HIGH PREVALENCE OF HANTAVIRUS INFECTION IN INDIAN COMMUNITIES OF THE PARAGUAYAN AND ARGENTINEAN GRAN CHACO

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Abstract. Serologic evidence of past infection with a Sin Nombre-like hantavirus(es) was demonstrated in 78 (40.4%) of 193 Indians living in western Paraguay and in 38 (17.1%) of 222 Indians inhabiting the Salta province of northern Argentina. In both populations seroprevalence increased with age, with the most striking increase occurring at 18 years of age in the Paraguayan population and at 35 years of age in the Salta population. The peak prevalences in both populations (66.6% and 44.0%, respectively) were seen in Indians > 53 years old. Although no sex difference was observed in the Paraguayan Indians, in the Salta population seroprevalence was greater in males than in females. Familiar clustering of the infection was observed. The data indicate that the Indian populations of the Gran Chaco are frequently exposed to and survive infection with a Sin Nombre-like virus(es). Possible explanations of this novel epidemiology are discussed.

Hantaviruses constitute a genus of trisegmented, negative-sense RNA viruses of the Bunyaviridae family. Each hantavirus is maintained in nature in single rodent reservoir species in which it causes a generalized, chronic, asymptomatic infection with virus shedding in urine, feces, and saliva. Transmission of hantaviruses to humans is believed to occur through the inhalation of aerosols contaminated with infectious rodent urine or feces or following the bite of an infected rodent.

A relatively large number of antigenically and genetically distinct hantaviruses have been detected in a wide array of rodent species throughout the world. At least four strains, termed Hantaan (HTN), Seoul (SEO) Puumala (PUU), and Dobrava/Belgrade (DOB), are causative agents of diseases characterized by hemorrhagic manifestations and renal involvement. These diseases, collectively named hemorrhagic fever with renal syndrome (HFRS), are endemic in several regions of Asia and Europe, but cases of HFRS have also occurred with varied frequency in other parts of the world, including South America. The most severe cases of HFRS are caused by the HTN and DOB viruses, whereas the milder forms of the disease are associated with the PUU and SEO viruses.

A novel hantavirus strain, designated Sin Nombre (SN) virus, was etiologically linked with an outbreak of a severe and often fatal respiratory disease termed hantavirus pulmonary syndrome (HPS) that occurred in 1993 in the southwestern United States. Other HPS-associated hantaviruses indigenous to North America, including the Black Creek Canal (BCC), Bayou (BAY) and New York viruses, have been identified. The reservoir species for all these viruses are rodents of the family Muridae, subfamily Sigmodontinae. As of August 1997, more than 185 cases of HPS with a mortality rate of approximately 45% have occurred in North America.

Between March 1995 and July 1997, 108 cases of HPS with a mortality rate of 48% were reported in Argentina (Weissenbacher MC, unpublished data). Approximately one-third of these cases occurred in and about El Bolson, a locality in the province of Rio Negro in southwestern Argentina. The remaining cases appeared in clusters in other parts of the country, including the northern province of Salta, the islands of the Parana River and Patagonia. The genome of an hantavirus named Andes was enzymatically amplified from tissues of one of the El Bolson cases and found to be molecularly related to but distinct from SN and other Sigmodontine rodent-borne hantaviruses. Prior to the El Bolson outbreak, several studies reported serologic evidence of hantavirus infection in humans and rodents of Argentina, and clinical cases compatible with HPS and HFRS were diagnosed retrospectively in several parts of the country.

In western Paraguay, a new SN-like virus termed Laguna Negra has been isolated from Calomys laucha and its genetic sequences have been matched with those obtained from tissue of HPS patients identified in the area. With the purpose of studying the epidemiology of emerging infectious diseases in isolated and relatively closed communities, a research team headed by one of us (JFF) collected serum samples as well as biographic and demographic data from a large number of Indians from several ethnic groups inhabiting Argentina and Paraguay. This paper reports our findings on the presence and distribution of hantavirus antibodies in these populations.

MATERIALS AND METHODS

Study populations. The Indians studied inhabit the following areas (Figure 1).

1) The northwestern part of the province of Salta in north-
ern Argentina, along the Pilcomayo river. This is a flat, low, and partially wooded tropical region within the territory called Gran Chaco. The Indians live in small huts made of dry branches, leaves and dried mud, clustered in small communities. Fishing, hunting, and gathering are their main sources of food. The Indian studied are of ethnic groups Chorote, Chulupi, and Wichi of the Mataco-Mataguayan linguistic family.

