PLOS Neglected Tropical Diseases

RESEARCH ARTICLE

Hantavirus infections and small mammal diversity in Chile: No differences between protected and unprotected areas highlight the need for public health strategies

Fernando Torres-Pérez[1]*, Nicolás Ferrada[1], Roxana Astudillo[1], Marcela Ferrés[2], Pablo A. Vial[3], Pablo A. Marquet[4], Alonso Parra[5], Gregory J. Mertz[6], R. Eduardo Palma[4,7]

1 Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile, **2** Departamento de Enfermedades Infecciosas e Inmunología Pediátricas, Pontificia Universidad Católica de Chile, Santiago, Chile, **3** Instituto de Ciencias e Innovación en Medicina, Universidad del Desarrollo, Santiago, Chile, **4** Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile, **5** Subsecretaría de Salud Pública, Ministerio de Salud, Santiago, Chile, **6** Department of Internal Medicine, University of New Mexico, Albuquerque, New Mexico, United States of America **7** Dirección de Postgrado, Universidad de O'Higgins, Rancagua, Chile

* fernando.torres@pucv.cl

Check for updates

OPEN ACCESS

Citation: Torres-Pérez F, Ferrada N, Astudillo R, Ferrés M, Vial PA, Marquet PA, et al. (2025) Hantavirus infections and small mammal diversity in Chile: No differences between protected and unprotected areas highlight the need for public health strategies. PLoS Negl Trop Dis 19(10): e0013668. https://doi.org/10.1371/journal.pntd.0013668

Editor: David Safronetz, Public Health Agency of Canada, CANADA

Received: June 30, 2025

Accepted: October 20, 2025

Published: October 30, 2025

Copyright: © 2025 Torres-Pérez et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data availability statement: Data are openly available in the manuscript and Supporting information.

Funding: This work was supported by ANID-FONDECYT Chile (#1230881,

Abstract

Background

Zoonotic viruses, such as *Orthohantavirus andesense* (ANDV; the causative agent of hantavirus cardiopulmonary syndrome, HCPS), pose significant public health risks at the human-wildlife interface. Understanding their eco-epidemiological dynamics is critical for elucidating the interplay between reservoir hosts, environmental factors, and spillover to humans. In Chile, the long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*) serves as the primary reservoir for ANDV. This study investigates whether protected areas (PA), which typically support higher biodiversity and stable ecosystems, exhibit lower ANDV seroprevalence compared to unprotected areas (UPA), where anthropogenic disturbances may alter host-pathogen dynamics.

Methodology

Between 2001–2008, we conducted small mammal sampling across 22 sites (11 PA and 11 UPA) in natural landscapes of Chile. Seroprevalence of ANDV was assessed via strip immunoassay, while small mammal diversity was evaluated using standardized trapping protocols and diversity indices. We used similarity percentage analysis to identify species contributing to community dissimilarities and applied Renyi diversity profiles to compare small mammal diversity between area types.

1171280, 1110664 to FT-P), the CONICYT-PIA (#ACT1408 to MF, FT-P), the Fogarty International Center Research Grant (#D43TW007131 to GJM, PAV, MF, REP, FT-P), and the National Institutes of Health ICIDR Chilean Hantavirus Grant (#1U19AI45452-01# to GJM, PAV, MF, PAM, REP). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Main Findings

We captured 627 small mammals (PA: 331, 14 species; UPA: 296, 10 species) across 12,898 trap-nights. Seroprevalence in *O. longicaudatus* was identical in PA and UPA (9.5%). No significant differences were found in the relative abundance or seropositivity of *O. longicaudatus* between area types. Ecological indices (Shannon-Wiener, Simpson, richness, evenness) and community composition (ANOSIM) also showed no significant differences. Rényi profiles indicated marginally higher diversity in PA, driven by greater richness and evenness.

Conclusions

These findings suggest that ecological factors, such as habitat type, climatic conditions, and/or human behavior, may play a more critical role in shaping viral prevalence than protection status alone. The study underscores the necessity for consistent public health interventions to mitigate the risk of hantavirus cardiopulmonary syndrome across all environments, particularly in regions where human activities intersect with natural habitats.

Author summary

This study looked at how a virus that causes a serious disease in humans, spread by wild rodents, behaves in different natural environments in Chile. The study focused on *O. longicaudatus*, the main reservoir of *Orthohantavirus andesense* (ANDV), and compared its presence and infection levels in areas with environmental protection and those without. Over eight years, we studied small mammals in 22 locations across the country. Surprisingly, we found no major difference in the number of infected animals or in the diversity of small mammals between protected and unprotected areas. This challenges the idea that protected areas alone reduce the risk of people catching this virus. The results suggest that other factors—such as the landscape, climate, or human activities—may have a stronger influence on how the virus spreads in nature. This work highlights the importance of monitoring and public health actions in all geographic areas, not just those without protection, to reduce the risk of disease for people who live in or visit natural areas.

Introduction

Zoonoses, infectious diseases capable of transmission between animals and humans, pose a persistent and dynamic global health threat [1]. These pathogens, frequently harbored within wildlife reservoirs, can cross into human populations via diverse routes, including direct contact, vector-mediated transmission, and inhalation of contaminated aerosols [2]. Among the most pressing concerns are emerging

infectious diseases, which can rapidly disseminate across regions and cause severe, often life-threatening outcomes [3]. RNA viruses play a significant role in this landscape, given their high mutation rates, adaptability, and potential for rapid evolution, making them key agents of zoonotic (re-)emergence [4]. Understanding the ecology, transmission, and pathogenesis of these viruses is therefore critical for addressing the challenges posed by zoonoses and emerging infectious diseases [5].

Orthohantaviruses (*Hantaviridae*) comprise a genus of segmented single-stranded RNA viruses, representing a significant global public health concern due to their widespread distribution and the severe clinical outcomes associated with their transmission [6]. In the Americas, these viruses pose a particularly urgent threat, as several genotypes are responsible for hantavirus cardiopulmonary syndrome (HCPS) [7,8], a severe and often fatal disease characterized by acute respiratory distress and cardiovascular collapse [9]. With a fatality rate of approximately 36%, HCPS is recognized as a serious emerging infectious disease, underscoring the need for continued research and public health vigilance [10]. Transmission of orthohantaviruses typically occurs through inhalation of aerosolized rodent excreta or secretions, with rodents serving as the primary reservoir hosts [11,12].

In Chile, the etiologic agent of HCPS is *Orthohantavirus andesense* (ANDV) [13], a unique virus that not only causes severe disease in humans but also is the only known hantavirus where person-to-person transmission has been demonstrated [14,15]. Of note, between 2001 and 2008, 453 cases of HCPS were confirmed in Chile, with a mortality rate of 32.2% (epi.minsal.cl). The primary reservoir of ANDV is the long-tailed pygmy rice rat *Oligoryzomys longicaudatus* (Cricetidae) a species that inhabits heterogeneous landscapes across Chile, spanning from 27°S to 54°S [16–18].

Chile's National System of Biodiversity and Protected Areas (SBAP) represents a unique ecological and epidemiological context for studying zoonotic diseases such as ANDV. The SBAP encompasses an extensive network of protected areas, including 44 national parks, 24 national reserves, 14 natural monuments, and 45 nature sanctuaries, covering approximately 18.6 million terrestrial hectares (https://datosturismo.sernatur.cl/siet/reporteDinamicoSNASPE). These protected areas (defined as specific, delimited geographic spaces aimed at preserving and conserving the country's biodiversity, as well as safeguarding natural, cultural heritage, and landscape value, both now and in the long term), attract over 3 million tourists annually, fostering interactions between wildlife and humans near human settlements. Such interactions create opportunities for pathogen spillover, particularly in regions where rodent populations are abundant and where human activities overlap with natural habitats [19].

