Tropical Medicine and International Health

VOLUME 6 NO 10 PP 811–816 OCTOBER 2001

Seasonal variation in prevalence of antibody to hantaviruses in rodents from southern Argentina

G. Cantoni[1], P. Padula[2], G. Calderón[3], J. Mills[4], E. Herrero[1], P. Sandoval[1], V. Martinez[2], N. Pini[3] and E. Larrieu[1,5]

1 *Secretaría de Estado de Salud, Provincia de Río Negro, Argentina*
2 *Instituto Nacional de Enfermedades Infecciosas Anlis/Malbrán, Buenos Aires, Argentina*
3 *Instituto Nacional de Enfermedades Virales Humanas, Pergamino, Argentina*
4 *Centers for Disease Control and Prevention, Division of Viral and Rickettsial Diseases, Atlanta, GA, USA*
5 *Facultad Ciencias Veterinarias, U. N. de la Pampa, General Pico, Argentina*

Summary We conducted a small mammal trapping study to investigate temporal variation in prevalence of infection in hantavirus reservoir populations in the Patagonian Andes mountain range, Rio Negro province, Argentina. Rodent blood samples collected in natural and periurban habitats and at the home of an hantavirus pulmonary syndrome (HPS) case patient were analysed by enzyme-linked immunosorbent assay. Organ tissue samples were tested by polymerase chain reaction (PCR) and nucleotide sequence analysis. Eight species of 1032 rodents were captured in 15 551 trap nights, giving an overall trap success of 6.6%. Hantavirus antibody was detected in 30 of 555 *Oligoryzomys longicaudatus* (reservoir of Andes virus), three of 411 *Abrothrix longipilis*, and one of 10 *Loxodontomys micropus*. Antibody prevalences in *O. longicaudatus* were 13.7% in spring 1996, 59.3% in summer 1996, 2.1% in autumn 1997, 12.4% in winter 1997 and 3.1% in spring 1997. A much higher antibody prevalence (33%) was found during trapping around the residence of an HPS case patient. Higher prevalences were found in older male *O. longicaudatus*. There was no apparent correlation of antibody prevalence with rodent population density, or of rodent population density or antibody prevalence with numbers of human cases. For an HPS case that occurred in our study area in 1997, we identified the probable rodent reservoir and likely site of exposure by matching the genetic sequences of virus obtained from a rodent and the HPS case patient.

keywords hantavirus pulmonary syndrome, Andes virus, reservoirs, transmission, ecology, *Oligoryzomys longicaudatus*, *Abrothrix longipilis*

correspondence Edmundo Larrieu, Laprida 240, 8500 Viedma, Argentina.
E-mail: elarrieu@salud.rionegro.gov.ar

Introduction

In 1993, an outbreak of a febrile illness characterized by pulmonary insufficiency in residents of the south-western United States resulted in the description of hantavirus pulmonary syndrome (HPS). The aetiologic agent of HPS was subsequently shown to be Sin Nombre virus (SNV), carried by the deer mouse, *Peromyscus maniculatus* (Nichol *et al*. 1993; Childs *et al*. 1994). Since the discovery of SNV, about 30 additional hantaviruses have been identified in the Americas, each associated with a primary host species in the rodent family Muridae. About half of these hantaviruses, all associated with the Murid subfamily Sigmodontinae, are known to cause HPS in humans (Peters 1998). Six sigmodontine hantavirus host species have been identified in Argentina. Among the most important of these are *Oligoryzomys flavescens*, host of Lechiguanas virus, and *O. longicaudatus*, host of Andes virus (Levis *et al*. 1995, 1997a,b; Lopez *et al*. 1996; Cantoni *et al*. 1997; Calderón *et al*. 1999).

The first case of HPS in the Andean region of Argentina was identified in November 1993. From 1993 to 1997, 38 HPS cases were recorded, with 11 cases in Esquel and El Hoyo (Chubut province), 26 in El Bolsón and Bariloche (Río Negro Province) and one in Neuquén Province. Of them, 60% of cases were male; 50% were fatal. Cases occur throughout the year, but are more frequent in spring. Nineteen recorded cases were part of an outbreak that

occurred in the Andean towns of El Bolsón and Bariloche from September to December 1996; 85% of cases in this outbreak ($n = 16$) resulted from person-to-person transmission of Andes virus (Enría *et al.* 1996; Wells *et al.* 1997; Padula *et al.* 1998; Yadón 1998).

