One Health 18 (2024) 100756

Contents lists available at ScienceDirect

One Health

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

Bioinformatics-based screening tool identifies a wide variety of human and zoonotic viruses in Trujillo-Peru wastewater

Brijen Miyani [a], Yabing Li [a], Heidy Peidro Guzman [a], Ruben Kenny Briceno [b], Sabrina Vieyra [b], Rene Hinojosa [b], Irene Xagoraraki [a,*]

[a] Department of Civil and Environmental Engineering, Michigan State University, East Lansing, MI, United States of America
[b] Institute for Global Health, Michigan State University, East Lansing, MI, United States of America

ARTICLE INFO

Keywords:
Bioinformatics
Trujillo-Peru
Viral pathogen screening
Viral diseases
Wastewater surveillance

ABSTRACT

Peru was one of the most affected countries during the COVID-19 pandemic. Moreover, multiple other viral diseases (enteric, respiratory, bloodborne, and vector-borne) are endemic and rising. According to Peru's Ministry of Health, various health facilities in the country were reallocated for the COVID-19 pandemic, thereby leading to reduced action to curb other diseases. Many viral diseases in the area are under-reported and not recognized. The One Health approach, in addition to clinical testing, incorporates environmental surveillance for detection of infectious disease outbreaks. The purpose of this work is to use a screening tool that is based on molecular methods, high throughput sequencing and bioinformatics analysis of wastewater samples to identify virus-related diseases circulating in Trujillo-Peru. To demonstrate the effectiveness of the tool, we collected nine untreated wastewater samples from the Covicorti wastewater utility in Trujillo-Peru on October 22, 2022. High throughput metagenomic sequencing followed by bioinformatic analysis was used to assess the viral diversity of the samples. Our results revealed the presence of sequences associated with multiple human and zoonotic viruses including Orthopoxvirus, Hepatovirus, Rhadinovirus, Parechovirus, Mamastrovirus, Enterovirus, Varicellovirus, Norovirus, Kobuvirus, Bocaparvovirus, Simplexvirus, Spumavirus, Orthohepevirus, Cardiovirus, Molliscipoxvirus, Salivirus, Parapoxvirus, Gammaretrovirus, Alphavirus, Lymphocryptovirus, Erythroparvovirus, Sapovirus, Cosavirus, Deltaretrovirus, Roseolovirus, Flavivirus, Betacoronavirus, Rubivirus, Lentivirus, Betapolyomavirus, Rotavirus, Hepacivirus, Alphacoronavirus, Mastadenovirus, Cytomegalovirus and Alphapapillomavirus. For confirmation purposes, we tested the samples for the presence of selective viruses belonging to the genera detected above. PCR based molecular methods confirmed the presence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), monkeypox virus (MPXV), noroviruses GI and GII (NoVGI and NoVGII), and rotavirus A (RoA) in our samples. Furthermore, publicly available clinical data for selected viruses confirm our findings. Wastewater or other environmental media surveillance, combined with bioinformatics methods, has the potential to serve as a systematic screening tool for the identification of human or zoonotic viruses that may cause disease. The results of this method can guide further clinical surveillance efforts and allocation of resources. Incorporation of this bioinformatic-based screening tool by public health officials in Peru and other Latin American countries will help manage endemic and emerging diseases that could save human lives and resources.

1. Introduction

Peru is the third largest South American country comprising of a diverse range of landscapes and a population of 31,331,228 [1]. Half of Peru is covered by the world's largest rainforest, the Amazon. Considering the country's unique landscapes and ecosystems, diverse range of wildlife, and large population, the country could be an epicenter for a wide variety of human and zoonotic diseases. The United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT program has included Peru, Bolivia, Brazil, and Colombia in training personnel for surveillance of outbreaks of infectious diseases associated with wildlife pathogens in the community [2].

