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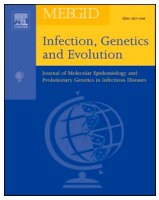

Molecular characterization of the gastrointestinal eukaryotic virome in elderly people in Belem, Para, Brazil

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ABSTRACT

Acute gastroenteritis is one of the main causes of mortality and morbidity worldwide, affecting mainly children, the immunocompromised and elderly people. Enteric viruses, especially rotavirus A, are considered important etiological agents, while long-term care facilities are considered favorable environments for the occurrence of sporadic cases and outbreaks of acute gastroenteritis. Therefore, it is important to monitor the viral agents present in nursing homes, especially because studies involving the elderly population in Brazil are scarce, resulting in a lack of available virological data. As a result, the causative agent remains unidentified in a large number of reported acute gastroenteritis cases. However, the advent of next-generation sequencing provides new opportunities for viral detection and discovery. The aim of this study was to identify the viruses that circulate among elderly people with and without acute gastroenteritis, living in residential care homes in Belém, Pará, Brazil, between 2017 and 2019. Ninety-three samples were collected and screened by immunochromatography and qPCR. After, the samples were analyzed individually or in pools by next generation sequencing to identify the viruses circulating in this population. In 26 sequenced samples, members of 13 eukaryotic virus families were identified. The most abundantly present virus families were *Parvoviridae*, *Genomoviridae* and *Smacoviridae*. Contigs displaying similarity to pegiviruses were also detected. Furthermore, a near-complete rotavirus A genome was obtained and could be classified as G3P[8] genotype with the equine DS-1-like genetic background. Complete sequences of the VP4 and VP7 genes of a rotavirus C were also detected, belonging to G4P[2]. This study demonstrates the first characterization of the gastrointestinal virome in elderly in Northern Brazil. A diversity of viruses was found to be present in patients with and without diarrhea, reinforcing the need to monitor elderly people residing in long-term care facilities, especially in cases of acute gastroenteritis.

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1. Introduction

Acute gastroenteritis (AG) is one of the leading causes of mortality and morbidity worldwide (Leung and Hon, 2021). Alongside several other possible etiologies, viruses are the main cause of diarrhea worldwide, especially in children (Hartman et al., 2019; Leung, 2020). The transmission of gastroenteric viruses occurs via the fecal-oral route, mainly by person-to-person contact and ingestion of contaminated food and water, as well as by fomites, aerosols and vomit droplets (Wikswo et al., 2015). Several groups of viruses have been reported to be responsible for causing AG. Among them, Group A rotavirus (RVA) stands out and is considered to be the main cause of AG in childhood, followed by norovirus (NoV), astrovirus (AstV) and adenovirus (AdV) (Akdag et al., 2020; Banyai et al., 2018; Dian et al., 2021; Farahmand et al., 2021; Olortegui et al., 2018). However, other less common viral pathogens are also related to cases of AG, such as sapovirus (SaV), rotavirus group C (RVC) and enteroviruses (Malik and Ghosh, 2020; Trovao et al., 2019; Zaki et al., 2021).

Diagnosis of AG viruses can be done by antigen detection (enzyme immunoassays- EIA) or conventional molecular methods, such as polymerase chain reaction (PCR), reverse transcription polymerase chain reaction (RT-PCR), multiplex RT-PCR or Real-Time PCR. Several EIAs are used to detect NoV, RVA, AstV and AdV viral antigens, but although these methods are considered sensitive and specific, their large-scale application is limited because of the high reagent costs (Chen et al., 2017; Miller et al., 2013). Of the molecular techniques, only Real-Time PCR offers quantitative data for the presence of viral genomes, while simultaneously offering superior detection sensitivity compared to other conventional PCR techniques (Aggarwala et al., 2017; Altan et al., 2017).

Molecular studies using PCR and sequencing techniques have contributed significantly to the improved detection of these viruses and to the knowledge of their epidemiology and clinical importance. However, despite the variety of available detection methods in surveillance laboratories, many clinical samples remain undiagnosed, even after having undergone several conventional tests (Aggarwala et al., 2017; Moore et al., 2015). This is the situation for up to 40% of AG cases, where the scope of the tests used is often insufficient to identify the causative virus and where episodes are often caused by yet uncharacterized viruses (Carding et al., 2017). Conversely, the technique of viral metagenomics, which performs the simultaneous analysis of all nucleic acids recovered in a single sample (with or without a step to enrich for viral particles), has the potential to revolutionize the detection of both known and new viruses (Osunmakinde et al., 2018). Instead of performing multiple tests, each one looking for a specific pathogen, metagenomics using next-generation sequencing (NGS) technology is able to identify all viruses present in the sample, and sometimes even allows the assembly of the (almost) complete genomes of known and novel viruses (Nooij et al., 2018). The resulting data sets can also be used to identify virulence genes and provide more complete information in cases of large outbreaks of AG (Altan et al., 2017). Furthermore, viral metagenomics is a valuable tool in detecting new viral species and strains. As NGS technologies improve, more studies involving this approach are being carried out in public health laboratories in different locations to investigate the causes of various diseases, mainly because metagenomics allows the detection of pathogens in low concentrations and can be performed directly on clinical specimens. The disadvantages of NGS technologies are that they take longer to perform, are more expensive, and are not as sensitive as PCR and qPCR (Greninger, 2018).

Yearly, 500,000 children - especially under 5 years old - die in developing countries due to diarrhea, with a hundred million more registered cases having a non-fatal outcome (Local Burden of Disease Diarrhea, 2020). Because of this significant impact on global human health, the role of enteric viruses in childhood AG has been studied extensively and is well defined (Chamberland et al., 2015; Elliott, 2007; Florez et al., 2020; Guarino et al., 2020; Parashar et al., 2013). However, little data about the occurrence of these viruses in the adult population is available, especially in elderly people. Nonetheless, the number of diarrheal cases in this population group can be very high in developing but also developed countries. For example, in the United States of America (USA), of the 179 million patients treated with diarrhea, 83% were elderly (Sell and Dolan, 2018; Wikswo et al., 2015). Because of the acquisition of age-related morbidities, contributing to a reduced independence, elderly are often cared for in Long-term Care Institutions (LCI). However, in these closed housing arrangements, the close contact between residents, employees and visitors can expedite the spread of pathogens when diseases are introduced (Harris et al., 2010; Inns et al., 2019; Strausbaugh et al., 2003). Consequently, outbreaks of AG in elderly care institutions have been reported globally, often attributable to viral pathogens. For example, in the United States, NoV was responsible for 79% of the outbreaks in long-term care facilities between 2009 and 2013 (Cardemil et al., 2012; Jayasekara et al., 2016; Luchs et al., 2017; Marshall et al., 2003; Meier, 2021). The detection of enteric viruses among the elderly in LCIs and the associated possibility of AG outbreaks adds to pre-existing concerns about the quality of life of this population, although the global impact of this disease burden on the internee geriatric population remains to be fully defined.

Studies involving the elderly population, although relevant, are scarce in Brazil, especially those concerning epidemiological and molecular research on viral gastroenteritis (IBGE, 2018). As the elderly represent one of the fastest growing segments of the Brazilian population, it is important to carry out better and more comprehensive surveillance studies to identify the viruses that circulate among these people, enabling a more detailed characterization of the impact of these agents on this population. Therefore, the aim of this study was to use a metagenomics approach to identify the viruses that circulate among elderly people living in private and public residential care homes in Belém, Pará, Brazil. It is noteworthy that this investigation, carried out over a period of two years, from March 2017 to June 2019, represents the first surveillance study in the Northern region of Brazil with the aim of identifying the viruses that circulate in this type of population.

2. Material and methods

2.1. Ethical authorization

This study was approved by the Ethics Committee of Human Research of the Evandro Chagas Institute (Comitê de Ética em Pesquisa (CEP)/Instituto Evandro Chagas ((IEC)) under the protocol number 1.942.979.

2.2. Sample collection

The study was conducted in two LCIs, one public and one private, over a two-year period. Initially, meetings were held with those responsible; caregivers and other employees, as well as with the elderly residents of these institutions. The objectives of the study were explained, how the research would be performed and what was expected to be achieved in the end. Two weekly visits (Tuesdays and Thursdays) were carried out to identify individual episodes or outbreaks of diarrhea and/or vomiting. During these visits, elderly residents (>65 years old) and their caregivers were instructed to collect their stool when they had more than three bowel movements per day, or more than normal, and/or episodes of vomiting. In addition, control samples of other residents were also obtained to check for possible asymptomatic infections. During these visits, all feces collected, and stored in a refrigerator available at the LCIs, were transported to the Laboratory of Norovirus and Other Gastroenteric Viruses at IEC, where they were kept at −30 °C until further use.

A total of 93 samples were collected - 50 (3 diarrheic and 47 non-diarrheic) from the private LCI and 43 (30 diarrheic and 13 non-diarrheic) from the public - from March 2017 to June 2019. All the

samples from the private institution were named with the initials PS and ordered numerically according to the time of collection (e.g. PS-45 was the forty-fifth sample collected). Likewise, the samples from the public institution received the initials LP (LP-01 to LP-43), in order to differentiate between samples from the two LCIs.

