MAJOR ARTICLE

Prospective Evaluation of Household Contacts of Persons with Hantavirus Cardiopulmonary Syndrome in Chile

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(See the editorial commentary by Montgomery et al., on pages 1553–5.)

Background. Andes virus (ANDV) infection, which has a case fatality rate of 37% in Chile, often occurs in household clusters and may be transmitted from person to person.

Methods. To determine the incidence and risk factors for additional household cases, we conducted a prospective study among recent household contacts of persons with hantavirus cardiopulmonary syndrome (HCPS) in Chile, including testing of serum for anti-hantavirus antibodies and blood cells for ANDV RNA by reverse-transcription polymerase chain reaction (RT-PCR).

Results. We enrolled 76 index case patients and 476 household contacts, of whom 16 (3.4%) developed HCPS; 32.6% of 92 cases occurred in household clusters. The risk of HCPS was 17.6% among sex partners of index case patients, versus 1.2% among other household contacts ($P < .001$). Person-to-person transmission was definite in 3, probable in 9, and possible in 2 of the 16 additional household case patients. We detected ANDV RNA by RT-PCR in peripheral blood cells 5–15 days before the onset of symptoms or the appearance of anti-hantavirus antibodies.

Conclusions. In recent household contacts of persons with HCPS in Chile, the risk of HCPS was greatest among sex partners. Among the household contacts who developed HCPS, viremia preceded the onset of symptoms and the appearance of anti-hantavirus antibodies by up to 2 weeks.

Hantavirus cardiopulmonary syndrome (HCPS) occurs throughout much of the Americas [1–7]. More cases (480) have been reported in Chile, where the case fatality rate is 37%, than in the United States (451), and Andes virus (ANDV), the etiologic agent of all known cases in Chile and of most cases in Argentina, is the only hantavirus for which person-to-person transmission has been documented [8–10]. In an outbreak in Argentina that included possible nosocomial transmission, person-to-person transmission also occurred in household clusters and in a group of people who took a day-long automobile trip [10]. Although we found no evidence of nosocomial transmission in Chile [11], approximately one-third of the first 100 cases in Chile occurred in household clusters, including one for which we isolated ANDV from a seronegative, asymptomatic child 2 days before he developed HCPS [12].

On the basis of these observations, we designed a prospective study to evaluate household contacts of index case patient with HCPS in Chile. The goals were to evaluate risk factors for the acquisition of ANDV infection and to determine whether viremia routinely preceded the onset of symptoms and the appearance of anti-hantavirus antibodies.

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METHODS

The human-experimentation guidelines of the US Department of Health and Human Services; of local, independent ethics committees in Chile; and of the Institutional Review Board of the University of New Mexico were followed in the conduct of the research. Study sites were established at tertiary care hospitals throughout central and southern Chile. HCPS was diagnosed on the basis of the presence of (1) an illness with a febrile prodrome followed by a cardiopulmonary phase with the development of bilateral pulmonary changes (as determined by chest radiography) with or without shock and (2) the detection of anti-hantavirus IgG and IgM antibodies. After giving written, informed consent, the index case patient, guardian, or legal next-of-kin identified all household contacts ⩾2 years of age who had resided in the same house for at least 1 night at any point from 30 days before to 7 days after the onset of symptoms in the index case patient. We then attempted to locate each household contact and confirm eligibility. At the first visit, the subject, parent, or guardian gave written, informed consent, and we performed a clinical evaluation, obtained blood samples, and administered a questionnaire that included demographic data, risk activities engaged in by the household contact, and the type of contact between the household contact and the index case patient.

The index case patient was the person who developed the first confirmed case in the household, and persons in whom all subsequent cases occurred were considered to be additional household case patients. If the presumed index case patient was later found to have the second case occurring in a household, the person with the first case was reclassified as the index case patient and the person with the subsequent case was reclassified as an additional household case patient. The latter classification was then used for all analyses, including the description of demographics and the analysis of risk factors for additional household case patients. We have chosen not to describe additional cases in the household as "secondary," because some additional cases resulted from acquisition via environmental exposures rather than via person-to-person transmission from index case patient.

Prospective follow-up of household contacts. Each household contact was clinically evaluated, and blood samples were obtained at least weekly for 28 days. Serum was tested immediately for anti-hantavirus antibodies. Blood obtained in an EDTA tube was allowed to stand without centrifugation, and cells were separated from plasma before both were frozen at −70°C. Clinical evaluation was recommended for any household contact who developed a febrile illness, including hospitalization unless the platelet count was normal.