A quick historical review of emerging infectious diseases of human pathogens points out that 60% or more have originated from animals

* Corresponding author.
E-mail address: xagorara@msu.edu (I. Xagoraraki).

https://doi.org/10.1016/j.onehlt.2024.100756
Received 5 March 2024; Received in revised form 9 May 2024; Accepted 13 May 2024
Available online 15 May 2024

[3]. Furthermore, 75% of human pathogens studied are known to be viruses that are associated with wildlife reservoirs [4]. SARS-CoV-2, apart from ebola hemorrhagic fever, nipah viral encephalitis, hantavirus pulmonary syndrome, H5N1 highly pathogenic avian influenza, and the pandemic 2009 H1N1 influenza virus are recent examples of emerging infectious diseases of wildlife origin [5,6]. The One Health approach (also known as 'One world, One health'), that focuses on the innate relation between human, animal, and environmental health, can provide solutions to effective monitoring and early detection of infectious viral diseases by incorporating environmental surveillance in addition to clinical testing [7].

Traditional disease detection systems are based on diagnostic analysis of clinical samples. This approach, however, assumes that patients are examined at a clinical setting after symptoms have developed. Testing every individual for active infections or immunity, especially in underserved communities, is impossible. Environmental based surveillance, such as wastewater testing, provides a means of collecting and analyzing community-composite-samples [7]. Wastewater testing may provide early warnings of potential upcoming viral outbreaks as well as predictions of fluctuations of established outbreaks for specific geographical areas. Wastewater-based epidemiology has the potential to identify multiple endemic respiratory, enteric, bloodborne and vector-borne diseases circulating in the community [13–16] as well as predicting viral outbreaks such as Covid-19 [8–12]. Early detection of emerging viral diseases in the community could help boost the preparedness of public health officials and save lives.

The immense burden of human and zoonotic viral infections in Peru is widely recognized. Respiratory diseases, from both viral and bacterial infections, were the leading cause of death across all age group from the year 2019 to 2020 (before the Covid-19 outbreak) [17]. Furthermore, Peru was one of the most affected countries during the Covid-19 pandemic. In Peru, from January 2020 to December 2023, as reported by the World Health Organization (WHO), there were 4,520,102 confirmed cases of Covid-19 with 221,564 deaths [18]. During the Covid-19 pandemic, Peru experienced the worst infection rate (99%) [92] and death rate (6400 deaths per million population) [93–95]. Moreover, multiple other viral diseases (enteric, respiratory, bloodborne, and vector-borne) are endemic and rising. Arboviruses, such as Dengue virus, have been on a rise in South American countries including Peru [19]. Other arboviruses like Chikungunya and Zika have also been reported in the region [20]. Many viral diseases in the area are underreported and not recognized. According to Peru's Ministry of Health, various health facilities in the country were reallocated for the COVID-19 pandemic, thereby leading to reduced action to curb other diseases.

The purpose of this study is to analyze wastewater samples collected at Trujillo-Peru, with a screening tool that involves high throughput metagenomic sequencing and bioinformatics analysis, to identify potential virus-related diseases circulating in the community. This method may supplement and guide clinical surveillance efforts. Incorporation of this bioinformatic-based screening tool by public health officials in Peru and other Latin American countries will help manage endemic and emerging diseases that could save human lives and resources.

2. Material and methods

2.1. Study area and wastewater sampling

Trujillo, located in northwestern part of Peru, is the capital city of La Libertad province. According to 2017 census [21], about 970 thousand people live in Trujillo making it the third largest city in the country. SEDALIB S.A (https://sedalib.com.pe/), a waste utility company, has divided the city's wastewater into five drainage collectors: Covicorti, Cortijo, Valdiva, Industrial Park, and the basin of La whitewashed. More than 90% of the city's wastewater is treated by these plants [22]. Covicorti is the largest wastewater treatment utility in Trujillo and collects wastewater from 3 districts of Trujillo-Peru: Trujillo, Victor Larco Herrera, and El Porvenir. Annually, it treats an average flow of 59,166 m^3 per day (685 L/s) and an organic load of 24,350 kg of chemical oxygen demand per day [23]. The utility discharges treated wastewater to the ocean along Playa de Buenos Aires. Wastewater samples were collected in triplicates from the Covicorti wastewater utility on October 22, 2022.

