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Case report

Evidence of circulation of Laguna Negra-like hantavirus in the Central West of Brazil: Case report

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ABSTRACT

Background: Hantavirus pulmonary syndrome has been reported with increasing frequency in some Brazilian regions, but information about viral genetic identification is still limited. Recently, the state of Mato Grosso, in the Legal Amazon of Brazil, experienced a growing number of hantavirus pulmonary syndrome (HPS) cases but the genetic characterization of the causative hantavirus is still missing.
Objectives: Our goal was to identify the hantavirus strain involved in a fatal HPS case in the Central region of Brazil.
Study design: Nested RT-PCR was conducted on blood clot samples from an HPS patient from Mato Grosso. PCR-positive samples were sequenced, and the resulting sequences were compared with reference samples. Viral antigens were detected by immunohistological analyses in lung and liver tissues.
Results: Analyses of the viral RNA isolated from the patient identified a Laguna Negra (LN)-like virus as the causative agent and histological analysis of lung sections were compatible with the genetic characterization.
Conclusions: This is the first report of circulation and human infection by a Laguna Negra-like hantavirus in Brazil.

1. Background

Hantaviruses are serologically related members of the family *Bunyaviridae* that occur worldwide in association with rodents and insectivore carriers. These viruses have been linked with two distinct diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).[1]

In Brazil, a surveillance program was implemented in 1993 to monitor HPS cases.[2] Afterwards, evidence for hantavirus infection was described in various regions. In order to identify the hantavirus strain involved with human infections, rodents have been sampled in diverse ecosystems and these studies have uncovered significant genetic diversity among the viruses described. Four different hantaviruses have been associated with HPS cases in Brazil so far, all of them associated with rodent species as hosts. Juquitiba-like virus is associated with *Oligoryzomys nigripes* and is found in the Atlantic rainforest and in the south and southeast, Araraquara virus is associated with *Necromys lasiurus* and is found in the savanna (cerrado) region and the central plateau, Castelo dos Sonhos virus is associated with *Oligoryzomys moojeni* and occurs in the Amazon region, and Anajatuba virus is associated with *Olygoryzomys fornesii* and is detected in the northeastern region.[3–10]

The state of Mato Grosso is located in the central west region of Brazil, it is part of legal Amazon and in its west borders Bolivia. Most of the state is covered by equatorial forest. In the west region the *cerrado*, formed by trees up 10 m high used to predominate. However, the *cerrado* has now been replaced by agricultural production and pasture in large parts of the state. Human population in the region has grown sharply in the last decade because of migration. Associated with the increase in human population, a growing number of HPS cases have been noted by the Brazilian Ministry of Health.

2. Objectives

The objective of this study is identifying the hantavirus strain related to a fatal HPS case in Mato Grosso state, Brazil and

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determined its phylogenetic relationship with circulating South American hantaviruses.

3. Study design

Serum and clot samples from a patient with suggestive symptoms of hantavirus infection were tested using specific IgM and IgG antibody assays (ELISA) and RT-PCR for viral RNA detection. Partial viral genomic S segment was sequenced and genotyped by phylogenetic reconstruction. In addition, histopathological and immunohistochemical studies were performed in tissues sections of lung and liver aiming to characterize the main target sites during hantavirus infection.

4. Results

The patient was a 19-year-old man farm worker, previously healthy, who lived in Campo Novo do Parecis, in the west of Mato Grosso (13°40′31″S 57°53′31″W). He was admitted to hospital reporting 3 days of flu-like symptoms, and after hospitalization, he developed malaise, high fever, myalgia, headache, dry cough, tiredness, dispnea, back pain, nausea, vomits, abdominal pain and diarrhea. The initial treatment was intravenous administration of one liter of normal saline solution and symptomatic drugs. Due to ongoing hypotension and hypoxia, the patient was maintained with oxygenoterapy. Vasopressors and inotropes drugs were used to uphold blood pressure and cardiac output. Three hours after his admission at the hospital, he had an asystolic cardiac arrest and resuscitation efforts were unsuccessful.

In the postmortem examination, the main histopathological features were seen in the lung, where microscopic examination revealed a moderate interstitial pneumonitis with evidence of marked pulmonary edema and very mild and focal lymphoid infiltrate. Extensive amounts of edema fluid were seen within the alveoli. Vascular thrombi, endothelial cell necrosis, focal hemorrhages, ischemic necrosis lesions, cellular responses of neutrophils and respiratory epithelium lesions were absent. Morphological changes of the endothelium, when present, consisted of swollen endothelial cells. Microscopic examination of the liver revealed a severe degree of congestion and clusters of hypertrophic and hyperplasic Kupffer cells.

