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Research Paper

Hantavirus Pulmonary Syndrome in the State of São Paulo, Brazil, 1993–1998

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ABSTRACT

Between 1993 and 1998, 10 cases of clinical hantavirus infection were diagnosed in Brazil. Hantavirus-specific IgM, or positive immunohistochemical analysis for hantavirus antigen, or positive reverse transcription–polymerase chain reaction results for hantavirus RNA were used to confirm nine of these cases; eight were hantavirus pulmonary syndrome (HPS), and one was mild hantavirus disease. The remaining clinical case of hantavirus infection was fatal, and no tissue was available to confirm the diagnosis. During the first 7 months of 1998, five fatal HPS cases caused by a Sin Nombre-like virus were reported from three different regions in the State of São Paulo, Brazil: two in March (Presidente Prudente Region), two in May (Ribeirão Preto Region), and one in July (Itapecerica da Serra Region). Epidemiologic, ecologic, and serologic surveys were conducted among case contacts, area residents, and captured rodents in five locations within the State of São Paulo in June of 1998. Six (4.8%) of 125 case contacts and six (5.2%) of 116 area residents had IgG antibody to Sin Nombre virus (SNV) antigen. No case contacts had a history of HPS-compatible illness, and only one area resident reported a previous acute respiratory illness. A total of 403 rodents were captured during 9 nights of trapping (1969 trap nights). All 27 rodents that were found to be positive for IgG antibody to SNV antigen were captured in crop border and extensively deforested agricultural areas where four of the 1998 HPS case-patients had recently worked. The IgG antibody prevalence data for rodents suggest that *Bolomys lasiurus* and perhaps *Akodon* sp. are potential hantavirus reservoirs in this state of Brazil. Key Words: *Akodon*—*Bolomys*—Brazil—Hantavirus—Hantavirus pulmonary syndrome—Rodent—São Paulo. Vector Borne Zoonotic Dis. 1, 181–190.

INTRODUCTION

NEW WORLD HANTAVIRUSES are an increasingly recognized group of rodent-borne viruses, some of which can cause hantavirus pulmonary syndrome (HPS). HPS was initially identified in the United States in 1993 as a cardiopulmonary syndrome with a high case-fatality rate. The disease is now known to be established as a series of pan-American zoonoses caused by any of several American hantaviruses, each associated with several different species of rodents belonging to the subfamily Sigmodontinae, family Muridae (Khan

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et al. 1996a). Fever, myalgia, headache, and gastrointestinal symptoms, with subsequent cardiopulmonary dysfunction and a 40–60% case-fatality ratio, characterize the disease in North America (Khan et al. 1996b).

In South America, endemic and periodic epidemic cases of HPS continue to be reported from Argentina (Levis et al. 1997), Chile (Toro et al. 1998), Paraguay (Williams et al. 1997), and Uruguay (Peters 1998). One case has been reported in a Chilean patient who had recently traveled to Bolivia and had an exposure history there (Espinoza et al. 1998). Hantavirus antibodies in asymptomatic humans in Bolivia (Weissenbacher et al. 1996) and several rodent species in Venezuela (Fulhorst et al. 1997) and Peru (Powers et al. 1999) suggest a more widespread distribution for hantaviruses in South America than that represented by currently recognized clinical disease. HPS was first identified in South America in 1993 in a family cluster in the State of São Paulo, Brazil (da Silva et al. 1997). Since then, sporadic cases have been recognized in Brazil and reflect a broad clinical spectrum of disease.

A Sin Nombre-like virus caused fatal HPS in five individuals in the State of São Paulo, Brazil, in 1998. The clustering of four cases, two each in the months of March and May, prompted a collaborative investigation by the Centro de Vigilancia Epidemiologica and the Instituto Adolfo Lutz, with invited participants from the U.S. Centers for Disease Control and Prevention (CDC) and the Museum of Southwestern Biology (MSB). We provide a summary of the cases that have occurred in Brazil since 1993 and report details on five of the cases identified in the State of São Paulo during 1998. Additionally, we summarize recent efforts to identify the rodent reservoir species and briefly describe potential contributing ecological associations in areas where human illnesses occurred.