Covicorti wastewater utility connects the grit removal tank and aeration tank by a long open 600 m aqueduct. Untreated influent grab wastewater samples were collected in triplicates at 100 m (A), 300 m (B), and 500 m (C) from the grit removal tank, for a total of 9 samples. For each sample, 200 mL of wastewater was collected in labeled corning polypropylene bottle. Samples were labeled as COVIA1, COVIA2, COVIA3, COVIB1, COVIB2, COVIB3, COVIC1, COVIC2, COVIC3. Samples were also collected at the Playa de Buenos Aires beach waters resulting in no signals for SARS-CoV-2 and hence no further analysis was conducted. Samples were shipped overnight on dry ice to the Environmental Virology Laboratory at Michigan State University (MSU) and stored in freezer (−80 °C) once delivered. Virus concentration were conducted within 72 h of sampling.

2.2. Virus concentration using Polyethylene Glycol (PEG) precipitation and RNA extraction

Polyethylene Glycol (PEG) precipitation was performed on grab samples using a previously described method [15,24]. This involved adding 1.17 g of sodium chloride and 8 g of polyethene glycol to 100 mL sample and mixing in 4 °C at 110 rpm for 2 h [24]. Samples were centrifuged for 45 mins at 4700 $\times g$ at 4 °C. The supernatant was discarded without disturbing the pellets. Virus pellets were resuspended with 1–4 mL of liquid sample. Final volumes were noted, and the concentrated samples were stored in the freezer (−80 °C) for subsequent RNA extraction. Type I water (Barnstead Nano pure water system) was used as a negative control (NTC) in PEG precipitation process.

Viral nucleic acids were extracted according to manufacturer's protocol using QIAGEN QIAamp Viral RNA Mini Kit (QIAGEN cat# 52904, Germantown, Maryland) to obtain sufficient viral genetic materials. PCR-grade water was included in the RNA extraction processes as a negative control (NTC).

2.3. High throughput metagenomic sequencing and downstream bioinformatics analysis

High throughput metagenomic sequencing and downstream bioinformatics was performed as described previously [15,16] to assess viral diversity in the samples. RNA extract from the triplicates of each site were combined to result in total of 3 samples (COVIA, COVIB, COVIC). The RNA samples were reverse transcribed to cDNA using a random-primer protocol developed to identify viral pathogens [25,26].

Viral cDNA of the wastewater samples ($n = 3$) were sent to the MSU Research Technology Support Facility's (RTSF) Genomics Core for library preparation and Illumina sequencing. Sequencing was conducted using the Illumina NovaSeq 6000 platform. Quality of the raw reads was assessed using FastQC [27] demonstrating quality scores greater than 30. An average of about 118 million reads and 35.4 Gb yield were obtained for each sample. Trimming, assembly and three different types of annotations were conducted using a previously described method [15,16]. Briefly, adapters and low-quality reads were trimmed with Trimmomatic [28]. The proportion of viral reads in the sample was determined by aligning the trimmed reads against the National Center for Biotechnology Information (NCBI) BLAST non-redundant database using Kaiju (v. 1.9.0) [29]. Contigs were assembled using Megahit [30] and aligned with NCBI RefSeq virus database (retrieved on December 1, 2022) with DIAMOND Blastx [31] to determine the virus composition. Furthermore, the assembled contigs were aligned again to custom Swiss-Prot human virus database using BLASTx to identify viral diversity at genus level [13]. MEGAN software was used for taxonomic annotation of

viruses at genus level [32].

2.4. Municipal wastewater characterization by quantification of pepper mild mottle virus (PMMoV) and crAssphage virus using digital droplet qPCR

PMMoV virus was quantified using a GT molecular ddPCR kit for the Bio-Rad QX200™ Droplet Digital™ PCR System (cat# 100320) following the manufacturer's protocol. The kit contains single plex assay mastermix and positive control of PMMoV. Briefly 5.5 μL One-Step RT-ddPCR Supermix (20×), 2.2 μL of Reverse Transcriptase, 1.1 μL of 300 mM DTT, 1 μL of PMMoV mastermix, and 9 μL RNA were mized. The total volume of reaction was made 22 μL by adding PCR-grade water. PMMoV was amplified using 50 °C for an hour, 95 °C for 10 mins, and 45 cycles (94 °C for 30 s and 55 °C for 1 min at slow ramp speed of 2 °C/s). The samples were subjected to a last extension cycle for 98 °C for 10mins followed by 4 °C for 30 min for droplet stabilization.

crAssphage virus was quantified using a GT molecular ddPCR assay kit (cat# 100285) using the manufacturer's protocol. Briefly, the mastermix contained 11 μL of 2× Supermix for Probes, 1 μL of crAssphage mastermix, and 10 μL RNA. crAssphage virus was amplified using 95 °C for 10 mins, and 45 cycles of (94 °C for 30 s and 58 °C for 1 min at slow ramp speed of 2 °C/s). The samples were subjected to a last extension cycle for 98 °C for 10 mins followed by 4 °C for 30 min for droplet stabilization. Each reaction consisted of samples run in triplicates, and NTCs from elution, extraction and ddPCR step.