2) The eastern part of the department of Boqueron in western Paraguay. This area, also in the Gran Chaco, is approximately 200 miles northeast of the Salta site and is ecologically similar. The Indians studied in this area live in communities surrounding the small towns of Yalve Sanga and Filadelfia. Many of the Indian men and women work in farms owned by Mennonite settlers of European descent. Women are primarily involved in cleaning houses and outbuildings used for storage of food, grain, and hay, as well as in milking cows, pitching hay, harvesting, and gardening. Men also perform some of these tasks but more often they spend their time working as herders, in construction, clearing scrubs, planting and harvesting crops, and driving farm equipment. In some of the communities the dwellings are made of brick and in others of branches, leaves, and dried mud. The predominant ethnic groups are the Chulupi, Lenqua, and Ayoreos, which belong to the Mataco-Mataguayan, Mascotan, and Zamucoan linguistic families, respectively.

3) Alumine. This is a mountainous region with a cold climate in the province of Neuquen in southwestern Argentina. The subjects examined in this region are the Mapuche Indians who raise sheep and cattle and practice some agriculture. Their dwellings are in most cases made of bricks and concrete.

Collection, processing, and testing of serum samples. Clotted and heparinized blood samples were collected during the period 1993–1995. The samples were centrifuged at 1,500 × g for 20 min within 4 hr after collection. Resulting serum/plasma samples were removed and stored at 4°C for several days. Upon arrival at the Laboratory of Virology, Universidad del Centro de la Provincia de Buenos Aires (Tandil, Argentina), the samples were aliquoted and stored at −20°C. Verbal informed consent was obtained from individuals in accordance to U.S. government guidelines in place at the time of this field work. The blood samples were collected as part of a collaborative agreement with the Ministerio de Salud Publica of Salta, Argentina and the Programa de Salud Indigena of the Asociacion de Servicios de Cooperacion Indigena Mennonite (Paraguay). The studies were also approved by the community chiefs.

Serum/plasma samples were examined for hantavirus antibodies using the following assays.

1) Strip immunoblot assay (SIA) (Chiron, Emeryville, CA). The strips contains four antigens of the SN virus and a recombinant nucleocapsid protein of the SEO virus (SEO rN). The SN virus antigens include synthetic peptides derived from the viral nucleocapsid N protein (SN Nep) and the G1 glycoprotein (SN Gpep), a full-length recombinant-expressed N protein (SN rN), and a recombinant-expressed G1 protein. The SIA strips were used according to the manu-
facturer's specifications. Twenty microliters of each serum sample was added to 1 ml of specimen diluent. The diluted serum was incubated with the strip for 4–6 hr. Bound antibodies were detected with horseradish peroxidase–conjugated goat anti-human heavy and light chain antibody for 10 min, followed by washing and development with an enzyme substrate. All steps were conducted at room temperature. Strips were rinsed with distilled, deionized water, and serum reactivities were read within 1 hr. Reactivity against the individual peptide and recombinant protein antigens was scored visually relative to IgG control bands with 1+ and 3+ reactivity. Following established scoring criteria, a serum was considered positive for antibodies to SN or a SN-related hantavirus if it reacted with a 1+ intensity with both SN Npep and SN rN. Reactivities with SN rN without reactivity with SN Npep were considered indeterminate. Sera showing any other reactivity or no reactivities were considered negative.

2) Enzyme-linked immunosorbent assay. The assay used was developed by the Centers for Disease Control and Prevention (CDC) (Atlanta, GA) for detection of IgG antibodies to the SN N protein. Specific bound antibody was detected using a anti-human IgG (heavy and light chains) peroxidase-conjugated goat IgG (Calbiochem-Novabiochem Co., San Diego, CA). Color was developed by addition of 2,2′-azino-bis(3-ethylbenzthiazolinesulfonic acid) (ABTS) substrate (Calbiochem-Novabiochem Co.). An IgG titer of 1:100 was interpreted as evidence of past hantavirus infection.