All samples were initially tested by immunochromatographic assay for norovirus, adenovirus and rotavirus when they arrived at the IEC. Afterwards, they were subjected to RNA/DNA extraction with the ReliaPrep Viral TNA Miniprep Kit, (Promega) and tested by Real-Time PCR for NoV, AdV and AstV, using the GoTaq Probe qPCR Kit and RT-qPCR Systems Kit (Promega) (See also Supplementary table 1) (Hernroth et al., 2002; Kageyama et al., 2003; Yokoi and Kitahashi, 2009). Specific RT-PCRs targeting RVA VP4 and VP7 were done using the OneStep RT-PCR kit (QIAGEN). For the VP7 PCR, 1.2 μM of each primer was used. For the VP4 PCR, 1.6 μM of each primer was used as well as 2 μM of a secondary, degenerated forward primer (Supplementary table 1). Samples were denatured at 94 °C for 2 min, followed by a reverse transcription step and 40 PCR cycles according to the manufacturer's instructions, using an annealing temperature of 50 °C.

2.3. Viral metagenomics

Fecal material (1 g) from 93 samples were lyophilized in a lyophilizer (Liotop L101), with an operating temperature of −54 °C in vacuum. After, the samples were stored at −30 °C until further processing.

The lyophilized samples were sent to the Viral Metagenomics Laboratory - Rega Institute KU Leuven, Leuven, Belgium, for viral metagenomics procedures. Only a subset of samples was used, to correct for the uneven distribution of samples throughout the collection period. From the diarrheic samples, 21 samples (18 from the public and 3 from the private LCI) that spanned the entire collection period were selected for individual sequencing (Table 1). Additionally, 15 samples were selected and combined into 5 pools as non-diarrheic controls. Each pool contained 3 samples collected in the same year (2017, 2018 or 2019) from the same institute. A 2017 pool for the public LCI was not included due to a lack of non-diarrheic samples. The samples were submitted to the preparation procedures described in the NetoVir protocol (Novel Enrichment Technique Of VIRomes) (Conceicao-Neto et al., 2015), following the steps described below:

2.3.1. Fecal specimen enrichment

The lyophilized fecal samples were rehydrated and homogenized using 2 mL of PBS pH 7.4% (GIBCO) in a MINILYS tissue homogenizer at 3.000 rpm for 1 min, followed by centrifugation at 17,000 x*g* for 3 min to collect a final volume of 150 μL supernatant, which was filtered using a 0.8-μm centrifugal filter (PES) at 17,000 xg for 1 min. 130 μL of the filtrate was placed in a sterile tube, together with 7 μL homemade buffer (1 M Tris, 100 mM $CaCl_2$ and 30 mM $MgCl_2$, pH 8), 2 μL benzonase and 1 μL micrococcal nuclease for nuclease treatment. The samples were incubated for 2 h at 37 °C and in the end, 7 μL 0.2 M EDTA was added to stop the reaction. The extraction of RNA/DNA was done using the QIAmp Viral RNA Mini Kit (QIAGEN), according to the manufacturer's instructions, without addition of carrier RNA.

2.3.2. Library preparation

For the random amplification, a PCR was performed with the Complete Whole Transcriptome Amplification Kit (Sigma-Aldrich) according to the manufacturer's instructions, using a thermocycler with the following programmed parameters: 94 °C/2 min, 17 cycles of 94 °C/30s and 70 °C/5 min. Amplicon purification was done with the MBS Spin PCRapace Kit (Stratec Molecular). Quantification of the samples was performed with the Qubit ™ dsDNA HS Assay Kit with the use of a Qubit Fluorometer. Preparation of the genomic library was done using the Nextera XT DNA Library kit (Illumina). AMPure magnetic beads were

Table 1
Illumina sequencing data overview.

Institution	Sample	Collection date	Type of sample	N° Raw reads	N° Trimmed reads	N° Total of contigs	N° Eukaryotic viral reads* (%) **	N° Viral contigs
Public	LP02	04/04/2017	DIARRHEAL	14,160,796	12,831,900	8517	1,636,450 (12.75)	417
	LP05	04/04/2017	DIARRHEAL	8,801,050	7,765,560	6671	1,127,308 (14.52)	137
	LP09	04/06/2017	DIARRHEAL	11,748,558	10,480,039	9379	5,719,902 (54.58)	466
	LP11	04/13/2017	DIARRHEAL	9,965,002	8,850,179	13,592	4,253,339 (48.06)	465
	LP15	04/15/2017	DIARRHEAL	14,330,692	12,723,448	6926	1,591,733 (12.51)	157
	LP16	04/19/2017	DIARRHEAL	14,167,040	12,581,397	38,556	2,820,462 (22.42)	811
	LP18	04/29/2017	DIARRHEAL	13,119,034	11,096,227	102,827	797,998 (7.19)	1542
	LP19	05/22/2017	DIARRHEAL	15,117,052	13,099,676	32,067	2,470,430 (18.86)	714
	LP21	07/07/2017	DIARRHEAL	1,061,730	828,532	12,499	157,264 (18.98)	195
	LP22	08/13/2017	DIARRHEAL	10,294,316	8,369,275	31,053	747,551 (8.93)	434
	LP23	10/29/2017	DIARRHEAL	13,561,446	11,152,308	22,439	2,451,639 (21.98)	288
	LP24	12/03/2017	DIARRHEAL	11,735,406	8,899,256	105,381	171,995 (1.93)	252
	LP25	01/18/2018	DIARRHEAL	7,321,942	6,295,076	3150	2,556,459 (40.61)	338
	LP26	02/21/2018	DIARRHEAL	5,498,194	4,691,336	12,946	776,423 (16.55)	226
	LP27	03/17/2018	DIARRHEAL	4,656,340	3,219,092	40,239	37,317 (1.16)	188
	LP28	04/26/2018	DIARRHEAL	1,572,988	1,348,794	3916	190,189 (14.10)	103
	LP42	06/09/2018	DIARRHEAL	4,674,976	3,488,387	61,313	16,538 (0.47)	185
	LP43	06/29/2018	DIARRHEAL	8,122,492	6,581,415	23,315	142,195 (2.16)	88
	POOL 01	05/13/2018 11/01/2018 11/08/2018	NON-DIARRHEIC CONTROL	10,346,002	8,811,949	26,158	5,770,149 (65.48)	291
	POOL 02	01/07/2019 03/20/2019 05/15/2019	NON-DIARRHEIC CONTROL	9,110,788	6,874,556	84,850	29,433 (0.43)	319
Private	PS18	07/21/2017	DIARRHEAL	5,134,310	4,228,844	51,530	552,581 (13.07)	273
	PS45	06/01/2019	DIARRHEAL	1,227,510	997,788	5505	36,240 (3.63)	195
	PS48	06/22/2019	DIARRHEAL	1,690,606	1,046,397	8135	3777 (0.36)	73
	POOL 03	04/04/2017 09/05/2017 11/29/2017	NON-DIARRHEIC CONTROL	14,186,114	12,176,792	51,788	288,958 (2.37)	508
	POOL 04	02/03/2018 06/13/2018 11/06/2018	NON-DIARRHEIC CONTROL	10,562,518	8,626,766	161,594	18,299 (0.21)	181
	POOL 05	02/18/2019 05/09/2019 07/10/2019	NON-DIARRHEIC CONTROL	8,730,090	7,392,166	117,312	944,010 (12.77)	526

* The number of viral reads was calculated by mapping back the trimmed reads to the contigs classified as 'eukaryotic viral' by DIAMOND.
** The percentage of viral reads was calculated in relation to the total obtained trimmed reads per sample.

used to purify the library. For quality control, the samples were analyzed with a Bioanalyzer 2100, using the High Sensitivity DNA kit (Agilent Technologies) according to the instruction manual. Sequencing was performed for 300 cycles on a HiSeqTM 2500 platform (Illumina).

2.4. Data analysis

Initially, human genome sequences, adapter sequences and low-quality reads were removed using Trimmomatic (Bolger et al., 2014). The remaining reads were de novo assembled using SPAdes 3.13.0 with the option MetaSPAdes (Bankevich et al., 2012) and taxonomic annotation was performed using DIAMOND v2.0.8 (Buchfink et al., 2015). The Refseq sequences for RVA and RVC were used to map the trimmed reads using the Burrows-Wheeler Alignment tool (BWA) (Li and Durbin, 2009), after which consensus sequences were obtained. For RVC, only partial genome segments were obtained and additional PCRs (primers in Supplementary Table S1), followed by Sanger sequencing, were used to further complete the VP4 and VP7 segments. PCRs were performed using the One Step RT-PCR kit (Qiagen) according to the manufacturer's instructions. The resulting nucleotide sequences were submitted to NCBI GenBank with the accession numbers MW715617 and MW715618, respectively. Additionally, nucleotide sequences for all 11 obtained RVA genes were submitted to NCBI GenBank with the accession numbers MW715606-MW715616.