MATERIALS AND METHODS

Epidemiologic surveillance and case definitions

State surveillance efforts were augmented with case definitions to cover the spectrum of infection from clinical HPS to asymptomatic hantavirus infection. HPS was defined as an acute febrile illness (temperature >38°C) characterized by unexplained acute respiratory distress syndrome or bilateral interstitial pulmonary infiltrates, with respiratory compromise requiring supplemental oxygen, or an unexplained illness resulting in death, in conjunction with an autopsy examination demonstrating noncardiogenic pulmonary edema without an identifiable specific cause of death (CDC 1997). Confirmed case-patients required laboratory evidence of infection for the presence of hantavirus-specific IgM and/or positive immunohistochemical (IHC) detection of hantavirus antigen and/or positive reverse transcription–polymerase chain reaction (RT-PCR) results for hantavirus RNA.

Thorough investigations of five previously suspected and confirmed cases were conducted to determine the site of potential exposure and characterize their clinical illnesses. The investigation included review of all available clinical charts, family interviews, and serologic testing for IgG antibodies to Sin Nombre virus (SNV) antigen of all contacts of confirmed HPS cases. A case contact was defined as a person who had been working, living, or pursuing recreational activities at the same location that was judged to be a potential exposure site for an HPS case.

Human antibody prevalence survey

We conducted a study of hantavirus antibody prevalence among residents of Nova Guataporanga, a small town (population 2,132 inhabitants) in an area where the terrain is marked by extensive deforestation for agricultural purposes, with patchy forested areas along stream borders. We sampled ~8% of the population, including 116 area residents and 47 case contacts. The residents and case contacts were contacted and recruited for the study by the local health department; participation was voluntary. Ten milliliters of blood was drawn from each study participant. Demographic data, including information about work activities, household location, time of residence, and history of acute respiratory illness, were obtained from participants with a standardized questionnaire.

Rodent sampling

Rodent captures and processing were conducted near likely sites of exposure for previously confirmed and suspected HPS case-patients. Rodents were captured using Sherman ($8 \times 9 \times 23$ cm; H. B. Sherman Trap Co.) and Tomahawk ($13 \times 13 \times 41$ cm; Tomahawk Trap Co.) live-capture traps in five principal areas of the State of São Paulo (Franca, Guariba, Juquitiba, Nova Guataporanga, and Tupi Paulista counties) during June 16–27, 1998. Traps were set in agricultural areas and in native Atlantic rainforest. The principal microhabitats in agroecosystems included crop fields (coffee bean, corn, grape vineyard, and sugarcane), crop border (uncultivated remnant native flora), and postharvest crop (crop stubble and plowed soils). Additionally, traps were set within and around residences, outbuildings, roadsides, and property fence lines.

Traps were baited with a combination of rolled oats and birdseed with or without peanut butter. Characteristics of the habitat (vegetation type and cover) were recorded at each trapping location. Captured rodents were collected each morning and processed according to a standardized protocol (Mills et al. 1995). Tentative species identification was made, and standard measurements and information were recorded (gender and reproductive data; mass; lengths of body, tail, hind foot, and ear; and evidence of external wounds or scars). After processing, each carcass was placed in 10% formalin for several days followed by immersion in 70% ethanol for preservation. Carcasses were sent to the MSB for cataloging and confirmation of identification.

Laboratory analysis

Human serum specimens in clinical cases of hantavirus infection were tested for antibodies reactive to SNV antigen by IgM and IgG enzyme-linked immunosorbent assay (ELISA) using a recombinant nucleocapsid protein antigen as previously described (Feldmann et al. 1993). SNV antigens were coated on microtiter plates at 1:100–1:6,400 in fourfold steps, antibody was allowed to bind, and then conjugate was added to detect bound antibody. When an autopsy was performed, IHC analysis for hantavirus antigens was performed on lung, liver, or kidney tissue by using a cross-reactive monoclonal antibody directed against conserved hantavirus nucleocapsid epitopes (Zaki et al. 1995). The IHC amplifying system used EnVision alkaline phosphatase to avoid activation by endogenous biotin and peroxidases (Sabattini et al. 1998). Tissue or blood clot samples for some HPS patients were examined for hantavirus RNA by RT-PCR (Johnson et al. 1999). Respective methods, reagents, and primers used in the IHC analyses and RT-PCR were as previously described (Zaki et al. 1995, Johnson et al. 1999). Rodent whole-blood specimens were similarly analyzed by ELISA for IgG antibodies to SNV antigen. Samples of lung tissue from all antibody-positive rodents were subjected to virus isolation attempts in cell culture following a standardized protocol (Elliott et al. 1994). Briefly, monolayers of Vero E-6 cells were inoculated with 0.1 ml of a 10% tissue suspension, maintained at 37°C, and blind-passaged three times at intervals of 2 weeks.

