subjects from the other communities whose mean ages ranged from 36.6 to 43 years ($P < 0.001$). Seroprevalence was higher on Isla de Cañas than in San Jose and Pocri, ($P < 0.001$), and these three communities had significantly higher rates than Jaquito ($P < 0.001$) (Table 1). The ratio of seropositive males to females in all communities combined was 55:45, and the increased representation of males was significant ($P = 0.05$). Adults described as farmers or housewives had slightly higher seroprevalence than students and adults with other occupations such as technicians, teachers, health care workers, and mill workers (Table 1), but this difference was not significant.

The seroprevalence for all four communities combined increased with age from 9.2% in children less than 11 years old to 22.8% in adults in the 41–50-year-old age group ($P < 0.001$) (Figure 3). In San Jose and Pocri, the seroprevalence was unchanged from the third to the seventh age deciles, but on Isla de Cañas the seroprevalence decreased significantly ($P < 0.05$) from 52% in the fifth decade to 24% in the sixth and seventh decades.

Risk factors. Combined bivariate analysis identified a significant correlation between seropositivity and 16 risk factors sought in the questionnaire in all four communities (Table 2) (www.gorgas.gob.pa/docs/cuestionario%2007102003). When the odds ratios were adjusted for sex, age, and locality known to be significant variables (Table 1), six variables remained independent of locality, including raising animals, raising poultry, handling animal food and grain, cleaning houses, sheds and barns, fishing, and handling wild rodents. Seven of 10 individuals who reported handling dead or live rodents were seropositive.

In step-wise logistic regression analysis using the variables identified from bivariate analysis (Table 2), only two variables, raising animals (odds ratio = 1.46) and fishing (odds ratio = 1.73), remained as independent activity-related variables ($P < 0.05$).

DISCUSSION

This population-based sample demonstrates the high prevalence of antibody to hantavirus in all three communities on the Azuero peninsula of Panama, in contrast to a community in the adjacent central region. The strain or strains of hantavirus associated with frequent seropositivity in Panama is not yet known. Two hantaviruses have been identified on the Azuero peninsula, Choclo virus of *O. fulvescens*[11] and Calabazo virus of another sigmodontine rodent (*Z. brevicauda*.).[11] Both of these rodents are abundant both in the field and in peridomestic habitats.[12] The high level of serodiagnostic cross-reactivity of nucleocapsid protein in both the SIA and EIA formats among all hantaviruses in the Americas renders tests based upon Sin Nombre N antigen sufficiently sensitive to detect most or all infections, but does not permit serologic distinction between viral strains. Only Choclo virus has been identified by RT-PCR in peripheral blood of all 12 hospitalized patients studied[11] (Pascale J, unpublished data). Future studies on strain-specific neutralizing antibody and sequencing RT-PCR amplimers from patients with mild hantavirus infection are needed to resolve the role of Calabazo virus in human infection. We speculate that if Calabazo virus infection accounts for a significant fraction of seroprevalence among humans, the frequency and clinical severity of Choclo virus infection could be profoundly altered in this population, a finding of relevance to vaccine development.

Risk of infection is a summation of potential exposures associated with the location and construction of the workplace and home, and personal activities in sites of rodent infestation. Outdoor agricultural activity in China[17,18] and forestry work in Europe[19] are associated with risk of hemorrhagic fever with renal syndrome, and is reflected in a high male:female infection ratio greater than 1.5:1. In contrast, in North America more frequent peridomestic exposure is supported by molecular epidemiology and a male:female ratio of

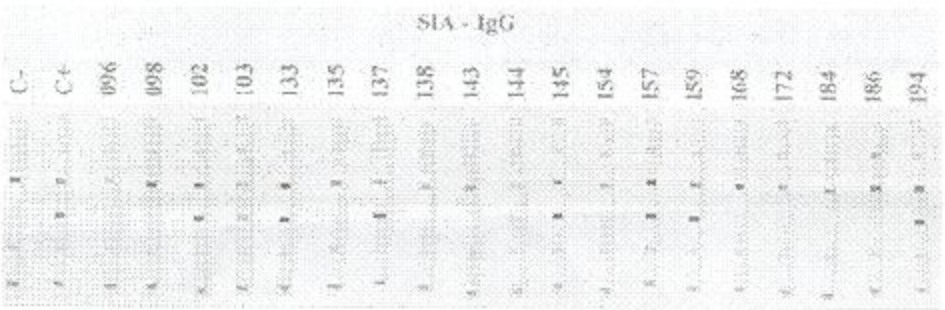

FIGURE 2. Hantavirus-specific IgG strip immunoblot assay (SIA). Tested subjects from the seroprevalence survey (three digit numbers) and positive (C+) and negative (C-) controls are shown. The four lines observed represent in order: top line (orientation marker), 3+ reaction control (human serum), hantavirus N protein antigen (specific reaction), and 1+ reaction control (human serum).

