Novel orthohepeviruses in wild rodents from São Paulo State, Brazil

William Marciel de Souza, Marilia Farignoli Romeiro, Gilberto Sabino-Santos Jr, Felipe Gonçalves Motta Maia, Marcilio Jorge Fumagalli, Sejal Modha, Marco Roberto Teixeira Nunes, Pablo Ramiro Murcia, Luiz Tadeu Moraes Figueiredo

A Virology Research Center, Ribeirão Preto Medical School, University of São Paulo, Av. Bandeirantes, 3900, Monte Alegre, 14049-900 Ribeirão Preto, SP, Brazil
b MRC-University of Glasgow Centre for Virus Research, Glasgow, United Kingdom
c Laboratory Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil
d Center for Technological Innovations, Evandro Chagas Institute, Ministry of Health, Ananindeua, Pará, Brazil

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ABSTRACT

The Hepeviridae comprise single-stranded positive-sense RNA viruses classified into two genera, Orthohepevirus and Piscihepevirus. Orthohepeviruses have a wide host range that includes rodents, but previous studies had been restricted to rodents of the Muridae family. In this study, we applied a high-throughput sequencing approach to examine the presence of orthohepeviruses in rodents from São Paulo State, Brazil. We also used RT-PCR to determine the frequency of orthohepeviruses in our sampled population. We identified novel orthohepeviruses in blood samples derived from Necromys lasiurus (1.19%) and Calomys tener (3.66%). Therefore, our results expand the host range and viral diversity of the Hepeviridae family.

1. Introduction

Hepatitis E virus (HEV) is a single-stranded, positive-sense RNA virus with a genome of 6.6–7.3 kb in length that belongs to the genus Orthohepevirus within the Hepeviridae family (Purdy et al., 2017; Smith et al., 2014). HEV is a leading cause of acute sporadic hepatitis and fulminant hepatic failure in humans (Kamar et al., 2014). Human infections can be acquired via contaminated water, consumption of undercooked or raw meat, direct contact with infected animals, and environmental contamination by animal manure run-off (Doceul et al., 2016). The disease caused by HEV is generally self-limiting with mortality rates of 0.5–3% in adults. In addition, recent studies have shown that HEV can infect neural cell lines, suggesting that HEV can cause in rare cases extrahepatic manifestations (Kamar et al., 2014; Zhou et al., 2017).

HEV is prevalent in a wide range of mammals and has been reported in developing and industrialized countries (Kamar et al., 2014). Our knowledge about the number of hosts harboring infections by members of the Hepeviridae family have been dramatically expanded in recent years, as well as the diversity of virus variants (Doceul et al., 2016). The Hepeviridae family is divided into two genera, the Piscihepevirus genus that includes a single species detected in salmonids (Smith et al., 2014), and the Orthohepevirus genus, comprised of four species: Orthohepevirus A includes viral strains known to infect humans, swine, camels, sheep, boars, deer, rabbits, and mongooses as well as rats; Orthohepevirus B comprises viral strains reported in birds; Orthohepevirus C includes viral strains described in rats, shrews, bandicoots, mink and ferrets, and Orthohepevirus D contains viral strains identified in bats (Purdy et al., 2017; Smith et al., 2014; Spahr et al., 2017). Currently, HEV exhibits a broad range of hosts including rodents of the Muridae family (Doceul et al., 2016; Lack et al., 2012; Li et al., 2013; Purcell et al., 2011). Here, we used a high-throughput sequencing (HTS) approach to identify and characterize orthohepeviruses present in blood samples derived from wild rodents in the northeastern region of São Paulo State, Brazil.

2. Materials and methods

2.1. Rodent samples

Between 2008 and 2013, blood samples were collected from 647 wild rodents in the rural area of Ribeirão Preto, São Paulo State, Brazil. Based on morphological features and cytochrome-b gene, the rodents were classified in five different species, Akodon montensis, Calomys tener, Oligoryzomys nigripes, Necromys lasiurus and Mus musculus (Bonvicino et al., 2008; Salazar-Bravo et al., 2013). Then, samples were divided into pools based on species and collection date (Table 1).
2.3. Genome characterization and phylogenetic analysis

RNA mini kit (Qiagen, USA) and stored at −80 °C. Subsequently, nucleic acids were quantified using a Qubit® 2.0 Fluorometer (Invitrogen, Carlsbad, USA) and the purity and integrity of nucleic acid samples were measured using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). cDNA synthesis was performed using SuperScript II (Invitrogen, Carlsbad, USA). Sequencing was performed using the TruSeq RNA sample preparation kit in an Illumina HiSeq. 2500 instrument (Illumina, USA) with a paired-end and 150-base-read protocol (Invitrogen, Carlsbad, USA). Sequencing was performed using the QIAamp viral RNA extraction kit (Qiagen, Germany) and converted to cDNA using Superscript III (Invitrogen, USA) with random hexamers (Invitrogen, USA), following the manufacturer’s instructions. Subsequently, PCR was performed using Platinum SuperFi DNA Polymerase (Thermo Fisher Scientific, USA), following the manufacturer’s instructions. Cycling conditions were: 98 °C for 30 s followed by 35 cycles at 98 °C for 10 s, 70 °C for 10 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Amplicons were visualized by gel electrophoresis in 1.5% agarose gels. All PCR products were verified by dyeoxy sequencing using ABI 3730 genetic analyzer (Applied Biosystems, USA).

