BRIEF REPORT

Expanded distribution of orthohantavirus Pergamino genotype: First record in Entre Rios province, Argentina

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Abstract   Hantavirus Pulmonary Syndrome (HPS) is an emerging infectious disease of the Americas. Eight native rodent species have been identified as HPS virus reservoirs in Argentina. The aim of this work was to detect the orthohantavirus genotypes present in a rodent community that inhabits a zone where a fatal HPS case occurred within an endemic locality of Central Argentina. We captured 27 rodents with a trapping effort of 723 trap nights. We detected 14.3% of infected *Akodon azarae* with the Pergamino genotype. This result expands the known distribution of this orthohantavirus. Although the Pergamino genotype has not been associated with human cases, the information about its distribution is relevant for risk assessment against potential changes in the virus infectivity.
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Ampliación de la distribución del *Orthohantavirus* genotipo Pergamino: Primer registro en la Provincia de Entre Ríos, Argentina

Resumen   El síndrome pulmonar por hantavirus (SPH) es una enfermedad infecciosa emergente en América. Ocho especies de roedores nativos han sido identificadas como reservorios del virus causante del SPH en la Argentina. El objetivo de este trabajo fue detectar los genotipos

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de *orthohantavirus* presentes en una comunidad de roedores que habita en una zona donde ocurrió un caso fatal de SPH, en una localidad endémica de Argentina central. Se capturaron 27 individuos con un esfuerzo de 723 trampas-noche. Se detectó un 14,3% de *Akodon azarae* infectados con el genotipo pergamino. Este resultado amplía el conocido rango de distribución de este *orthohantavirus*. A pesar de que el genotipo pergamino no ha sido asociado con casos humanos hasta el momento, la información sobre su distribución es relevante para analizar el riesgo ante un potencial cambio en la infectividad del virus.

Hantavirus Pulmonary Syndrome (HPS) is a zoonotic emerging infectious disease characterized by a rapid onset of pulmonary edema followed by respiratory failure and cardiogenic shock. It is caused by New World orthohantaviruses (family *Hantaviridae*, subfamily *Mammantavirinae*, genus *Orthohantavirus*) which are hosted by rodents of the family *Cricetidae*. In Argentina, since the characterization of the Andes virus (ANDV) in 1995[8], different variants or genotypes of this virus have been described. Eight native species have been identified as orthohantavirus hosts: *Oligoryzomys flavescens*, *O. nigripes*, *O. longicaudatus*, *O. chacoensis*, *O. occidentalis*, *Calomys callosus*, *Necromys obscurus* and *Akodon azarae*[4,6,7,14,17,21]. Individuals of other species, such as *Abrothrix hirta*, *A. olivacea*, *Loxodontomys micropus*, *Deltamys kempi* and *Oxymycterus rufus* have occasionally been found to be seropositive, though these cases are often considered the result of spillover events[3,15]. Three orthohantavirus hosts are known to be present in Central-East Argentina: *O. flavescens*, host of Lechiguanas (LECV), Buenos Aires (BASV) and Plata (PTV) genotypes in Buenos Aires[4,14] and Entre Ríos (only LECV)[21]; *Necromys obscurus*, host of Maciel (MACV) genotype in Buenos Aires; and *A. azarae*, host of Pergamino (PERV) genotype in Buenos Aires[14]. All these genotypes cause HPS with the exception of the PERV and MACV that have not yet been associated with human cases[14].

The PERV genotype was detected for the first time in *A. azarae* tissues from Buenos Aires, Argentina[7] and infected individuals were detected in subsequent investigations carried out in the same province[9,21]. However, all the studies conducted in other neighboring provinces such as Entre Rios and Santa Fe did not detect infected *A. azarae* despite the great capture effort[9,19,21].

Due to the fact that there is no specific treatment available for HPS, prevention is the only possible measure to reduce the morbidity and mortality caused by this disease. The identification of the reservoir hosts and the delimitation of both the geographical range of the host and that of the virus within the host range are crucial parts of the knowledge needed to design effective preventive measures[20]. The aim of this work was to detect the orthohantavirus genotypes present in a rodent community that inhabits a zone where a fatal HPS case occurred within an endemic locality of Central Argentina.