Rodent abundance, density, and seroprevalence are known to vary across landscapes due to a combination of habitat heterogeneity, climatic oscillations, and species-specific ecological factors [16,20,21]. While complex ecological interactions can either amplify or attenuate viral prevalence [21], protected areas may exhibit distinct eco-epidemiological patterns compared to unprotected areas. For instance, protected areas often harbor elevated rodent populations and species diversity due to reduced human disturbance, which could lead to higher pathogen prevalence [22]. However, increased competition or dilution effects among rodent species may also reduce the likelihood of high viral transmission [23], creating a scenario where protected areas exhibit lower disease prevalence despite higher rodent densities.

Given the ongoing threat of hantavirus transmission in protected areas - which are visited by millions of people each year who may potentially be exposed to ANDV - understanding rodent population patterns, viral prevalence, and their interplay across different habitat types is critical for developing effective public health strategies [10]. Elucidating these eco-epidemiological patterns are essential for protecting human health, particularly in regions where protected areas border human settlements and where tourism and recreational activities increase the likelihood of human-wildlife contact [24,25].

To address these gaps, we conducted an analysis sampling for 8 years to determine the relative abundance and seroprevalence of *O. longicaudatus* and the diversity of small mammals in Chile. By comparing protected areas (PA) with unprotected areas (UPA), we aimed to explore the eco-epidemiological patterns of ANDV infections and their principal

reservoir. Our findings provide novel insights into the factors influencing hantavirus transmission in diverse landscapes, offering valuable information for the development of targeted interventions to mitigate the risk of HCPS.

Materials and methods

Ethics statement

Permission to trap small mammals was obtained from the Servicio Agrícola y Ganadero (SAG, Chile; permits 17/2000, 7325/2005, 1056/1999), and Corporación Nacional Forestal (CONAF, Chile; permits 10–02/2002, 13–03/2003, 14–99/2004, 24/2004, 07–06/2006). All the National Institute of Health studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of New Mexico Health Sciences Centre under protocol number 14–101118-Field-HSC, and the Department of Health and Human Services of the National Institute of Health, Animal Welfare Assurance A5848-01. Small mammals trapping protocols and biosafety procedures for FONDECYT were reviewed and approved by institutional ethics and biosafety review boards at the Pontificia Universidad Católica de Chile (CBB 7/8/2005).

Sampling

We conducted 22 small mammal samplings across 11 protected and 11 unprotected sites spanning the Coquimbo to Magallanes administrative regions in Chile from 2001 to 2008 (Table 1). To approximate ecological consistency across sites and enhance the robustness of between-group comparisons, we chose sampling areas that fulfilled three criteria to minimize differences in climatic and biogeographic variables based on spatial proximity, temporal synchronicity and ecological similarity: i) the samplings had to be no more than six months apart between PA and UPA, ii) they had to be no more than 100 km apart and iii) they must be included within the same eco-geographic region [16,26].
Sampling sites ranged from Fray Jorge National Park, Coquimbo Region (-30º 22'S, -71º 23'W) to Fuerte Bulnes, Magallanes Region (-53º 37'S, -70º 55'W), covering a total of 2,597 kms from north to south (Fig 1). Our sampling scheme encompasses most of the latitudinal distribution of *O. longicaudatus* [27,28]. Small mammals were captured by using live Sherman traps (8 x 9 x 23 cm). The traps were installed with rolled oats and vanilla essence in meadows, thickets, and forests that are part of the habitat of *O. longicaudatus*. The traps were set for 2–6 consecutive nights, varying between 180–1080 traps per sampling site. Captured animals were identified by their external morphology. In the capture and manipulation of small mammals, processing and handling procedures adhered to in accordance with the established protocols [29] and the guidelines set forth by the American Society of Mammalogists [30].

Hantavirus detection

Captured small mammals were anesthetized, and blood samples were collected via retro-orbital sinus puncture using a heparinized capillary tube. The blood samples were transferred to cryovials, preserved in liquid nitrogen, and subsequently transported to the laboratory for analysis. Additionally, for each specimen, the heart, kidney, spleen, liver, and lung were excised, cryopreserved in liquid nitrogen, and stored for further study. Voucher specimens were fixed in 96% ethanol and deposited in the 'Colección de Flora y Fauna Professor Patricio Sánchez Reyes' at Pontificia Universidad Católica de Chile (Santiago, Chile), and the Division of Mammals at the Museum of Southwestern Biology, University of New Mexico (Albuquerque, NM). Antibodies against *Orthohantavirus andesense* were detected in blood samples using a strip immunoassay vacuum-blot test [31]. Briefly, 5 µL of small mammal blood was applied to a 1.6 × 5-cm nitrocellulose membrane with a 0.5-mm × 1.6-mm band containing ~300 ng of affinity-purified recombinant Andes virus N antigen, incubated overnight at room temperature in 1 mL volume. After washing, bound antibodies were detected using alkaline phosphatase−conjugated deer mouse anti-*Peromyscus leucopus* IgG (1:1,000 dilution) and revealed with the BCIP/NBT substrate system (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

Table 1. Type of area, trapping site, sampling year and *Orthohantavirus andesense* seroprevalence.

Type of area*	Site (Locality, County, Administrative Region)	Sampling Year	Latitude	Longitude	Night traps	Seroposi-tives (SIA)	Total of Micromam-mals captured	Seroprev-alence *(%)*
UPA	Bosque Nagué, Los Vilos, Coquimbo	2003	-31°50'S	-71°31'W	720	4	14	28,6
UPA	Rio Maipo, Santo Domingo, Valparaíso	2002	-33°22'S	-71°22'W	720	0	2	0,0
UPA	Fundo Lisboa, Alhué, Metropolitana	2003	-33°56'S	-71°01'W	570	0	8	0,0
UPA	Tregualemu, Pelluhue, Maule	2008	-35°56'S	-72°44'W	210	0	15	0,0
UPA	Retupel, Cauquenes, Maule	2003	-36°01'S	-72°27'W	300	1	12	8,3
UPA	Vilumanque, Concepción, Biobío	2003	-36°46'S	-73°01'W	218	0	64	0,0
UPA	Lautaro, Lautaro, Araucanía	2002	-38°31'S	-72°25'W	300	0	9	0,0
UPA	Fundo El Condor, Coyhaique, Aysén	2001	-45°33'S	-71°55'W	720	0	16	0,0
UPA	Lago Atravesado, Coyhaique, Aysén	2003	-45°40'S	-72°19'W	360	1	71	1,4
UPA	Porvenir, Porvenir, Magallanes	2008	-53°16'S	-70°09'W	300	1	56	1,8
UPA	Fuerte Bulnes, Punta Arenas, Magallanes	2006	-53°37'S	-70°55'W	600	1	29	3,4
PA	Fray Jorge, Ovalle, Coquimbo 2003	2003	-30°22'S	-71°23'W	720	1	99	1,0
PA	Cerro El Roble, Tiltil, Metropolitana	2003	-32°58'S	-71°01'W	300	0	14	0,0
PA	Quebrada de Córdoba, El Tabo, Valparaíso	2002	-33°26'S	-71°39'W	1080	0	3	0,0
PA	Reserva Nacional Los Ruiles, Chanco, Maule	2003	-35°50'S	-72°30'W	360	2	26	7,7
PA	Reserva Los Queules, Pelluhue, Maule	2008	-35°59'S	-72°41'W	180	1	17	5,9
PA	Parque Nacional Nahuelbuta, Angol, Araucanía	2003	-37°49'S	-73.01W	750	0	16	0,0
PA	Parque Nacional Huerquehue, Pucon, Araucanía	2002	-39°10'S	-71°43'W	990	1	13	7,7
PA	Parque Nacional Queulat, Cisnes, Aysén	2003	-44°27'S	-72°32'W	480	0	25	0,0
PA	Reserva Nacional Rio Simpson, Coyhaique, Aysén 2001	2001	-45°27'S	-72°19'W	500	2	21	9,5
PA	Parque Nacional Pali Aike, San Gregorio, Magallanes	2008	-52°06'S	-69°46'W	1080	0	38	0,0
PA	Reserva Nacional Magallanes, Punta Arenas, Magallanes	2006	-53°07'S	-71°01'W	840	0	59	0,0
TOTAL						15	627	2,4