2.4. Frequency of new orthohepeviruses

To determine the authenticity and frequency of viral genomes of novel orthohepeviruses, we designed primer sets to specifically amplify an ~800 bp long sequence between ORF1 and ORF2 gene of the viruses identified in this study (forward primer: 5′-GAGGGCCGGGCCTC(T/G)TTTGT-3′; reverse primer: 5′-CAGTGGGTTTAACGGCGGCG CAT-3′). Then, the viral RNA of individual rodents samples was extracted using the QIAamp viral RNA extraction kit (Qiagen, Germany) and converted to cDNA using Superscript III (Invitrogen, USA) with random hexamers (Invitrogen, USA), following the manufacturer’s instructions. Subsequently, PCR was performed using Platinum SuperFi DNA Polymerase (Thermo Fisher Scientific, USA), following the manufacturer’s instructions. Cycling conditions were: 98 °C for 30 s followed by 35 cycles at 98 °C for 10 s, 70 °C for 10 s and 72 °C for 30 s, followed by a final extension of 72 °C for 5 min. Amplicons were visualized by gel electrophoresis in 1.5% agarose gels. All PCR products were verified by dyeoxy sequencing using ABI 3730 genetic analyzer (Applied Biosystems, USA).

3. Results and discussion

The HTS analysis of fifteen pools of blood rodents generated a total of 9252,300 to 27,569,342 paired-end reads (Table 1). After assembly, we identified a nearly complete genome of a novel strain of HEV in the blood pool (Pool 5B – Table 1) of Hairy-tailed bolo mouse (Necromys lasiurus) with 6830 nucleotides (nt) and two partial genomes of other strain, one from ORF1 gene with 752 nt and another from capsid gene of 1500 nt in the blood pool (Pool 12B – Table 1) of Delicate vesper mouse (Calomys tener), which were tentatively been designated as Necromys HEV (NeHEV) and Calomys HEV (CaHEV), respectively (Fig. 1a). The nearly complete NeHEV sequence was 6830 nucleotide long and exhibits a typical genome organization associated with members of the Orthohepevirus genus, which contains three open-reading frames (ORFs) that encode for the non-structural polyprotein (ORF1), Capsid (ORF2) and ORF 3 protein (Ding et al., 2017; Kamar, 2014). Analysis of the non-structural polyprotein and capsid using BLASTX showed that the NeHEV and CaHEV shared between 67% and 76% amino acid identity with strains of orthohepevirus described in rodents from China (GenBank No. KY432901), and less than 62% amino acid identity with other representative orthohepeviruses.

Phylogenetic analysis using the RdRp gene showed that NeHEV and CaHEV did not cluster in any of the four species previously recognized for genus Orthohepeviridae. Instead, they formed a unique and monophyletic clade with orthohepeviruses derived various animal species including a fox captured in the Netherlands (Bodewes et al., 2013), a kestrel from Hungary (Reuter et al., 2016) and other strains of orthohepeviruses identified in rodents from China (Fig. 1b). This novel clade is more closely related to the Orthohepevirus C species that include viruses derived from Rodentia, Carnivora, and Soricomorpha (Smith et al., 2014). However, the novel clade that included the Necromys HEV and Calomys HEV have p-distances of less than 0.5 to other species of genus Orthohepevirus as showed in Fig. 2 (Smith et al., 2014).