Detection of anti-hantavirus antibodies. We used an ELISA with recombinant Sin Nombre virus (SNV) N antigen and Laguna Negra whole virus obtained from the US Centers for Disease Control and Prevention to detect IgG and IgM, respectively [13, 14]. All serum samples from patients positive by ELISA were also tested using a strip immunoblot assay for IgM and IgG antibodies with purified recombinant ANDV N antigen [15].

RNA extraction and cDNA first-strand transcription. Total RNA was extracted from 200 μL of plasma and 150 μL of peripheral blood cells by use of the High Pure Viral Nucleic Acid Kit (Roche Molecular Biochemicals), in accordance with the manufacturer's protocol. RNA (2.5 μL) was used as a template for cDNA synthesis with the primer 5′-CACACGAACAACAGCTCGTGA-3′ (nt 49–69), which was designed to serve as a primer for ANDV S segment using the Invitrogen first-strand synthesis kit as recommended by the manufacturer.

Qualitative, conventional, heminested reverse-transcription polymerase chain reaction (RT-PCR). The S open reading frame (ORF) cDNA was the template for PCR using primer S, located between nt 49 and 69, and primer R, located between nt 1107 and 1129, and the amplification product was used as a template for heminested PCR with primer S and primer A (nt 262–283). Amplification products were visualized by gel electrophoresis, and fragments of the expected sizes were sequenced for confirmation.

Quantitative RT-PCR. A real-time–based method was developed for ANDV quantification by use of a LightCycler instrument (Roche Molecular Biochemicals). The standard curve was generated from known copy numbers of the template, and the quantity in unknown samples was calculated as described elsewhere [16]. The PCR primers were 5′-CACACGAACAACAGCTCGTGA-3′ and 5′-TTAGATGATCATCAGGCTCAA-3′, which amplify a fragment of the ORF of the S segment between coordinates 37 and 271 bp (GenBank accession number NC003466). The specificity of the resulting product was confirmed by melting point analysis.

Statistical analysis. Simple statistics for frequencies and continuous variables were calculated for all variables in the database, including the demographic characteristics of the index case patients and household contacts, the types of contact with an index case patient, and risk activities. The frequency for each variable was compared using Fisher's exact test for contingency tables, the Mann-Whitney *U* test, and Student's *t* test. Relationships among risk factors were calculated using Spearman's correlation coefficient and variance inflation factors (VIFs). A basic logistic regression model was implemented to compare risk factors in household contacts who did and did not develop HCPS. We then used a conditional stepwise logistic regression model to identify important risk factors associated with the acquisition of HCPS. We used statistical packages SPSS (version 11.5) and SAS (version 9.1; SAS Institute) for all analyses.

RESULTS

Classification of index case patients and household contacts. From November 2001 through June 2005, we initially enrolled 421 household contacts in association with 76 confirmed index cases. Among these 421 household contacts, 405 remained seronegative during prospective follow-up. The remaining 16 household contacts were either seropositive at entry ($n = 6$) or subsequently developed HCPS ($n = 10$). Of the latter, 6 were followed for 1–4 weeks before they developed HCPS, whereas the remaining 4 could not be contacted until they had developed HCPS.

The 6 who were seropositive at entry had both IgM and IgG antibodies and a history of an illness with onset 14–20 days before the onset of symptoms in the next case patient in the family. Five of the 6 had sought medical evaluation during the acute illness, and 2 were hospitalized with a diagnosis of atypical pneumonia. The remaining 4 had a febrile illness with back pain ($n = 1$), myalgia ($n = 1$), abdominal pain ($n = 1$), and gastrointestinal symptoms and dehydration ($n = 1$). We performed RT-PCR on peripheral blood cells from the first available sample from these 6 case patients. We detected ANDV RNA in 4 case patients from blood obtained 24, 25, 32, and 35 days after symptom onset and were unable to detect ANDV RNA in 2 case patients from blood obtained 49 and 64 days after symptom onset. These 6 cases occurred in 4 family clusters—2 clusters with 2 cases and 2 clusters with 3 cases. We reclassified the first person to become ill as the index case patient and the second and third persons to become ill as additional household case patients.

Whenever HCPS was diagnosed in a household contact, we extended the period of observation for other household contacts and attempted to enroll additional contacts who met the entry criteria. In this manner, we enrolled 55 additional contacts for a total of 476 household contacts.

