Science of the Total Environment 645 (2018) 1334–1343

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Quito's virome: Metagenomic analysis of viral diversity in urban streams of Ecuador's capital city

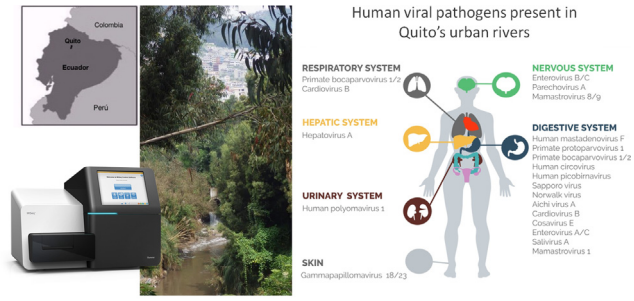
Laura Guerrero-Latorre [a,*], Brigette Romero [a], Edison Bonifaz [a], Natalia Timoneda [b], Marta Rusiñol [b], Rosina Girones [b], Blanca Rios-Touma [c]

[a] Grupo de investigación Biodiversidad, Medio Ambiente y Salud (BIOMAS), Facultad de Ingenierías y Ciencias Aplicadas (FICA), Ingeniería en Biotecnología, Universidad de las Américas, Quito, Ecuador
[b] Laboratory of Virus Contaminants of Water and Food, Department of Genetics, Microbiology and Statistics, University of Barcelona, Barcelona, Catalonia, Spain
[c] Grupo de investigación Biodiversidad, Medio Ambiente y Salud (BIOMAS), Facultad de Ingenierías y Ciencias Aplicadas (FICA), Ingeniería Ambiental, Universidad de las Américas, Quito, Ecuador

HIGHLIGHTS

- First viral metagomic study of highly impacted surface waters in Latin America
- The study describes human viral pathogens present in urban rivers of Quito.
- Several viral families detected containing emergent species firstly reported in Ecuador.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 May 2018
Received in revised form 16 July 2018
Accepted 16 July 2018
Available online 23 July 2018

Editor: Frederic Coulon

Keywords:
Urban river
Virome
Human viruses
Public health
Surface water quality

ABSTRACT

In Quito, the microbiological contamination of surface water represents a public health problem, mainly due to the lack of sewage treatment from urban wastewater. Contaminated water contributes to the transmission of many enteric pathogens through direct consumption, agricultural and recreational use. Among the different pathogens present in urban discharges, viruses play an important role on disease, being causes of gastroenteritis, hepatitis, meningitis, respiratory infections, among others.

This study analyzes the presence of viruses in highly impacted surface waters of urban rivers using next-generation sequencing techniques. Three representative locations of urban rivers, receiving the main discharges from Quito sewerage system, were selected. Water samples of 500 mL were concentrated by skimmed-milk flocculation method and the viral nucleic acid was extracted and processed for high throughput sequencing using Illumina MiSeq.

The results yielded very relevant data of circulating viruses in the capital of Ecuador. A total of 29 viral families were obtained, of which 26 species were associated with infections in humans. Among the 26 species identified, several were related to gastroenteritis: Human Mastadenovirus F, Bufavirus, Sapporovirus, Norwalk virus and Mamastrovirus 1. Also detected were: Gammapapillomavirus associated with skin infections, Polyomavirus 1 related to cases of kidney damage, Parechovirus A described as cause of neonatal sepsis with neurological affectations and Hepatovirus A, the etiologic agent of Hepatitis A. Other emergent viruses identified, of which its pathogenicity remains to be fully clarified, were: Bocavirus, Circovirus, Aichi Virus and Cosavirus.

* Corresponding author.
E-mail addresses: laura.guerrero.latorre@gmail.com (L. Guerrero-Latorre), bmromero@udlanet.ec (B. Romero), ebonifaz@udlanet.ec (E. Bonifaz), mrusinol@ub.edu (M. Rusiñol), rgirones@ub.edu (R. Girones), blanca.rios@udla.edu.ec (B. Rios-Touma).

https://doi.org/10.1016/j.scitotenv.2018.07.213

The wide diversity of species detected through metagenomics gives us key information about the public health risks present in the urban rivers of Quito. In addition, this study describes for the first time the presence of important infectious agents not previously reported in Ecuador and with very little reports in Latin America.

1. Introduction

Next generation sequencing studies have revealed the widespread of viruses in air, soil and water ecosystems, pointing out that viruses are the most abundant biological entities on Earth (Rastrojo and Alcamí, 2017; Rosario et al., 2018; Williamson et al., 2017). Although they are considered the simplest life forms, viral agents play an important role in human bodies as a part of our microbiome and being involved in acute infections and chronic diseases (Zárate et al., 2018). The interaction of humans with the surrounding environment (habitat, sanitation infrastructures, diet, leisure practices, etc.) has an impact on their viral exposure and at the same time, human viral excreta have also an impact on the environment polluting natural streams. Important viral pathogens causing diarrhoea, hepatitis, meningitis or associated to cancer are reported to be excreted by infected people during several days or even months, disseminating viruses in sanitation infrastructures (Rusiñol and Girones, 2017). Due to the representativeness of a specific human population, sewage, that collects excreta of thousand city's inhabitants, have been proposed as a tool for surveillance and monitoring of enteric viral pathogens among populations (Fernandez-Cassi et al., 2017b).

Latin America sanitation systems have improved last decades, however sewerage coverage is low, and <30% of wastewater is treated, causing severe microbial contamination in natural streams and increasing health risk for downstream populations (UNEP, 2016).

Quito is an example of a rapidly growing city (Ecuadorian human development index 0.789) but it still has very poor sanitation systems. Quito is the capital city of Ecuador, with a reported population of 2,597,989 inhabitants in 2016, expecting an increase of up to 7% by 2020 (INEC, 2016). The city is located 2800 m.a.s.l. and is crossed by several rivers that collect the urban sewage (93% coverage) without any treatment. As a big city, Quito produces 171 million m^3 of sewage per year at a mean flow of discharge 5413 L/s (EMAPS, 2016). Non-treated Quito wastewater contributes to the largest Ecuador's watershed, Esmeraldas river basin, that before ending up in the Pacific Ocean becomes the source of drinking water of Esmeraldas city (with approx. 160,000 inhabitants). It has been calculated that around 1% of the total Quito wastewater discharge impacts into the drinking water system source of Esmeraldas city (Voloshenko-Rossin et al., 2015).

Recent studies have used metagenomic platforms to describe viral pathogens in clinical samples from Peru, Chile, and Brazil. Two studies from Peruvian diarrheic children have shown the presence of circular Rep-encoding ssDNA (CRESS-DNA) viruses although the clinical association remains unclear (Altan et al., 2017; Phan et al., 2016). Also, studies in Brazil used metagenomic methods to analyze viral coinfections in Zika infected patients and revealed the presence of chikungunya virus and hepatitis A virus in different cases (Conteville et al., 2018; Sardi et al., 2016). Another study in Chile analysing the viral diversity in nasopharyngeal aspirates from acute lower respiratory tract infections, pointed out the extended presence of cyclovirus, anellovirus and adeno-associated viruses in these clinical samples (Phan et al., 2014).

Next generation sequencing of the viral population present in streams of Quito can give epidemiological data about waterborne viruses circulating among the capital's population and could identify reference pathogens and emerging strains. This information would also point out the importance of adapting treatment wastewater treatment systems and would enable the evaluation of potential health risks associated with using the downstream as a drinking water source.

The information obtained from these metagenomic studies has contributed to public health data, as many enteric diseases aetiologies are not usually reported. There are few studies describing enteric viruses among Ecuador's population but these have only reported Norovirus and Rotavirus prevalence in small populations (Gastañaduy et al., 2015; Vasco et al., 2014) and a small report about prevalence of Human Papillomavirus among women attended in a hospital of Southern Ecuador (Dalgo Aguilar et al., 2017). There are currently no reports on the presence, especially in the aquatic environment, about the presence of some and emerging enteric viruses related to human disease in the families *Picornaviridae*, *Astroviridae*, *Circoviridae*, and *Parvoviridae*.

In this study, we describe for the first time, the viral diversity in surface waters highly polluted by sewage discharge in an important Latin American city.

2. Methods

2.1. Sampling

In June 2017, during the dry season, 1 L of surface water was collected in three locations along urban streams of Quito. The water samples were taken with sterile bottles collecting in the middle of the riverbed at an average height of 50 cm (average river depth of 1.5 m). The location of the selected points was aimed to be representative of south (M1), centre (M2) and north (M3) parts of the city and its sewage contributions. The population density, the three sampling points located in the three principal sewage systems of the city and its main discharging points along the urban rivers Machangara and Monjas are shown in Fig. 1.

2.2. Concentration method and microbiological indicators analysis

A protocol for raw sewage viral concentration previously described was chosen for Quito urban rivers, as they are more similar to sewage matrices than freshwater. Collected water was concentrated for viral analysis using an adapted Skimmed Milk Flocculation using 500 mL of water (Fernandez-Cassi et al., 2017b). Briefly, the sample was preconditioned at pH 3.5 and conductivity above 1.5 mS/cm^2. Then, 5 mL of pre-flocculated skimmed milk solution were added. After 8 h of stirring, flocks were centrifuged at 8000 ×*g* for 40 min, and the pellet was suspended in 4 mL of phosphate buffer. The viral concentrate was kept at −80 °C until further use. Viral concentrates were used to analyze Human Adenovirus concentration by qPCR as a microbial indicator of human faecal contamination (Hernroth et al., 2002).

Additionally, *Escherichia coli* were quantified in each sample as faecal indicator bacteria (FIB) using serial dilutions on chromogenic agar (Chromocult, Merck).