TABLE 1
Hantavirus infection seroprevalence in Panama by demographic characteristics and location, 2001–2002*

Demography	Isla de Cañas		San José		Pocri		El Jaguito	
	IgG+/n	% (CI)	IgG+/n	% (CI)	IgG+/n	% (CI)	IgG+/n	% (CI)
Total	74/223	33.2 (27.0, 39.8)	80/487	16.4 (13.2, 20.0)	64/310	20.6 (16.3, 25.6)	10/326	3.1 (1.5, 5.6)
Sex								
Male	41/113	36.3 (27.4, 45.9)	43/220	19.5 (14.5, 25.4)	29/128	22.7 (15.7, 30.9)	4/151	2.6 (0.7, 6.6)
Female	33/110	30.0 (21.6, 39.5)	37/267	13.9 (9.9, 18.6)	35/182	19.2 (13.8, 25.7)	6/175	3.4 (1.3, 7.3)
Occupation								
Housewife	23/58	39.7 (27.0, 53.4)	23/165	13.9 (9.0, 20.1)	23/104	22.1 (14.6, 31.3)	5/89	5.6 (1.8, 12.6)
Student	14/65	21.5 (12.3, 33.5)	12/105	11.4 (6.0, 19.1)	6/58	10.3 (3.9, 21.2)	1/91	1.1 (0.0, 6.0)
Farmer	32/80	40.0 (29.2, 51.6)	16/64	25.0 (15.0, 37.4)	7/25	28.0 (12.1, 49.4)	0/17	0 ( )
Other	5/20	25.0 (8.7, 49.1)	29/153	19.0 (13.1, 26.1)	28/123	22.8 (15.7, 31.2)	4/129	3.1 (0.9, 7.7)

* CI = 95% confidence interval

1.0.[5,20,21] In Panama, the male:female ratio is close to unity at 1.2:1, both in this serosurvey (Table 1) and among symptomatic cases (17:14). The slight excess of men may reflect the exclusive participation by men in fishing, but men, women, and children participate in animal husbandry. Positivity for antibody to hantavirus is distributed across all reported occupations, again reflecting our observation that all adults and older children participate in peridomestic animal husbandry activities.

Since the rodent reservoir species are found throughout Panama, the ecologic bases for the concentration of rodent-borne hantaviruses on the Azuero peninsula is not entirely clear, but may be related to the drier climate, loss of natural forests, and replacement with intense agricultural land use associated with decreased biodiversity.[12] Open house construction with dirt floors, open animal food storage, and lack of municipal waste disposal may favor peridomestic rodent invasion and higher seroprevalence on Isla de Cañas. Conversely, the high seroprevalence of 20% on the mainland where houses are predominantly constructed with cement floors and walls is less consistent with the notion that most exposure occurs in the house itself. Although many agricultural activities correlated with seropositivity in bivariate analysis (Table 2), including working in and cleaning barns and handling animal feed grains, only animal husbandry emerged in logistic regression analysis as the single, independent agricultural risk factor. This information provides a focus for future detailed studies on rodent behavior and interventions. The correlation between hantavirus antibody positivity and bait fishing on the Pacific ocean is curious and may represent a marker for an unrelated risk activity. In the United States, early indications that outdoor agricultural activities such as hand plowing represented risk factors for Sin Nombre virus infection have not subsequently been borne out, and later investigations have increasingly implicated indoor exposures as more proximate risk activities.[5,21–23]