Characteristics of the case patients with HCPS and the case clusters. Of the 92 case patients with HCPS (76 index case patients and 16 additional case patients), 62 were male and 30 were female. The median age was 36 years (range, 1.5 months to 77 years); 11 patients (12%) were <16 years of age. Fifty cases were severe (requiring mechanical ventilation and/or vasoactive drugs), and 42 were mild (requiring neither mechanical ventilation nor vasoactive drugs; all but 5 of the patients with mild HCPS were hospitalized). The mortality was 21.7%. Thirty cases (32.6%) occurred in 14 household clusters, including 12 clusters with 2 cases and 2 clusters with 3 cases.

The demographic characteristics of the 76 index case patients, the 16 additional case patients, and the 460 household contacts who remained seronegative as well as the disease severity for the case patients are shown in table 1. The proportion of males was higher among the index case patients than among

Table 1. Characteristics of the index case patients, additional household case patients, and household contacts who remained seronegative.

Variable	Index case patients ($n = 76$)	Additional household case patients ($n = 16$)	Household contacts who remained seronegative ($n = 460$)	P[a]	
				Index vs. additional case patients	Additional case patients vs. contacts who remained seronegative
Sex					
Male	56 (73.7)	6 (37.5)	238 (51.7)	.008	.314
Female	20 (26.3)	10 (62.5)	222 (48.3)		
Age					
Mean ± SD, years	35.0 ± 16.1	37.3 ± 17.0	29.4 ± 18.9	.612	.100
Median (range), years	35.5 (0–77)	43 (2–67)	26 (2–94)	.315	.042
Subjects <16 years of age	9 (11.8)	2 (12.5)	132 (28.7)	1.000	.256
Disease severity					
Severe	44 (57.9)	6 (37.5)	NA	.172	NA
Mild	32 (42.1)	10 (62.5)	NA		
Mortality	18 (23.7)	2 (12.5)	NA	.508	NA
Ethnicity					
Hispanic	68 (89.5)	14 (87.5)	401 (87.2)	.656	1.000
Native American	7 (9.2)	2 (12.5)	53 (11.5)		
Other	1 (1.3)	0 (0)	6 (1.3)		

NOTE. Data are no. (%) of subjects, unless otherwise specified. NA, not applicable.

[a] By Fisher's 2-sided exact test for most comparisons; the Mann-Whitney U test was used for median age, and Student's t test was used for mean age. For ethnicity, the comparison is between Hispanics and Native Americans.

the additional household case patients ($P = .008$), and the median age of the additional household case patients was significantly greater than that of the household contacts who remained seronegative ($P = .042$). Otherwise, the demographic characteristics of the 3 groups were similar.

Mode of transmission and intervals between onset of symptoms. The median interval between symptom onset in the household clusters was 19.5 days (range, 4–30 days), and all but 2 intervals were 14 days or more (table 2). Person-to-person transmission was the only potential mode of acquisition for 3 additional household case patients (5, 8, and 10), with intervals of 16–23 days between symptom onset. There was close contact with the index case, including 2 of 3 who were sex partners, and the additional household case patient lived in an urban area without risk of environmental exposure. For 9 additional household case patients (3, 4, 7, 9, 11, 12, 13, 15, and 16) with intervals of 14–30 days, transmission is best explained by person-to-person transmission. There was close contact with the index case patient, including 7 of 9 who were sex partners, and a lack of major risk factors for environmental exposure. The mode of acquisition is less clear in additional household case patients 6 and 14, with intervals of 20 and 27 days, respectively, between symptom onset. Additional case patient 6 lived with the index case patient in a rural area but slept in a different room and denied having body fluid contact. Additional case patient 14, although not a sex partner, slept in the same room but also had strong environmental risk factors, including the demolition of a shed.

In the remaining 2 additional household case patients (1 and 2), transmission almost certainly resulted from common exposure to environmental sources rather than from person-to-person transmission. The intervals of 4 and 6 days between the onset of symptoms were shorter than the known incubation period for ANDV infection [17]. Additional case patients 1 and 2 were not sex partners with their index case patients and shared common exposure to environmental sources, including the demolition of a shed for additional case patient 2.

Risk factors for acquisition of HCPS by household contacts. We compared the exposure history for the 16 household contacts (3.4%) who developed HCPS and the 460 (96.6%) who remained uninfected. The risk of HCPS was 17.6% among sex partners, versus 1.2% among non–sex partners ($P < .001$). By univariate analysis based on the 95% confidence interval for relative risk, Fisher's exact test, and logistic regression (odds ratio), sleeping in the same bed or room; exposure to saliva (deep kissing), urine, and semen; and demolition of a shed were all also associated with an increased risk. Sleeping in a different room was associated with a decreased risk by uni-

Table 2. Characteristics of the index case patients and additional household case patients.