3) Western immunoblots using multiple recombinant-expressed hantavirus N antigens. The preparation of the antigens was described previously. Briefly, hantavirus N genes were amplified with primers containing restriction enzyme recognition sites for Hind III (sense primer) and Xho I (antisense primer). The amplification products were inserted into the Hind III and Xho I sites of the expression plasmid pET23b (Novagen Corp. Madison, WI). This creates an N-terminal fusion of the T7 epitope tag to the N protein. Following induction with isopropyl-β-D-thiogalactoside, a 55-kD recombinant N fusion protein was expressed in BL21DE3 cells. A hexahistidine tag at the C-terminus was used for a one-step affinity purification of the protein with nickel-nitriolate agarose (Qiagen, Chatsworth, CA).

Western blot assays were performed as described previously. Two dilutions (1:500 and 1:10,000) of the serum sample were incubated for 16 hr with nitrocellulose blots containing equimolar amounts (500 ng) of recombinant-expressed SN, RM, SEO, and PUU virus N antigens. Reactivities were scored on a visual scale from 1+ to 4+ at each serum dilution.

Statistical analysis. Multivariate logistic regression models were used to evaluate the dichotomous hantavirus antibody value (positive/negative) as a function of geographic location (Paraguayan, Salta), age (1–80 years) or sex (male and female). The variable age group years n (age_group n ) was defined for each age level n as 0 if the age was less than n, and 1 if the age was greater than n and was used to determine the age at which the odds ratio (ψ) for seropositivity is maximal. The relation of a subject’s status to that of family members, defined as spouse and/or parents, were examined in 145 subjects. Antibody status results of family members was defined as positive if a spouse, parent, or both were seropositive, and negative if all these family members were seronegative.

The multivariate logistic regression approach allows for the calculation of the odds ratio that measures the association of a variable (or set of variables) to the presence of hantavirus antibodies. The uncertainty of the odds ratio was assessed by calculation of the 95% confidence intervals using a standard formula.

RESULTS

Of the 193 samples from Paraguayan Indians tested in the SIA, 72 reacted with both the SN rN and Npep antigens, and were thus scored as positive for hantavirus antibodies. The 10 samples that showed an indeterminate SIA patterns were examined further with the multi-N antigen Western blot assay. Six of them reacted with N antigens of the SN, RM, SEO, and/or PUU viruses, and were therefore also scored as positive for hantavirus antibodies. Thus, 78 (40.4%) of the 193 Paraguayan Indians were positive for hantavirus antibodies.

Of 222 samples from the Salta Indians, 37 were positive and five were indeterminate by SIA. Western blot analyses of the indeterminate Salta samples showed that four were nonreactive and that one reacted with the RM N antigen. Thus, 38 (17.1%) of the 222 Salta Indians had hantavirus antibodies. Only one of the 82 Mapuche Indians living in Alumine was positive in the SIA.

All the samples from the Salta Indians and the SIA-positive Mapuche sample were examined for IgG antibodies in the CDC ELISA. Thirty-seven SIA-positive Salta samples and the Mapuche sample were positive by ELISA; the titers ranged from 1:400 to 1:6,400 in 30 samples and were 1:100 in the remaining seven samples. The other Salta samples were negative.

The distribution of hantavirus seropositivity according to age and sex in the Paraguayan and Salta populations is shown in Table 1. None of the samples collected from Indians 12 years of age in either population was positive. In the Paraguayan population, the antibody prevalence was 21.8% in the 13–20-year-old age group and ranged between 43.7% and 66.6% in the other age groups. In the Salta population, the prevalence was less than 8% in the 13–20, 21–28, and 29–36 years of age groups, and increased progressively thereafter and peaked (44%) in the Indians more than 53 years of age. In the Paraguayan Indians, males and females had almost identical prevalences (40.5% and 40.2%, respectively), but in the Salta population, males had a greater seroprevalence than females (26.6% versus 12.2%).

