CASE REPORT

First evidence of asymptomatic infection related to the Araucaria (Juquitiba-like) hantavirus

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SUMMARY

Hantavirus pulmonary syndrome (HPS) is a severe disease, transmitted to humans by inhalation of virus-contaminated aerosols from rodent excreta. Epidemiological, clinical and laboratory data confirmed a fatal HPS case and an asymptomatic infection in a household contact, both caused by Araucaria hantavirus, which has previously been found only in patients with HPS. This is the first report of asymptomatic infection related to a pathogenic hantavirus genotype, highlighting the need for additional studies on characterisation of viral and genetic mechanisms associated with this disease.

BACKGROUND

Hantaviruses belong to the Bunyaviridae family responsible for two different diseases: haemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in the Americas. The case death varies from 0.1% to 40%, depending on the virus involved. Rodents and insectivores serve as reservoirs for hantaviruses, which maintain lifelong and chronic infections in animals, and are generally species-specific.[1]

Southern Brazil has one of the highest incidences of HPS cases, and in this region two hantavirus genotypes were identified in IgG-positive rodents: Araucaria (Juquitiba-like) and Jaborá.[2] Since 2001, we have monitored all cases of HPS in Paraná State, and only the Araucaria genotype was found associated with HPS cases.

Following the notification of an HPS case, serum samples were collected from all the household contacts of the index patient and tested by hantavirus antibody detection. Immunoassays are carried out to detect IgG and IgM antibodies against hantavirus. Samples that yield IgM positive results are analysed by reverse transcriptase PCR (RT-PCR), for viral genomic S segment characterisation.

CASE PRESENTATION

The patient with the asymptomatic infection (case 2) was a 33-year-old man, living with his parents in a rural area of Paraná State (-25°52'27''S/50° 22'58''W), Southern Brazil. The mother (index case), who was 67 years old, was treated at a public health centre 4 days after the onset of asthenia, myalgia, headache, fever, abdominal pain and dry cough.

INVESTIGATIONS

Following initial drug treatment, the patient (case 2) was admitted to a hospital with hypoxia (SaO_2 of 57%; normal range: 97–99%) and low blood pressure, at 80/60 mm Hg (120/80 mm Hg). Chest X-ray showed diffuse bilateral alveolar infiltration. The clinical condition of this patient worsened, with SaO_2 falling to 47%, dyspnoea, sudoresis and fever, rapidly leading to death. Enzyme immunoassay (EIA) resulted in the detection of IgM and IgG antibodies against the recombinant N protein of the Araucaria hantavirus (figure 1).

Blood samples were collected from two household contacts and analysed by serological testing. Case 2 presented no symptoms of HPS, but serological tests detected IgM against hantavirus, indicating recent exposure. Changes in the levels of antibodies in this patient were monitored by taking two additional blood samples 2 weeks apart. EIA analysis of the new-paired blood samples clearly demonstrated seroconversion (figure 1).

Viral RNA was extracted from all the serum samples taken from index case and case 2 with the High Pure Viral RNA Kit (Roche Inc., Mannheim, Germany). The RNA was then used for nested RT-PCR, amplifying the hantavirus S segment, as previously described.[2] Viral genomic sequences of S segment from index case and case 2 encompassing 1107 and 1097 nucleotides, respectively, were analysed. They were not identical, exhibiting a 95.9% nucleotide identity and a 100% amino acidic identity.

Hantavirus genomic sequences isolated from the patients were aligned with S segment sequences from the main Brazilian and South American hantavirus genotypes using the ClustalW software (from BioEdit V.7.0.9 package). Sin Nombre virus (SNV) was included as outgroup species. Phylogenetic reconstruction was performed under Bayesian inference, using MrBayes software and showed that viral sequences from the index case and case 2 were placed in the Araucaria/Juquitiba-like clade, with a posterior probability of 1 (figure 2) sharing a common ancestor with Araucaria/Juquitiba-like viruses from Paraná and Santa Catarina states from Brazil and Paraguay. It is noteworthy that, although they were recovered from household contacts, the viral sequences reported here are different at the nucleotide level, ruling out the possibility of a laboratory cross-contamination and strongly suggesting that patients may have been infected in the living-environment. Partial nucleotide sequence of viral M segment from the index case was determined confirming the Araucaria genotype, but attempts to amplify the viral M segment sequences from case 2 were unsuccessful.

