ARARAQUARA HANTAVIRUS IN NECROMYS LASIURUS CAPTURED ON THE CAMPUS OF THE UNIVERSIDADE DE SÃO PAULO IN RIBEIRÃO PRETO, SP, BRAZIL

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ABSTRACT

Hantavirus is a genus including dozens of genotypes in the Bunyaviridae family. Human infection by Hantavirus occurs mainly from inhalation of aerosols from the droppings of infected wild rodents. American Hantaviruses produce a severe cardiopulmonary syndrome with a high fatality rate. The objective of this study was to identify rodents infected with Hantavirus on the campus of the Universidade de São Paulo in the municipality of Ribeirão Preto. A routine rodent trapping with 45 Sherman traps was performed; 15 rodents were captured and identified, using classic taxonomic classification. About 2/3 of the captured rodents were Necromys lasiurus. Three blood samples of N. lasiurus were IgG positive to Hantavirus in ELISA. The Hantavirus partial S and M gene sequences were recovered from the blood of one N. lasiurus and the phylogenetic analysis of nucleotide sequences showed that this rodent was infected with Araraquara Hantavirus. The presence of N. lasiurus infected with Hantavirus on the campus of the Universidade de São Paulo is a frightening problem. Measures for prevention of human infections by Hantavirus should be implemented on the campus, based on: reducing rodent shelter and food sources in and around buildings, standard sanitary precautions while rodent-contaminated areas are being cleaned up, and preventive measures, such as biosafety masks, for persons who have occupational exposure to wild rodents.

Keywords: Necromys lasiurus, Araraquara Hantavirus, wild rodents

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INTRODUCTION

Hantavirus is a genus that includes more than 20 species in the family Bunyaviridae. These viruses have a genome with trissegmented RNA and negative polarity, defined as small, medium, and large RNAs (Jonsson et al., 2010). Hantaviruses are rodent viruses that are transmitted to man by inhalation of aerosols containing excreta or by direct contact with infected rodents (Figueiredo, et al. 2009; Jonsson et al. 2010). These viruses can cause two serious illnesses in humans: hemorrhagic fever with renal syndrome (HFRS), which occurs in Asia and Europe, and cardio-pulmonary syndrome (HPCS) which is characterized by respiratory failure, shock, and high mortality, being an important public health problem in the Americas (Schmaljohn et al. 1997; Jonsson et al. 2008; Figueiredo et al. 2009; Jonsson et al. 2010).

In Brazil, about 1400 cases of HPCS have been reported since 1993, and fatalities occurred in 41% of these cases (Ministério da Saúde, 2011). The viruses known to cause HPCS in Brazil are Juquitibá, Araraquara (ARAV), Laguna Negra-like, Castelo dos Sonhos, and Anajatuba (Figueiredo et al. 2009; Jonsson et al., 2010). In cities of the state of São Paulo, Ribeirão Preto and neighboring cities, there occurred about 80 of these HPCS cases with a 50% case fatality rate (Ministério da Saúde, 2011). This infection is commonly detected in rodents by serology (antibodies anti-Hantavirus) and/or detection of the viral genome.

The natural reservoirs of Hantavirus in the New World are rodents of the family Cricetidae of the subfamilies Sigmodontinae and Arvicolinae (Schmaljohn et al. 1997; Jonsson et al. 2010). In the southeast and central plateau of Brazil, the Juquitiba virus is associated with the Oligoryzomys nigripes rodent and the Araraquara virus with Necromys lasiurus (Suzuki et al. 2004; Figueiredo et al. 2009). N. lasiurus lives in the Cerrado areas of the central plateau and southeastern Brazil, which includes the city of Ribeirão Preto (Figueiredo et al. 2009; Figueiredo et al. 2010).

In order to better understand the Hantavirus transmission to man and to control the occurrence of...
HCPS cases, it is important to check Hantavirus infection levels among wild rodent populations in the region of Ribeirão Preto. The Centro de Pesquisa em Virologia de Faculdade de Medicina [Virology Research Center of the Ribeirão Preto Medical School] in Ribeirão Preto, SP, Brazil has been studying and diagnosing cases of HCPS, as well as capturing wild rodents for the detection of the Hantavirus. The objective of this study was to identify wild rodents infected with Hantavirus on the campus of the Universidade de São Paulo in Ribeirão Preto.