RESULTS

Between November 1993 and July 1998, nine clinical cases of hantavirus infection in Brazil were confirmed by laboratory testing, including eight with fatal HPS and one with mild disease. One additional patient with clinical HPS died, but no tissue was available to confirm the presumptive diagnosis. He was the brother of two patients with laboratory-confirmed hantavirus infection, one with HPS and another with mild disease. Eighty percent of cases were in males, the average age was 33 years (range 15–55 years), and the case-fatality ratio was 90%. The occupational activities of the patients were reported as farmer (six), housewife (two), veterinarian (one), and policeman (one). The most common symptoms exhibited by the patients were fever, myalgia, dyspnea, and headache (Table 1).

Five of the fatal cases of HPS occurred in the State of São Paulo between March and July 1998. The cases were reported from three administrative regions in the state (Fig. 1): Presidente Prudente Region (one case each in the

TABLE 1. SYMPTOMS EXHIBITED BY HPS CASE-PATIENTS, SÃO PAULO STATE, BRAZIL, 1993–1998

1993–1998		
Symptoms	*Patients with/ patients examined*	*%*
Fever (per case definition)	10/10	100
Myalgia	10/10	100
Dyspnea	9/10	90
Headache	8/10	80
Cough	6/10	60
Abdominal pain	4/10	40
Hematemesis	2/10	20
Vertigo	2/10	20
Pulmonary hemorrhage	1/10	10

counties of Tupi Paulista and Nova Guataporanga), Ribeirão Preto Region (two cases in Guariba County), and Itapecerica da Serra Region (one case in Cotia County). Summaries of the clinical features of three cases, with a brief assessment of exposure risks, are presented here; clinical details of the two cases from the county of Guariba have previously been described (Figueiredo et al. 1999).

Clinical cases

Case 1 was a 51-year-old female homemaker with no significant medical history who became ill with a fever, headache, myalgia of the upper back, and retroorbital pain on March 7, 1998. She was clinically diagnosed with dengue fever on the fourth day of illness. Clinical laboratory testing on that day showed a white blood cell (wbc) count of 6,400/mm^3 with 75% neutrophils and 2% band forms (immature) and a hemoglobin content of 15.2 g/dl; her urinalysis showed 2+ blood, 25 wbc/high-power field (hpf), and 17 red blood cells (rbc)/hpf. She reportedly felt better the following day but re-