*UPA: Unprotected area; PA: Protected area

https://doi.org/10.1371/journal.pntd.0013668.t001

Statistical analysis

For every site we calculated the relative abundance of *O. longicaudatus* ((total of *O. longicaudatus* per site/ total traps nights used in the site) x 100), the trapping effort (total of small mammals captured per site/ total of traps nights used in the site), the relative seropositivity (total of seropositive *O. longicaudatus* per site/ (trapping effort x 100)), and the

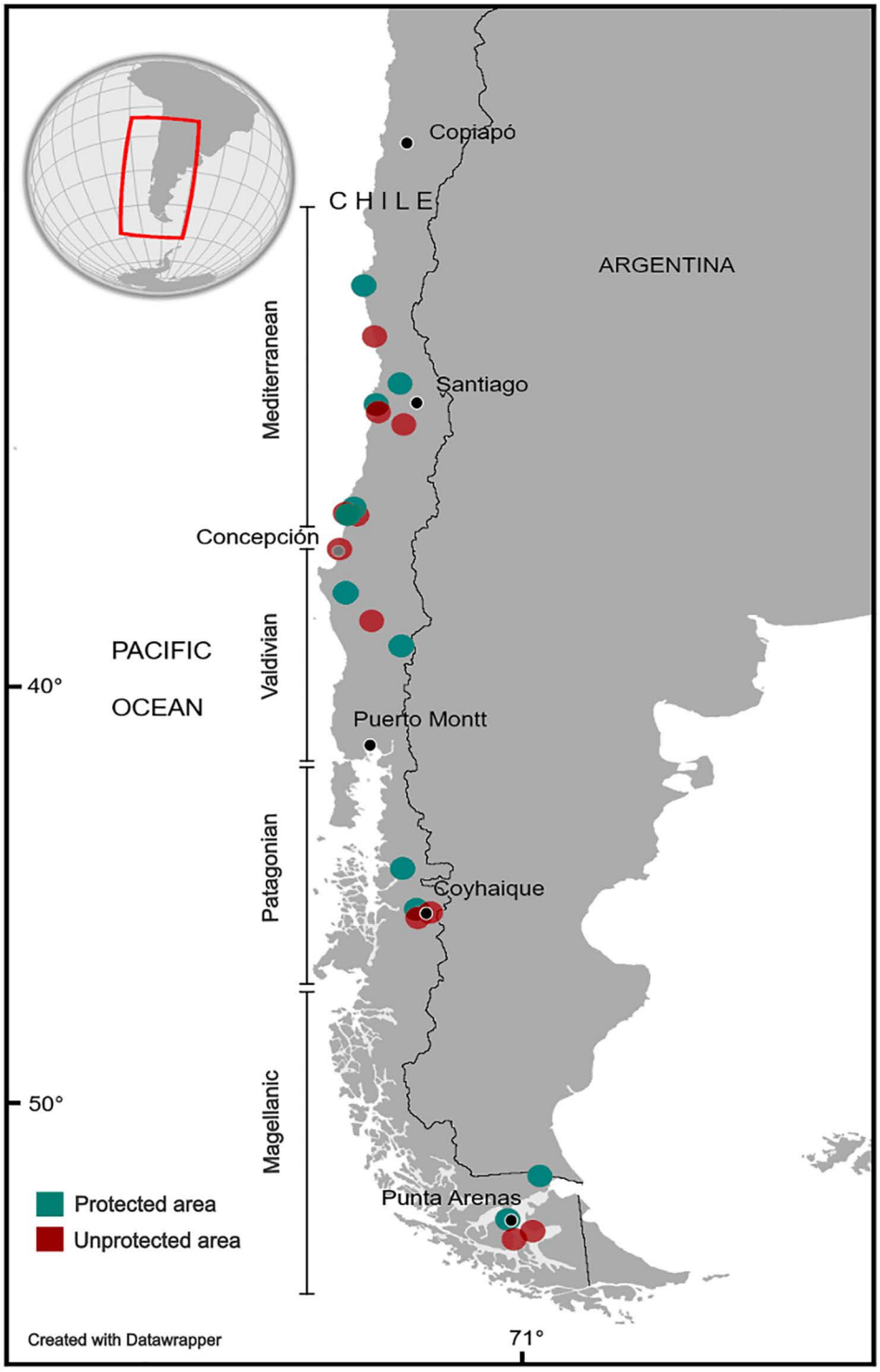


Fig 1. Small mammal trapping sites sampled in protected areas (PA, in green) and unprotected areas (UPA, in red) across Chilean ecoregions. Vertical lines indicate the approximate distribution of major eco-regions (Mediterranean, Valdivian rain forests, Patagonian rain forests, Magellanic sub-polar forests). Map created with Datawrapper.

https://doi.org/10.1371/journal.pntd.0013668.g001

seroprevalence (positive SIA *O. longicaudatus* per site/ total of *O. longicaudatus* captured per site). To determine the α diversity of small mammals on every site of PA and UPA we calculated the Shannon-Wiener diversity index ($H = -\Sigma pi \times \ln(pi)$, where pi is the relative proportion of species i in the community), the Simpson diversity index ($1 - \Sigma pi2$), and the richness (S) and evenness (H/Hmax, where $Hmax = \ln[S]$). We compared the relative abundance and relative seropositivity of *O. longicaudatus* seroprevalence, and diversity indexes using the Wilcoxon-Mann-Withney test between UPA and PA. To determine the β-diversity, we compare small mammal community composition between PA and UPA by performing a one-way analysis of similarity (ANOSIM), which is a permutational non-parametric test based on species abundance [32]; for this analysis we used a Bray-Curtis index as a similarity measure. To represent these results graphically, we applied a non-metric multidimensional scaling (nMDS) approach, which creates a two-dimensional abstract depiction of species composition similarity. The quality of the nMDS representation is assessed through ordination stress, a metric ranging from 0 to 1, where lower values correspond to better fit (stress values ≤ 0.2 are deemed indicative of good ordination). We additionally performed a Similarity Percentage (SIMPER) analysis to assess the extent to which individual species contributed to compositional dissimilarities. Renyi diversity profiles were applied to assess and compare multiple small mammals' diversity indices between types of areas [33,34]. The comparisons, ANOSIM, SIMPER, Rényi diversity profiles and nMDS were performed on R version 4.4.1 (R Development Core Team, 2021), using the "vegan" package for the diversity indexes and the ANOSIM, SIMPER and Rényi diversity profiles [35].

Results

We deployed 7,280 traps in PA, capturing 331 small mammal specimens that belonged to 14 species. In UPA, we used 5,618 traps, capturing 296 small mammal specimens of 10 species (S1 Table). Among the 63 specimens of *O. longicaudatus* captured in PA, six resulted positive for ANDV antibodies (seroprevalence of 9.5%). In UPA, 74 specimens of *O. longicaudatus* were captured, with seven seropositives (seroprevalence of 9.5%). Two additional seropositives were detected, one *Abrothrix hirta* in the Reserva Los Queules, Pelluhue (PA) and one *Rattus rattus* in Bosque Nague, Los Vilos (UPA) [36]. The site with the highest seroprevalence (28.6%) in UPA was Bosque Nague, Los Vilos (31°50'S), while in PA, Reserva Nacional Río Simpson, Coyhaique (45°27'S) had a seroprevalence of 9.5%. The comparison of the relative abundance ($p = 0.947$), and relative seropositivity ($p = 0.658$) of *O. longicaudatus*, revealed no significant differences between protected and unprotected areas (Fig 2).

