Short Report: Maripa Hantavirus in French Guiana: Phylogenetic Position and Predicted Spatial Distribution of Rodent Hosts

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Abstract. A molecular screening of wild-caught rodents was conducted in French Guiana, South America to identify hosts of the hantavirus Maripa described in 2008 in a hantavirus pulmonary syndrome (HPS) case. Over a 9-year period, 418 echimyids and murids were captured. Viral RNA was detected in two sigmodontine rodents, Oligoryzomys fulvescens and Zygodontomys brevicauda, trapped close to the house of a second HPS case that occurred in 2009 and an O. fulvescens close to the fourth HPS case identified in 2013. Sequences from the rodents had 96% and 97% nucleotide identity (fragment of S and M segments, respectively) with the sequence of the first human HPS case. Phylogenetic reconstructions based on the complete sequence of the S segment show that Maripa virus is closely related to Rio Mamore hantavirus. Using environmental descriptors of trapping sites, including vegetation, landscape units, rain, and human disturbance, a maximal entropy-based species distribution model allowed for identification of areas of higher predicted occurrence of the two rodents, where emergence risks of Maripa virus are expected to be higher.

Hantaviruses (Bunyaviridae) are distributed worldwide. Although certain types are non-pathogenic in humans, others are responsible for hemorrhagic fever with renal syndrome in the Old World and pulmonary syndrome in the New World. In Europe and North America, epidemiology of hantavirus is closely related to the population dynamics of their rodent hosts. In South America, the fine-scale relationships between hantaviruses and their hosts are far less well-understood in relation to the huge diversity of rodent taxa. In addition, the ecology of most species of rodents is almost unknown, and for many of them, their taxonomy continues to be debated. To date, knowledge on hantavirus diversity in the Amazonian region is sparse, with only a few reports on the Amazonas, Mato Grosso, and Para states of Brazil. In French Guiana, a French department located on the northeast coast of South America, the circulation of hantavirus was first suggested after a serological survey performed on a selected population of patients suffering from atypical pneumonia. In 2008, the occurrence of the first biological confirmed human case led to the identification of a new strain, Maripa virus, which was closely related to the Rio Mamoré species (GenBank accession number KC876041). This hantavirus was then responsible for three fatal human cases in 2009, 2010, and 2013.

Since 2005, an ecoepidemiological survey has been implemented in French Guiana to characterize rodent communities and putative hantavirus hosts. The objectives were to (1) investigate the presence of hantavirus in wild species without a priori targeting capture efforts on supposed areas of virus occurrence, (2) clarify the phylogenetic position of Maripa virus, and (3) identify more suitable habitats, with higher predicted occurrences for species identified as potential hosts and expected to be associated with higher emergence risks, by applying species distribution models (SDMs).

Rodents were sampled in 14 sites located in primary forests, edge and secondary forest habitats, and savannas for a total effort of 28,670 traps/night. The animals were identified using external and/or skull morphology and confirmed with the cytochrome oxidase I sequence. In total, 418 rodents (18 species) were captured and analyzed (Table 1).

Sera from 418 rodents were tested for immunoglobulin G (IgG) antibodies directed against Sin Nombre Virus (SNV) using an indirect enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with an SNV recombinant nucleocapsid antigen (positive antigen, lot SPR 569; Centers for Disease Control and Prevention [CDC], Atlanta, GA) or a recombinant non-viral antigen (negative antigen, lot SPR 568; CDC) diluted 1:2,000 in phosphate-buffered saline (PBS; 1 × ). Each tested serum was diluted 1:100 in 5% skim milk in PBS × containing 0.5% Tween 20. The presence of bound IgG from a wide variety of rodents was detected using an equal volume of two conjugates: anti-Rattus norvegicus and anti-Peromyscus leucopus (Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD). Positive control consisted of serum from deer mice known to be infected by SNV (lot 703110; CDC), whereas negative sera were collected from mice that previously tested negative for SNV. Serum specimens were considered SNV-positive if the net absorbance values (after subtraction of absorbance values with and without antigen) were greater than 0.2 for a 1:100 dilution. Among 418 rodents tested, none were found positive for the detection of IgG antibodies against SNV (Table 1).