2.3. Free DNA removal, nucleic acid extraction, library preparation and sequencing

Concentrates were firstly filtered through a 0.45-μm filter (Millipore) to remove bacterium-sized particles. Afterwards, filtered samples were processed for metagenomic analysis following previously described procedures (Fernandez-Cassi et al., 2017a).

DNAse treatment was applied to reduce free-DNA usually coming from great size genomes. For that purpose, 300 μL of each filtered concentrates were treated with 160 U of Turbo DNAse Ambion™ (Thermo

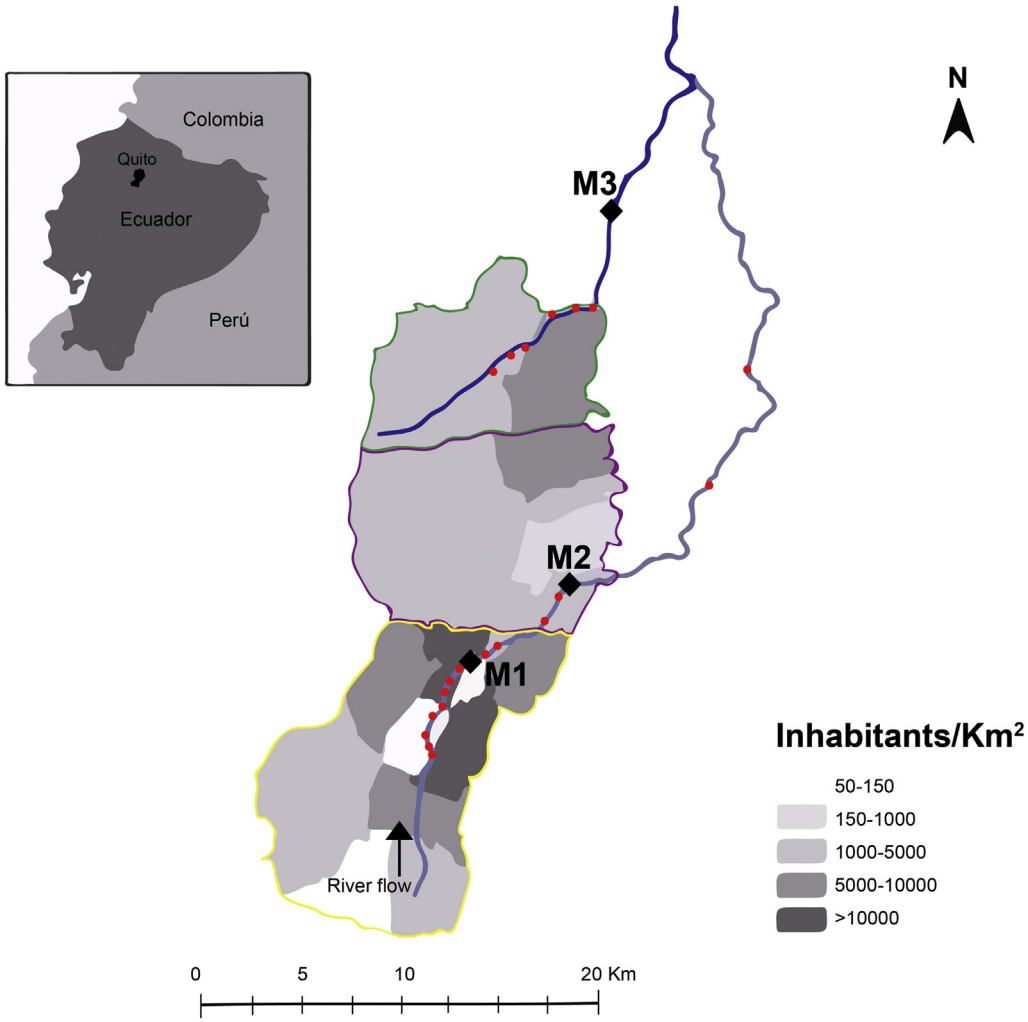


Fig. 1. Sampling locations along Quito. Urban rivers (blue) collecting all sewage discharges (red points) are represented in the map. M1 was collected in south discrict (yellow), M2 was collected in center district (pink) and M3 was collected after north district (green) of Quito.

Fisher Scientific, Massachusetts, USA) for 1 h at 37 °C to remove free DNA, then inactivated by incubating 10 min at 75 °C and centrifuged at 10.000 ×g for 1.5 min. Supernatants were kept at 4 °C.

Then, 280 μL of DNAse treated sample was extracted using Qiagen RNA Viral Mini Kit (Qiagen, Valencia, CA, USA) without the RNA carrier. Nucleic acids of each sample were eluted using 60 μL of AVE buffer from the same kit used for extraction.

In order to amplify DNA and RNA viruses, samples were retrotranscribed using random nonamer. Briefly, RNA templates were reverse transcribed using SuperScript III® (Thermo Fisher Scientific, Massachusetts, USA) Life Technologies and Primer A, which contains a 17-nucleotide-specific sequence followed by 9 random nucleotides for random priming (5′- GTTTCCCAGTCACGATANNNNNNNNN′- 3). A second cDNA strand was constructed using Sequenase 2.0 (USB/Affymetrix, Cleveland, OH, USA). To obtain sufficient DNA for library preparation, a PCR amplification step using Primer B (5′-GTTTCCCAG TCACGATA′-3) and AmpliTaqGold (Life Technologies, Austin, Texas, USA) was performed. After 10 min at 95 °C to activate DNA polymerase, the following PCR programme was used: 30 cycles of 30 s at 94 °C, 30 s at 40 °C, and 30 s at 50 °C with a final step of 60 s at 72 °C. PCR products were cleaned and concentrated in a small volume (15 μL) using the Zymo DNA clean and concentrator (Zymo research, USA). Amplified DNA samples were quantified using Qubit 2.0 (Life Technologies, Oregon, USA). The efficiency of the NA extraction and PCR amplification is controlled by running a gel electrophoresis to ensure a high quality and correct DNA concentration that has to overcome 1 μg/μL for library preparation.

Finally, libraries were constructed using a Nextera XT DNA sample preparation kit (Illumina Inc) according to the manufacturer's instructions. Samples were sequenced on Illumina MiSeq 2 × 250 bp and 2 × 300 bp, producing paired end reads.

2.4. Bioinformatic pipeline and quality filtering

The quality of raw and clean read sequences was assessed using the FASTX-Toolkit software, version 0.0.14 (Hannon Lab, http://www.hannonlab.org). Read sequences were cleaned and low complexity sequences discarded following previous protocols (Fernandez-Cassi et al., 2017a). Virome reads were assembled based on 90% identity over a minimum of 50% of the read length using CLC Genomics Workbench 4.4 (CLC bio USA, Cambridge, MA), and the resulting contig spectra were used as the primary input for the index.

Richness ratios were calculated using the Catchall software, version 4.0 (Allen et al., 2013). Of the models included in the package, the non-parametric model Chao1 was chosen, which was the model that provided the best results for the datasets. Heatmaps were generated using ggplot2 R graphics library (Kolde, 2015).

2.5. Viral description and typification analysis

Afterwards, contigs longer than 100 bp were queried for sequence similarity using BLASTN and BLASTX (Altschul et al., 1990) against the NCBI viral complete genomes database (Brister et al., 2015), the viral division from GenBank nucleotide database (Benson et al., 2017), and the viral protein sequences from UniProt (UniProt Consortium 2015, ftp://ftp.uniprot.org/pub/databases/uniprot/current_release). The species nomenclature and classification were performed according to the NCBI Taxonomy database standards. High-scoring pairs (HSPs) considered for taxonomical assessment had an *E*-value of 10–5 and minimum length of 100 bp. Based on the best BLAST result and 90% coverage cut-off, each sequence was classified into its likely taxonomic group.

Finally, viral sequences matching human viral species were analysed for typification when contigs were located in the typification regions established for this purpose due to its high genomic variability. The viral role on disease from the identified species was assessed and discussed based on the existing literature.

3. Results and discussion

3.1. Microbial contamination indicators

Three locations sampled along Quito rivers were highly impacted by microbial pollutants. Faecal indicator bacteria (FIB) values in M1, M2 and M3 locations exceeded the *E. coli* concentrations permitted for wastewater discharging effluents in natural water bodies (max. 2E+04 ufc *E. coli*/L) (Table 1) established by the Ecuadorian Ministry of Environment (MAE, 2015). Human Adenovirus, used as an indicator of human faecal contamination, was present in high and increasing concentrations along the riverflow, ranging from 6.2E+04, up to 8.22E+05 HAdV GC/L.

3.2. Metagenomic data

The 3 libraries analysed by MiSeq platform gave between 2.5 and 3.4 million sequences in each sample (Table 2). After cleaning and assembling by CLC the number of contigs obtained in M1, M2 and M3 were 105.311, 91.245 and 36.275, respectively. From those, the mean percentage which was assigned to any taxonomic viral family was 2.2%. Richness values, parameter that indicates the abundance and diversity of species detected per sample, were 580.8, 391.1 and 321.9 for M1, M2 and M3 respectively.

3.3. Viral families

Heatmap represented in Fig. 2 shows number of species from each viral family detected with at least 90% of identity towards the NCBI-complete genome database. A total of 29 viral families and 669 species matched with >90% identity towards complete viral genome databases. *Siphoviridae*, *Myoviridae* and *Podoviridae* were the most detected families, as previously reported in other viral sewage metagenomic studies (Cantalupo et al., 2011; Fernandez-Cassi et al., 2017b; Tamaki et al., 2012).

