

HANTAVIRUS PULMONARY SYNDROME IN TUCUMÁN PROVINCE ASSOCIATED TO AN UNEXPECTED VIRAL GENOTYPE

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Abstract We describe the characterization of the viral genotype involved in the first case of hantavirus pulmonary syndrome reported in Tucumán, a Northwestern province of Argentina. A 23-year-old woman, with no record of travel history and previously diagnosed with an antiphospholipid syndrome, died after 11 days of severe cardiopulmonary insufficiency. Among the four endemic regions of hantavirus pulmonary syndrome in Argentina, the Northwest Region has the highest incidence, exceeding 50% of all reported cases in the country. Until now, only Salta and Jujuy (2 out of the 6 provinces composing the Northwest Region), reported cases of hantavirus pulmonary syndrome, all of which occurred in the Yungas Forest area. Remarkably, the viral genotype characterized in this case showed higher nucleotide identity with the Andes-BsAs genotype most prevalent in Buenos Aires province, located 1400 km apart from Tucumán, than with any of the commonly found genotypes in the Northwest Region. The Andes-BsAs genotype has been associated with 30% lethality and interhuman transmission in Buenos Aires province. Interhuman transmission cannot be ruled out in the present case.

Key words: Bunyaviridae infections, Andes virus, Argentina

Resumen *Síndrome pulmonar por hantavirus en la provincia de Tucumán asociado a un genotipo viral inesperado.* Se describe la caracterización del genotipo viral del primer caso de síndrome pulmonar por hantavirus reportado en la Provincia de Tucumán. Una mujer de 23 años, con diagnóstico previo de síndrome antifosfolipídico y sin antecedente de viaje reciente, falleció tras 11 días de enfermedad. De las cuatro regiones endémicas para este síndrome, la región Noroeste es la de mayor incidencia del país, concentrando más del 50% de los casos informados en Argentina. Hasta el momento, solamente dos de las seis provincias que componen la región Noroeste, Salta y Jujuy, habían registrado síndrome pulmonar por hantavirus con casos en áreas pertenecientes a la Selva de las Yungas. Desde ahora, se suma a dicha región la provincia de Tucumán. Llamativamente, el genotipo viral caracterizado en este caso mostró mayor identidad con el genotipo más prevalente en la provincia de Buenos Aires llamado Andes-BsAs, y no como se esperaba, con los genotipos comúnmente encontrados en la región Noroeste. El genotipo Andes-BsAs se ha asociado a letalidad próxima al 30% y también a transmisión interhumana en la provincia de Buenos Aires. No se puede descartar transmisión interhumana en el presente caso.

Palabras clave: Bunyaviridae, virus Andes, Argentina

Hantaviruses are negative sense RNA viruses that belong to the Bunyaviridae family and contain tripartite RNA genomes; large, medium (M), and small (S) segments encoding for the RNA-dependent-RNA polymerase, the precursor of Gn and Gc membrane glycoproteins, and the nucleocapsid protein, respectively¹. The hantavirus pulmonary syndrome (HPS) was first described in 1993, during an outbreak of pulmonary disease in the United States². In Argentina, the first HPS outbreak was reported in 1995 when the Andes Virus was described³. HPS is a zoonotic disease transmitted to humans by inhalation of excreta or aerosolized particles generated by infected sigmodontinae

rodents, which are the natural reservoirs⁴. In Argentina and its five bordering countries, two hantavirus species causing HPS have been characterized: Andes Virus (ANDV) and Laguna Negra virus⁴. HPS has a great impact in human communities, since it mainly affects young and previously healthy people, it is a fast evolving illness from starting symptoms to death, and it has a high lethality rate, more than 40% in some regions⁵.