Table 1

<table>
<thead>
<tr>
<th>Pool</th>
<th>Species</th>
<th>N</th>
<th>Collection date</th>
<th>Number of reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>Akodon montensis</td>
<td>55</td>
<td>2008</td>
<td>27,569,342</td>
</tr>
<tr>
<td>2B</td>
<td>Akodon montensis</td>
<td>55</td>
<td>2008</td>
<td>24,339,326</td>
</tr>
<tr>
<td>3B</td>
<td>Akodon montensis</td>
<td>41</td>
<td>2009</td>
<td>16,698,848</td>
</tr>
<tr>
<td>4B</td>
<td>Akodon montensis</td>
<td>48</td>
<td>2012-2013</td>
<td>18,783,944</td>
</tr>
<tr>
<td>5B</td>
<td>Calomys tener</td>
<td>38</td>
<td>2008</td>
<td>27,037,352</td>
</tr>
<tr>
<td>6B</td>
<td>Calomys tener</td>
<td>37</td>
<td>2008</td>
<td>23,972,304</td>
</tr>
<tr>
<td>7B</td>
<td>Calomys tener</td>
<td>34</td>
<td>2009, 2012-2013</td>
<td>15,679,756</td>
</tr>
<tr>
<td>8B</td>
<td>Necromys lasiurus</td>
<td>59</td>
<td>2008</td>
<td>22,899,548</td>
</tr>
<tr>
<td>9B</td>
<td>Necromys lasiurus</td>
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<td>2008</td>
<td>2952,300</td>
</tr>
<tr>
<td>10B</td>
<td>Necromys lasiurus</td>
<td>58</td>
<td>2008</td>
<td>18,213,066</td>
</tr>
<tr>
<td>11B</td>
<td>Necromys lasiurus</td>
<td>59</td>
<td>2002, 2012-2013</td>
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</tr>
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<tr>
<td>13B</td>
<td>Oligoryzomys nigripes</td>
<td>43</td>
<td>2008-2009</td>
<td>15,813,430</td>
</tr>
<tr>
<td>14B</td>
<td>Oligoryzomys nigripes</td>
<td>20</td>
<td>2012-2013</td>
<td>24,796,054</td>
</tr>
<tr>
<td>15B</td>
<td>Mus musculus</td>
<td>24</td>
<td>2008-2009</td>
<td>16,661,928</td>
</tr>
</tbody>
</table>

* N: number of individual samples per pool.
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(a)  

Necromys HEV  
6,830 nt  

Calomys HEV  
752 nt (contig 1)  
1,500 nt (contig 2)  

(b)  

Orthohepeviruses  

HeV-C1  

HeV-C2  

New  

Orthohepeviruses  

Moose  

A  

B  

C  

D  

Little egret  

(caption on next page)
Orthohepevirus was identified in small mammals including shrews and rodents. In this novel clade, the dietary source of virus should also be considered as those birds feed on small mammals. In a previous study, it was suggested that the gastrointestinal tract could play an important role in the spread of this virus in the environment, but this feature needs to be elucidated.

Fig. 1. (a) Genome organization of the complete coding sequence of Necromys HEV and a partial genome of Calomys HEV. The length of the determined nucleotide sequences of the viral sequences are shown at the top. Solid-lined boxes and dashed-lined arrows indicate complete or partial sequence of ORFs, respectively. ORF1 encodes non-structural proteins including putative functional domains. MT: methyltransferase; Macro: Macro domain profile; Hel: helicase; RdRp: RNA-dependent RNA polymerase. ORF2 (green) encodes capsid protein and ORF3 (blue) encodes a small phosphoprotein with a multi-functional C-terminal region. (b) Maximum likelihood phylogenetic tree showing the evolutionary relationships of viruses identified in our study with representatives of the Hepeviridae family. Phylogenies are midpoint rooted for clarity of presentation. The scale bar indicates evolutionary distance in numbers of substitutions per amino acid site. Bootstrap values of 1000 replicates are shown in principal nodes. HEV sequences generated in this study are shown in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Hepaeviridae family (Purdy et al., 2017; Smith et al., 2014), we propose that the NeHEV and CaHEV should constitute strains of a new orthohepevirus species within the genus Orthohepevirus.

To determine the prevalence of NeHEV and CaHEV in Necromys lasiurus and Calomys tener, individual samples were screened by RT-PCR. A total of 3 out of 252 samples (1.19%) derived from Necromys lasiurus and 4 out of 109 (3.66%) samples derived from Calomys tener were PCR positive. As expected, positive samples were identified only in pools where the viruses had been identified by HTS.

The orthohepeviruses closely related to NeHEV and CaHEV were recently identified in common kestrels (Falco tinnunculus) and red-footed falcons (Falco vespertinus) from Hungary (Reuter et al., 2016). Furthermore, a previous study reported the mammalian HEV in fecal samples of Himalayan griffons (Gyps himalayensis) of Beijing Zoo in China (Li et al., 2015). Based on the high orthohepevirus viral load observed in kestrel feces it was suggested that the gastrointestinal tract might be the site of viral replication (Reuter et al., 2016). However, a dietary source of virus should also be considered as those birds feed on small mammals including shrews and rodents. In this novel clade, the orthohepevirus identified in fecal samples of fox (Vulpes vulpes) in Netherlands, suggests that this virus could be derived from their prey (e.g., rats) (Bodewes et al., 2013). Our results showed that rodents presented viremia, suggesting that viral replication might take place in these hosts and further indicate that rodents might act as natural reservoirs for this virus. Furthermore, infection of carnivore birds and foxes through the dietary route and presence of viruses in the feces could play an important role in the spread of this virus in the environment, but this feature needs to be elucidated.

In the current study, we detected the novel HEV in 1.19% samples of Hairy-tailed bolo mice and 3.66% in samples of Delicate vesper mouse from a rural area in São Paulo State, Brazil. Comparative genomic and phylogenetic analyses showed that NeHEV and CaHEV represent previously unrecognized orthohepevirus species. Our results expanded the ecology of HEV among wild rodents and now include members of the Cricetidae family.