This study was performed in a rural area of the Gualeguaychú department in Entre Ríos province, Argentina (33°03′53.1″S 58°52′39.1″W), which is located in Central-East Argentina, the second region in Argentina in terms of the number of HPS cases[1,10]. Based on a fatal HPS case that occurred in January 2018 the provincial health authorities requested the study of rodent assembles. In October 2018, small rodents were captured with live-traps baited with a mixture of fat, oats and peanut butter. Two hundred and forty one traps were located at 10 m intervals during three consecutive nights on a poultry farm where the HPS patient used to work(composed of five breeding sheds and two sheds for storing tools and animal feed), in the patient's dwelling (with breeding animals such as pigs in the peridomicile) and near the vegetated margins of the stream where the patient used to go fishing.

For each animal captured, we recorded the species according to external morphology, sex and body measurements. To detect hantavirus-specific immunoglobulin G (IgG) antibodies, enzyme-linked immunosorbent assays were performed on blood samples[13]. We used antibody presence as an indication of infection. The animals were sacrificed and the tissues were stored in a nitrogen tank to determine the orthohantavirus genotype. All animals were handled according to the Argentine law for the protection of animal welfare (Penal Code Law No. 14346) and safety precautions appropriate for handling the orthohantavirus reservoirs were followed.

Total RNA was extracted from the lung tissues of seropositive rodents using Trizol (Invitrogen) and purified by the RNAid kit (QbioGene). Partial S-segment was amplified by RT-PCR followed by a second round of nested PCR. Specific oligonucleotide primers, based on conserved regions of the ANDV virus genome, were used[2]. End-point PCR reactions were carried out in an Applied Biosystems 2720 Thermal Cycler. For the S segment, the targets were a fragment of 840 nt (position 86–926) in length, corresponding to the nucleocapsid protein coding region. The PCR products were separated by agarose gel electrophoresis, purified and sequenced in an automated sequencer ABI Prism R3500 Genetic Analyzer (Applied Biosystems, UK) using the ABI PRISM Big Dye Terminator Sequencing kit (Applied Biosystems, UK). The obtained sequences were analyzed and compared with published sequences using MEGA 10 software[5].
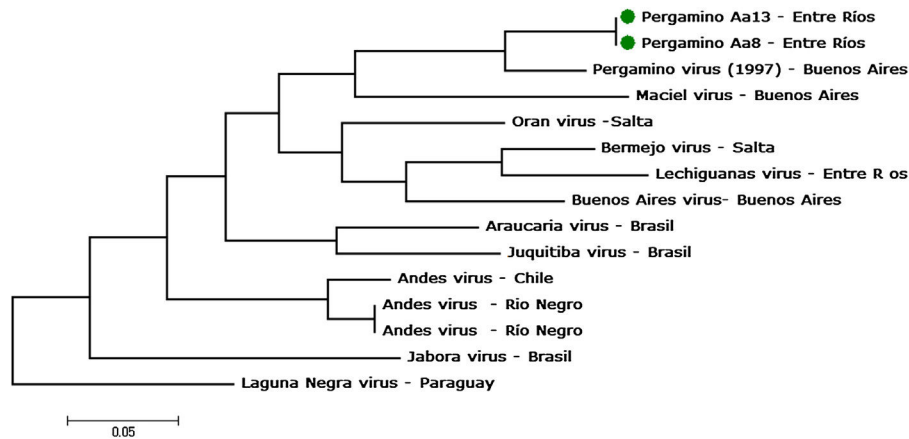
Figure 1 Molecular phylogenetic analysis by Maximum Likelihood method. Phylogenetic analysis of hantaviruses based on 853 bp of S-segment genome of Andes virus, and other orthohantaviruses characterized previously. Taxa in the branches of the tree indicate virus name and province of origin. GenBank accession numbers and reservoir rodent specie were: AF482717, Pergamino (*Akodon azarae*); AF482716, Maciel (*Necromys benefactus*); AF482715, Orán (*Oligoryzomys chacoensis*); AF482713, Bermejo (*Oligoryzomys occidentalis*); AF482714, Lechiguanas (*Oligoryzomys flavesc*ens and *O. nigripes*); AF482711, Buenos Aires (undescribed); AY740624, Araucaria (*O. nigripes*); KC422346, Juquitiba (*O. nigripes*); AF291702, AF004660 and AF324902 Andes (*Oligoryzomys longicaudatus*; HM582091, Jaborá (*Akodon montensis*) and NC038505 Laguna Negra (*Calomys laucha*). The model selection retained GTR+G+I as the best model that shows the lowest Akaike (6384.96) and BIC (6645.48) values.