For the RVC VP4 and VP7 segments and for all 11 RVA segments, alignments were made with appropriate reference strains as well as a selection of closely related sequences downloaded from the National Center for Biotechnology Information (NCBI) database. IQ-TREE v1.6.12 was used for model selection and subsequent maximum-likelihood phylogenetic tree inference, using 1000 ultra-fast bootstrap replications for each tree (Nguyen et al., 2015). The abundance table for eukaryotic viruses was plotted in RStudio with ggplot2 (R Core Team, 2020).

3. Results

All 93 samples tested by immunochromatography and Real Time-PCR were negative for RVA, NoV, AdV and AstV. For the 26 samples sequenced on an Illumina 2500 HiSeq (21 diarrheal specimens and 5 non-diarrheic pools of three samples each), 1,061,730 to 15,117,052 reads were obtained per sample or pool (Table 1). Following adapter trimming, between 828,532 and 13,099,676 reads remained and 3150 to 161,594 contigs could be assembled for each sample. The NGS analysis also revealed viral nucleotide sequences in all 26 samples tested but only for 18 (69.2%) of them contigs of eukaryotic viruses >500 bp could be assembled (Fig. 1). In 12 (66.7%) of these 18 specimens, two or more distinct eukaryotic viral species were detected. Based on DIAMOND classification, viruses related to unclassified parvorviruses (*Parvoviridae*), Chicken stool-associated gemycirculavirus (*Genomoviridae*) and Human smacovirus 1 (*Smacoviridae*) were the most prevalent, with their sequence being detected in 66.7% (12/18), 33.3% (6/18) and 27.8% (5/18) of the samples, respectively. Other eukaryotic RNA and

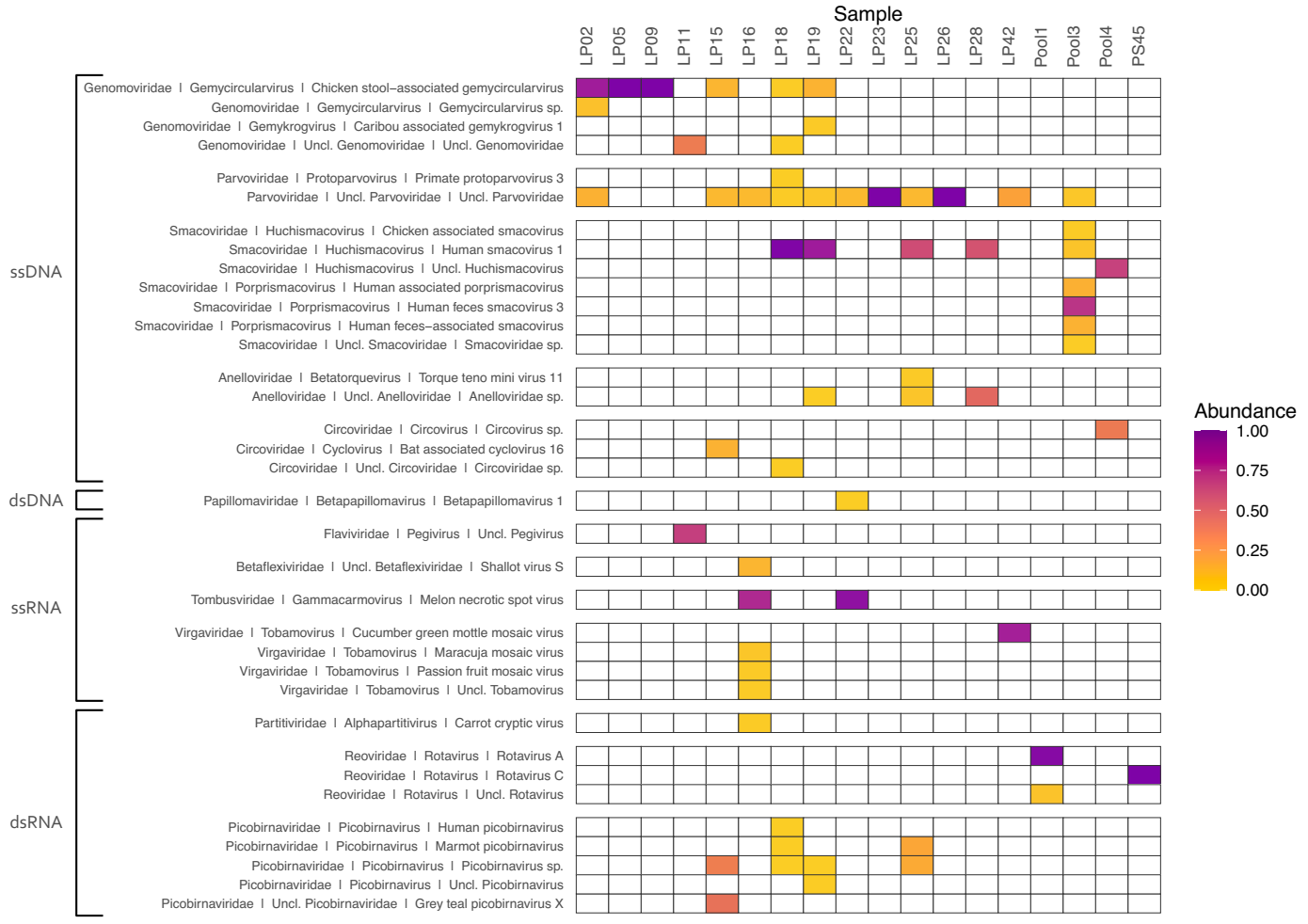

Fig. 1. Heatmap containing the viral sequences identified with NGS in fecal samples from elderly people residing in two long-term care institutions (public and private) in Belém, Pará, Brazil. Each row corresponds to a specific virus species that was detected in one of the samples or pools. Each column corresponds to an individual or sample pool. The colors represent the relative abundance of viral reads corresponding to the virus genome detected in each sample/pool.

DNA viruses that were detected at least once in these samples displayed similarity to members of the families *Picobirnaviridae*, *Reoviridae* (Human Rotavirus A and C), *Flaviviridae* (unclassified Pegivirus), *Papillomaviridae* (Betapapillomavirus 1) and *Anelloviridae* (Torque teno mini virus 11). Reads belonging to other eukaryotic viruses were also detected, but in insufficient quantity to assemble into larger contigs (>500 bp).

In total, 13 eukaryotic virus families were detected in the fecal samples. With the exception of four plant-associated virus families (*Betaflexiviridae*, *Partitiviridae*, *Tombusviridae* and *Virgaviridae*), only three families of RNA viruses were detected, one single-stranded (*Flaviviridae*) and two double-stranded (*Picobirnaviridae* and *Reoviridae*). The former was only found in one sample (LP11), wherein fragments of human pegivirus (HPgV), formerly known as GBV-C, were detected. Analysis of the obtained contigs (500 bp) using Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi), showed that the virus detected here belongs to genotype 2, with approximately 93% nucleotide identity with the closest related references (MN551063, MH053115, MH053121), a group of sequences detected previously in France (Jordier et al., 2019). Conversely, mammalian double-stranded RNA viruses were present in multiple samples, including multiple divergent picobirnavirus species, a rotavirus A and a rotavirus C. Besides the RNA virus families, at least one viral species was found for six DNA virus families. Five of these represent ssDNA viruses (*Genomoviridae*, *Parvoviridae*, *Smacoviridae*, *Anelloviridae* and *Circoviridae*), with the only detected dsDNA family being the family *Papillomaviridae*.

The two rotaviruses were detected in unrelated samples from the two LCIs: the RVA in a pool of non-diarrheic residents from the public LCI (POOL-01) and the RVC in a symptomatic sample from the private LCI (PS-45). Using two PCRs targeting the RVA VP4 and VP7 segments, we could trace back that the RVA detected in POOL-01 originated from sample LP29. To our knowledge, this virus is the first near-complete RVA genome obtained from an older patient living in a LCI in Brazil. Full or almost complete length contigs were obtained for all 11 RVA gene segments. For each segment, a phylogenetic tree was constructed using appropriate reference strains as well as the most similar non-redundant reference sequences available on GenBank, as determined by Blast. Phylogenetic analysis of the VP7 gene identifies this Brazilian strain as belonging to genotype G3, more specifically, the equine-like G3P[8] lineage, as this sequence was genetically related to previously detected equine-like G3P[8] strains from Brazil (KX469400, MT386432, MO386434) and other countries, such as Spain, Japan, and Hungary (Fig. 2A) (Akane et al., 2021; Arana et al., 2016; Doro et al., 2016; Guerra et al., 2016; Gutierrez et al., 2020). Comparably, phylogenetic analysis of the VP4 gene shows this gene clustering in the human genotype P[8], with 99.77–99.79% nucleotide similarity with samples from Spain and Hungary (Fig. 2B). In accordance with the classic binary nomenclature, based on the sequences of the VP7 and VP4 genes, the strain detected here can therefore be classified as a G3P[8] genotype. Detailed analysis of the complete genomic constellation showed that this strain was associated with the equine DS-1-like genetic background (I2-R2-C2-M2-A2-N2-T2-E2-H2). Accordingly, the remaining nine genes (VP1-VP3, VP6 and NSP1-NSP5) all clustered with other DS-like 1 strains from variable locations, showing nucleotide similarities ranging from 98.05–99.91% when compared to their respective closest references (Fig. 3A–I).