2.5. Quantification of SARS-CoV-2 and monkeypox virus using digital droplet qPCR

SARS-CoV-2 was quantified using Bio-Rad's One-Step RT-ddPCR Advanced kit with a QX200 digital droplet PCR (ddPCR) system (Bio-Rad, CA, USA) using a previously described method [10,11]. The assay contained 5.5 μL One-Step RT-ddPCR Supermix (20×), 2.2 μL of Reverse Transcriptase, 1.1 μL of 300 mM DTT, and 5.5 μL RNA. The final concentration of N1 and N2 gene forward and reverse primer was 900 nM, whereas the N1 and N2 probe was 250 nM. The total volume of reaction was made 22 μL by adding PCR-grade water. Each reaction consisted of samples run in triplicates, PEG precipitation NTC, extraction NTC and ddPCR controls (SARS-CoV-2 from Twist Bioscience Synthetic SARS-CoV-2 RNA Control 2 MN908947.3 as was used as positive control (PTC) and PCR-grade water as NTC). SARS-CoV-2 was amplified using 25 °C for 3 mins, 50 °C for an hour, 95 °C for 10 mins, and 40 cycles of (95 °C for 30 s and 55 °C for 1 min at slow ramp speed of 2 °C/s). The samples were subjected to a last extension cycle for 98 °C for 10mins. Limit of blank (LOB) and Limit of detection (LOD) were calculated according to manufacturer's guidelines (Bio-Rad) as shown previously [10,11]. LOB for SARS-CoV-2 N1 gene and N2 gene ddPCR was determined to be 0.09 and 0.08 copies/μL respectively [10,11]. Limit of detection (LOD) of 0.1 copies/μL with 72.92% confidence for the N1 gene ddPCR and 0.1 copies/μL with 81.25% confidence for the N2 gene ddPCR were determined as shown previously [10,11].

Monkeypox virus (MPXV) was quantified using digital droplet PCR (ddPCR) technology using Bio-Rad's ddPCR™ Supermix for Probes following the manufacture's protocol. The primers and probe used for amplifying are recommended by CDC [33] and were used in a previously published study [34]. The assay contained 10 μL ddPCR Supermix for probes (20×, No dUTP), 900 nM forward and reverse primer, 250 nM probe, and 8.7 μL sample. The total volume of reaction was made 22 μL by adding PCR-grade water. Monkeypox viral DNA was purchased from ATCC (ATCC Number: VR-3270SD). The stock concentration was diluted to 10^3 copies/μL and used as a PTC. PCR-grade water was used as an NTC. Each reaction consisted of samples and controls run in triplicates. MPXV was amplified using 95 °C for 10 mins, and 45 cycles of (94 °C for 30 s and 58 °C for 1 min at slow ramp speed of 2 °C/s). The samples were subjected to a last extension cycle for 98 °C for 10 mins followed by 4 °C for 30 min for droplet stabilization. Limit of blank (LOB) and Limit of detection (LOD) were calculated according to manufacturer's guidelines (Bio-Rad) for MPXV. Briefly, MPXV ddPCR assay was ran using PCR-grade water and the lowest value detected at 95% confidence was determined as LOB. Comparatively, for LOD, different dilutions of positive control were run using the MPXV assay. The positive control dilution for which 95% of ddPCR values were greater than the LOB, was determined to be the LOD of the method. LOB and LOD for MPXV was determined to be 0 copies/μL and 0.08 copies/μL (95% confidence) respectively.