Comparative analyses of ecological indices, including the Shannon-Wiener ($p = 0.212$), Simpson diversity ($p = 0.237$), species richness ($p = 0.611$), and evenness ($p = 0.293$) revealed no significant differences between protected and unprotected areas (Fig 3). No significant differences were detected in micromammal community composition between PA and UPA (ANOSIM $R = -0.063$, $p = 0.886$; Fig 4). Similarly, SIMPER analysis consistently failed to identify any species with a statistically significant contribution to the observed compositional dissimilarities (S2 Table). Rényi diversity profiles revealed that PA consistently exhibited a small higher micromammal diversity than UPA across all alpha values, indicating greater species richness (at low α) as well as higher evenness and lower dominance (at high α) in UPA (Fig 5).

Discussion

The findings of our study reveal unexpected insights into the eco-epidemiological patterns of *Orthohantavirus andesense* (ANDV) in Chile, particularly in relation to its primary reservoir, *Oligoryzomys longicaudatus*. We observed no significant differences in relative seropositivity between *O. longicaudatus* populations in protected areas (PA) and unprotected areas (UPA), with both areas exhibiting a seroprevalence of 9.5%. Similar to higher seroprevalences were reported previously in other protected areas in Argentina [22], whereas seroprevalences were lower in several previous studies in Chile [13,17,37,38], emphasizing the complexity of hantavirus prevalence [39,40]. Our findings emphasize that protection status alone may not be a reliable predictor of ANDV infection. This has important implications for public health interventions,

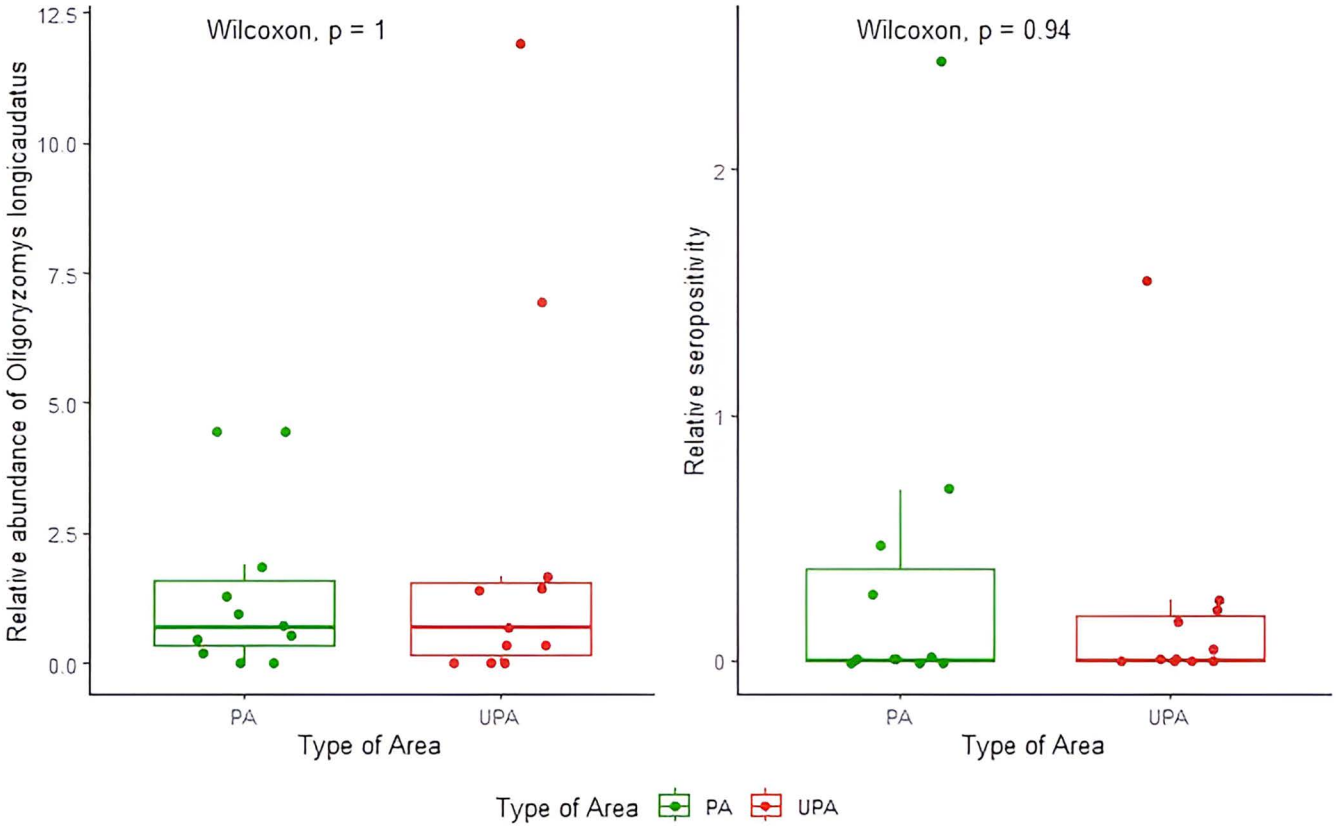


Fig 2. Comparison of relative abundance of *Oligoryzomys longicaudatus* (A) and relative seropositivity (B) between protected areas (PA) in green and unprotected areas (UPA) in red.

https://doi.org/10.1371/journal.pntd.0013668.g002

which must remain consistent across both protected and unprotected landscapes to effectively mitigate the risk of human infection, especially in sites that are not seroprevalence hotspots [41].

Although protected areas exhibited marginally higher small mammal diversity than unprotected areas (Rényi), the absence of significant differences across multiple ecological indices - such as the Shannon-Wiener and Simpson diversity measures, species richness, evenness, relative abundance and the analysis of similarity between PA and UPA - further supports that protection status alone may not directly influence ANDV infection rates in reservoir populations. Instead, our findings suggest that other ecological and environmental factors may play a more decisive role in shaping viral prevalence and rodent community structure. For instance, habitat type [16,42,43], climatic conditions [44–49], human behavior [50] have been shown to significantly influence rodent populations and pathogen dynamics. Together with ecological interactions [51–55], these may all play a more decisive role in shaping rodent diversity and viral prevalence [56]. Our findings align with previous studies emphasizing the importance of ecological heterogeneity, rather than protection status, in determining rodent community structure and pathogen transmission [24].