In response to the appearance of HPS, the Directorate of Environmental Health of the Provincial Public Health Council of Río Negro commenced studies of rodent populations in the province of Río Negro to: (1) identify the reservoir of Andes virus; (2) determine the geographical distribution of Andes virus associated with rodent populations in the Andean region of Rio Negro province; (3) estimate prevalence of infection in host populations; (4) evaluate seasonal variation in prevalence of hantavirus infection in reservoir populations; and (5) identify any additional potential hantavirus host species.

Materials and methods

Study area

Trapping was conducted in and around two towns (El Bolsón and San Carlos de Bariloche) in Bariloche Department, western Río Negro province, Argentina. Bariloche Department is located in the lake region of the Patagonian Andean mountain range; El Bolsón is in the southern part of the region near Lago Puelo (Chubut province); and Bariloche in the northern part, near Villa La Angostura (Neuquén province). The distance between the two towns is 120 km. The region borders Chile to the west; 70 km to the east is the Patagonian plateau.

The climate is cold and wet, with 200–1000 mm annual rainfall and monthly mean temperatures of 2 °C in winter and 18 °C in summer. The vegetation consists of dense southern beech (*Nothofagus* spp.) forests interspersed with brush areas with dog rose (*Rosa rubiginosa*), raspberry (*Rubus idaeus*) and broom (*Cytisus* sp.). Agricultural products include mushrooms, strawberries, hops and fine fruits. The gathering of wild fruits for homemade jam production is common. Bariloche and particularly El Bolsón are small rural towns with areas of dense brush and forest remnants even in the vicinity of the town centres.

Trapping

Rodent trapping was conducted during seven seasonal trapping events between January 1996 and December 1997 (Table 1). Sherman and Tomahawk live-capture traps were placed in trap lines of 10–20 traps at approximately 2-m intervals and baited with peanut butter and rolled oats. Traps were placed to sample the greatest possible variety of natural and disturbed habitat types including, forest, brush, streamside, lakeshore, and peridomestic environments in rural and periurban settings. Except for the summer 1997, which was a case follow-up investigation, traps were placed at approximately the same sites during each trapping event in order to detect seasonal changes in rodent population dynamics and infection prevalence. To minimize the risk of human infection with rodent-borne agents, rodent handling and sampling followed standardized safety protocols (Mills *et al.* 1995a,b). Briefly, captured rodents were anaesthetized using methoxyflourane before taking blood samples by cardiac puncture. Rodents were then killed by cervical dislocation and necropsied to obtain samples of liver, lung and kidney. All samples were preserved in liquid nitrogen until processed at the laboratories of the National Institute of Human Viral Diseases or at the Carlos Malbrán National Institute of Microbiology.

Laboratory analyses

Blood samples were processed by enzyme immunoassay (EIA) technique using SNV antigen (at the National Institute of Human Viral Diseases) or Andes virus antigen (at the Carlos Malbrán Institute of Microbiology) as described in detail by Feldmann *et al.* (1993) and Padula *et al.* (2000).

RNA extraction was performed on a blood clot from a case patient and lung tissue from antibody-positive *O. longicaudatus* captured near the residence associated with an HPS case in El Bolsón in 1997. Viral RNA was amplified by nested or seminested RT-polymerase chain reaction (PCR). PCR products were separated and purified in agarose gel, and later sequences were obtained by the cyclic chain elongation method employing dideoxynucleotides as terminators. Primers used for the amplification of different D and M fragments (G1 and G2) have been described previously (Padula *et al.* 2000).

To determine nucleotide and amino acid sequences, fragments of the region encoding for G1 (nts 1735–1984) and a region encoding for G2 (nts 2719–2943), relative to Andes virus M segment numbering, were analysed. Sequences were aligned using NALIGN and PALIGN of PCGENE software programs (Intelligenetics Inc., Mountain View, CA, USA). These sequences were compared with an Andes virus sequence obtained from a case patient associated with an outbreak of HPS involving person-to-person transmission in El Bolsón in 1996 (Padula *et al.* 1998).