There are seven different genotypes of ANDV associated with HPS cases, and rodents of the genus *Oligoryzomys* are their reservoir. Genetic comparison between Argentine genotypes and those of bordering countries showed less than 6% amino acid sequence divergence, indicating that these strains are variants of the same viral species^{4,6,7}. In Argentina, different ANDV genotypes have been characterized in four endemic areas geographically distant to each other: (i) the South-Andean region, where ANDV was originally described and the circulating genotype is AND-South (or AND-AH1); (ii) the

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Central region, which includes Buenos Aires province, where three pathogenic genotypes co-circulate: Andes-Lechiguanas, Andes-Buenos Aires (AND-BsAs) and Andes-Plata; (iii) the Northwest Region with Andes-Orán and Andes-Bermejo genotypes, and where Laguna Negra virus was also identified in rodents and a few HPS cases (Pires-Marczesky, et al 2010, personal communication); and (iv) the Northeast region, where two genotypes have been reported, Andes-Lechiguanas and Andes-Juquitiba⁵. Although other hantaviruses are present in the Central region, namely Pergamino and Maciel genotypes, these have not been associated to HPS cases and they have rodent reservoirs belonging to different genus, *Akodon azarae* and *Necromys benefactus* respectively⁸. The highest frequencies of HPS cases reported in the country were found in the Northwest Region, from 49.1% of total cases in the period 1995-2008 to over 50% more recently⁹ (Bassi SC, et al 2016, personal communication). The endemic area in the Northwest Region fits within the Yungas forest but does not cover the entire ecoregion⁵. This narrow area, also known as the Tucumano-Salteña jungle, extends from North to South all along Salta, Jujuy, Tucumán and Catamarca provinces. It is also the main area of *Oligoryzomys* distribution in the Northwest Region¹⁰ (Fig. 1).

We describe the diagnosis and characterization of the viral genotype of the first case of hantavirus pulmonary syndrome reported in Tucumán, a Northwestern province of Argentina. To obtain more information regarding the genetic identity of hantavirus in this case, the viral genome present in the blood of the patient was analyzed.

On February 29, 2016, a 23 year-old woman sought assistance in a primary health care center in Yerba Buena, Tucumán, referring flu-like symptoms that began 72 hours before. The patient presented with fever, headache, myalgia, nausea including vomiting and abdominal pain. The clinical status worsened the next days with higher fever and severe respiratory symptoms requiring mechanical ventilation. She was transferred to the intensive care unit, and her admission to a high complexity hospital in Buenos Aires City was prompted. The patient had a medical history of antiphospholipid syndrome, a systemic autoimmune disorder characterized by arterial and/or venous thrombosis. On admission, the clinical presentation was suggestive of septic shock, with a major respiratory component. She showed hemodynamic instability requiring inotropes and vasopressors in high doses. On physical examination, generalized edema with signs of insufficient distal perfusion was evidenced. Laboratory tests results were consistent with dehydration, showing hemoconcentration (56%), metabolic acidosis (pH 7.1, PCO_2 47, bicarbonate 14mm/l), hyperlactacidemia (3.5 mmol/l), elevated hepatic enzymes (GOT 142 U/l, GPT 89 U/l) and creatinine (0.78 mg/dl) with no signs of renal failure. Other abnormalities included leukocytosis (80100 cells/mm³) and thrombocytopenia (40000 cells/mm³). Broad spectrum antimicrobial treatment was administered, including antibiotics (Initially amoxicillin sulbactam, piperacillin tazobactam and clarithromycin; and then vancomycin, imipenem and colistin), antivirals (oseltamivir) and antimycotics (amphotericin B). Pulmonary edema was documented by chest X ray showing generalized interstitial bilateral infiltrates. At this point the hypoxemia became refractory and the respiratory distress increased, then connection to extracorporeal membrane oxygenation was instituted. In view of the worsening of oliguria with metabolic acidosis,