Cluster no.	Additional case patient no.	Sex, age in years of index case patient	Sex, age in years, no. in cluster of additional case patient	Relationship	Risk factors related to contact with the index case patient[a]	Environmental risk factors for the additional case patient[b]	Days between onset of symptoms in index and additional case patient
1	1	F, 63	M, 67, 2nd	Former couple	Unclear[c]	1	4
2	2	M, 37	M, 42, 2nd	Coworkers	SSR	2	6
3	3	M, 47	F, 43, 2nd	Marriage	SP, BFC, SSR, SSB	1	14
	4	F, 43	F, 8, 3rd	Mother/daughter	BFC, SSR, SSB	1	16
4	5	M, 46	F, 46, 2nd	Marriage	SP, SSR, SSB	0	16
	6	F, 46	M, 17, 3rd	Aunt/nephew	SDR[d]	1	20
5	7	M, 43	F, 43, 2nd	Marriage	SP, BFC, SSR, SSB	1	19
6	8	M, 39	F, 46, 2nd	Marriage	SP, SSR, SSB[d]	0	19
7	9	M, 46	F, 47, 2nd	Marriage	SP, BFC, SSR	1	19
8	10	M, 43	F, 2, 2nd	Father/daughter	SSR, SSB[d]	0	23
9	11	M, 42	F, 33, 2nd	Marriage	SP, BFC, SSR, SSB	1	23
10	12	M, 27	F, 24, 2nd	Couple	SP, BFC, SSR, SSB[d]	1	23
11	13	M, 22	F, 48, 2nd	Son/mother	BFC, SDR	1	25
12	14	M, 44	M, 50, 2nd	Coworkers	SSR	2	27
13	15	F, 34	M, 34, 2nd	Marriage	SP, BFC, SSR, SSB	1	29
14	16	F, 42	M, 47, 2nd	Marriage	SP, BFC, SSR, SSB	1	30

NOTE. In the 2 clusters with 3 case patients (clusters 3 and 4), the second case patient in the household was classified as the index case patient when the relationship with the third case patient in the household was analyzed. F, female; M, male.

[a] BFC, body fluid contact (saliva, semen, or urine); SDR, slept in different room; SP, sex partner; SSB, slept in same bed; SSR, slept in same room.

[b] Codes for potential environmental exposure to Andes virus–infected rodents: 0, no environmental risk, urban residence; 1, rural residence without major risk factor; 2, shared major risk factor (demolition of storage shed in rural area).

[c] Former couple slept in the same house in different beds. They denied sexual contact or body fluid contact with saliva (deep kissing), semen, or urine; information on whether they slept in the same or different room was not available.

[d] Information on body fluid contact with saliva, semen, or urine not available.

variate analysis (data not shown). By multivariate logistic regression analysis, only being a sex partner remained significant at a level of $P < .05$.

To focus on risk factors for person-to-person transmission, we repeated the risk analysis after excluding the 2 additional household case patients (1 and 2) whose infections clearly resulted from common environmental exposure (table 3). By univariate analysis, being a sex partner; exposure to saliva, urine, or semen; sleeping in the same room; sleeping in the same bed; assisting the index case patient; accompanying the index case patient to seek health care; and sleeping in the same room were associated with significantly increased risk (table 3). Exposure to respiratory secretions was not associated with increased risk, but sleeping in a different room was associated with significant protection (table 3). By conditional forward multivariate logistic regression analyses, only being a sex partner remained significant ($P < .001$). We then performed a backward conditional stepwise multivariate logistic regression analysis to determine which factors contributed to the risk. In this analysis, both being a sex partner ($P = .002$) and exposure to saliva ($P = .046$) remained significant, where those P values correspond to a likelihood ratio statistic based on conditional parameter estimates. Additional interaction between these 2 variables was not considered, and backward stepwise elimination likelihood ratio tests, based on maximum partial likelihood estimates, resulted in the same final model.

We investigated multicolinearity (which can result from strong correlation between independent variables) in our logistic regression model to avoid inflation of variances of parameters by use of VIFs. Sleeping in the same bed (VIF, 2.10), sleeping in different room (VIF, 1.67), exposure to urine (VIF, 1.14), and assisting the index case patient (VIF, 1.40) showed multicolinearity. These variables were excluded from our variable-selection procedures, so no further action was necessary. The correlation between being a sex partner and exposure to saliva was 0.54 ($P < .001$). Addition of the demographic variables age, sex, and ethnicity did not improve our model significantly. Parameters that we retained in our final model after backward selection are shown in table 4.