To assess more precisely the association of region, age and sex with hantavirus seroprevalence in the Paraguayan and Salta populations, a multivariate logistic regression analysis of the data was performed. Paraguayan Indians were 5.539 times more likely to be seropositive than Salta Indians (ψregion (P < 0.0001)) In the Salta population, for each year of age a subject was 1.052 (ψage (P < 0.0001)) times more likely to have hantavirus antibodies, while in the Paraguayan population a subject was 1.044 (ψage (P < 0.0003)) times more likely. The maximal odds ratio of age group (ψage-group ) in the Paraguayan population was with Indians 18 years or older having an odds for seropositivity of
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The striking age-dependent infection with such virus(es) observed in these populations is unprecedented. In the Paraguayan Indians, the most drastic increase in prevalence began at 18 years of age whereas in the Salta Indians this occurred at age 35, and prevalence increased progressively thereafter reaching its peak in those >53 years of age in both populations. The increase in seroprevalence could conceivably result from cumulative exposure over the lifetime of the host to a virus transmitted sporadically or could reflect a cohort effect whereby the risk of exposure has decreased during the last 2–4 decades. A decreased resistance to infection with age is another possible explanation. Further information is needed to assess these possibilities. An increase in hantavirus antibody prevalence with age has also been observed between hantavirus seropositivity and seroreactivity between the various ethnic groups. No correlation examined and no differences in seroprevalence were observed. Furthermore, in the >35-year-old age group of this population, being a male increased the chance of being seropositive by $\psi_{\text{sex}} = 3.060$ ($P < 0.0039$). In the group of 145 individuals whose family members were tested, having a seropositive member increased the chance of a subject being seropositive by 2.482 ($\psi_{\text{family member}}$) ($P < 0.0255$).

Seropositive Indians were found in all the communities examined and no differences in seroprevalence were observed between the various ethnic groups. No correlation was observed between hantavirus seropositivity and seronegativity of the samples reacted with the SEO virus N antigen, and one with the SEO virus N antigen. Most of the samples reacted with the SEO virus N and PUU virus N antigens but, with a few exceptions, they did so with less intensity than with the N antigens of the SN and RM viruses. One Paraguayan sample (A-322) reacted only with the SEO virus antigen by Western blot even though in the SIA it reacted with the SN virus recombinant N antigen. This is most likely due to a significant under-representation of the SEO virus N antigen in the SIA lot used (Hjelle B, DiNello R, unpublished data).

### DISCUSSION

The present study provides serological evidence of exceptionally high prevalence of endemic infection with a virus(es) antigenically similar to SN virus in Indian populations inhabiting the Paraguayan and Salta sectors of the Gran Chaco. The striking age-dependent infection with such virus(es) observed in these populations is unprecedented. In the Paraguayan Indians, the most drastic increase in prevalence began at 18 years of age whereas in the Salta Indians this occurred at age 35, and prevalence increased progressively thereafter reaching its peak in those >53 years of age in both populations. The increase in seroprevalence could conceivably result from cumulative exposure over the lifetime of the host to a virus transmitted sporadically or could reflect a cohort effect whereby the risk of exposure has decreased during the last 2–4 decades. A decreased resistance to infection with age is another possible explanation. Further information is needed to assess these possibilities. An increase in hantavirus antibody prevalence with age has also been observed between hantavirus seropositivity and seroreactivity between the various ethnic groups. No correlation examined and no differences in seroprevalence were observed. Furthermore, in the >35-year-old age group of this population, being a male increased the chance of being seropositive by $\psi_{\text{sex}} = 3.060$ ($P < 0.0039$). In the group of 145 individuals whose family members were tested, having a seropositive member increased the chance of a subject being seropositive by 2.482 ($\psi_{\text{family member}}$) ($P < 0.0255$).

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observed in European and Asian populations infected endemically with PUU, HTN and SEO viruses.26–28 The absence of sex differences in seroprevalence in the Paraguayan population suggests that both men and women are similarly exposed to host rodents. Thus, these Indians probably acquire the virus mainly by inhabiting and cleaning rodent-infested dwelling and by performing domestic, peri-domestic and farm activities, such as cleaning feed storage areas, that are not sex-specific and are recognized as risk factors for hantavirus infection.29,30 In contrast to the Paraguayan population, in the Salta population the frequency of seropositivity was significantly greater in men than in women, particularly in the age groups showing the highest prevalences, i.e., those more than 35 years old. Since in the Salta population hunting, gathering, and agricultural work are done primarily by men, their exposure to infected rodents in the field is probably greater than that of women. The antibody prevalence was also significantly higher in men than in women in PUU virus-infected populations and in people more than 60 years of age in populations exposed to the HTN and SEO viruses.26–28

Our data show that seropositive individuals were 2.482 times more likely than seronegative individuals to have a family member with hantavirus antibodies. This family clustering of the infection suggest that the Indians and their household relatives were similarly exposed to risk factors. However, this observation is also consistent with the possibility that seropositive family members are risk factors themselves. Indeed, recent studies suggest that person-to-person transmission may have accounted for at least some HPS cases occurring in South America.31