The absence of significant differences in seropositivity and diversity between PA and UPA suggests that viral transmission in *O. longicaudatus* is influenced by a complex interplay of factors that extend beyond species richness alone. Although examples are found for hantavirus infections [57], long term studies may reveal more complex dynamics on how biodiversity impacts mechanisms driving pathogens fluctuations [21,50]. This highlights the importance of integrating both

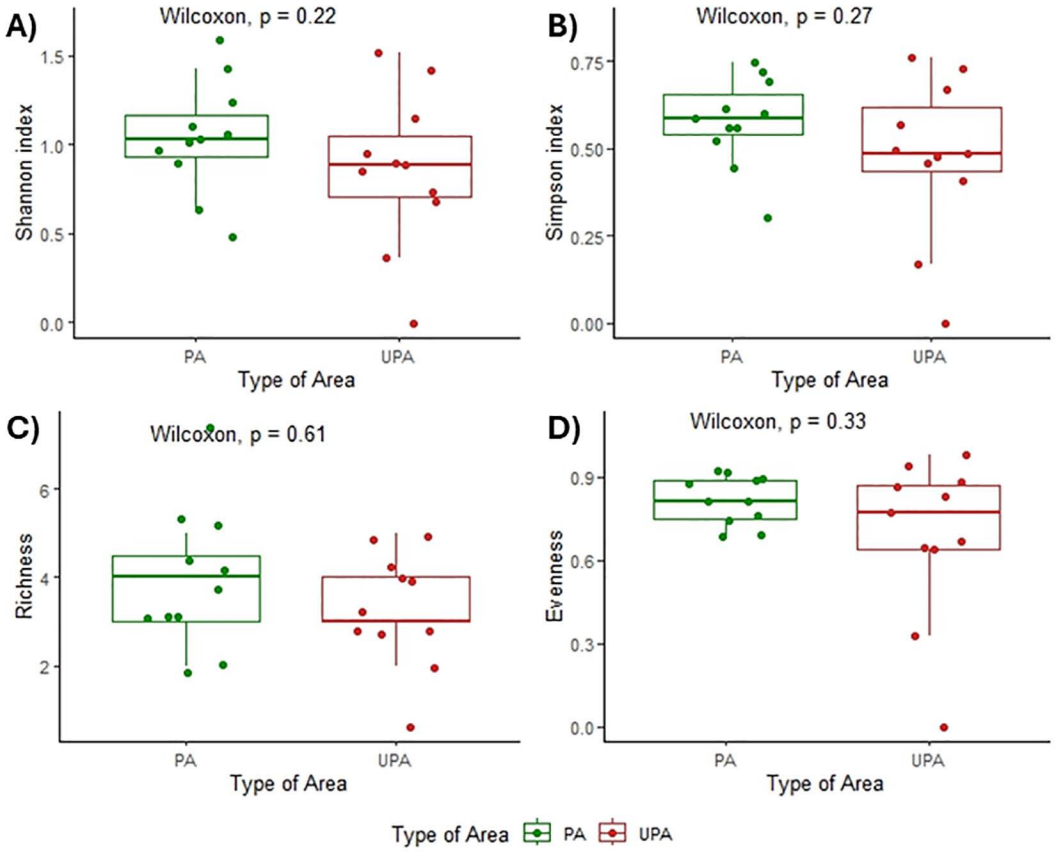


Fig 3. Comparison of overall Shannon-Wiener indexes (A), Simpson indexes (B), Richness (C) and Evenness (D) between protected areas (PA) in green and unprotected areas (UPA) in red.

https://doi.org/10.1371/journal.pntd.0013668.g003

viral and ecological factors into hantavirus surveillance programs [58], as well as the need for a more nuanced understanding of the mechanisms driving ANDV transmission.

Despite the relatively low number of HCPS cases during our sampling period, the high mortality rate (32.2%) highlights the significant threat posed by ANDV in Chile [59], particularly when compared to other RNA viral diseases. For instance, integrating hantavirus prevention into existing tourism infrastructure—such as signage at trailheads, visitor center briefings, and rental equipment hygiene protocols—could reduce exposure risks without requiring costly site-specific interventions. This is particularly concerning in areas where human activities intersect with natural habitats, as these interfaces create opportunities for pathogen spillover [57,58]. Collaborative efforts between public health agencies and park management could further standardize preventive measures, such as discouraging camping near rodent burrows or storing food in rodent-proof containers, across all recreational areas. Protected areas, which often attract significant tourism and recreational activities, represent a unique challenge in this scenario. While our study did not identify significant differences in seroprevalence or diversity between PA and UPA, it highlights the importance of considering broader ecological and anthropogenic factors in the development of targeted public health strategies [39].

This study provides important insights into ANDV seroprevalence and rodent communities across Chile, but certain limitations should be acknowledged. Our sampling for each locality was conducted at a single time point. This cross-sectional design limits our ability to account for temporal variability and infer transmission dynamics, as hantavirus seroprevalence is known to

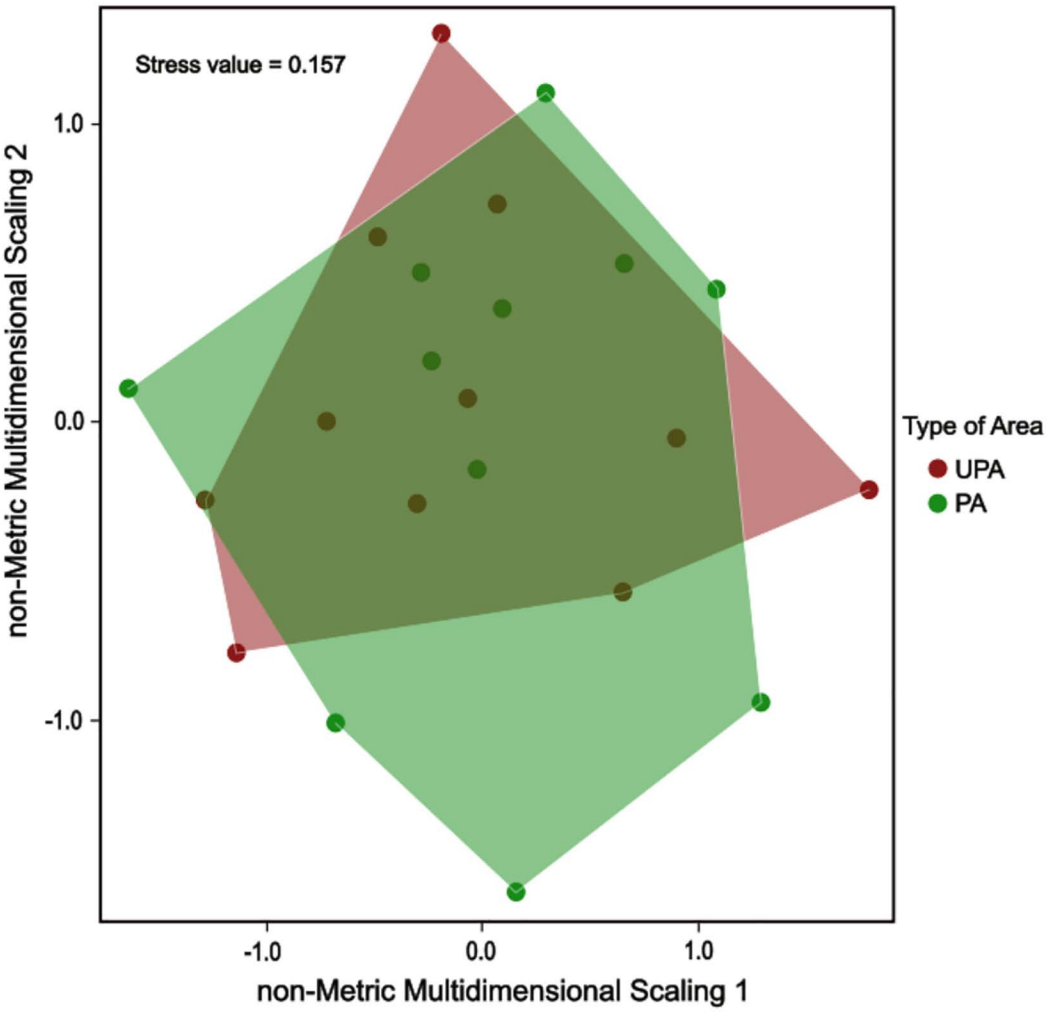


Fig 4. Comparison of the small mammal community's composition using the analysis of similarities (ANOSIM) between protected areas (PA) and unprotected areas (UPA).

https://doi.org/10.1371/journal.pntd.0013668.g004

lag behind changes in host population and community structure [21]. Although we applied rigorous geographic and ecological criteria to pair protected and unprotected areas, we did not include fine-scale measurements of environmental disturbance that could offer more detailed context. The study was also not designed to formally test the dilution effect. Lastly, some sites presented no seropositive individuals, which may reduce the statistical power of some comparisons—though these negative detections are themselves informative and contribute to understanding the broader spatial patterns of ANDV prevalence.