In the symptomatic sample PS-45, fragments of the 11 genetic segments of a RVC were detected, but due to limited read availability we were unable to assemble contigs covering the complete genome. To be able to classify this virus, specific primers were designed for the VP4 and VP7 genes based on the contigs that had been obtained by NGS. These primers were used for the amplification by RT-PCR and subsequent Sanger sequencing of the missing parts of the VP7 and VP4 gene segments to enable further characterization. Similarly to the RVA, phylogenetic trees were made for the VP7 and VP4 genes using the closest related references as identified by Blast. Based on the resulting maximum-like hood trees, the detected strain can be classified as a genotype G4P[2]. For both genes, the nucleotide sequences showed >98% similarities with Russian and Indian human RVC strains, respectively (Fig. 4A–B).

4. Discussion

Several studies have already demonstrated that LCIs present a great risk for the spread of viral agents such as norovirus, rotavirus, and other enteric viruses. However, the true incidence of viral infections in the elderly remains underdiagnosed because in some countries, including Brazil, there is no routine monitoring system focusing on this specific age group. The present investigation showed viral diversity in diarrheic and non-diarrheic samples obtained from elderly patients residing in two LCIs. As far as we know, this study, which spanned a two-year period (2017–2019), is the first to investigate the gastrointestinal virome in the elderly in northern Brazil through a metagenomics approach.

In the present study, viral metagenomics was performed on fecal samples, revealing the presence of 13 virus families. For some of these,

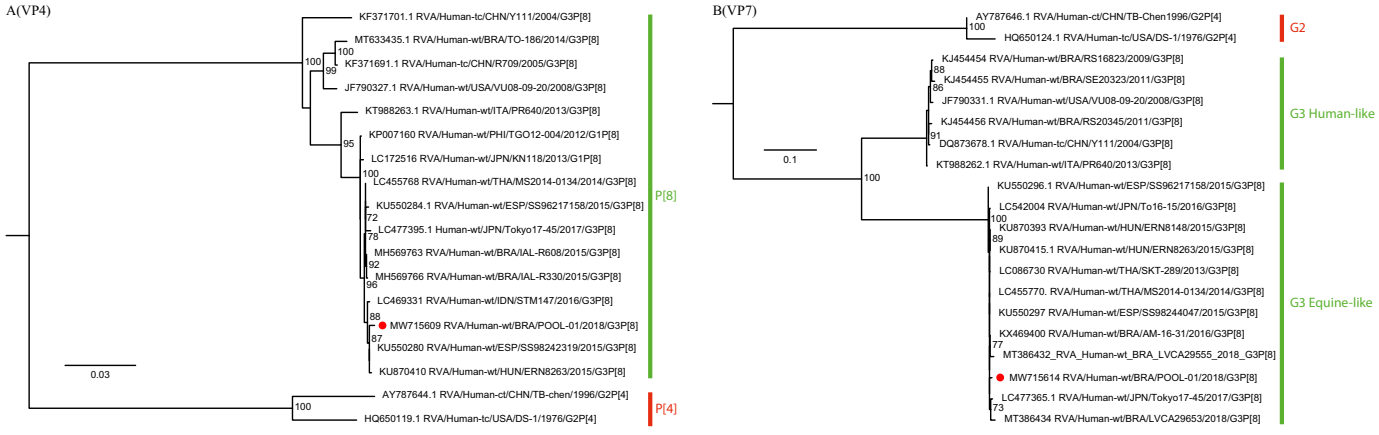

Fig. 2. Phylogenetic analysis based on VP4 (A) and VP7 (B) nucleotide (nt) sequences of rotavirus A strains. The green and red bars indicate the different G and P genotypes. The study strain is indicated with a filled, red circle. Reference strains were downloaded from GenBank and are labeled with their respective accession numbers. Both maximum-likelihood trees were constructed with IQ-TREE v1.6.12, using automated model selection (HKY + F + I for VP4; TPM2 + F + G4 for VP7) and 1000 bootstrap replicates. Only bootstrap values >70 are shown. The scale bar indicates the number of substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

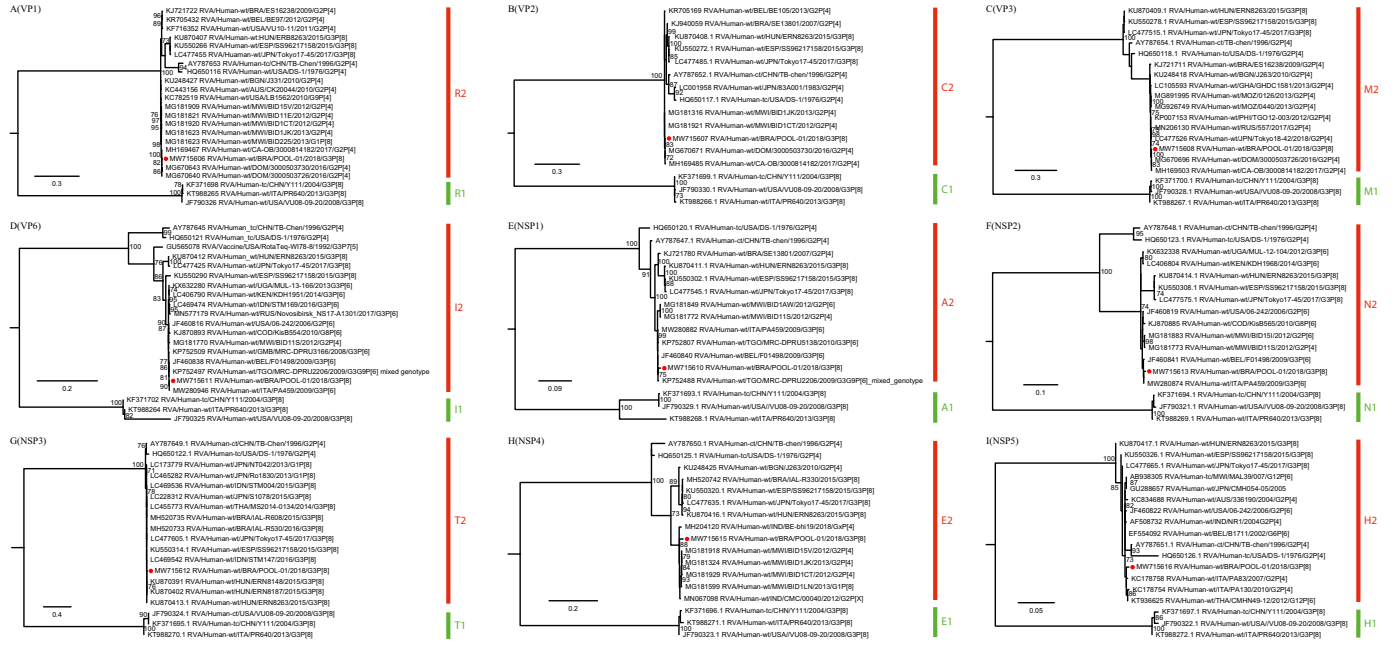

Fig. 3. Phylogenetic analysis based on VP1 to VP3 (A-C), VP6 (D) and NSP1 to NSP5(*E*-I) nucleotide (nt) sequences of rotavirus A strains. The green and red bars indicate the different lineages for each segment. The study strain is indicated with a filled, red circle. Reference strains were downloaded from GenBank and are labeled with their respective accession numbers. Both maximum-likelihood trees were constructed with IQ-TREE v1.6.12, using automated model selection (TIM + F + I for VP1, VP3; TN + F + I for VP2; HKY + F + I for VP6, NSP2, NSP3, NSP4; K3Pu + F + I for NSP1; HKY + F + G4 for NSP5) and 1000 bootstrap replicates. Only bootstrap values >70 are shown. The scale bar indicates the number of substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