Models were developed to describe substitution patterns and were selected by BIC (Bayesian Information Criterion). Models with the lowest BIC scores are considered to best describe the substitution pattern. For each model, AICc value (Akaike Information Criterion, corrected), Maximum Likelihood value (lnL), and the number of parameters (including branch lengths) are also estimated[12]. For estimating the ML values, a tree topology was automatically computed. The analysis involved 15 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated.

We captured a total of 27 individuals with a trapping effort of 723 trap nights. *Akodon azarae* was the most abundant species (14) followed by *Oxymycterus rufus* (7), *Calomys callidus* (5) and *Holochilus* sp. (1). The first two species were captured both on the farm and in the proximities of the stream, while *Calomys callidus* was captured only on the farm and *Holochilus* sp. only in the stream. No rodents were captured around the patient's dwelling.

Two individuals of *A. azarae* (14.3%) were infected. Viral RNA was recovered from rodent tissues, partial fragments of S and M genomic segments were amplified, the nucleotide sequence determined and the phylogenetic analysis performed. Amplifications of all partial fragments were successful and the comparative analysis with other sequences was published in GENBANK, and in accordance with the criterion established by the ICTV and Rivera et al.[16], showed the highest identities with the PERV genotype (strain 14403) for the S-segment fragment. The percentages of identity were 93% and 98% at nucleotide and amino acid level (accession numbers AF482717 and AAL82652, respectively, Fig. 1).

Despite not finding a pathogenic genotype that could be associated with the fatal HPS case recorded, the principal success of this research was to detect, for the first time, infected *Akodon azarae* with the PERV genotype in Entre Rios province. Although this genotype has not been associated with human cases, the information about its distribution could be relevant to assess risk in the case of changes in the infectivity of the virus[11], as was the case with the Tula virus, the pathogenic agent of hemorrhagic fever with renal syndrome[18]. Additionally, the LECV genotype (associated with *O. flavescens*) was detected in *O. nigripes* of Entre Rios[21] and Buenos Aires[3] and PERV genotype (associated with *A. azar*ae) in *Oxymycterus ruf*us in Buenos Aires[3], two non typical rodent species hosts, suggesting that host switching is frequent in this area. Due to these facts, permanent surveillance would be essential for the detection of the expansion of both reservoir host ranges and viral distribution in the reservoir population, as well as other factors associated with anthropogenic changes. These conditions should favor a potential impact on public health. Emerging infections are diseases that newly appear in a population, or existed previously but emerge due to dynamic interactions between rapidly evolving infectious agents and changes in the environment, such as perturbations, and changes in host's behavior that favor new ecological niches[11]. This first record of the PERV genotype in Entre Ríos expands the virus distribution known until now and complements the epidemiological monitoring and prevention of HPS in the province.

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Authors' contributions

IEGV and JGA designed the research; IEGV, JGA and EFB did the fieldwork; IEGV and EFB write of the manuscript; RMC, CMB and VPM did the serological and molecular analyses; IEGV, JGA and VPM funded the research.

Conflict of interest

The authors declare that they have no conflicts of interest.

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References

1. Alonso DO, Iglesias A, Coelho R, Periolo N, Bruno A, Córdoba MT, Filomarino N, Quipildor M, Biondo E, Fortunato E. Epidemiological description, case-fatality rate, and trends of Hantavirus Pulmonary Syndrome: 9 years of surveillance in Argentina. J Med Virol. 2019;91:1173–81.