From all assembled contigs (232831), 5262 matched putative viral sequences (2,3%). Among viral sequences a 87,9% (4630 contigs) was assigned to species infecting known hosts (Fig. 3). From those contigs, 2624 were viruses infecting prokaryote (60,18%), 798 were viruses infecting plants (18,3%), 400 were viruses infecting humans (9,17%), 338 were viruses infecting other vertebrate animals (7,75%), followed that a short number of invertebrate, prokaryotes and fungi viruses (3,03%, 1,47%, 0,09% respectively) (Table 2).

Table 1
Sample characteristics and microbial indicators.

Location	Date	pH	Cond (μS)	TDS (mg/L)	E.coli (ufc/L)	HAdV (GC/L)
M1	15/5/17	7,99	587	293	8,48E+06	6,20E+04
M2		8,23	596	298	7,21E+06	1,42E+05
M3		8	557	279	5,30E+06	8,22E+05

3.4. Human viral pathogens

Regarding potentially pathogenic humans viruses, 27 different pathogenic viral species included in 9 different families were detected (Table 3).

Human Mastadenovirus F (until 2013 assigned as Human adenovirus F) was detected in all samples, as expected because its previously reported ubiquity worldwide, being used as a human faecal indicator in several studies (Rames et al., 2016). Moreover, Human Mastadenovirus F includes human adenovirus (HAdV) 40 and 41 and are known to be one of the main causes of gastroenteritis in children worldwide (Brown et al., 2016; Osborne et al., 2015). However, there is not published data reporting gastroenteritis cases caused by adenovirus in Ecuador. Actually, we have detected high concentrations of Human Adenovirus in the three samples previously to metagenomic analysis, using it as a human faecal indicator (mean value 3,42E+05 GC/L).

Human polyomavirus 1 (also known as BKPyV) was detected only in M1. Infection with Human polyomavirus has been associated with diseases of the urinary tract including hemorrhagic cystitis and ureteral stenosis, especially in immunosuppressed patients (Helle et al., 2017). Its presence in the environment has been previously reported in Europe due to is capacity to cause lifelong chronic infections (Bofill-Mas et al., 2000). Regarding Latin America it has been only reported in Argentina and Brazil among kidney transplant recipients (Schiavelli et al., 2014; Zalona et al., 2011).

Gamapapillomavirus were detected in M2 sample, corresponding with central area of Quito. This genus of papillomavirus, infecting only humans, has been associated to skin infections (Li et al., 2009). The species matched correspond to a recently described papillomaviruses in Argentina and Sweden related to skin lesions (Chouhy et al., 2013; Johansson et al., 2013). Identities from the two contigs detected were the lowest reported towards databases (73 and 78%), indicating that genomes found in Quito could be a new HPV type (de Villiers et al., 2004). However, its presence was confirmed by nested-PCR using previously described primers set for HPV (Forslund et al., 1999).

Primate protoparvovirus 1, Primate bocaparvovirus 1 and 3 were detected in all samples analysed by several contigs and high identities. Primate protoparvovirus 1 specie, consists of three strains of bufavirus (bufavirus 1a, 1b and 2), which were identified initially by deep sequencing in childhood diarrhoea cases from Burkina Faso in 2012 (Phan et al., 2012). Bufavirus has been reported in sporadic human cases of diarrhoea in Tunisia, Bhutan, Finland, China and the Netherlands (Huang et al., 2015). Primate bocaparvovirus 1 and 3 include gorilla and human viruses. Its presence was firstly found in nasopharyngeal sample, so it was associated to respiratory infection (Allander et al., 2005). However, their role on gastrointestinal disease is still unclear as its prevalence is similar between patients with symptoms and healthy controls in most studies and double-infections caused by other viral agents are frequently seen (Ong et al., 2016). More recently, other types of human bocavirus have been associated to diarrhoea symptoms, confirming its enteric replication and spread via feces (Guido et al., 2016). Regarding its circulation in Latin America, many countries have reported its presence previously including Argentina, Mexico, Uruguay, Paraguay and Brazil in stool samples from acute gastroenteritis patients and sewage samples (Adamo, 2017; Martínez et al., 2015; Proenca-Modena et al., 2013; Salvo et al., 2018; Santos et al., 2010).

Two species related to *Circoviridae* family were detected in all samples analysed by multiple contigs. Circovirus infect several species of vertebrates although only the Porcine Circovirus 2 cause disease, the postweaning multisystemic wasting syndrome (PMWS) which affects

Table 2
Genomic characteristics per sample. Composition of the total viromes as determined by similarity to known viral sequences.

	M1	M2	M3	Total
No. of nucleotides obtained MiSeq	777,067,620	1,044,712,606	734,755,448	2,556,535,674
No. of sequences obtained MiSeq	2,581,620	3,470,806	2,441,048	8,493,474
No. of assembled contigs	105,311	91,245	36,275	232,831
Mean contig length (min-max)	538,72 (200–15.975)	475,82 (200–5.524)	459,57 (200–6.695)	
Richness estimated value (SE)	580,8 (45,9)	391,1 (39,9)	321,9 (42,3)	
No. Putative viral sequences (% from total contigs)	2.943 (2,79%)	1.574 (1,7%)	745 (2,05%)	5262 (2,3%)
No. viral sequences with known host (% assigned)		4360 (87,9%)		
Human		*400 (9,2%)*		
Animal vertebrates (no human)		*338 (7,6%)*		
Animal invertebrates		*132 (3%)*		
Plants		*798 (18,3%)*		
Fungi		*4 (0,1%)*		
Procariota		*2624 (60,2%)*		
Protist		*64 (1,5%)*		

growth rate and produces systemic inflammatory lesions (Delwart and Li, 2011). Sequences matching Human Circovirus VS6600022 were highly related to a sequence obtained in diarrhoea samples from patients in the Netherlands with not identified aetiology (Smits et al., 2014). Another group of contigs matched with 99% identity a human circo-like sequence detected in Peruvian children with unexplained diarrhoea. The virus discovered in Peru was distantly related to the *Circoviridae* family and authors referred to it as pecoviruses (Peruvian stool-associated circo-like viruses) (Phan et al., 2016).

Three other contigs matched towards a Human Picobirnavirus recently detected in 3 years old children with idiopathic diarrhoea from China by metagenomic analysis (Sun et al., 2016). Recent studies however, suggest that picobirnavirus could be a bacteriophage (Krishnamurthy and Wang, 2018).

Caliciviridae, one of the most important families of viruses causing diarrhoea worldwide, was present by matching 72 contigs with Sapporo virus and Norwalk virus species. Both species indistinguishably cause gastrointestinal symptoms including diarrhoea and vomiting (Oka et al., 2015).

Furthermore, a great group of species belonging to *Picornaviridae* family were detected as well in Quito. Firstly, Aichi virus A, a highly widespread waterborne virus that seems to play a role causing gastroenteritis (Kitajima and Gerba, 2015). Aichi virus, firstly described in Japan in 1989, has been detected in many areas of the world, but regarding Latin America, only in Brazil from children with gastroenteritis and in sewage samples from Venezuela and Uruguay (Alcalá et al., 2010; Burutarán et al., 2016; Santos et al., 2010).

Scaffolfd virus (SAFV), a member of Cardiovirus B specie, was detected in one sample with a high genomic similarity (identity 97%) to a Peruvian strain isolated from a 2 year old child in 2012 (Leguia et al., 2015). SAFVs have been associated to respiratory and gastrointestinal infections and are present in many populations studied around the world including Peru, Brazil and Bolivia (Drexler et al., 2008; Leguia et al., 2015; Nix et al., 2013).Cosavirus A and E, infecting humans, were detected in Quito samples. Human cosavirus were firstly described in 2008 among south asian children and have been highly detected in the feces of symptomatic and asymptomatic subjects, being unclear its role in human enteric disease (Stöcker et al., 2012; Tapparel et al., 2013).

Enteroviruses, important members of *Picornaviridae* family, were also detected in all samples analysed. Among the three human enterovirus species found, Enterovirus C was the most detected, however non poliovirus species were found, unexpected because attenuated poliovirus strains are usually detected where immunization takes places (WHO, 2003). Hepatovirus A (until 2014 assigned as Hepatitis A virus) was detected in one sample. The strain detected is probably a wild strain, because there are not immunization campaigns implemented in Ecuador for Hepatovirus A, as it is considered a intermedium endemic country (Jacobsen and Wiersma, 2010).

Parechovirus A (until 2014 assigned as Human Parechovirus) was detected in all samples analysed. Although most of the infections as for the enterovirus are subclinical, these viruses may affect mainly young children causing a sepsis-like illness that can lead to seizures or significant neurological impairment (Olijve et al., 2017).

