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Genome of a husavirus from Southern Brazil

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ABSTRACT

New viruses of the *Picornavirales* order have been discovered with the increase in the number of sequences obtained by high-throughput sequencing, as well as human stool-associated RNA virus (husavirus [HuV]), found in human stool samples. However, there is much to be clarified about HuV. Its cellular host, evolutionary history, and other biological characteristics are still unknown. Therefore, samples collected from human beings and environmental samples in a watershed in Southern Brazil were processed for the metagenomic library. Upon metagenomic analysis, we identified a HuV (husavirus LMM_67754 OP019707) genome with 8,846 bp, which was reported for the first time in Southern Brazil. The new genome presents only 37% of nucleotide identity with Brazilian strains and more than 90% with genomes from China, Vietnam, Venezuela, and the Netherlands. The HuV phylogeny presents significant differences among genomes, probably because multiple introductions of the virus may have occurred. Many questions still need to be answered about HuV. Therefore, more sequences and studies on this virus are necessary to improve the comprehension of the unknown origin of *Picornavirales.*

KEY WORDS: *Picornavirales.* HuV. Stool samples.

INTRODUCTION

Picornaviruses can cause different clinical manifestations, ranging from mild illnesses to serious conditions[1]. The *Picornavirales* order includes viruses with economic and medical importance, such as poliovirus (genus *Enterovirus*), which causes meningitis, encephalitis, rashes and cardiac and muscular diseases, including severe paralysis in children and teenagers; foot-and-mouth disease virus (genus *Aphthovirus*), which causes severe and highly contagious diseases in domestic ruminants with economically significant outbreaks, requiring extensive quarantines and the slaughter of infected animals; hepatitis A virus (genus *Hepatovirus*), which causes acute hepatitis in humans; rhinoviruses (genus *Enterovirus*), which cause the common cold in humans; and cardioviruses (genus *Cardiovirus*), which infect mice and other mammals and cause cardiac or neurologic disease. Thus, members of the *Picornavirales* order infect a wide range of hosts, including vertebrates, plants, arthropods, and unicellular organisms[2].

The *Picornavirales* contain non-enveloped viruses with icosahedral particles with a format of approximately 30 nm. Most members of the order have a genome comprised of a single molecule of positive-sense RNA ranging between 7,000 and 12,500 nt in length. Some members have a bipartite genome, with a RNA1 ranging between 5,800 and 8,400 nt and a RNA2 ranging between 3,200 and 7,300 nt[2].

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Metagenomics has been enabling the discovery of new viruses, including new members of the *Picornavirales* order; however, many of them have unknown pathogenicity and cellular origins, such as porcine stool-associated RNA virus (posavirus)[3,4], fish stool-associated RNA virus (fisavirus)[5], panda stool-associated RNA virus (pansavirus)[6] and human stool-associated RNA virus (husavirus)[7-11].

Husaviruses (HuV) were identified for the first time in 2015, from stool samples collected in 1984 and 1985, and were proposed to comprise a new genus in the *Picornavirales* order[12]. Afterwards, more studies reported the finding of HuV in stool samples, suggesting a wide distribution of these viruses worldwide and presenting lineages with significant differences in their phylogenetic and genomic organization[7-11]. However, the pathogenic effects on the host cell and the possible risks to human health are unknown. Considering the importance of many viruses of the *Picornavirales* order, in this study we reported a new HuV strain found in a stool sample from a person living in a rural area of Southern Brazil, which diverges from Brazilian strains and presents a low nucleotide identity.

MATERIALS AND METHODS

As part of a larger study, the environmental samples and feces of healthy adult individuals without symptoms of respiratory and/or gastrointestinal diseases were collected in the Rio dos Sinos watershed, from Rio Grande do Sul State, Southern Brazil, totaling 60 human stool samples, 12 surface water samples and 12 groundwater samples, divided into two seasons (February in the summer and August in the winter) and three different landscapes across the watershed: rural area (site 1: -29.774224, -50.332396 – site 2: -29.581236, -50.471425); rural and urban area (site 3: -29.497796, -50.776539 – site 4: -29.607082, -50.809638); urban and industrial area (site 5: -29.731074, -51.083373 – site 6: -29.876445, -51.243318). The geographic coordinates are from the sites where the surface water samples were collected, and the groundwater and human stool samples were collected close to the aforementioned sites.

For the analysis, the samples were organized into pools, separated by season and basin region, totaling 6 pools with 10 stool samples, 6 pools with 2 surface water samples and 6 pools with 2 groundwater samples. The preparation of the stool samples began by mixing the fecal matter in 1.5 mL of sterile saline solution. This mixture was homogenized by vortexing, incubated at 6 ºC for one hand low speed centrifuged to separate the supernatant, which was filtered with a 0.45 µm membrane. 1 mL of each stool sample was pooled, totaling 10 mL. The preparation of water samples was carried out by filtration, using a 0.45 µm membrane, and the pools were composed of two samples, 18 mL each. All pools were concentrated using an ultracentrifugation protocol[13].