2. Ciancaglini M, Bellomo CM, Torres Cabreros CL, Alonso D, Bassi SC, Iglesias AA, Martínez VP. Hantavirus pulmonary syndrome in Tucumán province associated to an unexpected viral genotype. Medicina (B Aires). 2017;77:81–4.

3. Colombo VC, Brignone J, Sen C, Previtali MA, Martin ML, Levis S, Monje L, González-Ittig RE, Beldomenico PM. Orthohantavirus genotype Lechiguanas in *Oligoryzomys nigripes* (Rodentia: Cricetidae): new evidence of host-switching. Acta Trop. 2019;191:133–8.

4. González-Ittig RE, Rivera PC, Levis SC, Calderón GE, Gardenal CN. The molecular phylogenetics of the genus *Oligoryzomys* (Rodentia: Cricetidae) clarifies rodent host–hantavirus associations. Zool J Linn Soc. 2014;171:457–74.

5. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870–4.

6. Levis S, Garcia J, Pini N, Calderon G, Ramirez J, Bravo D, Jeor SST, Ripoll C, Bego M, Lozano E. Hantavirus pulmonary syndrome in northwestern Argentina: circulation of Laguna Negra virus associated with *Calomys callosus*. Am J Trop Med Hyg. 2004;71:658–63.

7. Levis S, Morzunov SP, Rowe JE, Enria D, Pini N, Calderon G, Sabattini M, Jeor SCS. Genetic diversity and epidemiology of hantaviruses in Argentina. J Infect Dis. 1998;177:529–38.

8. López N, Padula P, Rossi C, Lázaro ME, Franze-Fernández MT. Genetic identification of a new hanta-virus causing severe pulmonary syndrome in Argentina. Virology. 1996;220:223–6.

9. Maroli M, Vadell MV, Padula P, Gómez Villafañe IE. Rodent abundance and hantavirus infection in protected area, East-Central Argentina. Emerg Infect Dis. 2018;24:131–4.

10. Martinez VP, Bellomo CM, Cacace ML, Suárez P, Bogni L, Padula PJ. Hantavirus pulmonary syndrome in Argentina, 1995–2008. Emerg Infect Dis. 2010;16:1853–60.

11. Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004;430:242–9.

12. Nei M, Kumar S. Molecular evolution and phylogenetics. New York: Oxford University Press; 2000.

13. Padula P, Rossi C, Della Valle M, Martinez P, Colavecchia S, Edelstein A, Miguel S, Rabinovich RD, Segura EL. Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. J Med Microbiol. 2000;49:149–55.

14. 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;48:267–81.

15. Piudo L, Monteverde MJ, Walker RS, Douglass RJ. Rodent community structure and Andes virus infection in sylvan and peridomestic habitats in northwestern Patagonia, Argentina. Vector Borne Zoonotic Dis. 2011;11:315–24.

16. Rivera PC, Gonzalez-Ittig RE, Gardenal CN. Preferential host switching and its relation with Hantavirus diversification in South America. J Gen Virol. 2015;96:2531–42.

17. Rivera PC, González-Ittig RE, Robainas Barcia A, Trimarchi LI, Levis S, Calderón GE, Gardenal CN. Molecular phylogenetics and environmental niche modeling reveal a cryptic species in the *Oligoryzomys flavescens* complex (*Rodentia Cricetidae*). J Mammal. 2018;99:363–76.

18. Schultze D, Lundkvist Å, Blauenstein U, Heyman P. Tula virus infection associated with fever and exanthema after a wild rodent bite. Eur J Clin Microbiol Infect Dis. 2002;21:304–6.

19. Vadell MV, Gómez Villafane IE. Environmental variables associated with hantavirus reservoirs and other small rodent species in two national parks in the Paraná Delta, Argentina: implications for disease prevention. EcoHealth. 2016;13:248–60.

20. Vadell MV, Carbajo AE, Massa C, Cueto GR, Gómez Villafane IE. Hantavirus pulmonary syndrome risk in Entre Ríos, Argentina. EcoHealth. 2019;16:558–69.

21. Vadell MV, Bellomo CM, San Martín A, Padula PJ, Gómez Villafañe IE. Hantavirus ecology in rodent populations in three protected areas of Argentina. Trop Med Int Health. 2011;16:1342–52.