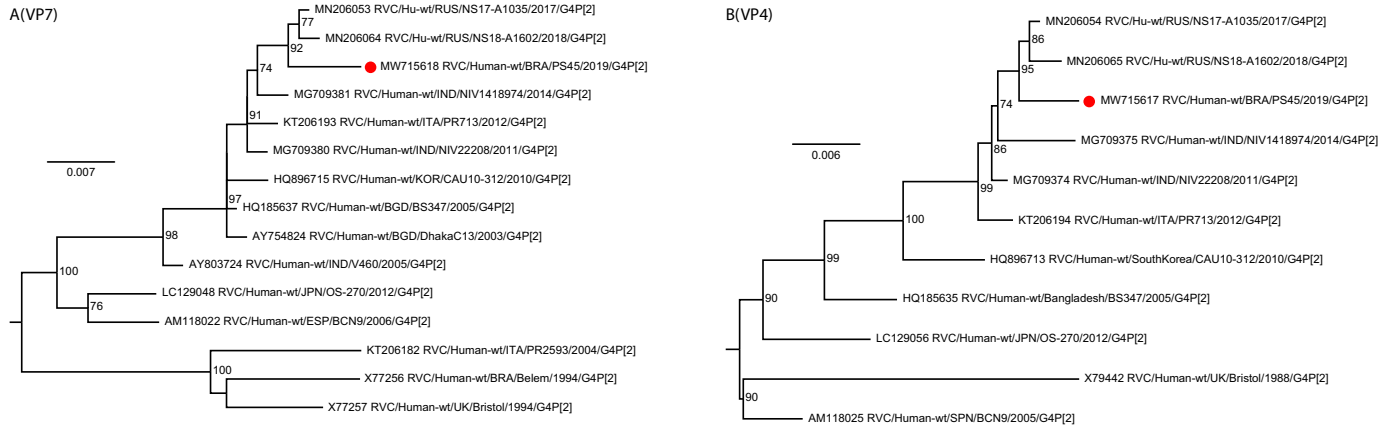

Fig. 4. Phylogenetic analysis based on VP7 (A) and VP4 (B) nucleotide (nt) sequences of RVC strains. The study strain is indicated with a filled, red circle. Reference strains were downloaded from GenBank and are labeled with their respective accession numbers. Both maximum-likelihood trees were constructed with IQ-TREE v1.6.12, using automated model selection (HKY + F + I for VP4; K3Pu + F + I for VP7) and 1000 bootstrap replicates. Only bootstrap values >70 are shown. The scale bar indicates the number of substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

their potential to infect humans remains to be unambiguously established, casting doubt on their relevance for human health. For example, gemycircularviruses belonging to the family *Genomoviridae* have been identified in feces of many animals and humans, as well as in plants, insects and environmental samples, but it is unknown if they are capable of infecting humans (Taboada et al., 2021). The same can also be said for members of the families *Picobirnaviridae* and *Smacoviridae*, which have been identified through metagenomic analyzes in fecal samples from humans and animals and might be implicated in cases of gastroenteritis, although the association with disease is not yet fully understood (Ng et al., 2015; Yinda et al., 2019). Conversely, some studies have provided evidence that smacoviruses and picobirnaviruses are not mammalian viruses but infect archaea and prokaryotes, respectively (Diez-Villasenor and Rodriguez-Valera, 2019; Ghosh and Malik, 2021; Varsani and Krupovic, 2018). However, despite some convincing evidence in the case of picobirnaviruses, these assumptions still remain hypothetical due to the lack of cell and animal models for their cultivation, which has prevented viral isolation and more complete clinical-epidemiological studies (Malik and Ghosh, 2020).

For other virus families detected here, the potential to infect humans is well established, although this does not necessarily imply a link with human disease. A prime example of this is the family *Anelloviridae*, of which Torque teno mini virus-11 (TTMV-11) was found, as well as several sequence fragments that resemble not yet classified members.

Anelloviruses in general have been detected in many diseases, like hepatitis, respiratory diseases, cancer and autoimmune disorders (Al-Qahtani et al., 2016; Spandole et al., 2015). Some studies have also reported anellovirus sequences, like TTMV-11 (detected in the sample LP25), in human melanoma and lymph nodes of patients with Hodgkin lymphoma (Pan et al., 2018). However, it is unclear if anelloviruses contribute to tumor formation (Figueiredo et al., 2007) or other human pathologies (Yu et al., 2020). Comparably, as is the case in this study, circoviruses, in particular members of the genus *Cyclovirus*, are commonly detected in metagenomics studies of different types of human samples (Zhao et al., 2019). However, even though they are known pathogens of pigs, birds and other animals, their human pathogenic potential has not yet been demonstrated (Klaumann et al., 2018; Nath et al., 2021).

In one of the symptomatic samples (LP-18), a species of parvovirus, known as protoparvovirus primate 3 or Cutavirus, was detected. The genus *Protoparovirus* contains several pathogens associated with diseases in humans and animals. However, more investigative studies on cutavirus infections are needed regarding its pathogenicity, to know its real impact on human health (Phan and Nagaro, 2020). According to the BLASTx results, the sequences detected here displayed 97.24% - 98.71% similarity with sequences also detected by viral metagenomics in Brazil, involving diarrhea of unknown cause from children (Phan and Nagaro, 2020). Nonetheless, this represents the first detection of this viral species in Brazil in this type of population. Fragments of Human pegivirus (HPgV) were also detected in this study. HPgV is transmitted among humans mainly through exposure to contaminated blood (Fama et al., 2020). This transmission profile suggests that HPgV coinfection with other viruses, such as HIV-1 and hepatitis C virus (HCV), is common (Coller et al., 2020; de Pina-Araujo et al., 2021). Several studies carried out in different populations in the last decades in Brazil have shown variable prevalence rates of HPgV infections, mainly in blood donors (Silva et al., 2020). However, data about the prevalence of HPgV and its circulating strains in the Northern Brazilian population are scarce, especially in the elderly population. HPgV is believed to be a non-pathogenic virus, but further studies are necessary to evaluate the unclear aspects related to HPgV infection (Tumbo et al., 2021).

Perhaps the most interesting result was the detection of different rotavirus species. Rotaviruses are considered an important cause of acute gastroenteritis in childhood, but they can also cause this disease in adults and the elderly, potentially with an underestimated prevalence due to the lower frequency of tests in this age group (Troeger et al., 2018). In our study, two rotaviruses were detected, a RVA in a pool of non-diarrheic samples from the public LCI (POOL-01) and a RVC in a symptomatic sample from the private LCI (PS-45). The true incidence of RVA and RVC infection in the elderly remains underestimated because in many countries, such as Brazil, there is no routine monitoring system in place with a focus on this specific age group (Cardemil et al., 2012; Luchs et al., 2017). In Brazil, two reports showed the implication of RVA as the cause of an outbreak in a residential geriatric institution in Sao Paulo (Luchs et al., 2017) and as a possible reason of GA cases in 4% of the patients >60 years of age who had diarrhea and sought outpatient care, in Rio Grande do Sul (Paesi et al., 2015).

The present investigation of RVA genotyping detected the G3P[8] equine strain on a DS-1-like genetic background (I2-R2-C2-M2-A2-N2-T2-E2-H2) in a pooled sample of non-diarrheic elderly. Emergent equine-like DS-1-like G3P[8] RVA strains were first identified in children with AG in Australia in 2013 (Kirkwood et al., 2014). From 2013 onwards, the equine-like G3P[8] DS-1-like genotype has spread and become endemic worldwide (Arana et al., 2016; Cowley et al., 2016; Gutierrez et al., 2020; Perkins et al., 2017). In Brazil, type Wa-like G3P[8] was the predominant G3 strain up until 2013–2014. In March of 2015, in the state of Paraná, the Equine-like G3P[8] similar to the DS-1 strain was first detected, after which it spread rapidly across the country (Luchs et al., 2019). Since then, it has been the only G3 strain detected in Brazil in areas under surveillance (Gutierrez et al., 2020). In northern Brazil, the first report of this strain was in 2016 in the Amazon region, in children hospitalized with severe gastroenteritis (Guerra et al., 2016). Interestingly, the previously detected Brazilian Amazon equine-like G3P[8] DS-1-like strains all had a distinct genotype constellation (G3-P[8]-I2-R2-C2-M2-A2-N1-T2-E2-H2), different from the one found in our study. This in accordance with the hypothesis of Luchs et al. (2019) that the Brazilian equine-like G3P[8] DS-1-like strains were introduced in the country at distinct points with a set of different co-circulating strains.

Besides RVA, we also detected RVC in one of our samples and obtained nearly full-length sequences of the genes encoding the RVC structural proteins, VP4 and VP7, representing the first set of sequences derived from an elderly person with diarrhea in Belém, Pará. RVC was previously detected in Belém, associated with gastroenteritis in children causing an outbreak, hospitalization and sporadic cases (Gabbay et al., 1999; Lobo Pdos et al., 2016). In addition, evidence of transmission of RVC from swine to humans as well as the endemic presence of both human and porcine RVCs in Belém has also been demonstrated previously (Gabbay et al., 2008). Lastly, an outbreak of group C rotavirus gastroenteritis in adults from Valentim Gentil, São Paulo state, has also been reported (Souza et al., 1998). The sample described in the present study grouped only with members of the human RVC G4P[2] genotype, the same type previously found in hospitalized samples from Belem collected between May 2008 and April 2011, and did not show evidence of recent animal ancestry (Lobo Pdos et al., 2016).