The nucleic acids were extracted from the concentrated pools with the commercial MagMAX™ CORE nucleic acid purification kit (Applied Biossystems, Waltham, Massachusetts, USA), using the automated KingFisher™ Duo Prime equipment (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). For stool samples, the RNA was treated with DNase, using the RQ1 RNase-Free DNase kit from Promega (Promega Corporation, Madison, Wisconsin, USA), following the manufacturer's instructions. First strand cDNA synthesis was performed with Superscript IV (Thermo Fisher Scientific Inc.) and second strand synthesis was performed with NEB Q5 polymerase (New England Biolabs, Ipswich, Massachusetts, USA), following the manufacturer's instructions. The shotgun metagenomic library was performed using the Illumina DNA Prep kit, according to the manufacturer's recommendations, and the sequencing was performed on the Illumina Miseq platform using the 600 cycle V3 reagent kit (Illumina Inc., San Diego, California, USA).

The new genome was assembled *de novo* using Genome Detective[14], and, to identify the mean coverage depth, the pair-end reads were mapped to KX673221 in Geneious Prime software (version 2022.2, Biomatters Inc., Auckland, New Zealand). For phylogeny, 24 HuV sequences were selected from GenBank and the inclusion criteria was to have a size greater than 7,000 bp and no gaps. Reference sequences of rasavirus (KX673232), posavirus (KX673273), fisavirus 1 (KM434233), basavirus (KX673228) and hepatitis A virus (M14707) were used as outgroups. Sequence alignment was performed in Geneious Prime software using MUSCLE, and the Maximum Likelihood phylogenetic analysis under the TIM2+F+G4 model was inferred in the IQ-TREE v2.1.2 webserver[15], applying 1,000 replicates.

ETHICS STATEMENT

The protocols for the use of stool samples were approved by the Research Ethics Committee at Feevale University, process Nº 19391019.8.0000.5348.

RESULTS

Upon metagenomic analysis of one pool of human stool samples collected from individuals living in a rural area, we identified a nearly full-length HuV genome (8,846 bp in length, 88.3% mean coverage depth), which we named husavirus LMM_67754 (GenBank access Nº OP019707). The genome of LMM_67754 encodes a putative polyprotein

of 2,788 aa. The assembled genome had a coverage of 99% (5,670 reads mapped to KX673221) and shared 93.96% nucleotide sequence identity with husavirus VSAD (MG571826). The HuV genome was found only in a pool of human stool samples. However, fragments of putative viruses from the *Picornavirales* order were detected in other samples. Three fragments (789–1652 bp) detected in three pools of human stool displayed 87–99% nt sequence identity with viruses of the *Picobirnaviridae* family. Three other fragments (404–880 bp) detected in three surface water samples displayed 81–89% nt identity with viruses of the *Picornaviridae* family, and one fragment (537 bp) displayed 75% nt identity with viruses of the *Marnaviridae* family. Lastly, one fragment (518 bp) detected in a groundwater sample displayed 76% nt identity with viruses of the *Picornaviridae* family, and two other fragments from the same sample (495 and 809 bp) displayed 90% and 75% nt identity, respectively, with viruses of the *Marnaviridae* family.

The new Brazilian HuV is more similar to the genomes from China, Vietnam, Venezuela, and the Netherlands (identity >90%) than to other Brazilian strains (identity around 37%) (Figure 1). The presence of HuV was not identified in the water samples taken in the same locality.

DISCUSSION

HuV has a wide geographic distribution[10]. The new HuV LMM_67754 OP019707 described here is the first reported from Southern Brazil. Until 2021, this virus had only been reported in the Central-western, Northeastern, and Northern regions[11]. Although all HuV sequences have been found in human stool samples, there are significant differences in their phylogenetic and genomic organization[10]. In this analysis, the HuV genome was found grouped in a clade with genomes from China, Vietnam, Venezuela, and the Netherlands, diverging with less than 10% nucleotide sequence identity among them (Figure 1). However, the identity with other Brazilian sequences is only around 37%, reinforcing the high divergence observed among HuV lineages[7] and suggesting that multiple introductions of the virus from different geographical regions may have occurred in Brazil.