Liver and lung tissues were analyzed by immunohistochemistry (IH) assays; the sections of paraffin-embedded tissues were stained with monoclonal antibody to JUQ-like virus (also known as ARAUV) nucleoprotein and a polymer-HRP anti-mouse secondary antibody (Envision$^{+®}$ System, DakoCytomation, Inc., CA, USA). The specificity of IH staining was confirmed by replacing first antibodies with phosphate-buffered saline (PBS) or isotypes-identical murine antibodies and a flavivirus monoclonal antibody (4G2). Control tissues included hantavirus infected cells as well as non-HPS autopsy tissues. Viral nucleoproteins were detected in the cytoplasm of the endothelial cells and in infiltrated mononuclear cells from lung (Fig. 1).

Serologic examination of blood samples, using JUQ-like virus recombinant nucleoprotein antigen[11] were positive for IgM and IgG. Viral RNA was extracted from blood clot using the High Pure Viral RNA kit (Roche Inc., Mannhein, GE), PCR products were synthesized by nested reverse transcriptase-PCR, primers were selected to amplify S and M segments, as previously reported.[12,13] One genomic fragment that covers the complete coding sequence of the nucleocapsid protein gene from the S segment was obtained (1.584 bp). The amplified PCR product was purified using Wizard PCR Preps. (Promega, Madison, WI, USA), and cloned into the pGEM-T easy vector (Promega, Madison, USA). Four independent clones were sequenced using an ABI 310 instrument. The nucleotide sequence had been deposited in the GenBank under the accession number

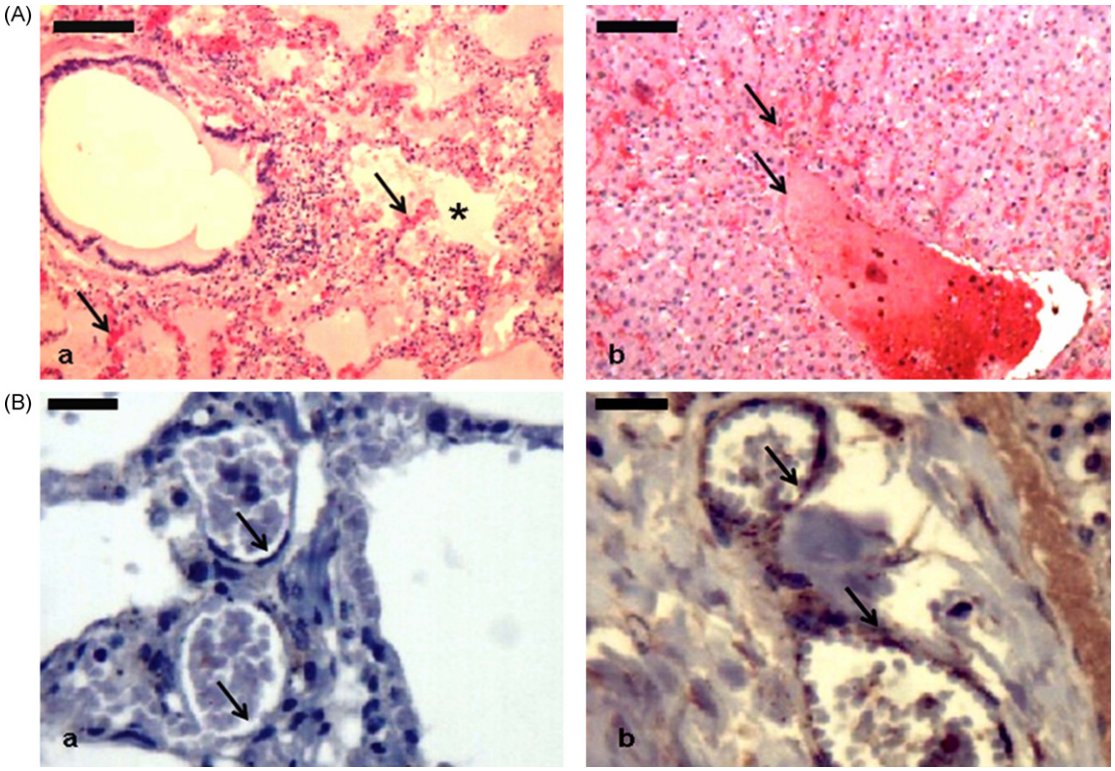

Fig. 1. Pathological findings in lung and liver tissues of a fatal case of HPS (A), and immunohistochemistry reaction (B). Hematoxilin–eosin reaction demonstrated severe capillary congestion (arrow) and extensive edema fluid in alveoli (*) (A-a). In the liver a severe degree of microvasculature congestion is noted (arrows) (A-b). The immunohistochemistry reaction confirms the HPS diagnosis, as demonstrated by the positive reaction with JUQ-like virus Mab 313/11E in contrast with the reaction using the isotype control 4G2 Mab (anti-flavivirus group-specific) (B-b and B-a).