Mamastrovirus genera associated to human disease is subdivided into four divergent species: *MAstV 1, MAstV 6, MAstV 8* and *MAstV 9. In Quito the highest number of contigs reported were associated to MastV 1 (classical human astrovirus), expected as* MAstV 1 is the most common specie of Mamastrovirus found, especially in children (Vu et al., 2017). Although the pathogenic MAstV 6 role in human health has not been clearly demonstrated, 10 sequences were detected firstly in Ecuador. The first description of MAstV 6 in Latin American was first reported in 2011 in children presenting acute diarrhoea (Xavier et al., 2015) and it has also been described in sewage samples from Uruguay

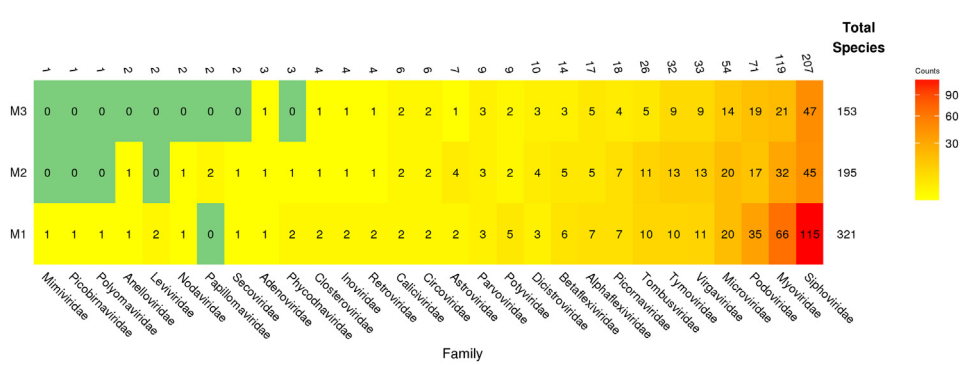


Fig. 2. Heatmap representing the abundance of viral families in the 3 city sites analysed (M1: south, M2: center, M3: north). The top row indicates the count sums of viral species classified by 29 families and the right column correspond to the count sums of viral species based on each sample. Data spanned from green (not detected) to red (high relative abundance), as illustrated by the colour scale.

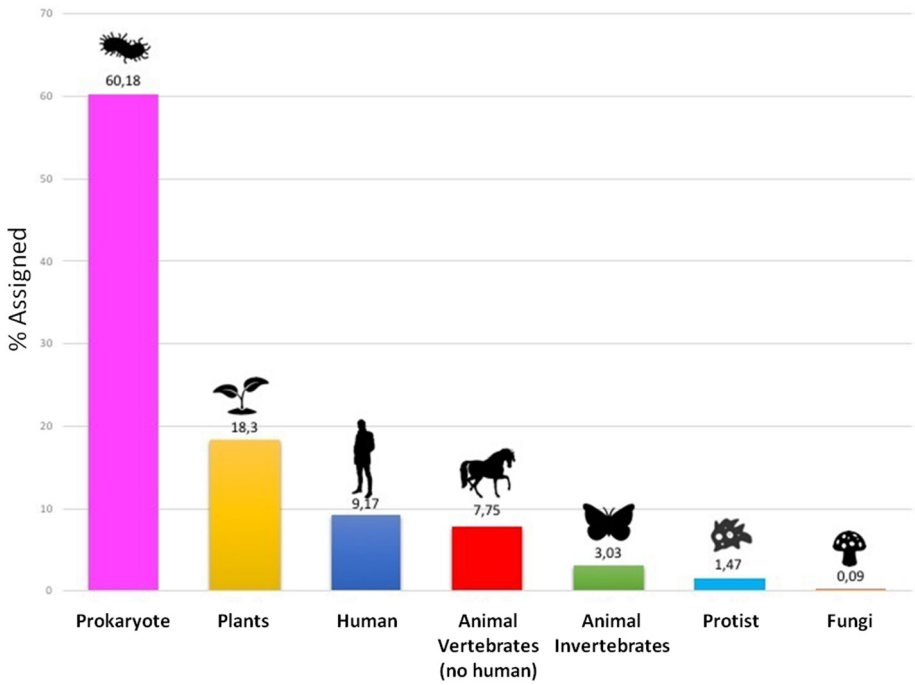


Fig. 3. Diagram representing relative abundance (%) of viral hosts from viral species detected in all samples analysed.

(Lizasoain et al., 2015). MAstV 8 and MastV 9, viruses isolated in children presenting non-polio acute flaccid paralysis or encephalitis (Vu et al., 2016), were also detected. This is the first evidence of those strains in Latin America.

Finally, any specie of *Reoviridae* family was detected in an endemic area of rotavirus maybe due to its low prevalence or seasonality. Although Rotavirus vaccination program established in 2007 in Ecuador reached the 100% coverage during 2010–2012, in the last years the immunization program has reduced significantly its coverage, and it dropped to 80% in 2016 (PAHO, 2018). Other metagenomic studies failed to detect rotavirus in endemic areas (Ng et al., 2012).

3.5. Typification of viral human species

In order to classify human viruses found into genotypes, we analysed viral contigs located into the typifying regions described for each viral specie (Table 4). This approach should also be considered as viruses can only be classified by genotype if we study

Table 3
Potentially pathogenic human viral families and species detected in Quito samples.

Viral family	Viral species	Sample			Total number of sequences	Maximum length	Maximum homology
		M1	M2	M3			
Adenoviridae	Human mastadenovirus F	1	1	3	5	706	100%
Polyomaviridae	Human polyomavirus 1	2	0	0	2	822	99%
Papillomaviridae	Gammapapillomavirus 18	0	1	0	1	365	73%
	Gammapapillomavirus 23	0	1	0	1	849	78%
Parvoviridae	Primate protoparvovirus 1	1	2	1	4	1147	100%
	Primate bocaparvovirus 1	5	3	2	10	1333	100%
	Primate bocaparvovirus 2	1	3	2	6	840	100%
Circoviridae	Human circovirus VS6600022	2	4	6	12	1119	99%
	Circovirus like	2	3	4	9	795	99%
Picobirnaviridae	Human picobirnavirus	3	0	0	3	874	98%
Caliciviridae	Sapporo virus	18	9	1	28	1154	98%
	Norwalk virus	21	20	3	44	1191	99%
Picornaviridae	Aichivirus A	0	6	0	6	581	98%
	Cardiovirus B	1	0	0	1	636	97%
	Cosavirus A	0	3	0	3	602	88%
	Cosavirus E	0	0	1	1	325	91%
	Enterovirus A	1	0	3	4	525	91%
	Enterovirus B	3	1	0	4	622	89%
	Enterovirus C	22	23	7	52	1348	97%
	Hepatovirus A	2	0	0	2	588	99%
	Parechovirus A	2	2	1	5	805	91%
	Salivirus A	2	8	0	10	1123	97%
Astroviridae	Mamastrovirus 1	29	26	7	62	3415	99%
	Mamastrovirus 6	6	4	0	10	2046	99%
	Mamastrovirus 8	0	2	0	2	411	97%
	Mamastrovirus 9	0	2	0	2	734	99%

Table 4
Typification analysis of human viral species. Genotype, type or serotypes are listed from contigs located into the typing region determined for each viral specie.

Family Specie	% typified contigs	Typing region	Position	Lenght	Blast identity (%)	Acc. Number	Genotype and subtype
Adenoviridae							
Human mastadenovirus F	40% (2/5)	Hexon protein	**17643-20414** [a]				
Contig_56367			19053-19750	698	99	KU162869.1	Human adenovirus F serotype 40
Contig_19441			18990-19217	227	99	KY316164.1	Human adenovirus F serotype 41
Papillomaviridae							
Gammapapillomavirus 18	100% (1/1)	L1 protein	**4917-6581** [b]				
Contig_49803			6099-7011	849	74	JX429973.1	Human papillomavirus type 156
Parvoviridae							
Primate protoparvovirus 1	75% (3/4)	VP1	**2397-4495** [c]				
Contig_78300			2934-3496	562	99	KU362763.1	Human bufavirus genotype 1
Contig_66586			3059-4205	1147	99	KX856937.1	Human bufavirus genotype 1
Contig_34693			2086-2602	481	99	KX856937.1	Human bufavirus genotype 1
Primate bocaparvovirus 1	50% (5/10)	VP1	**3023-5029** [d]				
Contig_61389			2675-3182	507	99	KM624026.1	Human bocavirus genotype 3
Contig_79350			4796-5230	435	99	JN086998.1	Human bocavirus genotype 3
Contig_46837			2631-3296	639	99	GU048665.1	Human bocavirus genotype 3
Contig_81024			2857-3159	303	100	KM624026.1	Human bocavirus genotype 3
Contig_25624			3670-4298	592	99	FJ948861.1	Human bocavirus genotype 3
Primate bocaparvovirus 2	83% (5/6)	VP1	**2961-4964** [e]				
Contig_48745			3891-4548	658	99	KY050744.1	Human bocavirus genotype 2
Contig_54132			3836-4506	638	100	KY050744.1	Human bocavirus genotype 2
Contig_63255			2630-3469	840	99	JQ964115.1	Human bocavirus genotype 2
Contig_31575			3626-4077	452	99	KY050744.1	Human bocavirus genotype 2
Contig_29823			3461-3786	326	87	HQ871668.1	Human bocavirus genotype 2
Caliciviridae							
Sapporo virus	39% (11/28)	VP1	**5173-6855** [f]				
Contig_100319			6171-6565	357	96	KP298674.1	Human sapovirus GI.1
Contig_27454			5338-5820	453	96	KP298674.1	Human sapovirus GI.1
Contig_42602			6520-6995	473	96	AB455796.1	Human sapovirus GI
Contig_47189			6146-6442	269	91	EU124657.1	Human sapovirus GI.2
Contig_21513			5653-6101	450	94	KM092508.1	Human sapovirus GI.2
Contig_42601			6035-6620	540	91	EU124657.1	Human sapovirus GI.2
Contig_42600			6157-6620	33	91	EU124657.1	Human sapovirus GI.2
Contig_104232			5916-6494	531	95	AY237420.2	Human sapovirus GII
Contig_36335			4529-5544	903	97	AB924385.1	Human sapovirus GV
Contig_64244			6393-7186	791	97	AB924385.1	Human sapovirus GV
Contig_89839			6713-6960	245	98	AB924385.1	Human sapovirus GV
Norwalk virus	30% (13/43)	ORF1	**3572-5101** [g]				
		ORF2	**5085-6692** [g]				
Contig_14305			5764-6077	319	98	KT732280.1	Norovirus GI.6
Contig_89122			5113-5431	318	98	KF944271.2	Norovirus GII.1
Contig_15510			5417-6204	787	99	KY806294.1	Norovirus GII.2
Contig_27177			5513-5934	421	99	MG002630.1	Norovirus GII.4
Contig_23524			6269-6853	584	99	KY905335.1	Norovirus GII.4
Contig_58560			5524-5847	323	99	KM386681.1	Norovirus GII.5
Contig_14582			4935-5677	739	99	KM267742.1	Norovirus GII.6
Contig_38983			5678-5932	254	99	KM036375.1	Norovirus GII.6
Contig_34549			6111-6484	373	95	KY424344.1	Norovirus GII.6
Contig_6769			4469-4713	244	100	KY485110.1	Norovirus GII.P16
Contig_6008			3858-4515	657	99	KY421159.1	Norovirus GII.P16
Contig_6009			3858-4515	656	99	KY421177.1	Norovirus GII.P16
Contig_100782			4185-4745	560	95	MF668937.1	Norovirus GII.Pg
Picornaviridae							
Enterovirus A	50%	VP2	**955-1728** [h]				
	(2/4)	VP3	**1729-2460** [h]				
contig_23332			1408-1689	281	92	AY697471.1	Enterovirus A76
contig_23329			1703-1978	275	91	AY697468.1	Enterovirus A76
Enterovirus B	50%	VP2	**949-1737** [i]				
	(2/4)	VP3	**1738-2451** [i]				
contig_46549			1847-2274	427	89	KJ957190.1	Echovirus 25
contig_47150			1401-1859	458	87	HM031191.1	Echovirus 25
Enterovirus C	44% (22/50)	VP1	**2480-3385** [j]				
		VP2	**950-1765** [j]				
		VP3	**1766-2479** [j]				
contig_91322			957-1447	490	86	JX174177.1	Coxsackievirus A1
contig_2722			693-1864	1171	89	KC785529.1	Coxsackievirus A1
Contig_56536			2671-3670	996	82	JX174176.1	Coxsackievirus A1
contig_61167			1022-1733	708	80	DQ995637.1	Coxsackievirus A13
Contig_80491			2690-2983	296	93	KX932039.1	Coxsackievirus A19
Contig_54025			2937-3820	880	89	KX932039.1	Coxsackievirus A19
Contig_35788			1384-2726	1342	90	KX932039.1	Coxsackievirus A19
Contig_67125			2675-3543	868	90	AB828290.1	Coxsackievirus A19
Contig_89878			2579-3151	575	93	KX932039.1	Coxsackievirus A19
Contig_10091			2936-3225	289	93	KX932039.1	Coxsackievirus A19
contig_19652			836-1711	872	89	AB828290.1	Coxsackievirus A19