2.6. Detection of noroviruses and rotavirus using conventional PCR, gel electrophoresis and sanger sequencing

The samples were tested for NoVGI, NoVGII and RoA using gene specific primers [35]. Reverse transcription (RT) was performed using Invitrogen SuperScript™ IV Reverse Transcriptase (cat# 18090010) protocol. A final concentration of 1 μM random primer (cat# 48190011 Invitrogen Thermo Fisher) was added in the reaction. cDNA was generated using a cycle of 23 °C for 10 min, 50 °C for 1 h, 94 °C for 5 min. Each reaction consisted of samples and NTC from all steps (PEG precipitation, extraction, RT). Norovirus G1 RNA (ATCC number: VR-3234SD) and human rotavirus RNA (ATCC number: VR-2018DQ) were included as RT PTCs.

PCR was performed using Invitrogen Platinum™ SuperFi II DNA Polymerase (cat# 12361010) protocol. The final concentrations of forward and reverse primers for NoVGI, NoVGII, and RoA were 1 μM, 0.8 μM, 1 μM respectively. 10 μL RNA and PCR-grade water were added to make a total volume of reaction of 20 μL. NoVGI was amplified at one cycle of 94 °C for 5 mins, 40 cycles (94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min) and a final extension cycle of 72 °C for 7 mins. NoVGII and RoA were amplified at one cycle of 94 °C for 3 mins, 35 cycles (94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min) and a final extension cycle of 72 °C for 7 mins. An additional NTC was included for PCR step to check for cross contamination.

The results were confirmed by running the samples on 1.5% agarose gels. All PTC for NoVGI and RoA assays were confirmed with gel electrophoresis. The samples were PCR purified using QIAquick PCR Purification Kit (QIAGEN cat#28104), quantified by Thermo Scientific Qubit Fluorometer and submitted to RTSF at Michigan State University for sanger sequencing. The result sequences were analyzed using FinchTV 1.5.0 version. After performing quality control, the sequences were blasted using NCBI BLASTn to confirm presence of the viral species. The top BLASTn result under default conditions was chosen as the best possible match. A phylogenetic tree was generated using similar hits from BLAST and other common human viruses within the genus. All sequences are aligned using UGENE software with the MUSCLE algorithm. The aligned regions are extracted for generation of a phylogenetic tree using MEGA11 software [36].

2.7. Clinical data collection

Clinical cases of viral diseases for the year 2022 for Peru, La Libertad, and Trujillo were retrieved from online publicly available sources [37–40].

3. Results

3.1. Municipal wastewater characterization

Water quality parameters of untreated wastewater were provided by the utility as shown in Table 1.

PMMoV co-occurs with multiple pathogens of interest and has been suggested as a promising index for enteric viruses [41]. Similarly, crAssphage has been detected abundantly in wastewater and has been proposed as a viral fecal indicator [42,43]. A combination of PMMoV

Table 1
Water Quality parameters at Covicorti wastewater treatment plant.

Description/test	Influent	Final Effluent
Sampling date	6/9/2022	6/9/2022
Biological oxygen demand (mg/L)	320.69	26.03
Chemical oxygen demand (mg O_2/L)	683.75	122.18
Total suspended solids (mg/L)	376	205
Oils and fats (mg/L)	43.7	5.2
Thermotolerant coliforms (MPN/100 mL)	1.7×10^8	3.4×10^5
pH	7.98	7.95

and crAssphage has been used by many researchers as a fecal and enteric virus indicator [44–47].

Levels of PMMoV and crAssphage in wastewater samples were tested and the results are shown in Fig. 1. All the samples tested positive for both viruses. Average concentrations of PMMoV and crAssphage ranged around 10^8 and 10^6 genomic copies per 100 mL of wastewater respectively as shown in Fig. 1. The results fall in the range found in untreated wastewater from all around the world [41].

3.2. Sequencing and bioinformatics analysis for viral diversity identification

A description of the metagenomic sequencing data after each bioinformatic analysis step is summarized in Table 2. After trimming, at least 80 million reads were obtained for each sample, among these reads, more than 1.2 million were classified as viruses with kaiju. To achieve substantial gains in taxonomic mapping, long contiguous sequences (contigs) generated by MEGAHIT, and more than 400 thousand contigs were obtained. When comparing these contigs against the NCBI RefSeq virus protein database, more than 17 thousand contigs were assigned to virus with MEGAN.

Virus composition in the family level was analyzed by comparing the assembled contigs against the NCBI RefSeq virus protein database. The results showed that the majority of the viral populations observed in the wastewater samples were affiliated to bacteriophage, and were members of *Myoviridae*, *Siphoviridae* and *Microviridae* families.