Results

Rodent populations and antibody prevalence

We captured 1032 rodents of eight species during 15 551 trap nights for an overall trap success of 6.6%

Table 1 Trap success rate, seroprevalence for hantavirus in rodents and human [hantavirus pulmonary syndrome (HPS) patients] in southern Argentina in the Patagonian Andean mountain range, 1996–1997 (prev = prevalence; *O.l.* = *Oligoryzomys longicaudatus*)

Trapping period	Trap-nights	Captures	Trap success	Adult (%)	Female (%)	Pregnant (%)	Overall Ab prev (%)	Ab prev *O.l.* (%)	Number *O.l.*	Number HPS cases*
Summer 1996	300	43	14.3	58	39	12	0	0	9	1
Spring 1996	700	93	13.2	55	46	0	7.5	13.7	51	4
Summer 1996/1997	9206	246	2.7	53	48	39	5.7	9.3	129	0
Fall 1997	3220	268	8.3	35	46	4	1.5	2.1	145	0
Winter 1997	1750	149	8.3	49	56	0	1.3	2.4	85	0
Spring 1997	250	221	90.4	22	54	0	2.3	3.1	130	0
Summer 1997	120	12	10.0	92	42	17	33.3	6	1	?
Total	15 551	1032	6.6	42	49	5	3.6	5.4	555	6

* Cases in which person-to-person transmission was demonstrated are not included.

Table 2 Number of captures and numbers antibody-positive for rodent species captured during seven trapping events in the Patagonian Andean mountain range of southern Argentina, 1996–1997

Rodent species	Captured (% of total)	Antibody-positive (%)*
Oligoryzomys longicaudatus	555 (53.8)	30 (5.4)
Abrothrix longipilis	411 (39.8)	3 (0.7)
A. olivaceus	44 (4.3)	0
Eligmodontia morgani	2 (0.2)	0
Loxodontomys micropus	10 (0.9)	1 (10)
A. xanthorhinus	4 (0.4)	0
Other species†	6 (0.6)	0
Total	1032 (100)	34 (3.3)

* Results of enzyme-linked immunosorbent assay using Sin Nombre or Andes virus as antigen.
† Other species include four *Mus musculus* and two *Rattus norvegicus*.

(Tables 1 and 2). The two most common species (*O. longicaudatus* and *Abrothrix longipilis*) comprised 94% of captures. Antibody reactive with SNV or Andes virus was detected most frequently in the two most common species: 5.4% of *O. longicaudatus* and 0.7% of *A. longipilis* (Table 2). The considerable variation in trap success over the course of the study indicated large temporal changes in rodent population density, with a population low represented by less than 3% trap success in summer 1996 and a population high with over 90% trap success in the spring of 1997 (Table 1). Trap success was higher ($P < 0.01$) in natural, relatively undisturbed areas (16–37%) than in urban and periurban areas of El Bolsón and Bariloche (1–11%).

The proportion of captures consisting of adult animals was highest (49–58%) in winter through summer, and relatively lower (35%) in the single autumn campaign. Autumn is the period when young animals from the summer breeding season (as indicated by relatively high numbers of pregnant females; Table 1) are likely to be entering the population. The exception was the spring of 1997, when a rodent population irruption resulted in the influx of a large number of juveniles (Table 1).

Hantavirus antibody prevalence in the dominant reservoir species (*O. longicaudatus*) was also highly variable, but there was no significant correlation with population density as indicated by trap success (Pearson's correlation coefficient = –0.2, $P = 0.60$). Six HPS cases occurred in the study area during the time of our investigation (Table 1); four of these during the spring of 1996. There was no statistically significant correlation between numbers of HPS cases and trap success (Pearson correlation coefficient = –0.8, $P = 0.60$) or HPS cases and antibody prevalence in *O. longicaudatus* (correlation coefficient = +0.3, $P = 0.40$).