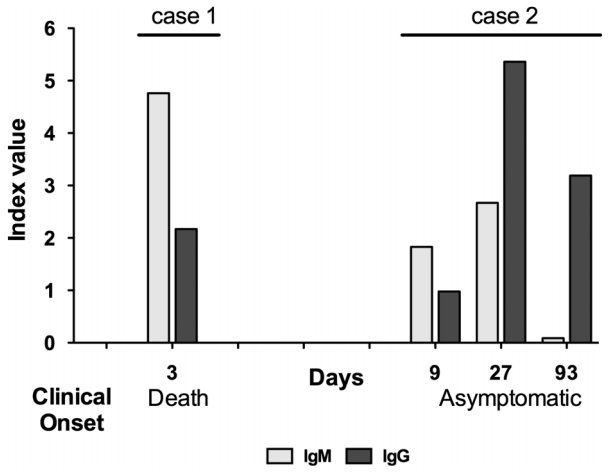

Figure 1 IgM and IgG antibodies against hantavirus, index values from one (index case) and three blood samples (case 2) taken at 14-day intervals.

OUTCOME AND FOLLOW-UP

The patient (case 2) continued without symptoms for at least 93 days, when the last sample was collected, and IgM levels were undetectable, while his mother (index case) passed away due to HPS in less than a week after presenting the symptoms.

DISCUSSION

This case provides the first evidence of asymptomatic infection related to the Araucaria hantavirus in Brazil. Asymptomatic infections with Puumala hantavirus (related to HFRS cases) have been reported, and it has been suggested that clinically mild disease and unapparent infections may be due to differences in the nature of exposure (eg, low levels of inoculum or inefficient transmission mechanisms) or individual genetic differences in mounting immune responses to infection. Alternatively, they may indicate the circulation of more than one hantavirus genotypes with variable virulence.[3] Genetic recombination between pathogenic and non-pathogenic hantaviruses in sympatry could also be one possibility for the development of asymptomatic infections.[4]

HPS cases have been seen to cluster in households; some outbreaks are associated with nosocomial transmission, and person-to-person transmission has been demonstrated in Argentina only for Andes virus (ANDV). It has been reported that 30% of the HPS cases in Chile are associated with household clusters,[5] the highest risk of transmission being between sexual partners. It seems unlikely that a person-to-person