To improve the detection of human viruses in the samples collected from the wastewater utility in Peru, assembled contigs were compared against a customized Swiss-Prot human virus protein database. Human viruses identified in less than 2 samples were excluded. Fig. 2 shows the diversity of viruses detected at genus level. Among the human viral contigs, *Orthopoxvirus* took a large proportion in all the samples,

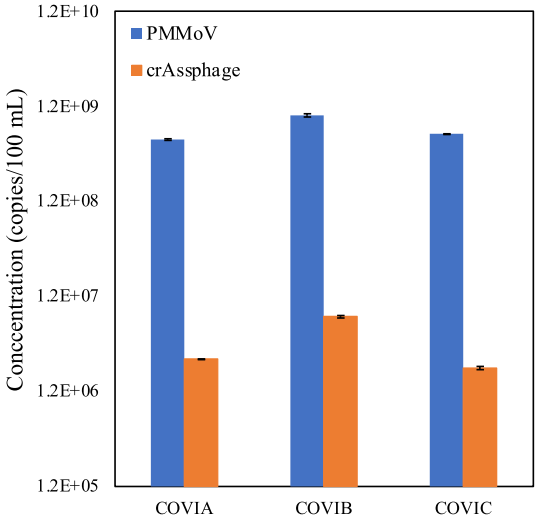

Fig. 1. Concentrations of enteric virus indicators (PMMoV and crAssphage) for wastewater characterization.

followed by *Hepatovirus*, *Rhadinovirus*, *Parechovirus*, *Mamastrovirus*, *Enterovirus*, *Varicellovirus*, *Norovirus*, *Kobuvirus*, *Bocaparvovirus*, *Simplexvirus*, *Spumavirus*, *Orthohepevirus*, *Cardiovirus*, *Molliscipoxvirus*, *Salivirus*, *Parapoxvirus*, *Gammaretrovirus*, *Alphavirus*, *Lymphocryptovirus*, *Erythroparvovirus*, *Sapovirus*, *Cosavirus*, *Deltaretrovirus*, *Roseolovirus*, *Flavivirus*, *Betacoronavirus*, *Rubivirus*, *Lentivirus*, *Betapolyomavirus*, *Rotavirus*, *Hepacivirus*, *Alphacoronavirus*, *Mastadenovirus*, *Cytomegalovirus* and *Alphapapillomavirus*.

3.3. Quantification of SARS-CoV-2 and MPXV

SARS-CoV-2 and MPXV virus assays were run in triplicates for all nine wastewater samples collected at the Covicorti wastewater utility, along with positive and negative controls. Fig. 3 shows SARS-CoV-2 and MPXV concentrations in copies/100 mL of wastewater. COVIB had the highest concentrations for both SARS-CoV-2 and MPXV with about 2500 and 700 copies/100 mL respectively. MPXV concentrations were almost three times less than SARS-CoV-2 as quantified by ddPCR.

3.4. Detection of noroviruses and rotavirus by PCR, gel electrophoresis, BLASTn analysis

Presence of NoVGI, NoVGII, and RoA was detected by PCR. For gel electrophoresis, all nine samples tested positive for NoVGI, NoVGII, and RoA. The amplicon size for NoVGI, NoVGII, and RoA was 330, 387, and 395 respectively [35]. The samples along with positive controls were PCR purified and submitted for sanger sequencing with forward primer and reverse primer separately. The resulting sequences were blasted against viral database on NCBI Blast website. The top 10 BLASTn sequences producing significant alignment results for all samples and positive controls amplified using gene specific primers (NoVGI, NoVGII, RoA) resulted in the respective viruses with at least 94% percent identity and 98% query coverage.

Using the BLASTn results, similar hits and other common human viruses were downloaded and aligned. Phylogenetic trees were generated using the aligned regions with MEGA11 software [36]. Phylogenetic trees generated with selected human viral related sequences identified in COVIC1, COVIC2, COVIC3 samples, reference sequences they are affiliated with, and PTCs purchased from ATCC, are shown in Fig. 4. All the nine sample BLASTn results were found to be closely associated with the respective viruses as shown in phylogenetic trees.