MATERIAL AND METHODS

Trapping and manipulation of rodents

From April to May 2011, three rodent captures were carried out at different places on the campus of the Universidade de São Paulo in Ribeirão Preto. All these places had a grass landscape including Brachiaria decumbens whose seeds are an important source of food for wild rodents. Forty-five Sherman traps containing vanilla extract mixed with corn and oatmeal flour as bait were prepared and placed in the grass in the afternoon, left overnight, and reviewed on the morning of the next day. Rodents eventually trapped were anesthetized, identified by morphological characteristics, and classified by age group (young, sub-adult and adult). The rodent blood was collected by puncturing the retro-orbital venous plexus, using heparinized capillary tubes, centrifuged at 15,000 x g for 10 minutes to obtain serum. The sera were employed for detection of Hantavirus genome by RT-PCR and of antibodies to Hantavirus by ELISA. Ear fragments were collected for future molecular characterization and confirmation of the rodent species. All the procedures for capturing and handling of wild animals were authorized by IBAMA (0115/07 SUPESP / Fauna / LIC) and by the Ethics Committee on Animal Experiments (CETEA) of the Faculdade de Medicina, Universidade de São Paulo in Ribeirão Preto (113/2006).

Serologic test

For detection of IgG antibodies to Hantavirus, an ELISA was performed using the recombinant nucleoprotein of ARAV (rN-ARAV) as antigen, as described, using ten microliters of each sample (Figueiredo et al. 2008; Figueiredo et al. 2009B; Machado et al. 2011). Sera were considered positive when the observed absorbance value was greater than the cut-off. This cut-off was calculated as the average absorbance value of the known negative samples added to three standard deviations.

Reverse transcription-polymerase chain reaction (RT-PCR)

The viral genome (viral RNA) was extracted from the rodent serum samples with a kit for viral RNA extraction (Qiagen, USA). A double RT-PCR that amplifies both, the 264 nt of the N gene in the viral RNA S segment and the 322 nt in the Gn gene of the viral M segment was performed as described (Morelli et al. 2004; Machado et al. 2009). The obtained amplicons were fractionated in agarose gel electrophoresis.

Nucleotide sequencing of amplicons and phylogenetic analysis

Amplicons obtained by the RT-PCR were purified with the QIAquick kit (Qiagen, USA) and subjected to nucleotide sequencing using BigDye Terminator v3.1 Cycle Sequencing 5X Kit (Applied Biosystems, USA). The resulting nucleotide sequences were analyzed using the ABI Prism* 3100 sequencer Genetic Analyzer (Applied Biosystems, USA). These sequences were confirmed using the BioEdit software and compared with those of S and M segments of other Hantavirus previously subtracted from the GenBank (National Center for Biotechnology Information - NCBI). Sequences from the following Hantavirus (including acronym and GenBank identification numbers) were used for the S segment: Araraquara virus (ARAV - EF564353.1; ARAV - EF571895.1; ARAV - EF564352.1; ARAV - EF564354.1; ARAV – EU170238; ARAV - EF564349.1 and ARAV - EF564350.1); Juquitiba virus (JUQV – EU373731 and JUQV – EF492472); Rio Mamore virus (RMV – AF133254.1); Laguna Negra virus (LNV – AF005727.1); Anajatuba virus (ANJV – DQ451829.1); Castelo dos Sonhos virus (CASV – HQ719472 and CASV – HQ719471); Bermejo virus (BMJV – AF482713.1); Lechiguanas virus (LECV – AF482714.1); Muleshoe virus (MULV – U54575.1); New York virus (NYV – U09488.1); Rio Segundo virus (RIOSV – U18100.1); Sin Nombre virus (SNV – L25784.1 and SNV – AF281851.1); Black Creek Canal virus (BCCV – L39949.1); Bayou virus (BAYV – L36929.1); and Haantan virus (HTNV – GU329991.1). For the M segment were used the following Hantavirus sequences (including acronym and GenBank identification numbers): Araraquara virus (ARAV – EU170185.1; ARAV – EU170182.1; ARAV – EU170167.1; ARAV – EU170166.1 and ARAV – EU170163.1; Castelo dos Sonhos (CASV – AF307326.1), Bermejo virus (BMJV – AF028025.1); Lechiguanas (LECV – AF028022.1), Oran virus (ORNV – AF028024.1), El Moro Canyon virus (EMCV – U26828.1), Sin Nombre virus (SNV – L37903.1 and SNV – L25783.1); Seoul virus (SEOV – AB457794.1).

Sequences of each segment were aligned using the CLUSTAL W software, available in the MEGA 5.5 program, and the percent of similarity and identity among sequences (Kumar et al., 2004) were determined. The model used in both phylogenetic analyses was of maximum composite likelihood distance, and the analyses were done by Neighbor-Joining (NJ) available in the MEGA 5.5 program.

RESULTS

A total of 15 wild rodents were captured and...
morphologically identified as the following species: 10 *Necromys lasiurus* (66.7%), 2 *Akodon sp.* (13.3%), 2 *Mus musculus* (13.3%) and 1 *Calomys sp* (6.7%). Most of the animals were female (13 - 80.0%), as shown in Table 1. The animal taxonomy will be confirmed by molecular characterization (to be published elsewhere).