Early detection of infection. Six of the 16 household contacts who developed HCPS were enrolled and provided blood samples before the development of prodromal symptoms. None had IgG or IgM antibody detected before the onset of prodromal symptoms. However, ANDV RNA was detected by RT-PCR in peripheral blood cells a median of 11 days before the cardiopulmonary phase in 6 additional household case patients

Table 3. Risk factors in 14 household contacts with hantavirus cardiopulmonary syndrome (HCPS) with definite ($n = 3$), probable ($n = 9$), or possible ($n = 2$) acquisition by person-to-person transmission from a household contact with HCPS and 460 household contacts who remained seronegative.

	Percentage (proportion) who acquired HCPS			*P*	
Risk variable[a] for additional household case patients	Subjects with risk variable	Subjects without risk variable	RR (95% CI)	Exact	By univariate logistic regression based on OR
Sex partner	17.6 (9/51)	1.2 (5/423)	14.93 (5.21–43.48)	<.001	<.001
Assisted index patient	5.6 (7/124)	1.4 (5/345)	3.90 (1.26–12.05)	.018	.018
Slept in same bed	12.7 (10/79)	1.0 (4/395)	12.50 (4.02–38.46)	<.001	<.001
Slept in same room	5.5 (12/217)	0.8 (2/257)	7.11 (1.61–31.25)	.004	.009
Slept in different room	0.8 (2/240)	5.2 (12/231)	0.16 (0.04–0.71)	.006	.015
Exposure to saliva (deep kissing)	12.7 (8/63)	0.7 (3/405)	17.14 (4.67–62.50)	<.001	<.001
Exposure to respiratory secretions	6.5 (2/31)	2.3 (10/438)	2.83 (0.65–12.35)	.185	.175
Exposure to vomit	6.7 (1/15)	2.4 (11/454)	2.75 (0.38–19.96)	.326	.328
Exposure to blood	0 (0/3)	2.6 (12/466)	NA	1.000	.999
Exposure to urine	21.4 (3/14)	1.8 (8/454)	12.16 (3.61–41.67)	.003	<.001
Exposure to semen	17.9 (5/28)	1.4 (6/440)	13.01 (4.26–40.28)	<.001	<.001
Accompanied index case patient to seek health care	4.8 (5/104)	1.4 (5/363)	3.49 (1.03–11.83)	.048	.045
Cleaned storage room	3.7 (2/54)	2.4 (10/415)	1.54 (0.35–6.83)	.637	.574
Cleaned barn	6.7 (1/15)	1.4 (6/443)	4.92 (0.63–38.37)	.209	.139
Domestic cleaning	3.3 (6/182)	1.7 (5/286)	1.89 (0.58–6.09)	.351	.289
Removed garbage/trash	4.2 (5/120)	1.2 (4/344)	3.58 (0.98–13.13)	.054	.054
Worked in agriculture or forestry	3.3 (3/92)	2.6 (10/378)	1.23 (0.35–4.39)	.725	.747
Demolished storage shed	9.1 (1/11)	2.2 (10/455)	4.14 (0.58–29.56)	.233	.173

NOTE. CI, confidence interval; NA, not applicable; OR, odds ratio; RR, relative risk.

[a] Exposure to index case patient or environmental sources.

Table 4. Final multivariate logistic regression model for predicting hantavirus cardiopulmonary syndrome on the basis of risk factors.

Variable	Parameter estimate	SE	Wald χ^2	P^a	P^b	OR (95% CI)
Intercept	−5.174	0.629	67.681	<.001	…	0.006
Sex partner	2.27	0.882	6.65	.010	.002	9.709 (1.724–54.674)
Saliva	1.619	0.883	3.359	.067	.046	5.049 (0.894–28.522)

NOTE. CI, confidence interval; OR, odds ratio.

[a] *P* values for Wald χ^2 test.

[b] *P* values are derived from a likelihood ratio statistic based on conditional parameter estimates from a stepwise model-selection procedure.

and 5, 7, 14, and 15 days before the onset of prodromal symptoms or anti-hantavirus antibodies in 4 additional household case patients (table 5). The time course of the development of ANDV RNA in peripheral blood cells, IgG and IgM antibodies, symptoms, and hospitalization in additional household case patient 16 is shown in table 6. Using real-time RT-PCR, we also tested blood cells from 132 samples obtained during follow-up from 52 seronegative, asymptomatic household contacts, including 34 samples from 12 sex partners of index case patients, but none was positive.