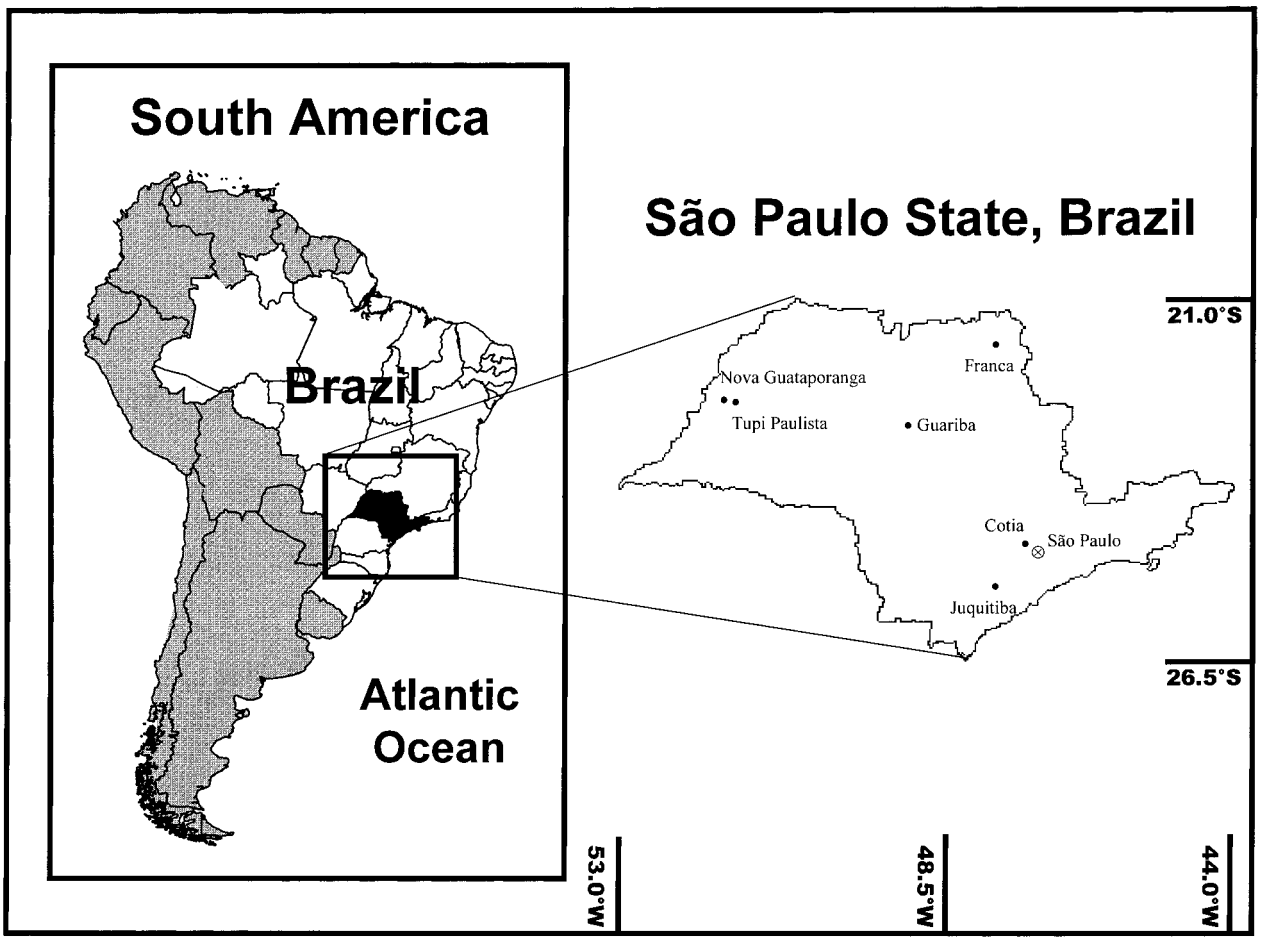

FIG. 1. Locations visited during HPS investigation to characterize the focal prevalence of hantavirus infection in sampled rodents, case contacts, and area residents in São Paulo State, Brazil, 1998.

turned on March 13 with a temperature of 36°C, pulse rate of 76/min, and a blood pressure of 90/60 mm Hg. No tachypnea was noted on admission, but she apparently became profoundly dyspneic after 1 h with shock and anuria (the volume of her intake fluid in this interval is unknown). This change prompted a chest radiograph that showed diffuse pulmonary edema. She was administered digitalis, furosemide, mannitol, steroids, an antibiotic, and oxygen. She died while in transit to a tertiary medical care center 3 h after hospitalization. No autopsy was performed. A blood specimen was tested and found to have IgM and IgG antibodies to SNV antigen but not to dengue viruses.

Case 1 resided in the urban area of Tupi Paulista County (population 13,282), Presidente Prudente Region, in a modern, well-constructed home. In the 6 weeks before her illness, she visited her 14.8-hectare farm (~5 km from her home) twice a week where she supervised the construction of a covered concrete storage shed with an attached storage room, bathroom, and living space. Her family grew grapes, sugarcane, coffee, and animal feed. During the investigation, rodent population density encountered near the farm was high (20% trap success, Table 2). Two employees of the farm and her husband did not have IgG antibody to SNV.