Antibody-positive *O. longicaudatus* were captured in natural as well as urban and periurban areas of El Bolsón and Bariloche. The antibody-positive population was 77% male animals and 87% adults (Table 3). Male animals were more likely to have hantavirus antibody than were females (odds ratio = 3.74, 95% CI = 1.28–11.69).

Table 3 Distribution by sex and age class for 30 hantavirus antibody-positive *Oligoryzomys longicaudatus* captured in the Patagonian Andean mountain range of southern Argentina, 1996–1997

	Male *n* (%)	Female *n* (%)	Total *n* (%)
Juveniles	2 (6.7)	2 (6.7)	4 (13.4)
Adults	21 (70.0)	5 (16.7)	26 (86.6)
Total (%)	23 (76.7)	7 (23.4)	30 (100)

Case follow-up investigation

Two of six *O. longicaudatus* captured near the residence of an HPS case patient in the summer of 1997 had antibody to a hantavirus (Table 1). Viral genome amplified from these two were 96% and 98% similar for the G1 and G2 portions of the M segment (Table 4); the sequence of the viral genome from the case patient was identical to that from one of the two *O. longicaudatus*; the G1 and G2 sequences from the case patient were 94% and 97% similar to the sequence from a case patient associated with the outbreak involving person-to-person transmission in the same town the previous year ('Epilink/96' sequence).

Discussion

We captured hantavirus antibody-positive *O. longicaudatus* throughout our study area, which encompassed the northern and southern extremes, as well as central portions, of the Andes mountain range within the province of Rio Negro. Establishment of the limits of the distribution of *O. longicaudatus* and of Andes virus within *O. longicaudatus* populations onto the Patagonian plateau to the east will require further study. Antibody-positive *O. longicaudatus* were captured in periurban as well as in more natural, undisturbed habitats, implying that there is some risk of exposure to hantaviruses even among urban residents of Bariloche and El Bolsón. However, given the lower trap success in periurban areas, a lower risk would be anticipated among town and city dwellers than among rural inhabitants.

The overall prevalence of hantavirus antibody in *O. longicaudatus* (5.4%) was similar to that found by other investigators in the Patagonian Andean mountain range (6.2%), but somewhat lower than that found for populations of the same species in northern Argentina (8%, Levis *et al.* 1997a). Antibody prevalences in sigmodontine hantavirus reservoirs in Argentina and other areas of the Americas are variable but remain in the range of 3–14% (Hjelle *et al.* 1996a; Levis *et al.* 1997a,b; Williams *et al.* 1997; Mills *et al.* 1998; Yahnke *et al.*, in press).

The finding of hantavirus antibody in blood samples from *A. longipilis* and *Loxodontomys micropus* could indicate that these species host previously unidentified hantaviruses, but this antibody more likely represents 'spillover' of Andes virus from the natural host (*O. longicaudatus*) into other rodents with which it comes into contact. The very low prevalence of infection in *A. longipilis* provides some evidence to support this hypothesis. A hantavirus infecting several *Akodon* (*Abrothrix*) *olivaceus* and one *A. longipilis* during a rodent irruption in southern Chile was found to be identical to Andes virus infecting *O. longicaudatus* captured in the same area (Toro *et al.* 1998). Nevertheless, more virus sequencing would be required to definitively rule out the possibility of additional viruses.

The lack of a correlation between trap success or antibody prevalence and numbers of HPS cases is somewhat surprising; but several factors must be considered. HPS cases were rare and sporadic; during most seasons there were no cases. Secondly, the rodent irruption in the spring of 1997 was an exceptional event that was very local in geographical scale. It was not associated with any HPS cases, but strongly influenced the correlation calculations. The highest

Table 4 Nucleotide and amino acid sequence similarities between partial M segments obtained from the case patient and two seropositive *Oligoryzomys longicaudatus* captured near the case home. Values above dashes are similarity values for nucleotide sequences; those below dashes are amino acid sequence similarities

	Case patient*		Rodent 1*		Rodent 2*		Epilink/96†	
	G1	G2	G1	G2	G1	G2	G1	G2
Case patient	–	–	100.00	100.00	96.43	98.23	94.05	96.90
Rodent 1	100.00	100.00	–	–	96.43	98.23	94.05	96.90
Rodent 2	100.00	100.00	100.00	100.00	–	–	95.24	95.13
Epilink/96	98.80	98.67	98.80	98.67	98.80	98.67	–	–