Total RNA was extracted from lung and/or kidney tissues of 236 rodents using the NucliSENS easyMAG bio-robot (bioMérieux, Marcy l’Etoile, France) (Table 1). Complementary DNA (cDNA) was prepared with the SuperScript III Reverse Transcriptase (Invitrogen; Life Technologies, Inc., Life Technologies, Carlsbad, NM) and random hexamers (Roche, Mannheim, Germany). Presence of the virus was
TABLE 1

Serological and molecular evidence of hantavirus infection in wild-caught rodents in French Guiana

<table>
<thead>
<tr>
<th>Species</th>
<th>SNV-positive IgG</th>
<th>RT-PCR results*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Holochilus sciureus</td>
<td>0/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Makalata didelphoides</td>
<td>0/17</td>
<td>0/5</td>
</tr>
<tr>
<td>Mesonyx hispidus</td>
<td>0/9</td>
<td>0/7</td>
</tr>
<tr>
<td>Mus musculus</td>
<td>0/23</td>
<td>0/5</td>
</tr>
<tr>
<td>Neotoma paracou</td>
<td>0/1</td>
<td>nd</td>
</tr>
<tr>
<td>Nectomys rattus</td>
<td>0/6</td>
<td>nd</td>
</tr>
<tr>
<td>Oecomys ayantepui</td>
<td>0/13</td>
<td>0/5</td>
</tr>
<tr>
<td>Oecomys bicolor</td>
<td>0/12</td>
<td>0/8</td>
</tr>
<tr>
<td>Oecomys rex</td>
<td>0/2</td>
<td>nd</td>
</tr>
<tr>
<td>Oecomys rutillus</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Oligoryzomys fulvescens</td>
<td>0/5</td>
<td>2/5†</td>
</tr>
<tr>
<td>Oryzomys megacephalus</td>
<td>0/7</td>
<td>0/5</td>
</tr>
<tr>
<td>Orzyomys yunganus</td>
<td>0/9</td>
<td>0/8</td>
</tr>
<tr>
<td>Proechimys cayennensis</td>
<td>0/201</td>
<td>0/112</td>
</tr>
<tr>
<td>Proechimys caviieri</td>
<td>0/56</td>
<td>0/33</td>
</tr>
<tr>
<td>Rattus rattus</td>
<td>0/10</td>
<td>0/9</td>
</tr>
<tr>
<td>Rhipidomys nitelae</td>
<td>0/7</td>
<td>0/6</td>
</tr>
<tr>
<td>Z. brevicauda</td>
<td>0/36</td>
<td>2/25†</td>
</tr>
<tr>
<td>Total</td>
<td>0/418</td>
<td>4/236</td>
</tr>
</tbody>
</table>

Taxonomy follows ref. 33. nd = not done.

*Based on the amplification of the 434-bp fragment of the S segment.
†Number of positive rodents/number of tested rodents.

investigated by nested reverse transcription polymerase chain reaction (RT-PCR) using primers previously designed for amplification of fragments of the S and M segments of New World hantaviruses. Amplification products were cloned using the TOPO-TA cloning system (Invitrogen, Life Technologies, Carlsbad, NM). Plasmids were sent for sequencing to Beckman Coulter Genomics (Takeley, United Kingdom). Sequences were aligned using MEGA 5.14 The phylogenetic tree was inferred from alignment of nucleotide sequences. The global time reversible (GTR) model, with \( \gamma \)-distribution and (I) proportion of invariable sites, was identified as the optimal model of nucleotide evolution (MrModeltest 2.2).15 Phylogenetic relationships were inferred with Bayesian approach performed with Mr. Bayes 3.1.b.16 Markov Chain Monte Carlo simulations were run for 1,000,000 generations with four simultaneous chains using a sample frequency of 1,000 and a burn-in of 25%. Validation of the inference was assessed based on the SD of split frequencies, which was less than the expected threshold value of 0.01; 4 of 236 animals analyzed molecularly were positive: two Zygodontomys brevicauda and two Oligoryzomys fulvescens (Cricetidae and Sigmodontinae) (Table 1). Three of those animals (2 Z. brevicauda and 1 O. fulvescens) were collected around the second human case of hantavirus pulmonary syndrome (HPS). Hantavirus sequences obtained on the S segment from the three rodents trapped in 2010 (GenBank accession numbers JN982967, JN982968, and JN982969) were identical on the 345 bp analyzed and are closely related (95.7% and 100% identity at the nucleotide level) to Maripa hantavirus detected from the first three rodents (Table 1). Three of those animals (2 Z. brevicauda and 1 O. fulvescens) found positive was captured around the fourth human case that occurred in 2013. The 345 bp of the S segment (GenBank accession number KJ209653) showed 95.7% identity at the nucleotide level and 99.1% identity at the amino acid level with the sequences identified from the three first rodents. This sequence is closely related to the first and second human cases (99.1% and 95.7% in nucleotides, respectively).