DISCUSSION

In the present study—the first prospective study of household contacts of index case patients with HCPS—we found that 16 (3.4%) of 476 household contacts developed HCPS. We were able to identify risk factors for the development of HCPS among household contacts of index patients with ANDV infection. Sex partners were at the greatest risk (17.6%), but person-to-person transmission also occurred in persons with close, nonsexual contact, such as sleeping in the same bed or room. We also demonstrated for the first time that viral RNA can routinely be detected in blood cells for up to 2 weeks before symptoms or anti-hantavirus antibodies are evident. These findings have several implications.

First, household contacts of patients with HCPS in areas where ANDV is present should be counseled that they are at risk of developing HCPS within the next 4 weeks and that they should seek medical attention at the first sign of fever. Second, risk factors identified in the present study could be used to identify household contacts at highest risk of developing HCPS, in order to prioritize efforts to contact household contacts and possibly to identify candidates for prevention studies. Passive postexposure administration of neutralizing antibody provided protection in an ANDV hamster model [18], so it is conceivable that postexposure treatment of household contacts with human plasma, γ globulin, or humanized monoclonal antibodies with high neutralizing activity might prevent severe or fatal illness.

Third, real-time RT-PCR of blood cells could be used to

Table 5. Detection of Andes virus (ANDV) RNA by reverse-transcription polymerase chain reaction (RT-PCR) in peripheral blood cells obtained from household contacts who were asymptomatic and seronegative at study entry.

Additional household case patient no. from table 3 (sex, age in years)	Viral level in first positive sample, ANDV copies/mL of sedimented peripheral blood cells	Days from first positive RT-PCR to		Days from onset of symptoms in index case patient to positive RT-PCR in contact
		Onset of prodromal symptoms	Onset of cardiopulmonary phase	
16 (M, 47)	28,244	14	18	18
15 (M, 34)	33,000	15	18	15
13 (F, 48)	18,342	7	10	18
10 (F, 2)	1,181,551	NA[a]	12	~14[b]
14 (M, 50)	116,754	5	8	22
9 (F, 47)	3882	NA[c]	7	19

NOTE. NA, not available.

[a] Case patient 10 was afebrile, asymptomatic, and seronegative when ANDV RNA was detected by RT-PCR, but the day of onset of prodromal symptoms could not be determined because of the subject's age.

[b] First positive PCR result on 1 June; the index case patient died on 21 May after several days of symptoms, but the exact date of the onset of symptoms was not known.

[c] Case patient 9 was seronegative when enrolled 7 days before the day of the onset of symptoms, but cells were not collected for analysis by RT-PCR at the enrollment visit.

Table 6. Natural history of Andes virus (ANDV) infection in additional household case patient 16, who was enrolled in prospective follow-up 7 days before detection of ANDV RNA in peripheral blood cells, 21 days before the onset of prodromal symptoms and the development of IgM antibody, and 25 days before hospitalization in the cardiopulmonary phase.

	Visit				Hospital		
Parameter	1	2	3	4	1	3	7
Date	3 Feb	10 Feb	17 Feb	24 Feb	28 Feb	2 Mar	6 Mar
Day	0	7	14	21	25	27	31
Prodromal symptoms	No	No	No	Yes	…	…	…
Cardiopulmonary phase	…	…	…	No	Yes	Yes	Yes
IgM	Neg	Neg	Neg	Pos	Pos	Pos	Pos
IgG	Neg	Neg	Neg	Neg	Pos	…	…
Quantitative RT-PCR[a]							
Plasma[b]	Neg	Neg	Neg	2040	6187	…	…
Peripheral blood cells[c]	Neg	28,244	34,756	87,637	63,383	297,099	304,211
Qualitative RT-PCR[d]							
Plasma	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Peripheral blood cells	Neg	Neg	Neg	Pos	Pos	Pos	Pos

NOTE. Neg, negative; Pos, positive; RT-PCR, reverse-transcription polymerase chain reaction.

[a] Determined by use of a LightCycler instrument (Roche Molecular Biochemicals).

[b] ANDV copies/mL of plasma.

[c] ANDV copies/mL of sedimented peripheral blood cells.

[d] Determined by gel electrophoresis.

detect infection before the onset of HCPS. HCPS occurs predominantly in rural areas, where access to real-time RT-PCR is limited, and family clusters are uncommon outside of Chile and Argentina. As such, testing is unlikely to be routinely available. However, testing might be feasible for some high-risk household contacts in Chile and Argentina or after high-risk rodent exposure, a laboratory accident, or intentional release.