Conclusions

This study contributes to a better understanding of the spatial patterns of ANDV seroprevalence and small mammals' community composition across Chile. While our results indicate no clear difference in infection prevalence between protected and unprotected areas, they highlight the need to focus prevention efforts on local hotspots of viral activity rather than legal land-use categories alone. The findings also underscore the importance of considering broader ecological and anthropogenic factors when designing hantavirus surveillance and mitigation strategies. Although the study design limits temporal resolution and

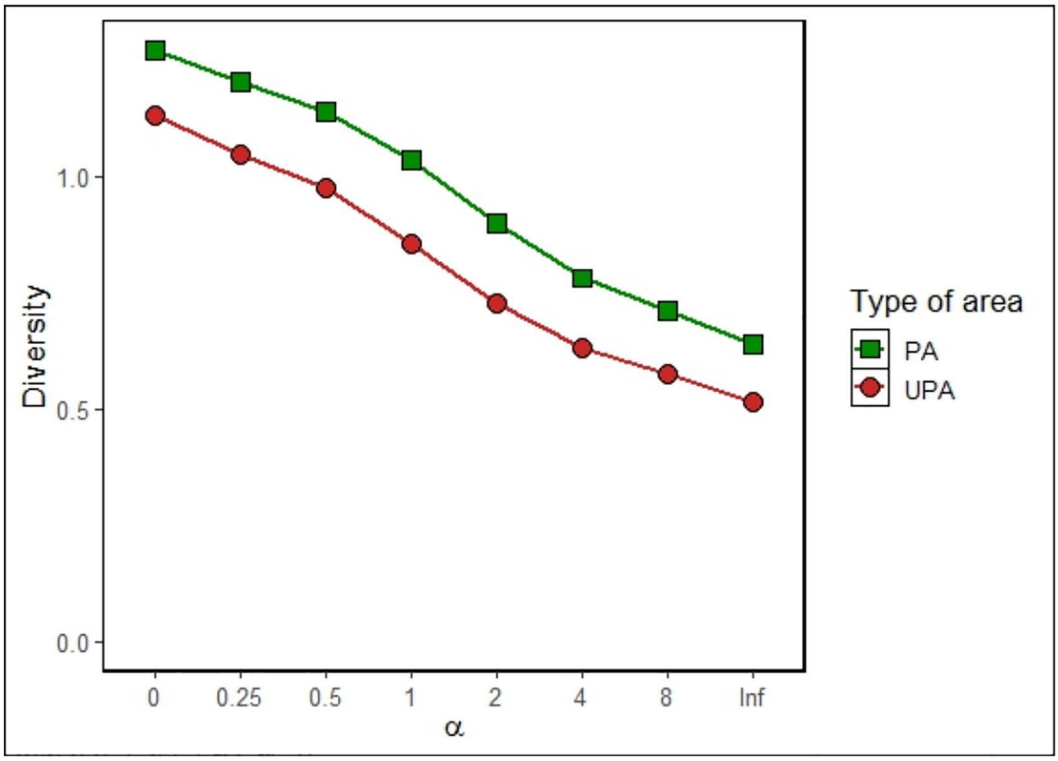


Fig 5. Rényi diversity profiles for Protected Areas (PA) in green and Unprotected Areas (UPA) in red. At lower alpha values, diversity is more influenced by species evenness, whereas higher alpha values emphasize the contribution of dominant species to overall diversity.

https://doi.org/10.1371/journal.pntd.0013668.g005

does not directly assess mechanisms such as the dilution effect, it provides a valuable baseline for future research. Addressing remaining knowledge gaps, such as the role of fine-scale environmental disturbances, climate variability, and rodent host ecology will be key to developing more targeted and effective public health interventions in HCPS endemic regions in Chile.

Supporting information

S1 Table. Type of area, trapping site, year, and relative abundance of small mammals captured in protected and unprotected areas in Chile.
(PDF)

S2 Table. SIMPER analysis showing the contribution of each species to the average Bray-Curtis dissimilarity in composition between protected (PA) and unprotected areas (UPA). The table includes the average contribution of each species (Average), standard deviation (Sd), contribution-to-variation ratio (Ratio), average abundance in group A (Ava) and group B (Avb), cumulative contribution to dissimilarity (Cumsum), and associated p-value. No species showed a statistically significant contribution to the dissimilarity between groups.
(PDF)

Acknowledgments

Nicolás Ferrada would like to thank the Pontificia Universidad Católica de Valparaíso for its support through a Master's scholarship.

Author contributions

Conceptualization: Fernando Torres-Perez, Roxana Astudillo, Marcela Ferrés, Pablo A. Vial, Pablo A. Marquet, Gregory J. Mertz, R. Eduardo Palma.

Data curation: Fernando Torres-Perez, Nicolás Ferrada, Roxana Astudillo, Alonso Parra, R. Eduardo Palma.

Formal analysis: Fernando Torres-Perez, Nicolás Ferrada, Roxana Astudillo, Pablo A. Marquet.

Funding acquisition: Fernando Torres-Perez, Marcela Ferrés, Pablo A. Vial, Pablo A. Marquet, Gregory J. Mertz, R. Eduardo Palma.

Investigation: Fernando Torres-Perez, Nicolás Ferrada, Roxana Astudillo, Marcela Ferrés, Pablo A. Vial, Pablo A. Marquet, Alonso Parra, Gregory J. Mertz, R. Eduardo Palma.

Methodology: Fernando Torres-Perez, Nicolás Ferrada, Roxana Astudillo, Marcela Ferrés, Pablo A. Marquet, Alonso Parra, Gregory J. Mertz, R. Eduardo Palma.

Project administration: Fernando Torres-Perez, Pablo A. Vial, Gregory J. Mertz, R. Eduardo Palma.

Resources: Fernando Torres-Perez, Marcela Ferrés, Pablo A. Vial, Alonso Parra, Gregory J. Mertz, R. Eduardo Palma.

Supervision: Gregory J. Mertz, R. Eduardo Palma.

Validation: Fernando Torres-Perez, Gregory J. Mertz, R. Eduardo Palma.

Writing – original draft: Fernando Torres-Perez, Nicolás Ferrada.

Writing – review & editing: Fernando Torres-Perez, Nicolás Ferrada, Roxana Astudillo, Marcela Ferrés, Pablo A. Vial, Pablo A. Marquet, Alonso Parra, Gregory J. Mertz, R. Eduardo Palma.