* G1 corresponds to nucleotides 1735–1984 of the M segment of Andes virus; G2 corresponds to nucleotides 2718–2943 of the M segment of Andes virus.
† Epilink/96 corresponds to an Andes virus sequence from a case patient in a cluster of cases thought to have occurred as a results of human-to-human transmission (Padula *et al.* 1998), GenBank accession numbers: AF042120 for G1 and AF042119 for G2.

antibody prevalence (33%) was in summer 1997, a period when there was only one HPS case. However, this prevalence is based on a sample size of only six *O. longicaudatus*, and the estimate may be biased because the survey was conducted in the vicinity of an HPS case residence, where unusual conditions might be expected.

Oligoryzomys longicaudatus is distributed the length of the Andean region, from the south of Tierra del Fuego to near the Bolivian border (Redford & Eisenberg 1992). They are nocturnal, frequently associated with brushy vegetation, and the diet is primarily granivorous. During the summer breeding season the female gives birth to three to five offspring with two, three or more successive parturitions. In winter the population diminishes, particularly if the weather is severe. The population begins to increase in spring, may decrease in mid-summer and peaks in autumn, with levels 5–10 times higher than in early spring (Mann 1978; Murúa *et al.* 1986; Guthmann *et al.* 1997).

Seasonal variation in trap success was largely as expected given published seasonal population dynamics. The rodent irruption in the vicinity of Villa La Angostura in the spring of 1997 was an impressive event that led to unusually high trap success, frequently involving the capture of multiple animals in a single trap. This phenomenon is a documented periodic occurrence, especially for populations of *O. longicaudatus* (Murúa *et al.* 1987, 1989). Such irruptions often follow synchronous flowering and seed set in local bamboo (*Chusquea* sp.) and may result in increased risk of human exposure to hantaviruses (Toro *et al.* 1998).

Matching genetic sequence information from viruses collected from rodents and humans has been used to determine the most likely site of exposure (Hjelle *et al.* 1996b). Our demonstration of 100% nucleotide identity between virus from an HPS case patient and an *O. longicaudatus* captured at the home of the case patient implicates *O. longicaudatus* as the responsible reservoir and the general area of the patient's residence as the likely site of exposure.

Acknowledgements

We thank D. Enría and T. Ksiazek for logistic and intellectual support, and N. Guthmann for her valuable collaboration.

References

Calderón G, Pino N, Bolpe J *et al.* (1999) Hantavirus reservoir hosts are associated with peridomestic habitats in Argentina. *Emerging Infectious Diseases* **5**, 792–797.

Cantoni G, Lázaro M, Resa A *et al.* (1997) Hantavirus pulmonary syndrome in the Province of Rio Negro, Argentina, 1993–1996. *Revista de Medicina Tropical Sao Paulo* **39**, 191–196.

Childs J, Ksiazek T, Spiropoulou C *et al.* (1994) Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *Journal of Infectious Diseases* **169**, 1271–1280.

Enría D, Padula P, Segura E *et al.* (1996) Hantavirus pulmonary syndrome in Argentina. Possibility of person-to-person transmission. *Medicina (Buenos Aires)* **56**, 709–711.

Feldmann H, Sanchez A, Morzunov S *et al.* (1993) Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Research* **30**, 351–367.

Guthmann N, Lozada M, Monjeau A & Heinemann K (1997) Population dynamics of five sigmodontine rodents of northwestern Patagonia. *Acta Theriologica* **42**, 143–152.

Hjelle B, Torrez Martinez N & Koster F (1996a) Hantavirus pulmonary syndrome-related virus from Bolivia. *Lancet* **347**, 57–59.

Hjelle B, Torrez-Martinez N, Koster F *et al.* (1996b) Epidemiologic linkage of rodent and human hantavirus genomic sequences in case investigations of hantavirus pulmonary syndrome. *Journal of Infectious Diseases* **173**, 781–786.