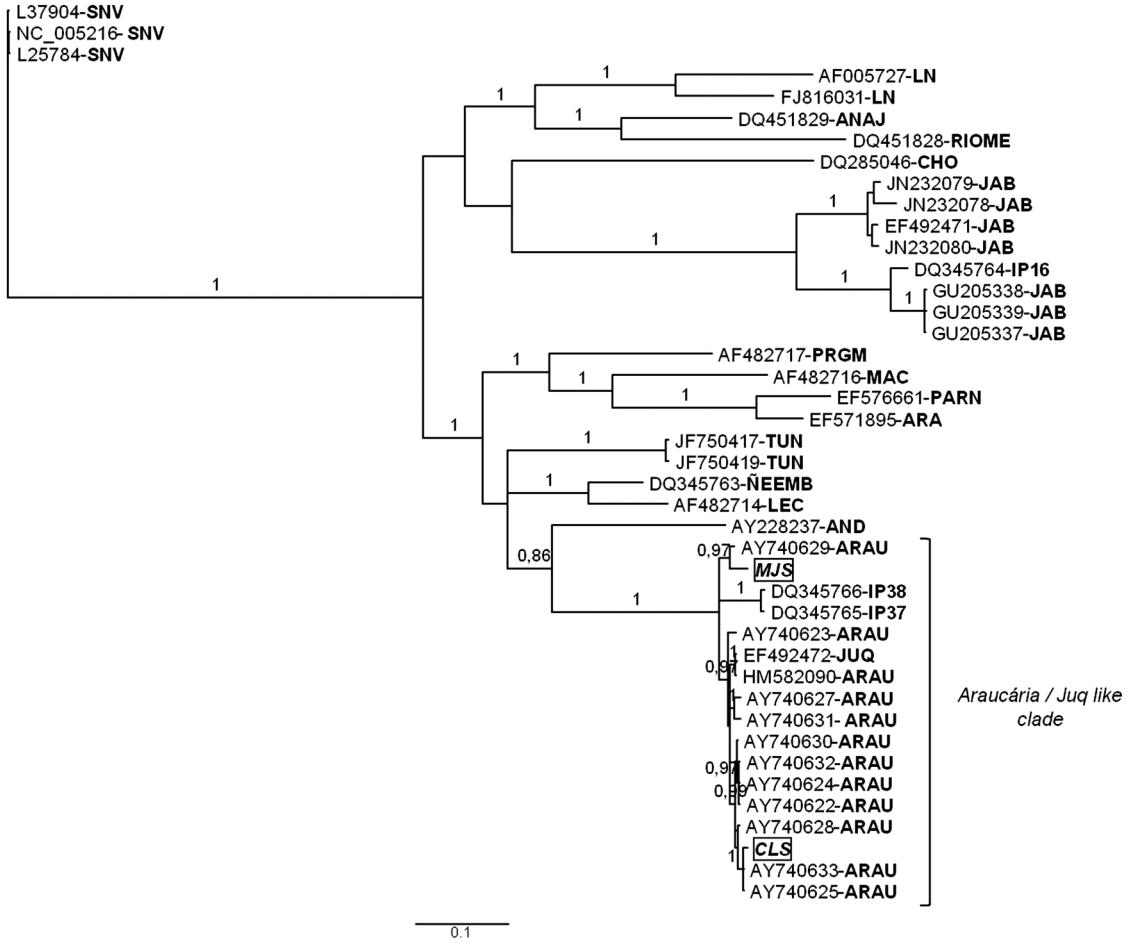

Figure 2 Phylogenetic tree of partial S segment sequences. Sequence editing and alignment were conducted with BioEdit V.7.0.9 package. The appropriate model of nucleotide substitution was estimated by using the ModelGenerator software, resulting in a GTR+I+G model. Phylogeny was constructed under Bayesian inference, using MrBayes software (V.3.1.2). Two runs of four chains, each were run for 2×106 generations; sampling trees every 100 generations. Burn-in was set to 25% and convergence was evaluated checking the average SD in partition frequency values across independent analyses (0.01 or less). Posterior probabilities are depicted above the nodes; scale bar indicates expected changes per site. GenBank accession numbers for the here reported sequences are JQ952598 (index case) and JQ952599 (case 2).

transmission has occurred in the patients described here, considering the viral genetic differences observed in the phylogenetic study. The husband of the index case tested negative for both IgM and IgG against hantavirus. Besides, household contacts concern individuals from the same conditions, exposed to the same environment, which may be the source of infection.

HPS has been reported in various regions in Brazil, and all cases reported to date in the South of the country have been caused by the Araucaria genotype. However, our analysis confirmed that the asymptomatic infection observed in this study was caused by Araucaria hantavirus. This finding highlights the possibility of other elements, such as genetic factors or viral attenuation, playing a role in disease development.

Hantavirus phenotype characterisation aiming to identify features associated with higher or lower illness severity indicated that in hamsters the disease could be attenuated by the recombination of genomic segments of the ANDV and SNV. ANDV infection induces a lethal HPS-like disease in hamsters, whereas SNV and all the other HPS-associated hantaviruses tested cause asymptomatic infections in these laboratory animals. A recombinant ANDV/SNV (L and S segments of SNV and M segment of ANDV) was generated and used to infect hamsters. This virus was highly infectious, but it did not cause HPS and was not lethal. Thus, the ANDV M genome segment is not sufficient to confer the lethal HPS phenotype associated with ANDV.[6]

In Paraná State, at least two sympatric hantaviruses genotypes, the pathogenic Araucaria and the non-pathogenic Jaborá genotypes, are known to co-circulate. We, therefore, cannot rule out the possibility of recombination between genomic segments generating a less pathogenic Araucaria variant. Alternatively, individual constraints or the presence of smaller numbers of infectious virus particles may be responsible for the findings. Further studies are required to determine the frequency of asymptomatic infection with pathogenic hantavirus strains and to identify the human and viral factors associated with disease development.

Learning points

- Hantavirus infections can reach a mortality rate of 40%, depending on the virus involved.
- This case provides the first evidence of asymptomatic infection related to the Araucaria hantavirus in Brazil.
- The fact that the patient had not developed any sign of disease stresses the possibility of other elements, such as genetic factors or viral attenuation, playing a role in disease development.

Contributors CNDDS and SMR designed the study. LDB and AD performed the experiments. SMR, AD and CNDS wrote the manuscript.

Competing interests None.

Patient consent No.

Provenance and peer review Not commissioned; externally peer reviewed.

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