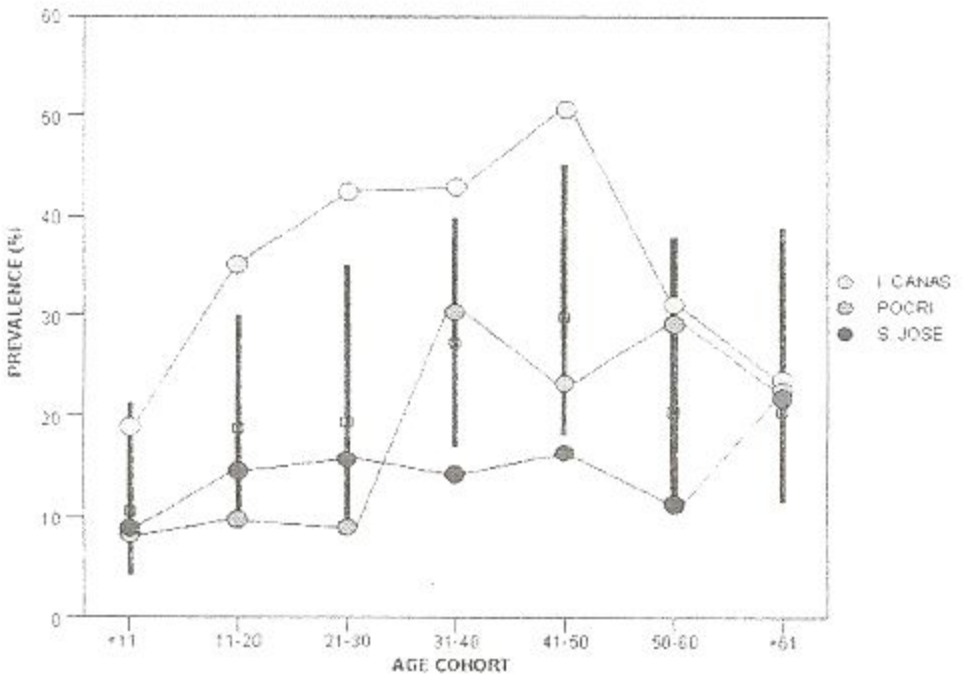

FIGURE 3. Prevalence of antibody to hantavirus by age (years) cohort shown as the mean only for each of three communities Isla (I.) de Cañas, Pocri, and San (S.) Jose and for the combined four communities (bar spanning 2 SD around the mean indicated by the square). The fourth community had too few seropositive individuals (20) to include by age cohort.

TABLE 2

Crude and adjusted odds ratios (ORs) and 95% confidence interval (CIs) of hantavirus seropositivity in Panama for significant risk factors, all communities combined, 2001–2002

Risk factors	Crude OR (95% CI)	Adjusted* OR (95% CI)
Cultivate vegetables or grains	2.11† (1.48, 3.01)	1.20 (0.79, 1.82)
Cut hay	2.43† (1.69, 3.51)	1.39 (0.91, 2.14)
Work in pastures or grain fields	1.93† (1.27, 2.92)	1.50 (0.43, 2.40)
Work in sheds or barns	2.55† (1.63, 4.00)	1.40 (0.84, 2.32)
Visited the fields	1.57† (1.14, 2.16)	1.05 (0.73, 1.51)
Breed domestic animals	1.73† (1.30, 2.31)	1.48‡ (1.09, 2.02)
Breed birds or fowl	1.64† (1.23, 2.18)	1.21‡ (1.03, 1.42)
Handle food for animals or birds	1.65† (1.31, 2.33)	1.46‡ (1.08, 1.99)
Clean houses or huts	2.54‡ (1.67, 3.86)	1.71‡ (1.09, 2.69)
Camping	1.67§ (1.11, 2.50)	1.45 (0.94, 2.23)
Fishing	2.24† (1.60, 3.13)	1.73‡ (1.15, 2.60)
Hunting	1.59† (1.12, 2.27)	1.21 (0.80, 1.82)
Saw rodents	1.53‡ (1.03, 2.27)	1.31 (0.85, 2.00)
Handled rodents	2.89† (1.44, 5.19)	2.10‡ (1.01, 4.37)
Rodent bite	3.53§ (1.33, 9.39)	2.68 (0.95, 7.57)

* Adjusted odds ratio by age, sex, and location.
* P value < 0.001, °P value < 0.01, °°P value < 0.05.
† $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.01$.

Positivity for antibody to hantavirus increased with age, beginning at four years, and is consistent with constant peridomestic exposure during the first 50 years of life. The seroprevalence of 9.2% among children (13.3% on Isla de Cañas), although not associated with known symptomatic disease, is the highest recorded in the literature. Symptomatic HCPS in young children has been demonstrated in South America,[7,24,25] but appears to be rare in North America.[26,27] Childhood infection in Chile is associated with intrafamilial person-to-person transmission (Ferres M, unpublished data), but there is no evidence of intrafamilial transmission of symptomatic infection in Panama. The significant decrease in seroprevalence among adults more than 50 years old may be explained by a recent rodent invasion of the island as the agricultural practices on the island changed from sugar cane to more diversified crops less than 50 years ago.

The coincidence of lower mortality rate and elevated prevalence of hantavirus antibody on the Azuero peninsula of Panama mirrors the same relationship in western Paraguay[9,10] and among the Mapuche in Chile.[4] Hantavirus fever, in the absence of pulmonary edema, is documented anecdotally, but no prospective studies have defined its frequency.[28,29] The identification of regions of high seroprevalence will make possible intensive prospective studies of transmission from rodent to humans not previously possible for other uncommon rodent-vectored infections such as arenaviruses, plague, and leptospirosis.

Received July 15, 2003. Accepted for publication October 26, 2003.

Acknowledgments: We are grateful for the support of the Ministry of Health and the Social Security System, the serosurvey team of the Gorgas Memorial Institute, and the communities participating in the surveys. Thomas Ksiazek provided a gift of Sin Nombre antigen for use in the enzyme-linked immunoassay. The Statistics Clinic of the University of New Mexico provided statistical advice. Karl Johnson provided valuable suggestions and support.

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 (AI-45452), and funds from the Gorgas Memorial Institute, Hantavirus Research Project No. 04-90-0075-8, Ministry of Health, Panama.

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