Fourth, our finding that ANDV RNA was present in peripheral blood cells for 5–15 days before the onset of symptoms or the appearance of IgM or IgG antibody significantly expands our understanding of the natural history and pathogenesis of HCPS and provides additional support for the concept that the life-threatening cardiopulmonary phase may be largely mediated by immune responses [19, 20]. We believe that the virologic and serologic data from additional household case patient 16 provides the most complete description of the natural history of human hantavirus infection in the literature, and for this reason we have shown the data for this patient in table 6.

Finally, our findings suggest that most additional cases in household clusters in Chile result from person-to-person transmission. Although acquisition was probably from exposure to rodents in 3 of the 16 cases and was unclear in 1, we believe that the remaining 12 cases resulted from person-to-person contact. The time intervals between the onset of symptoms were consistent with the incubation period for ANDV infection [8, 17], multiple variables associated with close contact with the index case patient were associated with increased risk of acquisition, and 3 of the 12 lived in urban areas where the only possible exposure to ANDV was from contact with the index case patient.

There are several limitations to our data. First, because the logistics of collecting samples at regional hospitals prevented optimal processing of samples for virus culture, we do not know whether the peripheral blood cells that were positive by RT-PCR would also have been positive by viral culture. Second, with 3 exceptions, we cannot completely exclude the possibility of common source acquisition. As such, our analysis of risk factors for person-to-person transmission (table 3) may include 1 or more cases acquired from common environmental sources rather than from person-to-person transmission. We considered limiting the analysis in table 3 to household contacts who were sex partners, but 1 definite, 2 probable, and 2 possible cases of person-to-person transmission in our study resulted from close contact without sexual contact, and others have clearly documented person-to-person transmission of ANDV infection that was not limited to sex partners of index case patients [10].

Third, our identification of exposure to saliva via deep kissing as a possible risk factor should be interpreted with caution. Although exposure to saliva was significant in the univariate analysis and remained significant in the backward stepwise multivariate logistic regression model, the correlation between being a sex partner and deep kissing was high ($P < .001$), and only being a sex partner remained significant in the conditional forward regression analysis. Further studies are needed to determine whether ANDV is present in human saliva and may be transmitted via deep kissing. Nonetheless, it is interesting

to note that ANDV has been detected by immunohistochemistry in salivary gland tissue and by amplification of genomic RNA from saliva from *Oligoryzomys longicaudatus,* the ANDV rodent reservoir [21]; that SNV RNA is present in saliva but is undetectable in most urine and fecal samples from deer mice [16]; and that hantaviruses may be transmitted from rodent bites [22–24]. Fourth, although exposure to semen and urine did not remain significant in the multivariate analysis, exposure to both was highly correlated with being a sex partner. As such, either the latter or the small sample size could have prevented identification of a significant association.

Exposure to hantavirus-carrying aerosols is thought to be the primary route of transmission from hantavirus-infected rodents to humans. In the present study, the lack of a significant association with exposure to respiratory secretions, the relative inefficiency of person-to-person transmission within households, and the increased risk among sex partners and others with close contact with the index case patient all argue that droplet or airborne transmission, if present, is likely to be an inefficient mode of person-to-person transmission. However, this mode of transmission cannot be completely excluded. These results are largely supported by those of Wells et al. [10] and Martinez et al. [8], which also suggest that person-to-person transmission requires protracted, relatively intimate interpersonal contact. Finally, the lack of association in the present study with risk variables such as work in forestry or cleaning storage rooms or barns should not be taken as evidence that these activities do not confer risk. Because most of the 16 household contacts with HCPS appear to have acquired infection from contact with the index case patient, we believe that we were left with little power to detect risks from environmental exposure among the few household contacts who acquired HCPS from environmental exposure. Although we believe that all 76 index case patients acquired ANDV infection from environmental exposure, we chose not to present data on risk factors among index patients in this analysis.

In summary, household clusters of ANDV infection are common in Chile, and most additional cases in household clusters result from person-to-person transmission. The risk of infection among household contacts of index case patients with HCPS was increased in sex partners, in those who engaged in deep kissing, and in those who participated in the demolition of a shed and was decreased in those who slept in different rooms. Finally, viral RNA could be detected in peripheral blood cells for up to 2 weeks before the onset of symptoms or the appearance of anti-hantavirus antibodies.