For phylogenetic purposes, we determined the complete sequence (length of 1,904 bp) of the S segment for the hantavirus detected in O. fulvescens captured in 2010 (GenBank accession number JN982968). The coding sequence of Maripa virus isolated from O. fulvescens presented 96.3% and 100% nucleotide identity and 99.8% and 100% amino acid identity with the two human strains of Maripa virus KC876041 and JQ611712, respectively. It presented 85.7% nucleotide identity (1,284 bp, S coding region) and 97.7% amino acid identity (428 amino acids analyzed) with the Bolivian Rio Mamoré strain. Phylogenetic analysis based on the S coding region showed that Maripa viruses are closely related to the Bolivian Rio Mamoré strain (posterior probability value [pp] = 1) (Figure 1). These two strains are closely associated with Laguna Negra and Alto Paraguay viruses (pp = 1).

Viral identification in O. fulvescens and Z. brevicauda in the immediate vicinity of fatal human cases suggests that these species could be potential reservoirs of the hantavirus Maripa strain. Additionally, capture data show the presence of O. fulvescens and Z. brevicauda around three of four human cases. Nevertheless, only O. fulvescens was found positive for hantavirus near two different cases. We can, thus, hypothesize a spillover from O. fulvescens to Z. brevicauda. The absence of SNV antibodies suggests either a very recent infection or a very low infecting dose unable to stimulate a humoral IgG response.18,19 Spillover and host switching could explain the infection of two different species: the ecology and respective reservoir performance of Z. brevicauda and O. fulvescens should be scrutinized. Considering that Z. brevicauda and O. fulvescens have already been identified as competent reservoirs of different hantaviruses20,21 and that it is now accepted that a single hantavirus strain can infect different rodent species,22–24 our results suggest that Maripa may be present in a rodent’s community of diverse sympatric and/or syntopic competence at the same collection site.

Among SDMs, maximum entropy analysis has a recognized efficiency in processing presence-only data and small datasets.25 MaxEnt 3.3.324 estimates the probability distribution of the maximum entropy of each environmental variable across the study area calculated with the constraint that the expected value of each environmental variable under this estimated distribution matches the empirical average generated from environmental values associated with species occurrence data.26 Based on the rodent trapping sessions carried out in French Guiana for 15 years, including published27 and unpublished vouchered specimens (Paris National Museum of Natural History and our own field collections), both positive species seem to be rather rare and unevenly distributed, with 28 records of Z. brevicauda and 14 records of O. fulvescens. The SDM was applied to all the cumulated records of the two species using the 116 sampling locations corresponding to all rodent trapping sessions. Four environmental layers were used; vegetation types were defined with high-definition remote sensing,28 landscape units,29 rainfall, and human footprint (representing the distribution and strength of pressures on natural habitats).30 To control the likely geographic bias of sightings distribution (i.e., trapping effort), the model was forced to use environmental layers restricted to the areas of sampling during the learning stage.31 Predicted areas of
Figure 1. Phylogenetic tree constructed using Bayesian methods (1,000,000 replicates; Mr. Bayes, version 3.1b) based on 1,308 bp of the S segment of 30 hantaviruses. The tree is based on the GTR + I + G model of nucleotide evolution. The virus names are associated with their accession numbers. The Maripa virus sequences generated in this study are in bold. The association between Maripa viruses and Rio Mamoré virus is labeled. Support for nodes is provided by the posterior probabilities of the corresponding clades. All resolved nodes have posterior probability greater than 0.8. Scale bar indicates nucleotide sequence divergence among hantavirus sequences. Focus corresponds to the phylogenetic tree based on 345 bp of the S segment of the hantavirus detected in *Oligoryzomys* captured around the four human cases.
This report is one of the first to predict more suitable habitats for the emergence of hantavirus in the Amazonian region dominated by forest habitats but including numerous patches of open habitats with substantial and increasing human-induced disturbance. The closest previous records of HPS were located more than 1,000 km away. The geographical relationship between the predicted distribution of the two host species reservoirs, \textit{Z. brevicauda} and \textit{O. fulvescens}, and the emergence risks must nevertheless be taken with caution. Indeed, hantavirus emergence is related to locally and temporally restricted rodent proliferation phenomena: these proliferations are caused by small-scale ecological events that the model may fail to detect.

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