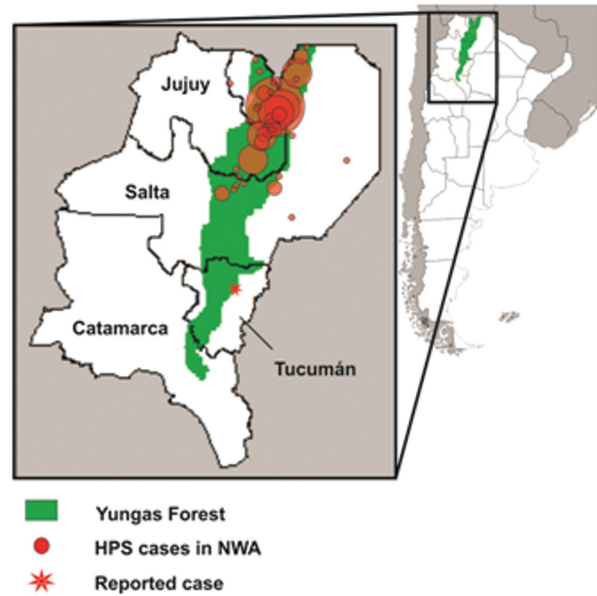


Fig. 1.— Geographic distribution of HPS reported cases in the Northwest region of Argentina and localization of Yungas forest. The numbers of HPS cases in each place are proportional to the size of the circles. NWA: Northwest Argentina.

hemodialysis was initiated. During a 5-day period the patient was transfused with red blood cells (50 U), platelets (110 U), fresh plasma (37 U), cryoprecipitates (56 U), and underwent one session of plasmapheresis. Refractory hypoxemia and cardiorespiratory failure occurred and sternotomy was urgently performed, revealing a cardiac tamponade. Manual cardiopulmonary resuscitation procedures, pericardial drainage and extracorporeal membrane oxygenation re-connection were performed. The patient persisted in refractory shock, unresponsive to resuscitation measures, evolving to death. Tests for influenza, dengue, chikungunya and zika viruses resulted negative.

A blood sample obtained 7 days after disease onset was received at the Hantavirus National Reference Laboratory (INEI-ANLIS Dr. C. Malbrán). The sample was tested for hantavirus antibodies by μ -capture IgM ELISA, and IgG ELISA, as previously described¹¹. RNA was obtained from 200 μ l of blood sample using Trizol (Invitrogen) and then purified with the RNAid kit (QBioGene, Solon) following the manufacturer's recommendations. Real time PCR (RT-qPCR, TaqMan Chemistry), was carried out for detection of the viral genome, using the equipment MyiQ single color PCR detection system (BioRad, Hercules, CA). Specific oligonucleotide primers and probe were designed based on the conserved region of viral S segment. The qScript™ One-Step qRT-PCR kit (Quanta BioSciences, USA) was used, with 1 mM primers, 100 nM probe, 12.5 μ l of buffer 2X, 2 μ l of RNA- and nuclease-free water to a final volume of 25 μ l. Each sample was analyzed in duplicate.

The blood sample did not present IgM or IgG specific antibodies against hantavirus detectable by ELISA but resulted positive for ANDV by real-time reverse-transcriptase-PCR. Considering the compatibility of the clinical presentation with the diagnosis of HPS and the positive result of real-time reverse-transcriptase assay

specific for ANDV, but taking also into account the negative ELISA results and the patient's non-endemic place of residence, a definitive diagnosis confirmation required end point reverse-transcriptase PCR assay and subsequent nucleotide sequence analysis.

For viral genome characterization, partial S and M segments were amplified by reverse-transcriptase PCR followed by a second round of nested or hemi-nested PCR. All end-point PCR reactions were carried out in an Applied Biosystems 2720 Thermal Cycler. For the M segment, the targets were two fragments of 461 nt (position 6 to 467) and 742 nt (position 2218 to 2961) in length, corresponding to the Gn and Gc envelope glycoproteins, respectively. For the S segment, the targets were two fragments of 952 nt (position 22 to 974) and 460 nt (position 1365 to 1818) in length, corresponding to the nucleocapsid protein coding region and the non-coding region, respectively. The primers were aligned to AND-South, sequence ID: AF324901 for the M segment and AF324902 for the S segment.