Case 2 was a 27-year-old male farmer who developed fever, headache, myalgia, backpain, and prostration on March 17, 1998. He also complained of a sore throat lasting a single day and had self-medicated with amoxicillin. On the third day of illness, he developed hematemesis and was admitted to a local hospital with concomitant severe abdominal pain, vertigo, confusion, and a blood pressure of 80/20 mm Hg. Clinical laboratory studies on admission showed a hemoglobin content of 20.1 g/dl, hematocrit of 59%, and wbc count of 18,900/mm^3 with 73% segmented neutrophils and 7% banded neutrophils. He died within 6 h of admission with profound shock and minimal dyspnea. A small volume of coffee-ground emesis was witnessed in the hospital, but no other bleeding manifestations were reported. Hantavirus infection was confirmed by detection of IgM and IgG antibodies to SNV antigen. No autopsy was performed.

TABLE 2. RODENT SPECIES AND TRAPPING LOCATION, SÃO PAULO STATE, BRAZIL, 1998, BY NUMBER CAPTURED AND NUMBER OF CAPTURED THAT WERE ALSO IGG ANTIBODY-POSITIVE (AB+) TO SNV ANTIGEN

	Trapping location by county					
Rodent species	*Tupi Paulista*	*Nova Guataporanga*	*Guariba*	*Franca*	*Juquitiba*	*Total*
M. musculus	18	3	19			40
R. rattus	5	2				7
Akodon sp.			87 (5)		4	91 (5)
Brucepattersonius soricinus					3	3
B. lasiurus	95 (18)	1	51 (4)	1	1	149 (22)
Calomys tener	*6*		25			31
Cavia fulgida					1	1
Delomys sublineatus					3	3
Oligoryzomys sp.			3	1	8	12
Oligoryzomys nigripes					9	9
Oligoryzomys stramineus			10	1	3	14
Oryzomys russatus					6	6
Oxymycterus hispidus					4	4
Thaptomys nigrita					3	3
Unknown[a]	4		26			30
Total captured	128 (18)	6	221 (9)	3	45	403 (27)
Trap nights	637	275	419	79	559	1,969
Trap success (%)	20.1	2.2	52.7	3.8	8.1	20.5
Predominant habitat type	Crop borders	Farmhouse	Crop borders	Pastures	Rainforest	

[a]Specific identification currently under study.

Case 2 resided in Nova Guataporanga County, Presidente Prudente Region, on a 15-hectare farm. Although he lived in a brick house with a poured concrete floor, it was open to the fields that surrounded the building on three sides. The house was also in close proximity to a vegetable garden, shed, pigsty, and an area where sugarcane was processed to make a local confection. He also worked at another satellite farm of 5 hectares, 15 km away, that housed a grain shed, which he had cleaned 3 days before becoming ill.

Cases 3 and 4 were from the county of Guariba. Hantavirus infection was diagnosed in both patients by detection of IgM and IgG antibodies to SNV antigen by ELISA, by positive IHC lung samples (both) and liver and kidney samples from Case 4, and by RT-PCR in the serum of Case 4 (Figueiredo et al. 1999).

Cases 3 and 4 both worked on a 450-hectare sugarcane farm in Guariba County, Ribeirão Preto Region. Case 4 spent the majority of each day on the farm, where he assisted his farmhand (Case 3) with harvesting the corn from the field and transporting it to a rodent-infested corn storage barn. Their major exposure to rodents was on a small cornfield where the corn was harvested and left under plastic tarps in the middle of a plowed field. An extraordinary number of rodents was reported by workers who lifted the tarp to load the corn harvest into trucks. The rodent population density during the investigation was very high (53% trap success, Table 2).

Case 5 was a 33-year-old military policeman who presented at an army hospital on July 3, 1998, with fever (38°C), myalgia, and dry cough of 3 days in duration. The physical examination was remarkable for dehydration, blood pressure of 70/50 mm Hg, and pulse rate of 100/min. Clinical laboratory studies on admission showed a hemoglobin content of 16.0 g/dl, hematocrit of 48%, and wbc count of 7,000/mm^3 with 80% segmented neutrophils and 4% banded neutrophils. Examination of urinary sediment showed visible blood, the serum urea nitrogen level was 57 mg/dl, and the creatinine level was 1.6 mg/dl. After 10 h he developed dyspnea, and the chest radiographs showed bilateral interstitial infiltrates concurrent with a platelet count of 80,000/mm^3. The patient's condition continued to deteriorate over the next several days. His creatinine and serum urea nitrogen levels increased and on July 10 were 10.5 and 332 mg/dl, respectively. He developed pulmonary hemorrhage; he had generalized edema on July 11 and died on July 15. Hantavirus infection was diagnosed by detection of IgM antibodies to SNV antigen by ELISA and positive IHC in tissue samples of lung and heart.