Enteric human virome from isolated areas such as villages can present higher levels of picornavirus genomes

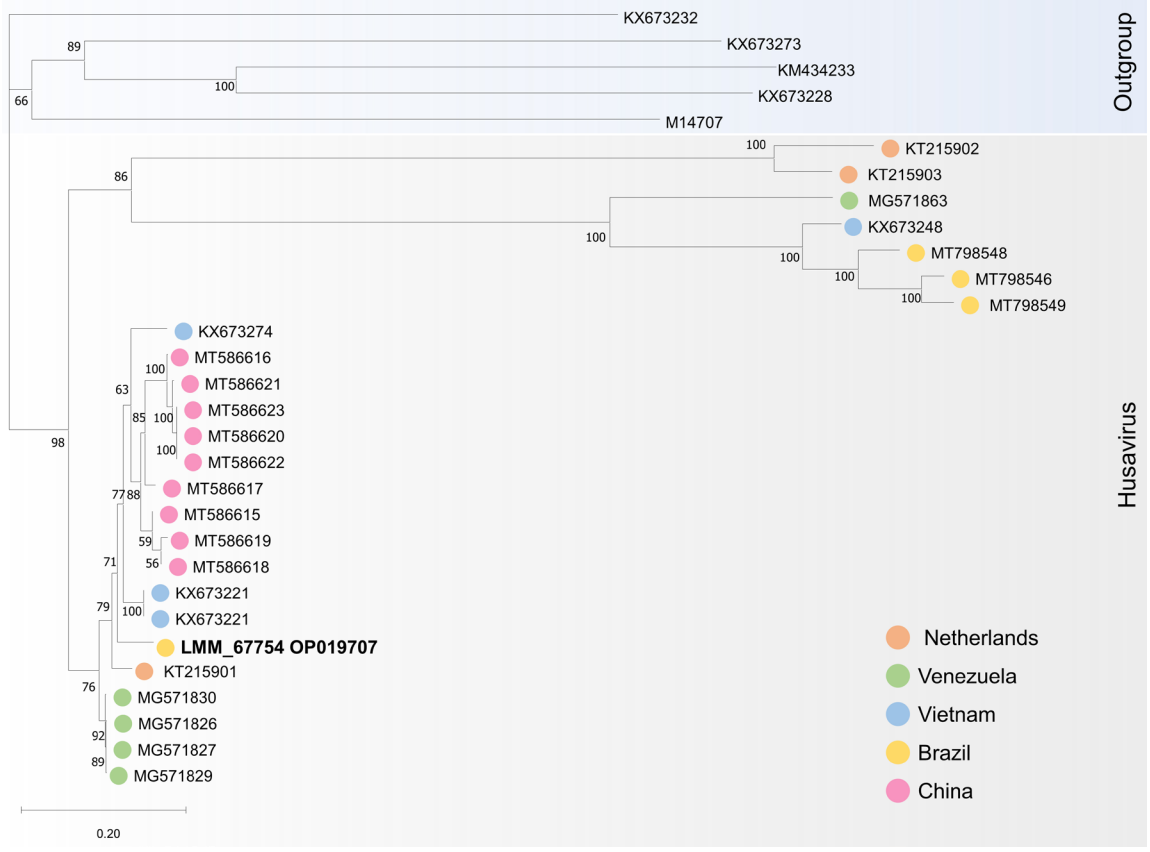

Figure 1 - Maximum-likelihood phylogenetic tree based on full-length HuV genomes. The gray block includes 25 HuV genomes. Yellow dots indicate sequences from Brazil, including HuV LMM_6775 (OP019707); orange, from the Netherlands; green, from Venezuela; blue, from Vietnam; and pink, from China. In the blue block are the outgroup sequences composed of reference genomes from rasavirus (KX673232), posavirus (KX673273), fisavirus 1 (KM434233), basavirus (KX673228) and hepatitis A virus (M14707). Bootstrap values (1,000 replications) are indicated at each branch. The scale bar indicates nucleotide substitutions per site.

than those from urban areas[9]. Corroborating with this hypothesis, the HuV genome reported in this study was found in stool samples from a rural area, composed of small properties with low population density, where agriculture and small dairy, pig and poultry farms predominate. Researchers reported the greatest number of such *Picornavirales* reads in isolated villages and also showed that children's feces from those areas can present a higher parasitic burden (protozoan and nematode DNA) compared with children from urban areas[9]. With this correlation, they presented a hypothesis that HuV hosts could be nematodes. However, there are currently no data to confirm this hypothesis. In addition, all genomes available to date were found in human stool samples and another study showed that all children's stool with HuV were free of helminths of any kind[11]. In contrast to the non-association with parasites, others picornaviruses with unknown origin, such as posaviruses, have been identified in porcine stool samples, sharing high identities with viruses infecting nematodes and arthropods (primarily insects). However, the possibility of direct porcine infection cannot be disregarded yet[3,4].

CONCLUSION

Although HuV was detected in human fecal samples four decades ago, it was only properly described in 2015. Still, its cellular host tropism and other biological characteristics remain unknown. Therefore, studies that describe new genomes are relevant for answering questions about HuV, besides helping to improve our understanding of the evolutionary history of the *Picornavirales* order. There are still few available sequences and data on HuV, so we must draw attention to it because many members of the *Picornavirales* order have great clinical and economic importance.

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