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 sequences of previous HPS cases in the country using MEGA 6.06 software.

Amplifications of all partial fragments were successful (2615 nt) and the comparative analysis with other sequences published in GENBANK showed maximum similarity with AND-BsAs: 98.6%, 96.7%, 96.1% and 95.9% for the nucleocapsid protein coding region, the S segment non-coding region, the Gn glycoprotein and the Gc glycoprotein, respectively. Furthermore, this relationship was confirmed by phylogenetic analysis with other AND-BsAs cases and genotypes present in Argentina (Fig. 2).

According to the comparative analysis of a partial fragment of Gn (nt 2721 to 2946) with our genetic database of AND-BsAs, this case showed highest similarity (97.8%) with the southernmost Buenos Aires case, which occurred in spring 2002 in Balcarce, an area located ~1400 km

away from Tucumán where very few cases have been reported. This is unusual, because an inverse relationship between hantavirus genetic similarity and geographic distance has been observed among HPS cases in various endemic regions⁷. We evaluated nucleotide similarities in Gc glycoprotein sequences of 21 HPS cases occurred in Buenos Aires province corresponding to the AND-BsAs genotype. The lowest nucleotide similarity was 90.7%, corresponding to the two most geographically distant cases. Similarly, Andes-Lechiguanas viruses previously associated to HPS cases reported in Misiones province showed the highest similarity (90.2%) with an Andes-Lechiguanas case occurred in Buenos Aires province, displaying again an inverse correlation between genetic similarity and geographic distance⁷.

This case represents one of the most severe clinical manifestations of HPS reported in Argentina to present, showing respiratory insufficiency with treatment-refractory hemodynamic compromise followed by death. The patient was a young woman previously diagnosed with an autoimmune disorder which most probably worsened the HPS evolution. On day 7 after disease onset the patient did not present detectable ANDV-specific antibody response. HPS patients typically mount a strong IgM response very early after the appearance of symptoms and IgG antibodies become detectable with 1 or 2 days of delay^{4,11}. Previous findings of our group showed that low or undetectable ANDV specific IgG titers are strong predictors of death^{5,11}. The case herein presented is the first HPS case detected only by viral genome amplification, with undetectable humoral response at day 7 of illness, although the viral load was similar to loads typically detected in patients infected with AND-BsAs genotype¹². Complete absence of specific IgG and barely detectable IgM have been observed in fatal cases of Ebola virus infection¹³. In our case, however, we cannot rule out the possibility that blood transfusions had diluted the antibodies turning them undetectable.

To our knowledge, this is the first genetically confirmed HPS case reported in Tucumán province. The fact that the viral genotype resulted to be AND-BsAs was utterly unexpected. Indeed, the most prevalent HPS-causing genotype