Table 4 (*continued*)

Family Specie	% typified contigs	Typing region	Position	Lenght	Blast identity (%)	Acc. Number	Genotype and subtype
contig_81902			1944-2472	528	89	AB828290.1	Coxsackievirus A19
contig_12945			1544-1874	330	88	AB828288.1	Coxsackievirus A19
contig_9842			1933-2395	462	91	AB828290.1	Coxsackievirus A19
Contig_70355			2693-3265	572	89	AB828290.1	Coxsackievirus A19
Contig_65082			910-1443	532	82	KU183495.1	Coxsackievirus A24
contig_39567			1428-2577	1146	80	EF015033.1	Coxsackievirus A24
Contig_79066			2186-2702	519	79	EF015033.1	Coxsackievirus A24
Contig_56057			2850-4002	1152	81	EF555644.1	Enterovirus C99
Contig_66760			2820-3827	1009	83	EF015011.1	Enterovirus C99
contig_34711			1861-2459	595	83	EF555644.1	Enterovirus C99
Contig_38531			2815-3775	963	83	KF129411.1	Enterovirus C99
Hepatovirus A	50% (1/2)	VP1	**2208-3107** [k]				
Contig_99111			3003-3307	305	99	MF175366.1	Hepatovirus genotype IA
Astroviridae							
Mamastrovirus 1	23% (14/62)	ORF2	**4289-6673** [l]				
Contig_92012			6266-6673	456	97	HQ398856.2	Human astrovirus genotype 1
Contig_63238			4450-4696	241	93	HQ398856.2	Human astrovirus genotype 1
Contig_44034			5514-5810	297	99	HQ398856.2	Human astrovirus genotype 1
Contig_4556			4789-5558	724	98	HQ398856.2	Human astrovirus genotype 1
Contig_258			2696-5935	3003	98	HQ398856.2	Human astrovirus genotype 1
Contig_59279			4412-4674	263	98	KX932051.1	Human astrovirus genotype 2
Contig_7859			4649-5169	481	98	JX087964.1	Human astrovirus genotype 2
Contig_97655			5076-5696	606	99	KF668570.1	Human astrovirus genotype 3c
Contig_11722			4237-4553	317	100	KU318561.1	Human astrovirus genotype 4
Contig_31067			4840-5558	640	98	AB025812.1	Human astrovirus genotype 4
Contig_26370			6072-6474	409	96	KF039913.1	Human astrovirus genotype 4
Contig_21700			4425-5056	608	98	AB025806.1	Human astrovirus genotype 4
Contig_31066			4701-4951	243	98	AB025812.1	Human astrovirus genotype 4
Contig_11721			4180-4553	373	99	AF292073.1	Human astrovirus genotype 8

a NC_001454.1; b NC_008189.1; c JX027295.1; d NC_012564.1; e NC_012042.1; f NC_006554.1; g NC_029646.1; h AY697458.1; i NC_001472.1; j NC_002058.3; k NC_001489.1; l NC_030922.1

annealings in the specific typing region determined for its high genomic variability.

Mastadenovirus F contigs located into hexon region were matching to type 40 and 41, strains associated with acute gastroenteritis and very common found in sewage matrices (Iaconelli et al., 2017).

Regarding sequences from Gammapapillomavirus, only one sequence could be confirmed as a Human Papilloma type 156. The strain was first identified in 2010 in Argentina, from a 83-year-old male skin sample with basal cell carcinoma in the upper lip (Chouhy et al., 2010). However, the same strain was found in healthy skin subjects so its role in pathogenesis is still unclear (Chouhy et al., 2013).

Three contigs related to protoparvovirus species were assigned to Human Bufavirus 1 genotype (BuV1). This genotype has been associated to gastroenteritis in several studies although many authors suggest that more studies need to be conducted to better characterise their pathogenic role in humans (Ayouni et al., 2016; Väisänen et al., 2017). The presence of this genotype has not been reported before in America, as only BuV3 was reported in a Peruvian study (Phan et al., 2016).

Primate bocaparvovirus 1 and 3 were assigned to Human Bocavirus 2 and 3 respectively. This is likely to occur since Human Bocavirus subtypes 2, 3 are associated to gastrointestinal infections; therefore they are excreted from the gastrointestinal tract (Salvo et al., 2018).

Among the Calicivirus detected in samples analysed, Sapporo virus (SaV) genogrups I, II and V, were detected in Quito according to previous studies in Latin America (Costa et al., 2017; Sánchez et al., 2018). Regarding Norovirus or Norwalk virus (NoV) genotypes, high diversity was observed in sewage samples, with NoV GI.6 and GII.1, GII.2, GII.4, GII.5, GII.6 and GII.p16. Two other studies in Ecuador reported NoV genotype description including GI.3, GII.1, GII.4, GII.6 and G.16 genotypes (Gastañaduy et al., 2015; Lopman et al., 2015).

A great group of Enterovirus C strains were reported, mostly found in healthy patients (CA1, CA13, CA19, CA24) (Faleye et al., 2016). However, Enterovirus C99, found in 4 sequences (81–83% identity), seems to be related with Acute Flacid Paralysis cases in children from West Africa (Fernandez-Garcia et al., 2017).

From Enterovirus A we identified EV-76 strain, firstly found in France (1991) from a patient suffering of gastroenteritis (Oberste et al., 2005) Finally, the unique Enterovirus B strain identified, Echovirus 25 (E-25), has been associated to important clinical symptoms ranging from minor herpangina, skin rash, hand, foot, and mouth disease (HFMD), severe acute flaccid paralysis (AFP), and encephalitis to aseptic meningitis (Li et al., 2015). Regarding Latin America, E-25 has been only reported in few cerebrospinal fluid specimens from meningitis cases in Brazil (dos Santos et al., 2006).

A partial sequence in VP1 from Hepatovirus A matched with IA genotype with an identity of 99%. This is the first evidence of IA genotype in Ecuador, but it has already been reported as the most abundant genotype in Latin America (Prado et al., 2012; Sulbaran et al., 2010).

Human Astrovirus types 1,2,3,4 and 8 were detected in Quito, showing a great diversity among this important gastroenteritis viral specie. This genotype characterization is firstly described in Ecuador but strains have been previously detected in other Latin American countries among clinical specimens in Brazil and Venezuela as well as in environmental waters from Argentina and Uruguay (González et al., 2011; Lizasoain et al., 2015; Masachessi et al., 2018; Resque et al., 2007; Siqueira et al., 2017).

4. Conclusions

Among the human viral families detected in Quito's urban streams, important human pathogens have been detected, including members of the *Parvoviridae*, *Caliciviridae*, *Adenoviridae*, *Polyomaviridae*, *Papillomaviridae*, *Picornaviridae* and *Astroviridae* families.

The detection of the wide diversity of viral species and genotypes described in Quito urban steams is a very useful information to health practitioners in order to consider more aetiological agents circulating among patients.