3.5. Publicly available health records of clinical confirmed viral disease in the area

Table 3 contains all publicly available clinical cases for Peru, La Libertad, and Trujillo [37–40]. During 2022, Trujillo had 2, 5, 21, 151, and 148, cases of chicken pox, monkeypox, dengue, acute diarrheal disease, and acute respiratory infections respectively. There were no reported cases of Yellow fever, Hepatitis B, Zika, Chikungunya in Trujillo for the year 2022. Comparatively, Peru as a whole country had 3, 811, 10, 164 cases of Yellow fever, Hepatitis B, Zika, and Chikungunya respectively. Of all diseases acute diarrheal, acute respiratory, and Dengue cases are the highest in the country of Peru, the state of La Libertad, and the city of Trujillo. It is important to note that most viral diseases in the area are unreported.

4. Discussion

Since the COVID-19 pandemic, multiple efforts have focused on wastewater surveillance. Most studies focused on selected known pathogens, such as SARS-CoV2 [11,48,49] or MPXV [50–52]. In this study we apply a tool that includes broad screening of viruses at the genus level [13,15,16]. The first level of screening includes testing wastewater, or other environmental samples, with high throughput sequencing followed by bioinformatics analysis. If signals identify

Table 2
Sequencing yield, number of clean reads, viral reads and viral contigs of each wastewater sample.

Sample	Yield (Gbp)	Number of clean reads	Number of viral reads	Percent of viral reads (%)	Number of contigs	Number of viral contigs	Percent of viral contigs (%)
COVIA	36.6	118,495,760	6,068,836	5.12	884,618	140,316	15.86
COVIB	33.2	107,865,545	11,395,312	10.56	704,133	114,245	16.22
COVIC	32.0	104,061,454	3,720,569	3.58	669,569	83,766	12.51

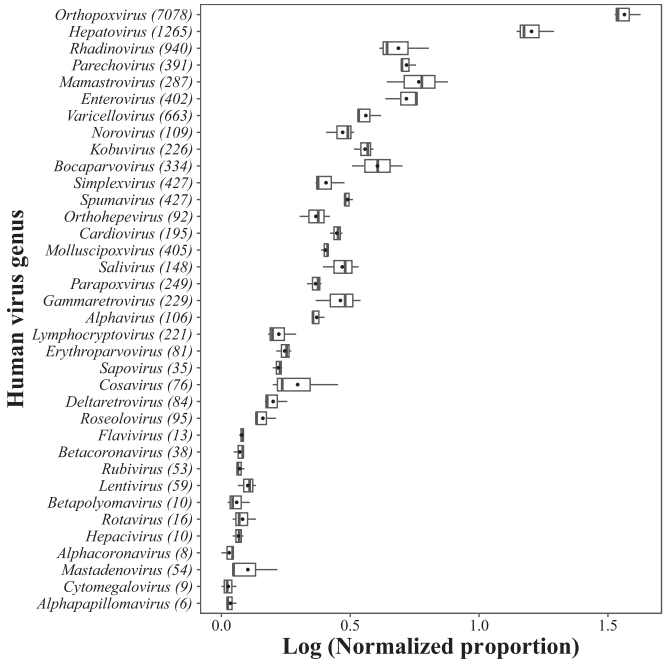

Fig. 2. Log (Normalized proportion) of human viruses in wastewater collected from Trujillo-Peru. Contigs in three samples (COVIA, COVIB and COVIC) were combined together to do the calculation. Numbers in the brackets after the genus names are the sum of human viral contigs identified in the three samples.

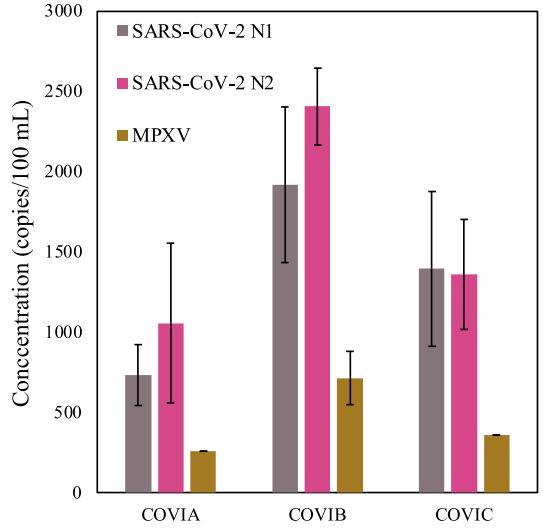

Fig. 3. Confirmation of selected human viruses (SARS-CoV-2 [N1 and N2 genes] and monkeypox viruses) in wastewater collected from Trujillo-Peru by digital droplet PCR.