When the sequence of the VP7 gene of the strain described here (PS-45) was analyzed phylogenetically with a set of representative sequences available through GenBank, its clustering was observed close to strains from Russia and India. On the other hand, the only available complete sequence from Brazil, X77256, clusters separately from our sequence, close to the Bristol strain. Comparably, also for VP4, our strain was most similar to strains from Russia, as well as sequences from Italy, India and Bangladesh. It should be noted that not all available RVC VP7 and VP4 sequences from Brazil were included in the tree because of their limited length. However, all of them show only limited similarity with our sequence (<96.5%), highlighting its unique lineage.

Different studies in developed countries in the elderly population have demonstrated evidence that these patients can experience severe cases of diarrhea caused by RV, NoV and other viral infections, potentially leading to hospitalization, the acquisition of subsequent nosocomial infections and a higher chance of mortality when associated with other co-morbidities (Anderson et al., 2012; Beck-Friis et al., 2019; Meier, 2021; Yandle et al., 2021). In Brazil, there is no proper monitoring system investigating adult or elderly cases of AG in hospitals, nursing homes or LCIs, nor in the general population, and the official number of cases is probably an underestimation. In our study, we detected some known diarrheal viruses but also many other viruses in our NGS data. Although there is insufficient data to conclusively support the role of most of these viruses, like the smacoviruses and parvoviruses, as the causative agents of AG, their role as potential contributor to some of the patient's symptoms cannot be excluded. However, even when taking into account these poorly characterized viruses, a causative agent could not be identified for several diarrheic cases. While some were probably of bacterial origin, it is also likely, especially in the public LCI, that some were of non-infectious origin. Many of the patients in these institutions are of high age, with many underlying illnesses, requiring the intake of preventive/therapeutic drugs. Furthermore, the reduced mobility and/or cognitive ability of patients often results in malnutrition or patients being kept on a liquid diet, all factors that might affect the quality/consistency of the patient's stool and the number of bowel movements.

5. Conclusions

In summary, this study demonstrated the first diversity description of the gastrointestinal virome in institutionalized elderly from Brazil. Our results show the presence of a wide viral diversity, with several viruses

of clinical interest being present in patients with and without diarrhea. The presence of RVA and RVC demonstrates the circulation of pathogenic enteric viruses in this population and reinforces the need to monitor elderly people residing in LCIs, especially in cases of acute gastroenteritis.

CRediT authorship contribution statement

Thayara Morais Portal: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Bert Vanmechelen:** Methodology, Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Lore Van Espen:** Formal analysis, Data curation. **Daan Jansen:** Methodology, Formal analysis, Data curation, Writing – review & editing. **Dielle Monteiro Teixeira:** Methodology, Investigation. **Emanuella Sarmento Alho de Sousa:** Methodology, Investigation. **Victor Pereira da Silva:** Methodology, Investigation. **Juliana Silva de Lima:** Methodology, Investigation. **Tammy Katlhyn Amaral Reymão:** Methodology, Investigation. **Carina Guilhon Sequeira:** Conceptualization. **Ana Maria Revorêdo da Silva Ventura:** Conceptualization. **Luciana Damascena da Silva:** Conceptualization, Writing – review & editing, Project administration. **Hugo Reis Resque:** Conceptualization, Project administration. **Jelle Matthijnssens:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Yvone Benchimol Gabbay:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2022.105241.

References

Aggarwala, V., Liang, G., Bushman, F.D., 2017. Viral communities of the human gut: metagenomic analysis of composition and dynamics. Mob. DNA 8, 12.
Akane, Y., Tsugawa, T., Fujii, Y., Honjo, S., Kondo, K., Nakata, S., Fujibayashi, S., Ohara, T., Mori, T., Higashidate, Y., Nagai, K., Kikuchi, M., Sato, T., Kato, S., Tahara, Y., Kubo, N., Katayama, K., Kimura, H., Tsutsumi, H., Kawasaki, Y., 2021. Molecular and clinical characterization of the equine-like G3 rotavirus that caused the first outbreak in Japan, 2016. J. General Virol. 102.
Akdag, A.I., Gupta, S., Khan, N., Upadhayay, A., Ray, P., 2020. Epidemiology and clinical features of rotavirus, adenovirus, and astrovirus infections and coinfections in children with acute gastroenteritis prior to rotavirus vaccine introduction in Meerut, North India. J. Med. Virol. 92, 1102–1109.
Al-Qahtani, A.A., Alabsi, E.S., AbuOdeh, R., Thalib, L., El Zowalaty, M.E., Nasrallah, G. K., 2016. Prevalence of anelloviruses (TTV, TTMDV, and TTMV) in healthy blood donors and in patients infected with HBV or HCV in Qatar. Virol. J. 13, 208.
Altan, E., Del Valle Mendoza, J., Deng, X., Phan, T.G., Sadeghi, M., Delwart, E.L., 2017. Small circular rep-encoding single-stranded DNA genomes in peruvian diarrhea virome. Genome Announce. 5.
Anderson, E.J., Katz, B.Z., Polin, J.A., Reddy, S., Weinrobe, M.H., Noskin, G.A., 2012. Rotavirus in adults requiring hospitalization. J. Infect. 64, 89–95.
Arana, A., Montes, M., Jere, K.C., Alkorta, M., Iturriza-Gomara, M., Cilla, G., 2016. Emergence and spread of G3P[8] rotaviruses possessing an equine-like VP7 and a DS-1-like genetic backbone in the Basque Country (North of Spain), 2015. Infect. Gene. Evol. 44, 137–144.
Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V. M., Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev, M.A., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J. Comp. Biol. 19, 455–477.
Banyai, K., Estes, M.K., Martella, V., Parashar, U.D., 2018. Viral gastroenteritis. Lancet 392, 175–186.
Beck-Friis, T., Andersson, M., Gustavsson, L., Lindh, M., Westin, J., Andersson, L.M., 2019. Burden of rotavirus infection in hospitalized elderly individuals prior to the introduction of rotavirus vaccination in Sweden. J. Clin. Virol. 119, 1–5.
Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
Buchfink, B., Xie, C., Huson, D.H., 2015. Fast and sensitive protein alignment using DIAMOND. Nat. Methods 12, 59–60.
Cardemil, C.V., Cortese, M.M., Medina-Marino, A., Jasuja, S., Desai, R., Leung, J., Rodriguez-Hart, C., Villarruel, G., Howland, J., Quaye, O., Tam, K.I., Bowen, M.D., Parashar, U.D., Gerber, S.I., Rotavirus Investigation, T., 2012. Two rotavirus outbreaks caused by genotype G2P[4] at large retirement communities: cohort studies. Ann. Intern. Med. 157, 621–631.
Carding, S.R., Davis, N., Hoyles, L., 2017. Review article: the human intestinal virome in health and disease. Aliment. Pharmacol. Ther. 46, 800–815.
Chamberland, R.R., Burnham, C.A., Storch, G.A., Jackups, R., Doern, C.D., 2015. Prevalence and seasonal distribution of norovirus detection in stools submitted from pediatric patients for enteric pathogen testing. J. Pediatric Infect. Dis. Soc. 4, 264–266.
Chen, S.Y., Tsai, C.N., Lee, Y.S., Lin, C.Y., Huang, K.Y., Chao, H.C., Lai, M.W., Chiu, C.H., 2017. Corrigendum: intestinal microbiome in children with severe and complicated acute viral gastroenteritis. Sci. Rep. 7, 46820.
Coller, K.E., Bruce, V., Cassidy, M., Gersch, J., Frankel, M.B., Vallari, A., Cloherty, G., Hackett Jr., J., Evans, J.L., Page, K., Dawson, G.J., 2020. Chronic human pegivirus 2 without hepatitis C virus co-infection. Emerg. Infect. Dis. 26, 265–272.
Conceicao-Neto, N., Zeller, M., Lefrere, H., De Bruyn, P., Beller, L., Deboutte, W., Yinda, C.K., Lavigne, R., Maes, P., Van Ranst, M., Heylen, E., Matthijnssens, J., 2015. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. Sci. Rep. 5, 16532.
Cowley, D., Donato, C.M., Roczo-Farkas, S., Kirkwood, C.D., 2016. Emergence of a novel equine-like G3P[8] inter-genogroup reassortant rotavirus strain associated with gastroenteritis in Australian children. J. General Virol. 97, 403–410.
de Pina-Araujo, I.I.M., Horta, M.A., do Amaral Mello, F.C., Soares, C.C., 2021. Human pegivirus 1 in Cabo Verde: prevalence and genotypic distribution among HIV-infected individuals. Arch. Virol. 166, 1345–1353.
Dian, Z., Sun, Y., Zhang, G., Xu, Y., Fan, X., Yang, X., Pan, Q., Peppelenbosch, M., Miao, Z., 2021. Rotavirus-related systemic diseases: clinical manifestation, evidence and pathogenesis. Crit. Rev. Microbiol. 1–16.
Diez-Villasenor, C., Rodriguez-Valera, F., 2019. CRISPR analysis suggests that small circular single-stranded DNA smacoviruses infect Archaea instead of humans. Nat. Commun. 10, 294.
Doro, R., Marton, S., Bartokne, A.H., Lengyel, G., Agocs, Z., Jakab, F., Banyai, K., 2016. Equine-like G3 rotavirus in Hungary, 2015 - is it a novel intergenogroup reassortant pandemic strain? Acta Microbiol. Immunol. Hung. 63, 243–255.
Elliott, E.J., 2007. Acute gastroenteritis in children. Bmj 334, 35–40.
Fama, A., Larson, M.C., Link, B.K., Habermann, T.M., Feldman, A.L., Call, T.G., Ansell, S. M., Liebow, M., Xiang, J., Maurer, M.J., Slager, S.L., Nowakowski, G.S., Stapleton, J. T., Cerhan, J.R., 2020. Human pegivirus infection and lymphoma risk: a systematic review and meta-analysis. Clin. Infect. Dis. 71, 1221–1228.
Farahmand, M., Moghoofei, M., Dorost, A., Shoja, Z., Ghorbani, S., Kiani, S.J., Khales, P., Esteghamati, A., Sayyahfar, S., Jafarzadeh, M., Minaeian, S., Khanaliha, K., Naghdalipour, M., Tavakoli, A., 2021. Global prevalence and genotype distribution of norovirus infection in children with gastroenteritis: A meta-analysis on 6 years of research from 2015 to 2020. Rev. Med. Virol. e2237.
Figueiredo, C.P., Franz-Vasconcelos, H.C., Giunta, G., Mazzuco, T.L., Caon, T., Fernandes, A.L., Simoes, C.M., Antunes, V.L., Niel, C., Barardi, C.R., 2007. Detection of torque Teno virus in Epstein-Barr virus positive and negative lymph nodes of patients with Hodgkin lymphoma. Leuk. Lymphoma 48, 731–735.
Florez, I.D., Nino-Serna, L.F., Beltran-Arroyave, C.P., 2020. Acute infectious diarrhea and gastroenteritis in children. Curr. Infect. Dis. Rep. 22, 4.