The results obtained describe for the first time the circulation in Ecuador of a great variety of viral species causing gastroenteritis: Mastadenovirus F, Human Bocavirus, Human Bufavirus, Cosavirus, Sappovirus, Aichi Virus and Astrovirus. Other virus newly detected in Ecuador are associated to important clinical syndromes affecting urinary tract, skin tissues, and meningitis by Human Polyomavirus 1 (BKPyV), Gamapapillomavirus, Parechovirus A and Echovirus 25 respectively.

Moreover, the information produced on the long list of important viral pathogens and emerging viral strains present in urban rivers of Ecuador's capital city should contribute to alert local governments and to establish sanitation measures to prevent viral transmission across the river basin.

This study provides more evidence on the benefits of the metagenomics public health surveillance systems based on excreted viruses in sewage and superficial waters and the viability of developing data bases of the viruses circulating in different human populations.

Acknowledgements

This work was supported by the Universidad de las Americas, Quito-Ecuador (Dirección de Investigaciones, AMB·BRT.17.01). Special thanks to Xavier Amigo and Nature Experience for its invaluable service during the sampling campaign.

References

Adamo, M.P., 2017. Human Bocavirus 1: role in the acute respiratory infection and epidemiology in Cordoba, Argentina. Rev. Fac. Cien. Med. Univ. Nac. Cordoba 74, 134–143.

Alcalá, A., Vizzi, E., Rodríguez-Díaz, J., Zambrano, J.L., Betancourt, W., Liprandi, F., 2010. Molecular detection and characterization of Aichi viruses in sewage-polluted waters of Venezuela. Appl. Environ. Microbiol. 76, 4113–4115. https://doi.org/10.1128/AEM.00501-10.

Allander, T., Tammi, M.T., Eriksson, M., Bjerkner, A., Tiveljung-Lindell, A., Andersson, B., 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proc. Natl. Acad. Sci. U. S. A. 102, 12891–12896. https://doi.org/10.1073/pnas.0504666102.

Allen, H.K., Bunge, J., Foster, J.A., Bayles, D.O., Stanton, T.B., 2013. Estimation of viral richness from shotgun metagenomes using a frequency count approach. Microbiome 1, 5. https://doi.org/10.1186/2049-2618-1-5.

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 Announc. 5 (e00822-17). https://doi.org/10.1128/genomeA.00822-17.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Ayouni, S., Estienney, M., Hammami, S., Neji Guediche, M., Pothier, P., Aouni, M., Belliot, G., de Rougemont, A., 2016. Cosavirus, Salivirus and Bufavirus in diarrheal Tunisian infants. PLoS One 11 (e0162255). https://doi.org/10.1371/journal.pone.0162255.

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2017. GenBank. Nucleic Acids Res. 45 (D37–D42). https://doi.org/10.1093/nar/gkw1070.

Bofill-Mas, S., Pina, S., Girones, R., 2000. Documenting the epidemiologic patterns of polyomaviruses in human populations by studying their presence in urban sewage. Appl. Environ. Microbiol. 66, 238–245.

Brister, J.R., Ako-adjei, D., Bao, Y., Blinkova, O., 2015. NCBI viral genomes resource. Nucleic Acids Res. 43 (D571–D577). https://doi.org/10.1093/nar/gku1207.

Brown, J.R., Shah, D., Breuer, J., 2016. Viral gastrointestinal infections and norovirus genotypes in a paediatric UK hospital, 2014–2015. J. Clin. Virol. 84, 1–6. https://doi.org/10.1016/j.jcv.2016.08.298.

Burutarán, L., Lizasoain, A., García, M., Victoria, M., 2016. Detection and molecular characterization of aichivirus 1 in wastewater samples from Uruguay. Food Environ. Virol. 8, 13–17. https://doi.org/10.1007/s12560-015-9217-1.

Cantalupo, P.G., Calgua, B., Zhao, G., 2011. Raw sewage harbors diverse viral populations. MBio 2, 1–11. https://doi.org/10.1128/mBio.00180-11.Editor.

Chouhy, D., Gorosito, M., Sánchez, A., Serra, E.C., Bergero, A., Fernandez Bussy, R., Giri, A.A., 2010. New generic primer system targeting mucosal/genital and cutaneous human papillomaviruses leads to the characterization of HPV 115, a novel Beta-papillomavirus species 3. Virology 397, 205–216. https://doi.org/10.1016/j.virol.2009.11.020.

Chouhy, D., Bolatti, E.M., Piccirilli, G., Sanchez, A., Fernandez Bussy, R., Giri, A.A., 2013. Identification of human papillomavirus type 156, the prototype of a new human gammapapillomavirus species, by a generic and highly sensitive PCR strategy for long DNA fragments. J. Gen. Virol. 94, 524–533. https://doi.org/10.1099/vir.0.048157-0.

Conteville, L.C., de Filippis, A.M.B., Nogueira, R.M.R., de Mendonça, M.C.L., Vicente, A.C.P., 2018. Metagenomic analysis reveals hepatitis a virus in suspected yellow fever cases in Brazil. Mem. Inst. Oswaldo Cruz 113, 66–67. https://doi.org/10.1590/0074-02760170260.

Costa, L.C.P. das N., Siqueira, J.A.M., Portal, T.M., Sousa Júnior, E.C., da Linhares, A.C., Gabbay, Y.B., Resque, H.R., 2017. Detection and genotyping of human adenovirus and sapovirus in children with acute gastroenteritis in Belém, Pará, between 1990 and 1992: first detection of GI.7 and GV.2 sapoviruses in Brazil. Rev. Soc. Bras. Med. Trop. 50, 621–628. https://doi.org/10.1590/0037-8682-0198-2017.

Dalgo Aguilar, P., Loján González, C., Córdova Rodríguez, A., Acurio Páez, K., Arévalo, A.P., Bobokova, J., 2017. Prevalence of high-risk genotypes of human papillomavirus: women diagnosed with premalignant and malignant pap smear tests in Southern Ecuador. Infect. Dis. Obstet. Gynecol. 2017, 1–7. https://doi.org/10.1155/2017/8572065.

Delwart, E., Li, L., 2011. Rapidly expanding genetic diversity and host range of the Circoviridae viral family and other Rep encoding small circular ssDNA genomes. Virus Res. 164, 114–121. https://doi.org/10.1016/j.virusres.2011.11.021.

dos Santos, G.P.L., Skraba, I., Oliveira, D., Lima, A.A.F., de Melo, M.M.M., Kmetzsch, C.I., da Costa, E.V., da Silva, E.E., 2006. Enterovirus meningitis in Brazil, 1998–2003. J. Med. Virol. 78, 98–104. https://doi.org/10.1002/jmv.20509.

Drexler, J.F., Luna, L.K. de S., Stöcker, A., Almeida, P.S., Ribeiro, T.C.M., Petersen, N., Herzog, P., Pedroso, C., Huppertz, H.I., Ribeiro, H. da C., Baumgarte, S., Drosten, C., Drosten, C., 2008. Circulation of 3 lineages of a novel Saffold cardiovirus in humans. Emerg. Infect. Dis. 14, 1398–1405. https://doi.org/10.3201/eid1409.080570.

EMAPS, 2016. Memoria de Sostenibilidad. Quito.

Faleye, T.O.C., Adewumi, M.O., Coker, B.A., Nudamajo, F.Y., Adeniji, J.A., 2016. Direct detection and identification of enteroviruses from faeces of healthy Nigerian children using a cell-culture independent RT-seminested PCR assay. Adv. Virol. 2016, 1–12. https://doi.org/10.1155/2016/1412838.

Fernandez-Cassi, X., Timoneda, N., Gonzales-Gustavson, E., Abril, J.F., Bofill-Mas, S., Girones, R., 2017a. A metagenomic assessment of viral contamination on fresh parsley plants irrigated with fecally tainted river water. Int. J. Food Microbiol. 257, 80–90. https://doi.org/10.1016/j.ijfoodmicro.2017.06.001.

Fernandez-Cassi, X., Timoneda, N., Martínez-Puchol, S., Rusiñol, M., Rodriguez-Manzano, J., Figuerola, N., Bofill-Mas, S., Abril, J.F., Girones, R., 2017b. Metagenomics for the study of viruses in urban sewage as a tool for public health surveillance. Sci. Total Environ., 1–11 https://doi.org/10.1016/j.scitotenv.2017.08.249.

Fernandez-Garcia, M.D., Kebe, O., Fall, A.D., Ndiaye, K., 2017. Identification and molecular characterization of non-polio enteroviruses from children with acute flaccid paralysis in West Africa, 2013–2014. Sci. Rep. 7 (3808). https://doi.org/10.1038/s41598-017-03835-1.

Forslund, O., Antonsson, A., Stenquist, B., Göran Hansson, B., Nordin, P., 1999. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J. Gen. Virol. 80, 2437–2443. https://doi.org/10.1099/0022-1317-80-9-2437.

Gastañaduy, P.A., Vicuña, Y., Salazar, F., Broncano, N., Gregoricus, N., Vinjé, J., Chico, M., Parashar, U.D., Cooper, P.J., Lopman, B., 2015. Transmission of norovirus within households in Quininde, Ecuador. Pediatr. Infect. Dis. J. 34, 1031–1033. https://doi.org/10.1097/INF.0000000000000783.

González, G.G., Liprandi, F., Ludert, J.E., 2011. Molecular epidemiology of enteric viruses in children with sporadic gastroenteritis in Valencia, Venezuela. J. Med. Virol. 83, 1972–1982. https://doi.org/10.1002/jmv.22185.

Guido, M., Tumolo, M.R., Verri, T., Romano, A., Serio, F., De Giorgi, M., De Donno, A., Bagordo, F., Zizza, A., 2016. Human bocavirus: current knowledge and future challenges. World J. Gastroenterol. 22, 8684–8697. https://doi.org/10.3748/wjg.v22.i39.8684.