**Table 1.** Identification of wild rodents captured at the Universidade de São Paulo in Ribeirão Preto and title of serum antibody anti-hantavirus, using the recombinant nucleoprotein of Araraquara virus.

<table>
<thead>
<tr>
<th>Identification Number</th>
<th>Species</th>
<th>Genus</th>
<th>Age Classification</th>
<th>Antibodies title/10 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td><em>Ákodon sp.</em></td>
<td>Male</td>
<td>Adult</td>
<td>NR</td>
</tr>
<tr>
<td>R2</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>256</td>
</tr>
<tr>
<td>R3</td>
<td><em>Mus musculus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R4</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R5</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R6</td>
<td><em>Calomys sp.</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R7</td>
<td><em>Necromys lasiurus</em></td>
<td>Male</td>
<td>Subadult</td>
<td>1024</td>
</tr>
<tr>
<td>R8</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R9</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R10</td>
<td><em>Ákodon sp.</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R11</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R12</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R13</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Young</td>
<td>512</td>
</tr>
<tr>
<td>R14</td>
<td><em>Necromys lasiurus</em></td>
<td>Female</td>
<td>Subadult</td>
<td>NR</td>
</tr>
<tr>
<td>R15</td>
<td><em>Mus musculus</em></td>
<td>Male</td>
<td>Young</td>
<td>NR</td>
</tr>
</tbody>
</table>

**NR.** Nonreactive.

Sera of three *N. lasiurus*, R2, R7, R13, showed IgG antibodies to Hantavirus in the ELISA at 256, 1024 and 512 titers/10 microliters, respectively (Table 1). However, Hantavirus RNA was amplified by RT-PCR from the serum of only one of these animals. Fragments of the S and M segments of a Hantavirus were amplified with the R13 serum, as shown in Figure 1.

DNA amplicons derived from the R13 serum had their nucleotides sequenced. A 264 nt sequence of the N gene in the S viral RNA segment, located between nucleotides 261 and 583, showed an identity of 95% and a similarity of 98% were found as compared with the ARAV sequence (EU170182.1). Nucleotide sequences were phylogenetically analyzed with sequences from the GenBank. This analysis generated two phylogenetic trees, showing that the two sequences of segments S and M from detected Hantavirus in R13 rodent clustered with sequences of the ARAV, as shown in Figures 2 and 3.
DISCUSSION

The ecosystem changes generated by urban growth and agricultural activity have led to profound changes in the species and the populations of wild rodents. These changes have extinguished some rodent species and others, called opportunistic, have adapted to the new environment and approached man in search for food (Mills 2005). Many wild rodents are natural reservoirs of microorganisms that cause human disease, for example, the Hantaviruses, emerging viruses that have gradually become a public health problem in Brazil (Schmaljohn et al. 1997; Figueiredo et al. 2009; Jonsson et al. 2010).

The campus of the Universidade de São Paulo in Ribeirão Preto is located on an old coffee plantation in the western part of the city and has a wide territorial extension (5.8 million m²). Despite the ever-expanding construction of buildings, many sites preserve their native vegetation or grass areas that include B. decumbens, a plant whose seeds are a favorite food for wild rodents. Fifteen rodents were captured on the campus and 80% of them were N. lasiurus, one of the most abundant small mammals found in the Cerrado (savannah) areas of the southeastern and central plateau of Brazil. N. lasiurus is also the rodent-reservoir of Araraquara Hantavirus (Suzuki et al. 2004; Figueiredo et al. 2009; Jonsson et al. 2010).

It was observed that 1/3 of the captured N. lasiurus were infected by Hantavirus, based on a positive IgG-ELISA. However, Hantavirus genome was found in only one animal. Little is known about Hantavirus infection in N. lasiurus, but it should be noted that the other two animals completely eliminated the virus. Alternatively, it is possible that the animals, after a long period of infection, could maintain a low viral load, not allowing detection of the agent by RT-PCR (Moreli et al. 2004; Schönrich et al. 2008; Easterbrook et al. 2008). The phylogenetic analysis showed that the Hantavirus infecting one of the captured N. lasiurus was the ARAV, which is the most common cause of HCPS in the Ribeirão Preto region (Figueiredo et al. 2009; Figueiredo et al. 2010). This is worrying, because this Hantavirus, based on case-fatality ratio, is probably the most virulent among all Hantaviruses worldwide (Figueiredo et al. 2009).

Considering that there are rodents infected with Hantavirus on the campus of the Universidade de São Paulo in Ribeirão Preto, even though a human Hantavirus case was never reported on the campus, there is a potential risk of infection for the local population. Thus, prophylactic measures should be taken by the campus population, especially by gardeners, stoneworkers, and animal facility workers in order to avoid Hantavirus infections. These measures include the reduction of rodent shelters and food sources in and around buildings, and standard precautions for persons who have occupational exposure to wild rodents, including wearing safety masks when rodent-contaminated areas are being cleaned up.

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