References

1. Cutler SJ, Fooks AR, van der Poel WHM. Public health threat of new, reemerging, and neglected zoonoses in the industrialized world. Emerg Infect Dis. 2010;16(1):1–7. https://doi.org/10.3201/eid1601.081467 PMID: 20031035
2. Mahapatra CS. Hantaviruses as Emergent Zoonoses: A Global Threat. Emerging Human Viral Diseases, Volume I. Springer Nature Singapore. 2023. p. 377–400. https://doi.org/10.1007/978-981-99-2820-0_15
3. Baker RE, Mahmud AS, Miller IF, Rajeev M, Rasambainarivo F, Rice BL, et al. Infectious disease in an era of global change. Nat Rev Microbiol. 2022;20(4):193–205. https://doi.org/10.1038/s41579-021-00639-z PMID: 34646006
4. Holmes EC. The evolution and emergence of RNA viruses. Oxford: Oxford University Press. 2009.
5. Holmes EC. The Ecology of Viral Emergence. Annu Rev Virol. 2022;9(1):173–92. https://doi.org/10.1146/annurev-virology-100120-015057 PMID: 35704744
6. Chen R-X, Gong H-Y, Wang X, Sun M-H, Ji Y-F, Tan S-M, et al. Zoonotic Hantaviridae with Global Public Health Significance. Viruses. 2023;15(8):1705. https://doi.org/10.3390/v15081705 PMID: 37632047
7. Alonso DO, Kehl SD, Coelho RM, Periolo N, Poklépovich Caride T, Sanchez Loria J, et al. Orthohantavirus diversity in Central-East Argentina: Insights from complete genomic sequencing on phylogenetics, Geographic patterns and transmission scenarios. PLoS Negl Trop Dis. 2024;18(10):e0012465. https://doi.org/10.1371/journal.pntd.0012465 PMID: 39383182
8. Hjelle B, Torres-Pérez F. Hantaviruses in the americas and their role as emerging pathogens. Viruses. 2010;2(12):2559–86. https://doi.org/10.3390/v2122559 PMID: 21994631
9. Vial PA, Ferrés M, Vial C, Klingström J, Ahlm C, López R, et al. Hantavirus in humans: a review of clinical aspects and management. Lancet Infect Dis. 2023;23(9):e371–82. https://doi.org/10.1016/S1473-3099(23)00128-7 PMID: 37105214
10. Ortiz N, Pinotti JD, Andreo V, González-Ittig RE, Gardenal CN. Orthohantavirus rodent hosts and genotypes in Southern South America: A narrative review. PLoS Negl Trop Dis. 2025;19(9):e0013489. https://doi.org/10.1371/journal.pntd.0013489 PMID: 40924751
11. Botten J, Mirowsky K, Ye C, Gottlieb K, Saavedra M, Ponce L, et al. Shedding and intracage transmission of Sin Nombre hantavirus in the deer mouse (Peromyscus maniculatus) model. J Virol. 2002;76(15):7587–94. https://doi.org/10.1128/jvi.76.15.7587-7594.2002 PMID: 12097572
12. Padula P, Figueroa R, Navarrete M, Pizarro E, Cadiz R, Bellomo C, et al. Transmission study of Andes hantavirus infection in wild sigmodontine rodents. J Virol. 2004;78(21):11972–9. https://doi.org/10.1128/JVI.78.21.11972-11979.2004 PMID: 15479837
13. Toro J, Vega JD, Khan AS, Mills JN, Padula P, Terry W, et al. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. Emerg Infect Dis. 1998;4(4):687–94. https://doi.org/10.3201/eid0404.980425 PMID: 9866751

14. Ferres M, Vial P, Marco C, Yanez L, Godoy P, Castillo C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in chile. J Infect Dis. 2007;195(11):1563–71. https://doi.org/10.1086/516786 PMID: 17471425
15. Padula PJ, Edelstein A, Miguel SD, López NM, Rossi CM, Rabinovich RD. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. Virology. 1998;241(2):323–30. https://doi.org/10.1006/viro.1997.8976 PMID: 9499807
16. Torres-Pérez F, Palma RE, Hjelle B, Ferrés M, Cook JA. Andes virus infections in the rodent reservoir and in humans vary across contrasting landscapes in Chile. Infect Genet Evol. 2010;10(6):820–5. https://doi.org/10.1016/j.meegid.2009.07.004 PMID: 19632357
17. Torres-Pérez F, Palma RE, Boric-Bargetto D, Vial C, Ferrés M, Vial PA, et al. A 19 Year Analysis of Small Mammals Associated with Human Hantavirus Cases in Chile. Viruses. 2019;11(9):848. https://doi.org/10.3390/v11090848 PMID: 31547341
18. Palma RE, Boric-Bargetto D, Torres-Pérez F, Hernández CE, Yates TL. Glaciation effects on the phylogeographic structure of Oligoryzomys longicaudatus (Rodentia: Sigmodontinae) in the southern Andes. PLoS One. 2012;7(3):e32206. https://doi.org/10.1371/journal.pone.0032206 PMID: 22396751
19. Muschetto E, Cueto GR, Cavia R, Padula PJ, Suárez OV. Long-Term Study of a Hantavirus Reservoir Population in an Urban Protected Area, Argentina. Ecohealth. 2018;15(4):804–14. https://doi.org/10.1007/s10393-018-1360-3 PMID: 30128613
20. Lima M, Marquet PA, Jaksic FM. El Niño events, precipitation patterns, and rodent outbreaks are statistically associated in semiarid Chile. Ecography. 1999;22(2):213–8. https://doi.org/10.1111/j.1600-0587.1999.tb00470.x
21. Luis AD, Kuenzi AJ, Mills JN. Species diversity concurrently dilutes and amplifies transmission in a zoonotic host-pathogen system through competing mechanisms. Proc Natl Acad Sci U S A. 2018;115(31):7979–84. https://doi.org/10.1073/pnas.1807106115 PMID: 30012590
22. Vadell MV, Bellomo C, San Martín A, Padula P, Gómez Villafañe I. Hantavirus ecology in rodent populations in three protected areas of Argentina. Trop Med Int Health. 2011;16(10):1342–52. https://doi.org/10.1111/j.1365-3156.2011.02838.x PMID: 21733047
23. Keesing F, Ostfeld RS. Dilution effects in disease ecology. Ecol Lett. 2021;24(11):2490–505. https://doi.org/10.1111/ele.13875 PMID: 34482609
24. Maroli M, Vadell MV, Padula P, Villafañe IEG. Rodent Abundance and Hantavirus Infection in Protected Area, East-Central Argentina. Emerg Infect Dis. 2018;24(1):131–4. https://doi.org/10.3201/eid2401.171372 PMID: 29260665
25. Vadell MV, Burgos EF, Lamattina D, Bellomo C, Martínez V, Coelho R, et al. Orthohantaviruses in Misiones Province, Northeastern Argentina. Emerg Infect Dis. 2024;30(7):1454–8. https://doi.org/10.3201/eid3007.240183 PMID: 38916725
26. Medina RA, Torres-Perez F, Galeno H, Navarrete M, Vial PA, Palma RE, et al. Ecology, genetic diversity, and phylogeographic structure of andes virus in humans and rodents in Chile. J Virol. 2009;83(6):2446–59. https://doi.org/10.1128/JVI.01057-08 PMID: 19116256
27. Belmar-lucero S, Godoy P, Ferrés M, Vial P, Palma RE. Range expansion of Oligoryzomys longicaudatus (Rodentia, Sigmodontinae) in Patagonian Chile, and first record of Hantavirus in the region. Rev chil hist nat. 2009;82(2). https://doi.org/10.4067/s0716-078x2009000200008
28. Palma RE, Rivera-Milla E, Salazar-Bravo J, Torres-Pérez F, Pardiñas UFJ, Marquet PA, et al. Phylogeography Of Oligoryzomys Longicaudatus (rodentia: Sigmodontinae) In Temperate South America. Journal of Mammalogy. 2005;86(1):191–200. https://doi.org/10.1644/1545-1542(2005)086<0191:poolrs>2.0.co;2
29. Mills JN, Yates TL, Childs JE, Parmenter RR, Ksiazek TG, Rollin PE, et al. Guidelines for Working with Rodents Potentially Infected with Hantavirus. Journal of Mammalogy. 1995;76(3):716. https://doi.org/10.2307/1382742
30. Sikes RS, Gannon WL. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy. 2011;92(1):235–53. https://doi.org/10.1644/10-mamm-f-355.1
31. Yee J, Wortman IA, Nofchissey RA, Goade D, Bennett SG, Webb JP, et al. Rapid and simple method for screening wild rodents for antibodies to Sin Nombre hantavirus. J Wildl Dis. 2003;39(2):271–7. https://doi.org/10.7589/0090-3558-39.2.271 PMID: 12910753
32. Clarke KR. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology. 1993;18(1):117–43. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x
33. Renyi Diversity Profiles with vegan, BiodiversityR and ggplot2. https://rpubs.com/Roeland-KINDT/694066. Accessed 2025 June 22.
34. Tóthmérész B. Comparison of different methods for diversity ordering. J Vegetation Science. 1995;6(2):283–90. https://doi.org/10.2307/3236223
35. Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB. vegan: Community Ecology Package. 2013.
36. Lobos G, Ferres M, Palma Re. Presencia de los géneros invasores Mus y Rattus en áreas naturales de Chile: un riesgo ambiental y epidemiológico. Rev chil hist nat. 2005;78(1). https://doi.org/10.4067/s0716-078x2005000100008
37. Torres-pérez F, Navarrete-droguett J, Aldunate R, Yates Tl, Mertz Gj, Vial Pa, Et Al. Peridomestic Small Mammals Associated With Confirmed Cases Of Human Hantavirus Disease In Southcentral Chile. Am J Trop Med Hyg. 2004;70(3):305–9. https://doi.org/10.4269/ajtmh.2004.70.305
38. Ortiz JC, Venegas W, Sandoval JA, Chandía P, Torres-Pérez F. Hantavirus en roedores de la Octava Región de Chile. Historia Natural. 2004;77:251–256. https://doi.org/10.4067/S0716-078X2004000200005
39. Carver S, Mills JN, Parmenter CA, Parmenter RR, Richardson KS, Harris RL, et al. Toward a Mechanistic Understanding of Environmentally Forced Zoonotic Disease Emergence: Sin Nombre Hantavirus. Bioscience. 2015;65(7):651–66. https://doi.org/10.1093/biosci/biv047 PMID: 26955081
40. Tian H, Stenseth NC. The ecological dynamics of hantavirus diseases: From environmental variability to disease prevention largely based on data from China. PLoS Negl Trop Dis. 2019;13(2):e0006901. https://doi.org/10.1371/journal.pntd.0006901 PMID: 30789905