Gabbay, Y.B., Jiang, B., Oliveira, C.S., Mascarenhas, J.D., Leite, J.P., Glass, R.I., Linhares, A.C., 1999. An outbreak of group C rotavirus gastroenteritis among children attending a day-care Centre in Belem, Brazil. J. Diarrhoeal Dis. Res. 17, 69–74.
Gabbay, Y.B., Borges, A.A., Oliveira, D.S., Linhares, A.C., Mascarenhas, J.D., Barardi, C. R., Simoes, C.M., Wang, Y., Glass, R.I., Jiang, B., 2008. Evidence for zoonotic transmission of group C rotaviruses among children in Belem, Brazil. J. Med. Virol. 80, 1666–1674.
Ghosh, S., Malik, Y.S., 2021. The true host/s of picobirnaviruses. Front. Vet. Sci. 7.
Greninger, A.L., 2018. The challenge of diagnostic metagenomics. Expert. Rev. Mol. Diagn. 18, 605–615.
Guarino, A., Aguilar, J., Berkley, J., Broekaert, I., Vazquez-Frias, R., Holtz, L., Lo Vecchio, A., Meskini, T., Moore, S., Rivera Medina, J.F., Sandhu, B., Smarrazzo, A., Szajewska, H., Treepongkaruna, S., 2020. Acute gastroenteritis in children of the world: what needs to be done? J. Pediatr. Gastroenterol. Nutr. 70, 694–701.
Guerra, S.F.S., Soares, L.S., Lobo, P.S., Penha Junior, E.T., Sousa Junior, E.C., Bezerra, D. A.M., Vaz, L.R., Linhares, A.C., Mascarenhas, J.D.P., 2016. Detection of a novel equine-like G3 rotavirus associated with acute gastroenteritis in Brazil. J. General Virol. 97, 3131–3138.
Gutierrez, M.B., Fialho, A.M., Maranhao, A.G., Malta, F.C., Andrade, J., Assis, R.M.S., Mouta, S., Miagostovich, M.P., Leite, J.P.G., Machado Fumian, T., 2020. Rotavirus a in Brazil: molecular epidemiology and surveillance during 2018-2019. Pathogens 9.
Harris, J.P., Lopman, B.A., O'Brien, S.J., 2010. Infection control measures for norovirus: a systematic review of outbreaks in semi-enclosed settings. J. Hospital Infect. 74, 1–9.
Hartman, S., Brown, E., Loomis, E., Russell, H.A., 2019. Gastroenteritis in Children. Am. Fam. Physician 99, 159–165.
Hernroth, B.E., Conden-Hansson, A.C., Rehnstam-Holm, A.S., Girones, R., Allard, A.K., 2002. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. Appl. Environ. Microbiol. 68, 4523–4533.
IBGE, 2018. Perfil dos idosos responsáveis pelos domicílios no Brasil 2000. Accessed on November 27 2018,. http://www.ibge.gov.br/home/estatistica/populacao/perfildoso/tabela1_1.shtm.
Inns, T., Wilson, D., Manley, P., Harris, J.P., O'Brien, S.J., Vivancos, R., 2019. What proportion of care home outbreaks are caused by norovirus? An analysis of viral causes of gastroenteritis outbreaks in care homes, north East England, 2016–2018. BMC Infect. Dis. 20, 2.
Jayasekara, L., Leone, C.M., Sharp, J., Fraser, A., 2016. Preventing and controlling human noroviruses in South Carolina long-term care facilities: an analysis of institutional policies and procedures. Am. J. Infect. Control 44, 24–29.
Jordier, F., Deligny, M.L., Barre, R., Robert, C., Galicher, V., Uch, R., Fournier, P.E., Raoult, D., Biagini, P., 2019. Human pegivirus isolates characterized by deep sequencing from hepatitis C virus-RNA and human immunodeficiency virus-RNA-positive blood donations, France. J. Med. Virol. 91, 38–44.
Kageyama, T., Kojima, S., Shinohara, M., Uchida, K., Fukushi, S., Hoshino, F.B., Takeda, N., Katayama, K., 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J. Clin. Microbiol. 41, 1548–1557.
Kirkwood, C.D., Roczo-Farkas, S., Australian Rotavirus Surveillance, G, 2014. Australian Rotavirus Surveillance program annual report, 2013. In: Communicable Diseases Intelligence Quarterly Report, 38, pp. E334–E342.
Klaumann, F., Correa-Fiz, F., Franzo, G., Sibila, M., Nunez, J.I., Segales, J., 2018. Current knowledge on porcine circovirus 3 (PCV-3): a novel virus with a yet unknown impact on the swine industry. Front. Vet. Sci. 5, 315.
Leung, A.K.C., 2020. Viral Gastroenteritis in Children. Accessed on November 28, 2020. http://online.epocrates.com/u/2911794/Viral.
Leung, A.K., Hon, K.L., 2021. Paediatrics: how to manage viral gastroenteritis. Drugs Context 10.
Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics 25, 1754–1760.
Lobo Pdos, S., Guerra Sde, F., Siqueira, J.A., Soares Lda, S., Gabbay, Y.B., Linhares, A.C., Mascarenhas, J.D., 2016. Phylogenetic analysis of human group C rotavirus in hospitalized children with gastroenteritis in Belem, Brazil. J. Med. Virol. 88, 728–733.
Local Burden of Disease Diarrhoea, C, 2020. Mapping geographical inequalities in childhood diarrhoeal morbidity and mortality in low-income and middle-income countries, 2000-17: analysis for the global burden of disease study 2017. Lancet 395, 1779–1801.
Luchs, A., Madalosso, G., Cilli, A., Morillo, S.G., Martins, S.R., de Souza, K.A.F., Namiyama, G.M., Goncalves, C.R., Carmona, R.C.C., Timenetsky, M., 2017. Outbreak of G2P[4] rotavirus gastroenteritis in a retirement community, Brazil, 2015: an important public health risk? Geriatr. Nurs. 38, 283–290.
Luchs, A., da Costa, A.C., Cilli, A., Komninakis, S.C.V., Carmona, R.C.C., Boen, L., Morillo, S.G., Sabino, E.C., Timenetsky, M., 2019. Spread of the emerging equine-like G3P[8] DS-1-like genetic backbone rotavirus strain in Brazil and identification of potential genetic variants. J. General Virol. 100, 7–25.
Malik, Y., Ghosh, S., 2020. Etymologia: Picobirnavirus. Emerg. Infect. Dis. J. 26, 89.
Marshall, J., Botes, J., Gorrie, G., Boardman, C., Gregory, J., Griffith, J., Hogg, G., Dimitriadis, A., Catton, M., Bishop, R., 2003. Rotavirus detection and characterisation in outbreaks of gastroenteritis in aged-care facilities. J. Clin. Virol. 28, 331–340.
Meier, J.L., 2021. Viral acute gastroenteritis in special populations. Gastroenterol. Clin. N. Am. 50, 305–322.
Miller, R.R., Montoya, V., Gardy, J.L., Patrick, D.M., Tang, P., 2013. Metagenomics for pathogen detection in public health. Genom. Med. 5, 81.
Moore, N.E., Wang, J., Hewitt, J., Croucher, D., Williamson, D.A., Paine, S., Yen, S., Greening, G.E., Hall, R.J., 2015. Metagenomic analysis of viruses in feces from unsolved outbreaks of gastroenteritis in humans. J. Clin. Microbiol. 53, 15–21.
Nath, B.K., Das, S., Roby, J.A., Sarker, S., Luque, D., Raidal, S.R., Forwood, J.K., 2021. Structural perspectives of beak and feather disease virus and porcine circovirus proteins. Viral Immunol. 34, 49–59.
Ng, T.F., Zhang, W., Sachsenroder, J., Kondov, N.O., da Costa, A.C., Vega, E., Holtz, L.R., Wu, G., Wang, D., Stine, C.O., Antonio, M., Mulvaney, U.S., Muench, M.O., Deng, X., Ambert-Balay, K., Pothier, P., Vinje, J., Delwart, E., 2015. A diverse group of small circular ssDNA viral genomes in human and non-human primate stools. Virus Evol. 1, vev017.
Nguyen, L.T., Schmidt, H.A., von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol. Biol. Evol. 32, 268–274.
Nooij, S., Schmitz, D., Vennema, H., Kroneman, A., Koopmans, M.P.G., 2018. Overview of virus metagenomic classification methods and their biological applications. Front. Microbiol. 9, 749.
Olortegui, M.P., Rouhani, S., Yori, P.P., Salas, M.S., Trigoso, D.R., Mondal, D., Bodhidatta, L., Platts-Mills, J., Samie, A., Kabir, F., Lima, A., Babji, S., Shrestha, S.K., Mason, C.J., Kalam, A., Bessong, P., Ahmed, T., Mduma, E., Bhutta, Z.A., Lima, I., Ramdass, R., Moulton, L.H., Lang, D., George, A., Zaidi, A.K.M., Kang, G., Houpt, E. R., Kosek, M.N., Network, M.-E., 2018. Astrovirus infection and diarrhea in 8 countries. Pediatrics 141.
Osunmakinde, C.O., Selvarajan, R., Sibanda, T., Mamba, B.B., Msagati, T.A.M., 2018. Overview of trends in the application of metagenomic techniques in the analysis of human enteric viral diversity in Africa's environmental regimes. Viruses 10.
Paesi, S., Modena, N., Roesch-Ely, M., 2015. Detection of rotavirus and norovirus in the elderly population of Caxias do Sul, Rio Grande do Sul, Brazil, from 2010 to 2012. Sci. Med. 25, ID19519.
Pan, S., Yu, T., Wang, Y., Lu, R., Wang, H., Xie, Y., Feng, X., 2018. Identification of a torque teno mini virus (TTMV) in Hodgkin's lymphoma patients. Front. Microbiol. 9, 1680.
Parashar, U.D., Nelson, E.A., Kang, G., 2013. Diagnosis, management, and prevention of rotavirus gastroenteritis in children. Bmj 347, f7204.
Perkins, C., Mijatovic-Rustempasic, S., Ward, M.L., Cortese, M.M., Bowen, M.D., 2017. Genomic characterization of the first equine-like G3P[8] rotavirus strain detected in the United States. Genome Announce. 5.
Phan, T., Nagaro, K., 2020. Cutavirus: a newly discovered parvovirus on the rise. Infect. Gene. Evol. 80, 104175.
R Core Team, 2020. R: A language and environment for statistical computing. Accessed on,. https://www.R-project.org/.
Sell, J., Dolan, B., 2018. Common gastrointestinal infections. Primary Care 45, 519–532.
Silva, A.S.N., Silva, C.P., Barata, R.R., da Silva, P.V.R., Monteiro, P.D.J., Lamarao, L., Burbano, R.M.R., Nunes, M.R.T., de Lima, P.D.L., 2020. Human pegivirus (HPgV, GBV-C) RNA in volunteer blood donors from a public hemotherapy service in Northern Brazil. Virol. J. 17, 153.
Souza, D.F., Kisielius, J.J., Ueda, M., Gabbay, Y.B., Carmona, R.C., St Timenetsky Mdo, C., Mascarenhas, J.D., Takimoto, S., Tanaka, H., 1998. An outbreak of group C rotavirus gastroenteritis among adults living in Valentim Gentil, Sao Paulo State, Brazil. J. Diarrhoeal Dis. Res. 16, 59–65.
Spandole, S., Cimponeriu, D., Berca, L.M., Mihaescu, G., 2015. Human anelloviruses: an update of molecular, epidemiological and clinical aspects. Arch. Virol. 160, 893–908.
Strausbaugh, L.J., Sukumar, S.R., Joseph, C.L., 2003. Infectious disease outbreaks in nursing homes: an unappreciated hazard for frail elderly persons. Clin. Infect. Dis. 36, 870–876.
Taboada, B., Moran, P., Serrano-Vazquez, A., Isa, P., Rojas-Velazquez, L., Perez-Juarez, H., Lopez, S., Torres, J., Ximenez, C., Arias, C.F., 2021. The gut virome of healthy children during the first year of life is diverse and dynamic. PLoS One 16, e0240958.
Troeger, C., Khalil, I.A., Rao, P.C., Cao, S., Blacker, B.F., Ahmed, T., Armah, G., Bines, J. E., Brewer, T.G., Colombara, D.V., Kang, G., Kirkpatrick, B.D., Kirkwood, C.D., Mwenda, J.M., Parashar, U.D., Petri Jr., W.A., Riddle, M.S., Steele, A.D., Thompson, R.L., Walson, J.L., Sanders, J.W., Mokdad, A.H., Murray, C.J.L., Hay, S.I., Reiner Jr., R.C., 2018. Rotavirus vaccination and the global burden of rotavirus diarrhea among children younger than 5 years. JAMA Pediatr. 172, 958–965.
Trovao, N.S., Shepherd, F.K., Herzberg, K., Jarvis, M.C., Lam, H.C., Rovira, A., Culhane, M.R., Nelson, M.I., Marthaler, D.G., 2019. Evolution of rotavirus C in humans and several domestic animal species. Zoonoses Public Health 66, 546–557.
Tumbo, A.M., Schindler, T., Dangy, J.P., Orlova-Fink, N., Bieri, J.R., Mpina, M., Milando, F.A., Juma, O., Hamad, A., Nyakarungu, E., Chemba, M., Mtoro, A., Ramadhan, K., Olotu, A., Makweba, D., Mgaya, S., Stuart, K., Perreau, M., Stapleton, J.T., Jongo, S., Hoffman, S.L., Tanner, M., Abdulla, S., Daubenberger, C., 2021. Role of human Pegivirus infections in whole plasmodium falciparum sporozoite vaccination and controlled human malaria infection in African volunteers. Virol. J. 18, 28.
Varsani, A., Krupovic, M., 2018. Smacoviridae: a new family of animal-associated single-stranded DNA viruses. Arch. Virol. 163, 2005–2015.
Wikswo, M.E., Kambhampati, A., Shioda, K., Walsh, K.A., Bowen, A., Hall, A.J., Centers for Disease, C., Prevention, 2015. Outbreaks of acute gastroenteritis transmitted by person-to-person contact, environmental contamination, and unknown modes of transmission–United States, 2009–2013. In: Morbidity and Mortality Weekly Report. Surveillance Summaries, 64, pp. 1–16.