Despite the strikingly high hantavirus seroprevalence in the Paraguayan population, from the information we obtained in interviews with the Indians and the director of the Yalve Sanga Hospital, which provided health services to most of the Paraguayan communities involved in our study, the frequency of clinical manifestations compatible with HPS or HFRS in this population had been extremely rare or absent during at least the five preceding years. In a previous study, 23 HPS cases with a relatively low case-fatality rate (13%) were detected in the Filadelfia area.31 Interestingly, despite the observation in this study that the prevalence of hantavirus antibodies was higher among the Indians, most of the affected patients were Mennonites and other people who were not indigenous to the region. Likewise, from the information we obtained it seems that clinical features commonly associated with hantavirus infection also occur very rarely if at all among the Salta Indian population we examined. As of July 1997, 52 cases diagnosed as HPS have been identified in Oran, a locality of the Salta province which is about 100 miles from the area inhabited by these Indians but, as in the Filadelfia area, most, if not all the cases occurred in non-Indians (Enria D, Cacase ML, unpublished data).

It is reasonable to presume that viruses that cause less severe acute clinical disease will be associated with more frequent asymptomatic infections, such as those detected retrospectively in serologic surveys. The case-fatality rates of the diseases caused by hantaviruses differ markedly, ranging from 0% to 1% and 5–10% for diseases associated with the PUU and HTN viruses, respectively, to nearly 50% for the SN virus-associated HPS.26–28,32 As might be expected, the seroprevalence of hantavirus infection has been found to be higher among populations in which viruses associated with HFRS (12–19%) are endemic than among populations endemically infected with the highly pathogenic SN virus (<1%).

Thus, our findings suggest that the pathogenicity of the hantavirus(es) prevalent in the areas inhabited by the Paraguayan and Salta Indians examined herein is low. From our findings and those of Williams and others,17 it could be inferred that compared with the other ethnic groups living in the same areas, the Indians are more resistant to hantavirus diseases. However, we cannot rule out the possibility that these diseases have not been recognized among the Indian groups because they received comparatively less medical care than the non-Indian groups. One of the main objectives of our current studies is to conduct a more thorough study of the relationship between hantavirus seroprevalence and frequency of hantavirus-associated illnesses in the Indians and non-Indian populations living in the areas of Paraguay and Salta included in this paper.

The hantaviruses infecting the Paraguayan and Salta Indian populations and their animal reservoirs remain to be identified. The carrier rodent of the Andes virus, O. longicaudatus (Enria D, unpublished data), occurs in the area of Salta where the HPS cases have been detected. This virus and the Laguna Negra virus, which was detected in HPS cases occurring in western Paraguay, are phylogenetically related to the HPS-associated BCC and BAY viruses, which occur in the United States.25,26,17 Several other South American hantaviruses have been shown to cluster as a clade within this monophyletic group, which we refer to as oryzomine-complex viruses.14,16 A recombinant N antigen of RM virus, which is a close relative of the Andes virus and of other South American hantaviruses, was prepared14 and used in
Western blot assays alongside with other hantavirus N antigens to try to determine whether the virus(es) that infect the Indians of Paraguay and Salta is (are) more closely related to the BCC-BAY-Andes virus complex than to other hantaviruses such as SN virus. Interestingly, among the weakly reactive Paraguayan samples that were scored as indeterminate in the SIA, there were several that exhibited preferential reactivity to SN virus N antigen relative to the other viral N antigens by Western blot. We believe that the utility of the multiple N antigen Western blot in identifying the infecting serotype is limited for such weakly reactive samples, since the reacting antibodies could be directed against a hantavirus for which there is no close counterpart on the Western blot membrane. To screen for possible reactivities to highly divergent hantaviruses, we incorporated PUU and SEO virus N antigens on the Western blot membranes, and were able to obtain evidence for infection for a SEO-like virus in one sample. The preferential reactivity with RM virus antigen exhibited by several Indian serum samples supports the hypothesis that the hantavirus(es) in circulation both in western Paraguay and Salta are related to the oryzomine-complex viruses.

Genetically homogenous human populations living in relatively closed communities and having an extraordinarily high prevalence of hantavirus infection, such as those identified herein, are exceptionally well-suited for comprehensive studies on the natural history and pathogenicity of hantaviruses.

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