Levis S, Calderón G, Briggiler A & Enría D (1995) Infeción por un hantavirus en pobladores y roedores de las Islas Lechiguanas del delta del río Paraná. In: *Proceedings of the First Latin American Congress of Zoonoses,* Buenos Aires, Argentina.

Levis S, Pini N, Calderón G, Mills J, Cantoni G & Enria D (1997a) Cross-sectional studies of hantavirus infections in rodent populations in Argentina. In: *Proceedings of the Fourth International Conference on HFRS and Hantavirus.* Centers for Disease Control & Prevention, Atlanta, GA.

Levis S, Rowe J, Morzunov S, Enría D & Jeor S (1997b) New hantaviruses causing hantavirus pulmonary syndrome in central Argentina. *Lancet* **349**, 998.

Lopez N, Padula P, Rossi C & Lazaro M (1996) Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology* **6**, 220–223.

Mann G (1978) *Los pequeños mamíferos de Chile. Gayana, Zoología (Chile)* **40**, 1–342.

Mills J, Childs J, Ksiazek G & Peters C (1995a) *Methods for Trapping and Sampling Small Mammals for Virologic Testing.* US Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta, GA.

Mills J, Yates T, Childs Y, Parmenter R & Peters C (1995b) Guidelines for working with rodents potentially infected with hantavirus. *Journal of Mammals* **76**, 716–722.

Mills J, Johnson J, Ksiazek T *et al.* (1998) A survey of hantavirus antibody in small-mammal populations in selected United States national parks. *American Journal of Tropical Medicine and Hygiene* **58**, 525–532.

Murúa R, Gonzalez L & Meserve P (1986) Population ecology of *Oryzomys longicaudatus philippi* (Rodentia: Cricetidad) in southern Chile. *Journal of Animal Ecology* **55**, 281–293.

Murúa R, Rojas M & González L (1987) Ciclo reproductivo anual de *Akodon olivaceus* y *Oryzomys longicaudatus*. *Medio Ambiente* **8**, 44–51.

Murúa R, González L, González M & Jofré C (1989) Efectos del florecimiento del arbusto *Chusquea quila* Kunth (Poaceae) sobre la demografía de poblaciones de roedores de los bosques templados fríos del sur Chileno. *Bol Soc Biol (Chile)* **67**, 37–42.

Nichol S, Spiropoulou C, Morzunov S *et al.* (1993) Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* **262**, 914–917.

Padula P, Edelstein A, Miguel S, Lopez M, Rossi C & Rabinovich R (1998) Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* **15**, 323–330.

Padula P, Rossi C, Della Valle M *et al.* (2000) Development and evaluation of a solid phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. *Journal of Medical Microbiology* **49**, 149–155.

Peters C (1998) Hantavirus pulmonary syndrome in the Americas. In: *Emerging Infections 2* (eds W Scheld, W Craig & J Hughes). ASM Press, Washington, DC, pp. 17–64.

Redford K & Eisenberg J (1992) *Mammals of the Neotropics, Vol. 2: The Southern Cone*. University of Chicago Press, Chicago, pp. 268–269.

Toro J, Vega J, Khan A *et al.* (1998) An outbreak of hantavirus pulmonary syndrome, Chile, 1997. *Emerging Infectious Diseases* **4**, 687–694.

Wells R, Sosa S, Yadon Z, Enria D *et al.* (1997) An unusual hantavirus outbreak in southern Argentina: person to person transmission? *Emerging Infectious Diseases* **2**, 1–6.

Williams J, Bryan R, Mills J *et al.* (1997) An outbreak of hantavirus pulmonary syndrome in western Paraguay. *American Journal of Tropical Medicine and Hygiene* **57**, 274–282.

Yadón Z (1998) Epidemiología del síndrome pulmonar por hantavirus en la Argentina (1991–1997). *Medicina (Buenos Aires)* **58**, 25–26.

Yahnke C, Meserve P, Ksiazek T & Mills J (2001) Patterns of infection with Laguna Negra virus in wild populations of *Calomys laucha* in the central Paraguayan Chaco. *American Journal of Tropical Medicine and Hygiene* **65**(4).