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References

1. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. Emerg Infect Dis **1997**; 3:95–104.
2. Duchin JS, Koster F, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med **1994**; 330:949–55.
3. Hallin GW, Simpson SQ, Crowell RE, et al. Cardiopulmonary manifestations of the hantavirus pulmonary syndrome. Crit Care Med **1996**; 24:252–8.
4. Sotomayor V, Aguilera X. Epidemiology of human infection with hantavirus in Chile. Rev Chil Infect **2000**; 17:220–32.
5. Torres-Pérez F, Navarrete-Droguett J, Aldunate R, et al. Peridomestic small mammals associated with confirmed cases of human hantavirus disease in southcentral Chile. Am J Trop Med Hyg **2004**; 70:305–9.
6. Mertz GJ. *Bunyaviridae:* bunyaviruses, phleboviruses, nairoviruses, and hantaviruses. In: Richman DD, Whitley RJ, Hayden FG, eds. Clinical virology. 2nd ed. Washington, DC: American Society for Microbiology Press, **2002**:921–48.
7. Pini N. Hantavirus pulmonary syndrome in Latin America. Curr Opin Infect Dis **2004**; 17:427–31.
8. Martinez VP, Bellomo C, San Juan J, et al. Person-to-person transmission of Andes virus. Emerg Infect Dis **2005**; 11:1848–53.
9. Padula PJ, Edelstein A, Miguel SD, Lopez NM, Rossi CM, Rabinovich RD. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology **1998**; 241:323–30.
10. Wells RM, Sosa Estani S, Yadon ZE, et al. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? Emerg Infect Dis **1997**; 3:171–4.
11. Castillo C, Villagra E, Sanhueza L, Ferres M, Mardones J, Mertz GJ. Prevalence of antibodies to hantavirus among family and healthcare worker contacts of persons with hantavirus cardiopulmonary syndrome

in Chile: lack of evidence for nosocomial transmission of Andes virus to healthcare workers in Chile. Am J Trop Med Hyg **2004**; 70:302–4.
12. Galeno H, Mora J, Villagra E, et al. First human isolate of hantavirus (Andes virus) in the Americas. Emerg Infect Dis **2002**; 8:657–60.
13. Rossi C, Ksiazek T. Virus detection and identification with serological tests. II. Enzyme-linked immunosorbent assay (ELISA). In: Lee HW, Calisher C, Schmaljohn C, eds. Manual of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome. Seoul: WHO Collaborating Centre for Virus Reference and Research (Hantavirus), **1999**: 87–91.
14. Ksiazek TG, Peters CJ, Rollin PE. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. Am J Trop Med Hyg **1995**; 52:117–23.
15. Bharadwaj M, Nofchissy R, Goade D, Koster F, Hjelle B. Humoral immune responses to the hantavirus cardiopulmonary syndrome. J Infect Dis **2000**; 182:43–8.
16. Botten J, Mirowsky K, Ye C, et al. Shedding and intracage transmission of Sin Nombre hantavirus in the deer mouse *(Peromyscus maniculatus)* model. J Virol **2002**; 76:7587–94.
17. Vial PA, Valdivieso F, Mertz G, et al. The incubation period of hantavirus cardiopulmonary syndrome. Emerg Infect Dis **2006**; 12:1271–3.
18. Hooper JW, Larson T, Custer DM, Schmaljohn C. A lethal disease model for hantavirus pulmonary syndrome. Virology **2001**; 289:6–14.
19. Mertz GJ, Hjelle BL, Williams TM, Koster FT. Host responses in the hantavirus cardiopulmonary syndrome. In: Saluzzo JR, Dodet B, eds. Factors in the emergence and control of rodent-borne viral diseases. New York: Elsevier, **1999**:133–7.
20. Kilpatrick ED, Terajima M, Koster FT, Catalina MD, Cruz J, Ennis FA. Role of specific DE8+ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. J Immunol **2004**; 172:3297–304.
21. Padula P, Figueroa R, Navarrete M, et al. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol **2004**; 78: 11972–9.
22. Merino C, Arias A, Castillo C. First case of hantavirus cardiopulmonary syndrome secondary to a rodent bite. Rev Chil Enf Respir **2002**; 18: 199–205.
23. St Jeor SC. Three-week incubation period for hantavirus infection. Pediatr Infect Dis J **2004**; 23:974–5.
24. Douron E, Moriniere B, Matheron S, et al. HFRS after a wild rodent bite in the hautesavoie and risk of exposure to a hantaan-like virus in a Paris laboratory. Lancet **1984**; 1:676–7.