Yandle, Z., Coughlan, S., Dean, J., Hare, D., De Gascun, C.F., 2021. Indirect impact of rotavirus vaccination on viral causes of acute gastroenteritis in the elderly. J. Clin. Virol. 137, 104780.
Yinda, C.K., Vanhulle, E., Conceicao-Neto, N., Beller, L., Deboutte, W., Shi, C., Ghogomu, S.M., Maes, P., Van Ranst, M., Matthijnssens, J., 2019. Gut virome analysis of cameroonians reveals high diversity of enteric viruses, including potential interspecies transmitted viruses. mSphere 4.
Yokoi, H., Kitahashi, T., 2009. [Astrovirus RNA detection using real-time reverse transcription-polymerase chain reaction]. Kansenshogaku zasshi. J. Japan. Assoc. Infect. Dis. 83, 120–126.
Yu, T., Pan, S., Zhang, Y., Pei, J., Liu, J., Xie, Y., Feng, X., 2020. Occurrence and quantification of Anelloviruses and herpesviruses in gingival tissue in Chinese Shanghai sub-population. BMC Oral Health 20, 196.
Zaki, M., Shrief, R., Hassan, R., 2021. Molecular study of sapovirus in acute gastroenteritis in children: a cross-sectional study [version 2; peer review: 1 approved with reservations]. F1000Research 10.
Zhao, L., Rosario, K., Breitbart, M., Duffy, S., 2019. Eukaryotic circular rep-encoding single-stranded DNA (CRESS DNA) viruses: ubiquitous viruses with small genomes and a diverse host range. Adv. Virus Res. 103, 71–133.