Andes virus infections in the rodent reservoir and in humans vary across contrasting landscapes in Chile

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A R T I C L E   I N F O

A B S T R A C T

Hantavirus cardiopulmonary syndrome (HCPS) is an emerging infectious disease first reported in Chile in 1995. Andes hantavirus (ANDV) is responsible for the more than 500 cases of HCPS reported in Chile. Previous work showed that ANDV is genetically differentiated in Chile across contrasting landscapes. To determine whether the reservoir rodent (Oligoryzomys longicaudatus) populations are also geographically segregated, we conducted range-wide spatial genetic analyses of O. longicaudatus in Chile using the mitochondrial DNA cytochrome b gene. Given that landscape structure influences the incidence of hantavirus infections, we also tested 772 O. longicaudatus specimens for antibodies to ANDV captured during the period 2000–2006. Population genetic analyses of O. longicaudatus are largely congruent with those reported for ANDV, with the host primarily differentiated according to three defined ecoregions, Mediterranean, Valdivian rain forest and North Patagonian rain forest. Significant differences in the relative prevalence of anti-ANDV antibodies in rodent samples also were found across the three ecoregions. We relate these results to the number of reported human HCPS cases in Chile, and discuss the importance of landscape differences in light of ANDV transmission to humans and among rodent populations.

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1. Introduction

Hantavirus cardiopulmonary syndrome (HCPS) is an emerging infectious disease mainly known in North America from the outbreak of Sin Nombre virus (SNV) in the Four Corners region of the United States in 1993 (Nichol et al., 1993; Hjelle et al., 1994). The disease produces a variable number of human cases every year, with a case-fatality ratio between 30 and 50%. Rodents (Muridae and Cricetidae) are considered the reservoirs of hantavirus; however, recent discoveries have also documented a number of species of shrews (Soricomorpha) from at least three continents as hosts of viruses of unknown pathogenicity (Arai et al., 2007; Klempa et al., 2007; Song et al., 2009). Hantaviruses are transmitted to humans through rodent excreta and secretions (Botten et al., 2002; Padula et al., 2004). In Chile, the related Andes virus (ANDV) is responsible for all cases of HCPS, and the long-

tailed pygmy rice rat (Oligoryzomys longicaudatus) appears to be the main reservoir (Toro et al., 1998; Medina et al., 2009). ANDV epidemiology is complicated slightly because person-to-person transmission has been well documented in Argentina and Chile although this is unique among hantaviruses (Martinez et al., 2005; Ferres et al., 2007). Since the first documented outbreak in Chile in 1995, serological surveys of hantavirus have confirmed the presence of ANDV from 30° 56′S to 53° 37′S (Toro et al., 1998; Torres-Pérez et al., 2004; Belmar-Lucero et al., 2009). This wide latitudinal range spans contrasting geographic features and landscapes ranging from a Mediterranean heterogeneous vegetation mosaic (Mediterranean ecoregion) to mixed evergreen-deciduous Temperate Forests (Valdivian and North Patagonian rain forest ecoregions) (Armesto et al., 2007; Veblen, 2007). Across these diverse ecotypes, strong differences in population structure and density have been documented for several species of small mammals (Murua et al., 1986; Simonetti and Aguero, 1990; Cofre et al., 2007). In a previous study, we reported that ANDV in Chile is segregated into distinct lineages that correspond to the limits of ecoregions (Medina et al., 2009). Populations of O. longicaudatus, however, seem to show a relatively homogeneous genetic structure (Palma et al., 2005), although ecogeographic subdivision

has not been fully assessed. The observation that there exist areas wherein genetic lineages are subdivided according to different ecotypes may be the result of complex geographical, historical, and/or ecological processes with differential selective regimes or random genetic drift acting on populations (Wright, 1931; Hartl and Clark, 2007).

Environmental features influence the geographic distribution of diseases by affecting ecological properties of host and vectors (Linard et al., 2007). Landscape structure influences the incidence of hantavirus infections (Langlois et al., 2001; Glass et al., 2007; Heyman et al., 2009) and when combined with other environmental factors, may prove to be a strong determinant in the transmission of the virus to humans (Linard et al., 2007; Zhang et al., 2009). Variable host population structure has implications for viral population demographics and transmission (Adler et al., 2008). Given differences in ecology of *O. longicaudatus* populations across their latitudinal range in Chile, transmission rates of ANDV may also differ, resulting in differences in potential rates of infection of rodent hosts. Consequently, humans may be differentially exposed to viral infection.

In this study, we used molecular data from the rodent (*O. longicaudatus*) to explore genetic discontinuities across Chile, and discuss the importance of host population structure in the transmission of virus among rodents and to humans. We also compare the seroprevalence of ANDV in *O. longicaudatus* across the latitudinal gradient, and provide a quantitative assessment of the distribution of incident and fatal cases of HCPS during the period 2000–2006 in Chile. We predict differences in *O. longicaudatus* population structure across the ecogeographic regions in south-central Chile (congruent with ANDV genetic structure), and that

prevalence of ANDV-seropositive *O. longicaudatus* differs across those ecoregions. Our study highlights the value of combining information from host population structure, epidemiology, viral phylogeography, and geography to gain insights into the transmission and persistence of infectious diseases.