Case 5 worked in an office in the city of São Paulo (population 15 million), and he was born in Tupã County (population 61,138), where his family still resides. He traveled to and stayed at his parents' house on May 9. On June 11, he went with his girlfriend to a park in the city of Cotia, 20 km from São Paulo City. They spent all day in the park where native vegetation predominates. His girlfriend did not have antibody to SNV.

Human antibody prevalence survey

Hantavirus IgG antibody tests conducted among contacts (Table 3) of five recently confirmed HPS case-patients found six (4.8%) positive results among 125 contacts from the counties of Tupi Paulista (two), Nova Guataporanga (two), and Cotia (two). None had a history of illness compatible with HPS. Hantavirus IgG antibody alone was detected in six (5.2%) serum samples from 116 area residents in the county of Nova Guataporanga; only one reported an acute respiratory illness in the past year.

Rodent antibody prevalence and ecologic surveys

A total of 1,969 Sherman and Tomahawk traps were set over 9 nights, with an average of 219 traps per night. Four hundred three ro-

Table 3. IgG Antibody Positivity among Contacts of Confirmed HPS Case-Patients in Selected Counties of São Paulo State, Brazil, 1998

County	*No. positive/no. tested*	*% positive*
Tupi Paulista	2/8	25.0
Nova Guataporanga	2/47	4.3
Guariba	0/11	0
Cotia	2/59	3.4
Total	6/125	4.8

dents representing at least 15 species and 12 genera were captured from five locations (Table 2). Two rodent species, *Akodon* sp. and *Bolomys lasiurus*, had IgG antibody to SNV antigen. The 91 *Akodon* specimens captured appear to be of an undescribed species, most likely related to *Akodon cursor*. In Tupi Paulista County, 128 rodents were captured in 4 nights, and 95 (74.2%) were *B. lasiurus*; 18 (18.9%) were IgG antibody-positive to SNV antigen. In Guariba County, 221 rodents were captured in 3 nights; 4 (7.8%) of the 51 *B. lasiurus* and 5 (5.7%) of the 87 *Akodon* sp. were antibody-positive. In the other trapping locations, a combined 54 rodents were captured, and none were positive for antibody to SNV antigen. All virus isolation attempts on antibody-positive rodents were unsuccessful.

The greatest trapping success (Table 2) was achieved in the counties of Tupi Paulista (20.1%) and Guariba (52.7%). In Tupi Paulista County, the predominant habitat in which 89% of rodents were captured was typified by dense aerial coverage of grasses (*Panicum* sp.) 1.5–2.0 m in height and matted vegetation ground cover. In Guariba County, 90% of rodents were captured in border habitats adjacent to post-harvest corn fields (plowed soil). These vegetation borders were only 5–10 m wide and hundreds of meters long and covered primarily with remnant native forest with a mix of tall grasses and scattered trees and shrubs including castor bean (*Ricinus communis*). The predominant species captured in this border habitat were *Akodon* sp. (38.9%) and *B. lasiurus* (23.5%). By comparison, in Juquitiba County, trapping was conducted in the less disturbed primary Atlantic rainforest. Trap success was lower (8.1%), but rodent species richness and diversity were higher, representing at least 11 species. *Akodon* sp. and *B. lasiurus* were found in much lower densities, and rodents were more evenly dispersed within the forested area as well as along the bordering habitat. Approximately 49% of the rodents were captured along the edge of primary Atlantic rainforest, usually along roads and fence lines. Members of the genus *Oligoryzomys* accounted for 43.5% of these animals.

In the two remaining locations, Franca and Nova Guataporanga Counties, rodent trapping was less successful. Only three rodents were captured in Franca from an area adjacent to grazing pastures that was composed of small native shrubs and trees with patches of elephant grass (*Pennisetum purpureum*). Trapping in Nova Guataporanga County was concentrated around a farmhouse and outlying buildings adjacent to pastures that abutted the secondary forest where five of six captured rodents were *Mus musculus* and *Rattus rattus* (Table 2).