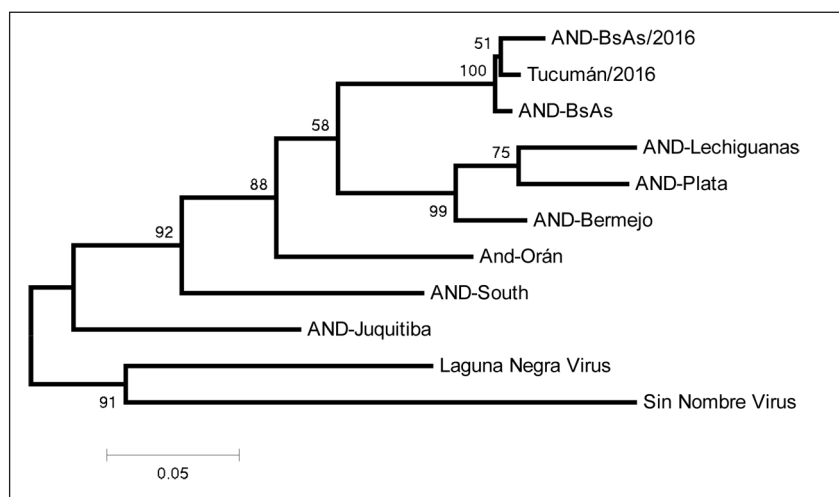


Fig. 2.— Phylogenetic trees were generated by ML method (MEGA 6), using the Tamura 3-parameter model of evolution, based on the alignment of 900 nt nucleocapsid protein sequences. Bootstrap values obtained by 2000 iterations are shown.

in nearby provinces is Andes-Orán and the patient had no recent travel history outside Tucumán province. Apart from sporadic cases of HPS related to Laguna Negra virus and Andes-Bermejo, no other pathogenic hantavirus genotype had been reported to date in the Northwest area^{7,14}. Instead, AND-BsAs, the genotype characterized in the present study, is commonly associated with HPS cases within the Central region of Argentina, mainly in Buenos Aires province, located more than 1000 km away from Tucumán.

The Gn encoding fragment of the M segment (nt 2721 to 2946) has previously shown to be useful for genotyping, being approximately 90% its lower limit of within-genotype nucleotide similarity⁴. Our group observed a correlation between degree of similarity and geographic distance among cases (data not shown). A similar observation was made for the AND-South genotype in HPS cases occurred in Neuquén province¹⁵.

The occasional presence of certain genotypes in areas that are geographically distant from their original description has been previously reported. This is the case of the Andes-Lechiguanas genotype, which is prevalent in the Central region but it was also characterized in a HPS case in the Northeast region. Still, this latter variant showed a remarkable nucleotide divergence when compared to the Andes-Lechiguanas strains of the Central region, a finding that was concordant with the geographic distance between these regions⁶. Other HPS cases in which the infecting genotype was prevalent in a different area than the patient's place of residence had a history of travel to the corresponding endemic area or had been in contact with HPS patients from endemic areas¹⁵. Considering there was minimal genetic divergence between the variant characterized in the present study and those found in Buenos Aires province, an interhuman transmission event should be considered as highly likely. The AND-BsAs genotype, as AND-South, has already been associated with person to person transmission¹⁵. In this scenario, a non-diagnosed person infected with hantavirus in the Central region could plausibly have traveled to Tucumán and have infected the study case. Since there are remote areas of Buenos Aires where few HPS cases have been reported, HPS suspicion would hardly arise facing a mild clinical presentation. The lack of awareness about the disease in areas with low or null records of HPS might well lead to underdiagnosis and to incorrectly consider certain areas as non endemic.

Although AND-BsAs has not been associated to any rodent reservoir to present, its reservoir is thought to be a member of the *Oligoryzomys* genus⁹. Several species of this genus have been reported in Tucumán province, principally in the Yungas forest and grasslands¹⁰. Considering the possibility of local infection, the putative risk zone for this case is the rural environment of the Yungas forest. HPS is most probably underdiagnosed in several regions of the country⁵, particularly in the Northwest Region, where HPS cases would be expected to occur all along the Yungas forest, including Tucumán and Catamarca provinces. However, to confirm these areas as endemic for hantavirus, further efforts must be directed to determine the viral prevalence in local rodent populations. Such studies would help to determine more precisely the circulating viral genotypes and therefore the risk of infection with pathogenic genotypes. Thus, the

status of Tucumán province regarding HPS endemicity would eventually be clarified.

Besides of its rapid progression and unfavorable evolution, HPS presents overlapping clinical manifestations with other acute respiratory diseases. To that effect, it is imperative to consider hantavirus in the differential diagnosis of severe acute respiratory conditions, as septic shock of pulmonary origin, in endemic and even in non-endemic regions.

Conflict of interests: None to declare

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