Helle, F., Brochot, E., Handala, L., Martin, E., Castelain, S., Francois, C., Duverlie, G., 2017. Biology of the BKPyV: an update. Viruses 9, 327. https://doi.org/10.3390/v9110327.

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.

Huang, D.-D., Wang, W., Lu, Q.-B., Zhao, J., Guo, C.-T., Wang, H.-Y., Zhang, X.-A., Tong, Y.-G., Liu, W., Cao, W.-C., 2015. Identification of Bufavirus-1 and Bufavirus-3 in feces of patients with acute diarrhea, China. Sci. Rep. 5 (13272). https://doi.org/10.1038/srep13272.

Iaconelli, M., Valdazo-González, B., Equestre, M., Ciccaglione, A.R., Marcantonio, C., Della Libera, S., La Rosa, G., 2017. Molecular characterization of human adenoviruses in urban wastewaters using next generation and sanger sequencing. Water Res. 121, 240–247. https://doi.org/10.1016/J.WATRES.2017.05.039.

INEC, 2016. INEC presenta sus proyecciones poblacionales cantonales|Instituto Nacional de Estadística y Censos. WWW Document. URL. http://www.ecuadorencifras.gob.ec/inec-presenta-sus-proyecciones-poblacionales-cantonales/ (accessed 11.28.17).

Jacobsen, K.H., Wiersma, S.T., 2010. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. Vaccine 28, 6653–6657. https://doi.org/10.1016/J.VACCINE.2010.08.037.

Johansson, H., Bzhalava, D., Ekström, J., Hultin, E., Dillner, J., Forslund, O., 2013. Metagenomic sequencing of "HPV-negative" condylomas detects novel putative HPV types. Virology 440, 1–7. https://doi.org/10.1016/j.virol.2013.01.023.

Kitajima, M., Gerba, C.P., 2015. Aichi virus 1: environmental occurrence and behavior. Pathog. (Basel, Switzerland) 4, 256–268. https://doi.org/10.3390/pathogens4020256.

Kolde, R., 2015. CRAN — Package Pheatmap. WWW Document. URL. https://cran.r-project.org/web/packages/pheatmap/index.html.

Krishnamurthy, S.R., Wang, D., 2018. Extensive conservation of prokaryotic ribosomal binding sites in known and novel picobirnaviruses. Virology 516, 108–114. https://doi.org/10.1016/j.virol.2018.01.006.

Leguia, M., Loyola, S., Rios, J., Juarez, D., Guevara, C., Silva, M., Prieto, K., Wiley, M., Kasper, M.R., Palacios, G., Bausch, D.G., 2015. Full genomic characterization of a Saffold virus