DISCUSSION

HPS was first identified in the State of São Paulo, Brazil in 1993, and sporadic cases continue to occur. The description of the cases from São Paulo show that it is difficult to evaluate the magnitude of HPS because only two of the five cases identified were suspected of hantavirus infection during their initial clinical presentation. The high case-fatality rate, hantavirus antibody prevalence among case contacts (6/125), and hantavirus antibody prevalence among inhabitants of Nova Guataporanga County (6/116) suggest that surveillance had not detected the full spectrum of disease caused by hantavirus infection in this region. Our finding of 5% prevalence of SNV antibody among inhabitants of Nova Guataporanga was higher than that found for any of the localities included in another serosurvey in the State of São Paulo, 1.23% overall (Holmes et al. 2000). This indicates that much hantavirus infection in the area goes undetected and that the risk of infection varies on a regional scale within the state. Many of the more severe cases of HPS may have been provisionally determined to be dengue fever or leptospirosis, especially in São Paulo State where many cities have active transmission of dengue fever. Improved surveillance will be required to determine the burden of hantavirus-associated disease. Future epidemiological studies of case contacts should include serologic testing for IgM in addition to IgG antibodies to SNV antigen. Surveillance should be enhanced by educating physicians through bulletins and lectures about the epidemiological features of the disease.

Variants of HPS have been reported, including disease caused by Bayou and Black Creek Canal viruses, which seem to have more renal involvement (Hjelle et al. 1996). Disease caused by Andes virus has been associated with flushing of the head and neck in Argentina (Wells et al. 1997) and hemorrhagic manifestations in Chile (Toro et al. 1987). In São Paulo State, the disease manifestations are also variable. In the majority of cases (90%), the most important sign was dyspnea, but three cases developed hemorrhage (gastrointestinal and pulmonary). Other cases were initially diagnosed as dengue hemorrhagic shock syndrome because of the prominent hypotension. Because some of the cases have been characterized only by bleeding with evidence of renal involvement, the case definition in Brazil should be broadened to include hemorrhagic manifestations.

We found high densities of several species of sigmodontine rodents in geographic locations associated with the 1998 cases of HPS in Tupi Paulista and Guariba Counties. Both areas have been extensively deforested for crop production and livestock grazing. The remaining forested areas and uncultivated linear border habitats in Guariba had a very high density of rodents as compared with the adjacent sugar cane and plowed cornfields. The remnant native forest vegetation appears to serve as a primary refuge for rodents, and the adjacent postharvest cornfields provide abundant food in this area. Similar border habitats have been shown to support higher small-mammal population densities, species diversity, and richness than adjacent crop fields in Mexico (Mellink 1991) and Argentina (Mills et al. 1992, Ellis et al. 1997).

Within the State of São Paulo, all cases of HPS had identifiable risk factors for hantavirus infection. The factors responsible for the occurrence of disease have not been well defined and may not be the same in the different regions of the state. Cases 3 and 4, with onsets of illness 11 days apart, probably acquired their infections while harvesting, transporting, and storing corn in common rodent-infested environments. Conversely, Cases 1 and 2, with onsets of illness 10 days apart, lived 15–20 km from each other, did not know one another, and did not share any common exposure activities. Case 5 had less well-defined exposure risks but did engage in recreational activities that may have put him at risk for hantavirus infection.

The preliminary clinical and serologic data for disease in humans, ranging from asymptomatic persons to rapidly fatal cases, combined with the ecological data, suggest that multiple hantaviruses cocirculate in the State. The rodent antibody prevalence data suggest that *B. lasiurus* and perhaps *Akodon* sp. are potential hantavirus reservoirs in this region of Brazil. The finding that all antibody-positive rodents in this study were associated with agroecosystems (specifically crop borders and adjacent fields), and not primary or secondary rainforest habitats, implies that anthropogenic disturbances (extensive deforestation) may be creating favorable habitats for reservoir species and that human risk is consequently higher in these environments.

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ABBREVIATIONS

CDC, Centers for Disease Control and Prevention; ELISA, enzyme-linked immunosorbent assay; hpf, high-power field; HPS, hantavirus pulmonary syndrome; IHC, immunohistochemical; MSB, Museum of Southwestern Biology; rbc, red blood cells; RT-PCR, reverse transcription–polymerase chain reaction; SNV, Sin Nombre virus; wbc, white blood cell(s).

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