2. Materials and methods

2.1. Mitochondrial DNA sequences and spatial genetic analyses

A total of 108 *O. longicaudatus* from 11 localities in Chile ranging from 30°S to 46°S (Fig. 1A) were used for mitochondrial DNA cytochrome *b* (cyt-*b*) amplification and sequencing following procedures explained previously (Palma et al., 2005). Sequences were edited using BioEdit Sequence Alignment Editor (Hall, 1999), and aligned using Clustal W implemented in BioEdit. Sequences are deposited in GenBank (Accession numbers GQ282502–GQ282603, AF346566, AF346568, AY275692, AY275693, AY275698, AY275699).

A spatial analysis of molecular variance was performed in SAMOVA v.1.0 (Dupanloup et al., 2002). This method uses a simulated annealing approach to identify groups of populations (K), which are geographically homogeneous and maximally differentiated by maximizing F_{CT} (the proportion of the total genetic variance due to differences among groups of populations). F_{CT} values were calculated by running the program sequentially (100 random initial conditions), and forcing the data into k groups (where $k = 2$–9). Population subdivision was estimated using the fixation index (Fst) between all population pairs generated in Arlequin 3.1 (Excoffier et al., 2005). For detecting dissimilar
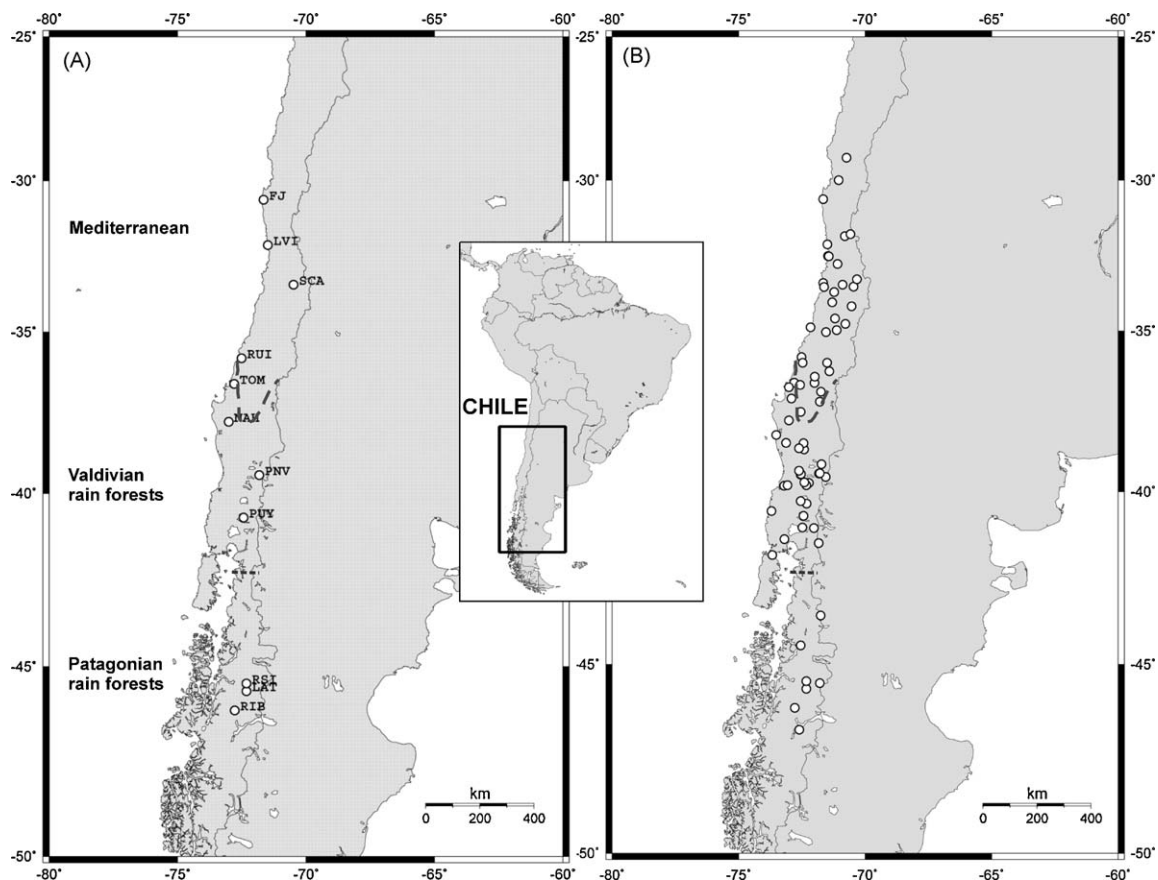

Fig. 1. Map of the sampled localities of *O. longicaudatus* used for (A) molecular analyses, and (B) seropositivity to ANDV in Chile. Locality names are explained in Table 1. Dashed lines represent approximate limits of ecoregions. Maps were generated using Online Map Creator (www.aquarius.ifm-geomar.de).