isolated in Peru. Pathog. (Basel, Switzerland) 4, 816–825. https://doi.org/10.3390/pathogens4040816.
Li, L., Barry, P., Yeh, E., Glaser, C., Schnurr, D., Delwart, E., 2009. Identification of a novel human gammapapillomavirus species. J. Gen. Virol. 90, 2413–2417. https://doi.org/10.1099/vir.0.012344-0.
Li, H., Meng, Y., Pang, L., Liang, J., Lu, H., Wang, Q., Liang, P., Cao, J., Liu, S., Cheng, J., 2015. Complete genome sequence of a new recombinant echovirus 25 strain isolated from a neonatal patient with hand, foot, and mouth disease complicated by encephalitis in Beijing, China. Virus Genes 50, 505–508. https://doi.org/10.1007/s11262-015-1186-9.
Lizasoain, A., Tort, L.F.L.F.L., García, M., Gómez, M.M.M., Leite, J.P.G.P.G., Miagostovich, M.P.P., Cristina, J., Colina, R., Victoria, M., 2015. Environmental assessment reveals the presence of MLB-1 human astrovirus in Uruguay. 119. https://doi.org/10.1111/jam.12856.
Lopman, B.A., Trivedi, T., Vicuña, Y., Costantini, V., Collins, N., Gregoricus, N., Parashar, U., Sandoval, C., Broncano, N., Vaca, M., Chico, M.E., Vinjé, J., Cooper, P.J., 2015. Norovirus infection and disease in an Ecuadorian birth cohort: association of certain norovirus genotypes with Host FUT2 secretor status. J. Infect. Dis. 211, 1813–1821. https://doi.org/10.1093/infdis/jiu672.
MAE, 2015. Norma de calidad ambiental y de descarga de efluentes al recurso de agua. Ministerio del Ambiente Ecuatoriano, pp. 1–37.
Martínez, M.A., de los Dolores Soto-del Río, M., Gutiérrez, R.M., Chiu, C.Y., Greninger, A.L., Contreras, J.F., López, S., Arias, C.F., Isa, P., 2015. DNA microarray for detection of gastrointestinal viruses. J. Clin. Microbiol. 53, 136–145. https://doi.org/10.1128/JCM.01317-14.
Masachessi, G., Pisano, M.B., Prez, V.E., Martínez, L.C., Michelena, J.F., Martínez-Wassaf, M., Giordano, M.O., Isa, M.B., Pavan, J.V., Welter, A., Nates, S.V., Ré, V., 2018. Enteric viruses in surface waters from Argentina: molecular and viable-virus detection. Appl. Environ. Microbiol. 84 (e02327-17). https://doi.org/10.1128/AEM.02327-17.
Ng, T.F.F., Marine, R., Wang, C., Simmonds, P., Kapusinszky, B., Bodhidatta, L., Oderinde, B.S., Wommack, K.E., Delwart, E., 2012. High variety of known and new RNA and DNA viruses of diverse origins in untreated sewage. J. Virol. 86, 12161–12175. https://doi.org/10.1128/JVI.00869-12.
Nix, W.A., Khetsuriani, N., Penaranda, S., Maher, K., Venczel, L., Cselko, Z., Freire, M.C., Cisterna, D., Lema, C.L., Rosales, P., Rodriguez, J.R., Rodriguez, W., Halkyer, P., Ronveaux, O., Pallansch, M.A., Oberste, M.S., 2013. Diversity of picornaviruses in rural Bolivia. J. Gen. Virol. 94, 2017–2028. https://doi.org/10.1099/vir.0.053827-0.
Oberste, M.S., Maher, K., Michele, S.M., Belliot, G., Uddin, M., Pallansch, M.A., 2005. Enteroviruses 76, 89, 90 and 91 represent a novel group within the species human enterovirus A. J. Gen. Virol. 86, 445–451. https://doi.org/10.1099/vir.0.80475-0.
Oka, T., Wang, Q., Katayama, K., Saif, L.J., 2015. Comprehensive review of human sapoviruses. Clin. Microbiol. Rev. 28, 32–53. https://doi.org/10.1128/CMR.00011-14.
Olijve, L., Jennings, L., Walls, T., 2017. Human Parechovirus: an increasingly recognized cause of Sepsis-like illness in young infants. Clin. Microbiol. Rev. 31 (e00047–17). https://doi.org/10.1128/CMR.00047-17.
Ong, D.S.Y., Schuurman, R., Heikens, E., 2016. Human bocavirus in stool: a true pathogen or an innocent bystander? J. Clin. Virol. 74, 45–49. https://doi.org/10.1016/j.jcv.2015.11.027.
Osborne, C.M., Montano, A.C., Robinson, C.C., Schultz-Cherry, S., Dominguez, S.R., 2015. Viral gastroenteritis in children in Colorado 2006-2009. J. Med. Virol. 87, 931–939. https://doi.org/10.1002/jmv.24022.
PAHO, 2018. IM Coverage. WWW Document. URL. http://ais.paho.org/imm/IM_JRF_COVERAGE-web.asp (accessed 2.15.18).
Phan, T.G., Vo, N.P., Bonkoungou, I.J.O., Kapoor, A., Barro, N., O'Ryan, M., Kapusinszky, B., Wang, C., Delwart, E., 2012. Acute diarrhea in West African children: diverse enteric viruses and a novel parvovirus genus. J. Virol. 86, 11024–11030. https://doi.org/10.1128/JVI.01427-12.
Phan, T.G., Luchsinger, V., Avendano, L.F., Deng, X., Delwart, E., 2014. Cyclovirus in nasopharyngeal aspirates of Chilean children with respiratory infections. J. Gen. Virol. 95, 922–927. https://doi.org/10.1099/vir.0.061143-0.
Phan, T.G., da Costa, A.C., Mendoza, J. del V., Bucardo-Rivera, F., Nordgren, J., Ryan, M.O., Deng, X., Delwart, E., da Costa, A.C., del Valle Mendoza, J., Bucardo-Rivera, F., Nordgren, J., O'Ryan, M., Deng, X., Delwart, E., 2016. The fecal virome of South and Central American children with diarrhea includes small circular DNA viral genomes of unknown origin. Arch. Virol. 161, 959–966. https://doi.org/10.1007/s00705-016-2756-4.The.
Prado, T., Fumian, T.M., Miagostovich, M.P., Gaspar, A.M.C., 2012. Monitoring the hepatitis A virus in urban wastewater from Rio de Janeiro, Brazil. Trans. R. Soc. Trop. Med. Hyg. 106, 104–109. https://doi.org/10.1016/j.trstmh.2011.10.005.
Proenca-Modena, J.L., Martinez, M., Amarilla, A.A., Espínola, E.E., Galeano, M.E., Fariña, N., Russomando, G., Aquino, V.H., Parra, G.I., Arruda, E., 2013. Viral load of human bocavirus-1 in stools from children with viral diarrhoea in Paraguay. Epidemiol. Infect. 141, 2576–2580. https://doi.org/10.1017/S095026881300023X.
Rames, E., Roiko, A., Stratton, H., Macdonald, J., 2016. Technical aspects of using human adenovirus as a viral water quality indicator. Water Res. 96, 308–326. https://doi.org/10.1016/J.WATRES.2016.03.042.
Rastrojo, A., Alcamí, A., 2017. Aquatic viral metagenomics: lights and shadows. Virus Res. 239, 87–96. https://doi.org/10.1016/J.VIRUSRES.2016.11.021.
Resque, H.R., Munford, V., Castilho, J.G., Schmich, H., Caruzo, T.A.R., Rácz, M.L., 2007. Molecular characterization of astrovirus in stool samples from children in São Paulo, Brazil. Mem. Inst. Oswaldo Cruz 102, 969–974.
Rosario, K., Fierer, N., Miller, S., Luongo, J., Breitbart, M., 2018. Diversity of DNA and RNA viruses in indoor air as assessed via metagenomic sequencing. Environ. Sci. Technol. 52, 1014–1027. https://doi.org/10.1021/acs.est.7b04203.
Rusiñol, M., Girones, R., 2017. Summary of excreted and waterborne viruses|global water pathogen project. In: Rose, J.B., Jiménez-Cisneros, B. (Eds.), Global Water Pathogens Project (Global Water Pathogen Project).
Salvo, M., Lizasoain, A., Castells, M., Bortagaray, V., Castro, S., Colina, R., Tort, F.L., Victoria, M., 2018. Human Bocavirus: detection, quantification and molecular characterization in sewage and surface waters in Uruguay. Food Environ. Virol., 1–8 https://doi.org/10.1007/s12560-017-9334-0.
Sánchez, G.J., Mayta, H., Pajuelo, M.J., Neira, K., Xiaofang, L., Cabrera, L., Ballard, S.B., Crabtree, J.E., Kelleher, D., Cama, V., Bern, C., Oshitani, H., Gilman, R.H., Saito, M., Sapovirus Working Group, 2018. Epidemiology of sapovirus infections in a birth cohort in Peru. Clin. Infect. Dis. https://doi.org/10.1093/cid/cix1103.
Santos, N., Peret, T.C.T., Humphrey, C.D., Albuquerque, M.C.M., Silva, R.C., Benati, F.J., Lu, X., Erdman, D.D., 2010. Human bocavirus species 2 and 3 in Brazil. J. Clin. Virol. 48, 127–130. https://doi.org/10.1016/j.jcv.2010.03.014.
Sardi, S.I., Somasekar, S., Naccache, S.N., Bandeira, A.C., Tauro, L.B., Campos, G.S., Chiu, C.Y., 2016. Coinfections of Zika and Chikungunya viruses in Bahia, Brazil, identified by metagenomic next-generation sequencing. J. Clin. Microbiol. 54, 2348–2353. https://doi.org/10.1128/JCM.00877-16.
Schiavelli, R., Bonaventura, R., Rial, M.C., Petrone, H., Pujol, G.S., Gaite, L.J., Acosta, M., Gutierrez, A., Acosta, F., Valdez, G., Raffaele, P., Chanta, G., Perez, M., Potes, L., Suso, E., Cremades, G., Ibañez, J., Imperiali, N., Luxardo, R., Castellanos, M., Maggiora, E., Carreño, C.A., Cobos, M., Marinic, K., Sinchi, J.L., Otero, A.B., Freire, M.C., 2014. First epidemiologic study in Argentina of the prevalence of BK viruria in kidney transplant patients. Transplant. Proc. 46, 3010–3014. https://doi.org/10.1016/j.transproceed.2014.07.009.
Siqueira, J.A.M., Oliveira, D. de S., de Carvalho, T.C.N., Portal, T.M., Justino, M.C.A., da Silva, L.D., Resque, H.R., Gabbay, Y.B., 2017. Astrovirus infection in hospitalized children: molecular, clinical and epidemiological features. J. Clin. Virol. 94, 79–85. https://doi.org/10.1016/j.jcv.2017.07.014.
Smits, S.L., Schapendonk, C.M.E., van Beek, J., Vennema, H., Schürch, A.C., Schipper, D., Bodewes, R., Haagmans, B.L., Osterhaus, A.D.M.E., Koopmans, M.P., 2014. New viruses in idiopathic human diarrhea cases, the Netherlands. Emerg. Infect. Dis. 20. https://doi.org/10.3201/eid2007.140190.
Stöcker, A., de Carvalho Dominguez Souza, B.F., Ribeiro, T.C.M., Netto, E.M., Araujo, L.O., Corrêa, J.I., Almeida, P.S., Peixoto de Mattos, A., Ribeiro, H., Da, C., Pedral-Sampaio, D.B., Drosten, C., Drexler, J.F., 2012. Cosavirus infection in persons with and without gastroenteritis. Brazil. Emerg. Infect. Dis. 18, 656–659. https://doi.org/10.3201/eid1804.111415.
Sulbaran, Y., Gutierrez, C.R., Marquez, B., Rojas, D., Sanchez, D., Navas, J., Rovallo, E., Pujol, F.H., 2010. Hepatitis A virus genetic diversity in Venezuela: exclusive circulation of subgenotype IA and evidence of quasispecies distribution in the isolates. J. Med. Virol. 82, 1829–1834. https://doi.org/10.1002/jmv.21856.
Sun, G., Qinbo Zang, B., Yu, Gu, Guoping Niu, B., Chen Ding, B., Peiying Zhang, B., 2016. Viral metagenomics analysis of picobirnavirus-positive feces from children with sporadic diarrhea in China. Arch. Virol. 161, 971–975. https://doi.org/10.1007/s00705-015-2726-2.
Tamaki, H., Zhang, R., Angly, F.E., Nakamura, S., Hong, P.Y., Yasunaga, T., Kamagata, Y., Liu, W.T., 2012. Metagenomic analysis of DNA viruses in a wastewater treatment plant in tropical climate. Environ. Microbiol. 14, 441–452. https://doi.org/10.1111/j.1462-2920.2011.02630.x.
Tapparel, C., Siegrist, F., Petty, T.J., Kaiser, L., 2013. Picornavirus and enterovirus diversity with associated human diseases. Infect. Genet. Evol. 14, 282–293. https://doi.org/10.1016/J.MEEGID.2012.10.016.
UNEP, 2016. A Snapshot of the World's Water Quality: Towards a Global Assessment. Nairobi, Kenya.
Väisänen, E., Fu, Y., Hedman, K., Söderlund-Venermo, M., 2017. Human protoparvoviruses. Viruses 9, 354. https://doi.org/10.3390/v9110354.
Vasco, G., Trueba, G., Atherton, R., Calvopiña, M., Cevallos, W., Andrade, T., Eguiguren, M., Eisenberg, J.N.S., 2014. Identifying etiological agents causing diarrhea in low income Ecuadorian communities. Am. J. Trop. Med. Hyg. 91, 563–569. https://doi.org/10.4269/ajtmh.13-0744.
de Villiers, E.-M., Fauquet, C., Broker, T.R., Bernard, H.-U., zur Hausen, H., 2004. Classification of papillomaviruses. Virology 324, 17–27. https://doi.org/10.1016/j.virol.2004.03.033.
Voloshenko-Rossin, A., Gasser, G., Cohen, K., Gun, J., Cumbal-Flores, L., Parra-Morales, W., Sarabia, F., Ojeda, F., Lev, O., 2015. Emerging pollutants in the Esmeraldas watershed in Ecuador: discharge and attenuation of emerging organic pollutants along the San Pedro–Guayllabamba–Esmeraldas rivers. Environ. Sci. Process. Impacts 17, 41–53. https://doi.org/10.1039/C4EM00394B.
Vu, D.-L., Cordey, S., Brito, F., Kaiser, L., 2016. Novel human astroviruses: novel human diseases? J. Clin. Virol. 82, 56–63. https://doi.org/10.1016/j.jcv.2016.07.004.
Vu, D.-L., Bosch, A., Pintó, R., Guix, S., 2017. Epidemiology of classic and novel human Astrovirus: gastroenteritis and beyond. Viruses 9, 33. https://doi.org/10.3390/v9020033.
WHO, 2003. Guidelines for environmental surveillance of poliovirus circulation. Vaccine Assess. Monit. Dep. Vaccines Biol. 3.
Williamson, K.E., Fuhrmann, J.J., Wommack, K.E., Radosevich, M., 2017. Annual review of virology viruses in soil ecosystems: an unknown quantity within an unexplored territory. Annu. Rev. Virol 4, 201–219. https://doi.org/10.1146/annurev-virology.
Xavier, M. da P.T.P., Carvalho Costa, F.A., Rocha, M.S., de Andrade, J. da S.R., Diniz, F.K.B., de Andrade, T.R., Miagostovich, M.P., Leite, J.P.G., Volotão, E. de M., 2015. Surveillance of human Astrovirus infection in Brazil: the first report of MLB1 Astrovirus. PLoS One 10 (e0135687). https://doi.org/10.1371/journal.pone.0135687.
Zalona, A.C.J., Lopes, G.S., Schrago, C.G., Gonçalves, R.T., Zalis, M.G., Varella, R.B., 2011. Molecular characterization of BK polyomavirus subtypes in renal transplant recipients in Brazil. J. Med. Virol. 83, 1401–1405. https://doi.org/10.1002/jmv.22117.
Zárate, S., Taboada, B., Yocupicio-Monroy, M., Arias, C.F., 2018. The human virome. Arch. Med. Res. 0. https://doi.org/10.1016/j.arcmed.2018.01.005.