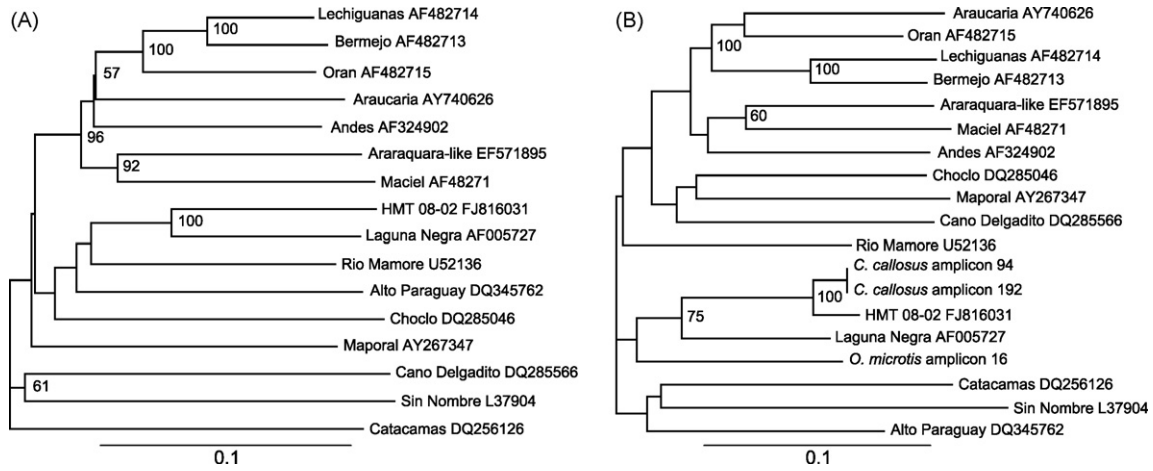

Fig. 2. Panel A. Neighbor-joining tree based on an 1.287 bp alignment of the complete coding sequence of the gene encoding for the nueclocapsid protein (N), depicting phylogenetic relationships between viral sequences from the human patient in our study (HMT 08/02), and a reference panel obtained from Genbank. Panel B. Neighbor-joining tree based on a 291-basepairs alignment of the gene encoding for the nueclocapsid protein (N), depicting phylogenetic relationships between viral sequences from the human patient in our study (HMT 08/02), and a reference panel obtained from Genbank, with 3 additional sequences from Carroll et al.[14] In the two cases sequences collected in the human patient HMT 08/02 were similar to sequences from the Laguna Negra virus (from western Paraguay and Bolivia). Sequences were aligned with ClustalW[19] and in MEGA version 4[20] using the maximum composite likelihood estimates of the Tamura-Nei distance.[21,22] Support for the nodes was evaluated with 1000 bootstrap pseudoreplicates.[23]

FJ816031. A comparison of the sequence from nucleocapsid gene showed that the closest match to the new sequence was the Laguna Negra virus, which was 86% similar at the nucleotide level, and 96% at the amino acid level. These results were confirmed by neighbor-joining phylogenies (Fig. 2).

Experiments involving human samples were approved by Ethical Committee from Brazilian Ministry of Health (CONEP) under protocol no. 10573.

5. Discussion

In Brazil, despite the increasing training of medical personnel to recognize the clinical symptoms of HPS, mortality rate is still high (~40%). The demarcation of hantavirus transmission areas and the characterization of circulating virus should contribute to better direct control measures. To identify its causative agent, we studied a fatal HPS case from the state of Mato Grosso, in the central region of Brazil. This area is of special interest because it presents a growing number of hantavirus infections, and borders Bolivia, where hantavirus circulation is well documented.[14–16]

In an ecological assessment in Santa Cruz, Bolivia, Carroll et al.[14] found two rodent species with antibodies for hantavirus, *Oligoryzomys microtis* and *Calomys callosus.* The viral nucleotide sequences isolated from two *C. callosus* were 87–88% similar to the Laguna Negra virus and 99% identical to viral sequences obtained from HPS patients in this area, implicating *C. callosus* as the host of Laguna Negra virus. More recently, IgG anti-hantavirus positive *Calomys launcha* rodents were detected in Mato Grosso.[17] Our phylogenetic analysis from the hantavirus of a fatal HPS case grouped this virus with the Laguna-Negra hantavirus (Fig. 2).

The observed pulmonary histopathological features are in agreement with those described by Zaki et al.[18] consisting of an interstitial pneumonitis with a variable mononuclear cell infiltrate, edema, and focal hyaline membranes. IHC analysis showed widespread presence of hantaviral antigens in endothelial cells of the microvasculature in the lung. Hantaviral antigens were also observed within follicular dendritic cells, macrophages, and lymphocytes.

In this report we confirmed the circulation of a Laguna Negra-like hantavirus virus in the west of Brazil and emphasize the importance of monitoring the seroprevalence for hantaviruses in rodent hosts from different regions of Brazil. In addition, the genetic characterization of the involved viruses, is critical to implementing adequate control measures to protect the exposed populations in regions which were previously free of hantavirus infection.

Conflict of interest

The authors have declared that no competing interests exist.

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