41. Tortosa F, Perre F, Tognetti C, Lossetti L, Carrasco G, Guaresti G, et al. Seroprevalence of hantavirus infection in non-epidemic settings over four decades: a systematic review and meta-analysis. BMC Public Health. 2024;24(1):2553. https://doi.org/10.1186/s12889-024-20014-w PMID: 39300359
42. Langlois JP, Fahrig L, Merriam G, Artsob H. Landscape structure influences continental distribution of hantavirus in deer mice. Landsc Ecol. 2001;16:255–66.
43. Prist PR, D Andrea PS, Metzger JP. Landscape, Climate and Hantavirus Cardiopulmonary Syndrome Outbreaks. Ecohealth. 2017;14(3):614–29. https://doi.org/10.1007/s10393-017-1255-8 PMID: 28620680
44. Klempa B. Hantaviruses and climate change. Clin Microbiol Infect. 2009;15(6):518–23. https://doi.org/10.1111/j.1469-0691.2009.02848.x PMID: 19604276
45. Clement J, Vercauteren J, Verstraeten WW, Ducoffre G, Barrios JM, Vandamme A-M, et al. Relating increasing hantavirus incidences to the changing climate: the mast connection. Int J Health Geogr. 2009;8:1. https://doi.org/10.1186/1476-072X-8-1 PMID: 19149870
46. Ferro I, Bellomo CM, López W, Coelho R, Alonso D, Bruno A, et al. Hantavirus pulmonary syndrome outbreaks associated with climate variability in Northwestern Argentina, 1997-2017. PLoS Negl Trop Dis. 2020;14(11):e0008786. https://doi.org/10.1371/journal.pntd.0008786 PMID: 33253144
47. Carbajo AE, Vera C, González PL. Hantavirus reservoir Oligoryzomys longicaudatus spatial distribution sensitivity to climate change scenarios in Argentine Patagonia. Int J Health Geogr. 2009;8:44. https://doi.org/10.1186/1476-072X-8-44 PMID: 19607707
48. Luis AD, Douglass RJ, Mills JN, Bjørnstad ON. The effect of seasonality, density and climate on the population dynamics of Montana deer mice, important reservoir hosts for Sin Nombre hantavirus. J Anim Ecol. 2010;79(2):462–70. https://doi.org/10.1111/j.1365-2656.2009.01646.x PMID: 20015212
49. Meserve PL, Yunger JA, Gutierrez JR, Contreras LC, Milstead WB, Lang BK, et al. Heterogeneous Responses of Small Mammals to an El Nino Southern Oscillation Event in Northcentral Semiarid Chile and the Importance of Ecological Scale. Journal of Mammalogy. 1995;76(2):580–95. https://doi.org/10.2307/1382367
50. Pei S, Yu P, Raghwani J, Wang Y, Liu Z, Li Y, et al. Anthropogenic land consolidation intensifies zoonotic host diversity loss and disease transmission in human habitats. Nat Ecol Evol. 2025;9(1):99–110. https://doi.org/10.1038/s41559-024-02570-x PMID: 39558089
51. Astorga F, Escobar LE, Poo-Muñoz D, Escobar-Dodero J, Rojas-Hucks S, Alvarado-Rybak M, et al. Distributional ecology of Andes hantavirus: a macroecological approach. Int J Health Geogr. 2018;17(1):22. https://doi.org/10.1186/s12942-018-0142-z PMID: 29929522
52. Mills JN, Amman BR, Glass GE. Ecology of hantaviruses and their hosts in North America. Vector Borne Zoonotic Dis. 2010;10(6):563–74. https://doi.org/10.1089/vbz.2009.0018 PMID: 19874190
53. Milholland MT, Castro-Arellano I, Garcia-Peña GE, Mills JN. The Ecology and Phylogeny of Hosts Drive the Enzootic Infection Cycles of Hantaviruses. Viruses. 2019;11(7):671. https://doi.org/10.3390/v11070671 PMID: 31340455
54. Palma RE, Polop JJ, Owen RD, Mills JN. Ecology of rodent-associated hantaviruses in the Southern Cone of South America: Argentina, Chile, Paraguay, and Uruguay. J Wildl Dis. 2012;48(2):267–81. https://doi.org/10.7589/0090-3558-48.2.267 PMID: 22493103
55. Murua R, Gonzalez LA, Meserve PL. Population Ecology of Oryzomys longicaudatus philippii (Rodentia: Cricetidae) in Southern Chile. The Journal of Animal Ecology. 1986;55(1):281. https://doi.org/10.2307/4708
56. Heyman P, Thoma BR, Marié J-L, Cochez C, Essbauer SS. In Search for Factors that Drive Hantavirus Epidemics. Front Physiol. 2012;3:237. https://doi.org/10.3389/fphys.2012.00237 PMID: 22934002
57. Suzán G, Marcé E, Giermakowski JT, Mills JN, Ceballos G, Ostfeld RS, et al. Experimental evidence for reduced rodent diversity causing increased hantavirus prevalence. PLoS One. 2009;4(5):e5461. https://doi.org/10.1371/journal.pone.0005461 PMID: 19421313
58. Holmes EC, Rambaut A, Andersen KG. Pandemics: spend on surveillance, not prediction. Nature. 2018;558(7709):180–2. https://doi.org/10.1038/d41586-018-05373-w PMID: 29880819
59. Reyes R, Yohannessen K, Ayala S, Canals M. Estimates of the spatial distribution of the relative risk of mortality of the main zoonoses in Chile: Chagas disease, hydatidosis, Hantavirus cardiopulmonary syndrome and leptospirosis. Rev Chilena Infectol. 2019;36(5):599–606. https://doi.org/10.4067/S0716-10182019000500599 PMID: 31859801