potential presence of viruses of concern, further PCR testing targeting the specific virus follows. Final confirmation with clinical surveillance is recommended. We have successfully applied this method in samples collected in Detroit MI to identify endemic and emerging diseases in the area [13–16,53]. Application of such a screening tool has the potential to identify endemic and emerging diseases that may otherwise be missed by regular clinical testing.

The screening analysis presented in this study revealed the presence of a wide variety of human and zoonotic, respiratory, bloodborne, enteric and vector-borne viruses circulating in the population (Fig. 2). For example, genera *Betacoronavirus* and *Orthopoxvirus* (that includes species SARS-CoV-2 and MPXV respectively) were detected by high throughput sequencing (Fig. 2). To further investigate and validate these findings SARS-CoV-2 and MPXV were quantified with ddPCR (Fig. 3). Clinical cases of COVID-19 and Mpox reported by Peru Ministry of Health (Table 3) confirm our findings. Until November 2022, Peru reported the greatest number of MPX cases among countries where the disease is not endemic (like Africa) [54]. Monkeypox virus is shed from skin rashes while rinsing and showering as well as from human fecal matter which ultimately ends up in wastewater treatment plant [96–98]. Similarly, NoV GI, NoV GII, and RoA viruses that belong to genera *Norovirus* and *Rotavirus* were selected for further testing and confirmation to target part of the large number of diarrheal cases detected in Trujillo-Peru (Table 3). Confirmation tests for SARS-CoV2, MPXV, norovirus and rotavirus validate the utility of the method. Importantly, the genomic sequences shown in Fig. 2 reveal the potential presence of a wider range of human and zoonotic viruses.

Many of the viral-related sequences identified in this study (Fig. 2) correspond to viruses that have been detected in wastewater or other environmental samples in Latin American countries. For example, *Orthopoxvirus* was the largest genus detected in all three wastewater samples tested in this study. MPXV, a viral species in genus *Orthopoxvirus*, has been detected in wastewater in Chile [55]. Vaccinia virus, another viral species of genus *Orthopoxvirus* caused outbreaks in animals and workers in the dairy industry in Brazil [56–58] and Colombia [59]. *Hepatovirus* genus, consisting of different types of hepatitis viruses, was the second largest genera to be detected in our samples. Hepatitis A and E virus has been detected in wastewaters in Brazil [60], Venezuela [61], Argentina [62,64], Colombia [63], and Ecuador [65]. SARS-CoV-2 (belonging to genus *Betacoronavirus* which was detected in our samples) has been detected in wastewater in Peru [66,67], Brazil [68], and Ecuador [69].

Gastroenteritis related viruses belonging to genera *Cosavirus*, *Kobuvirus*, *Mamastrovirus, Norovirus, Rotavirus, Salivirus, and Sapovirus* were detected in our samples. Cosavirus was detected in river waters in Argentina [70] and Ecuador [65] and wastewater samples in Venezuela [71]. Aichivirus (belonging to genus *Kobuvirus*) was tested positive in wastewater samples of Uruguay [72] and Ecuador [65]. Astroviruses were present in wastewater of Brazil [73], Venezuela [61], Ecuador [65], and Uruguay [74]. Wastewater of Latin American countries like Brazil [75], Chile [76], Argentina [77], Nicaragua [78], Uruguay [79], Ecuador [65], and Venezuela [61] tested positive for norovirus. Comparatively, rotavirus was detected in wastewater samples of Uruguay [80], Brazil [81], and Argentina [82]. Klassevirus and sapovirus were detected in river waters [83] and wastewater [65,84] of Brazil and Ecuador respectively.

Genera such as *Bocaparvovirus* and *Mastadenovirus* have been known to be associated with both gastroenteritis and respiratory illnesses. Human bocaparvovirus has been detected in wastewaters of Uruguay [85,86] and Ecuador [65], whereas various types of human

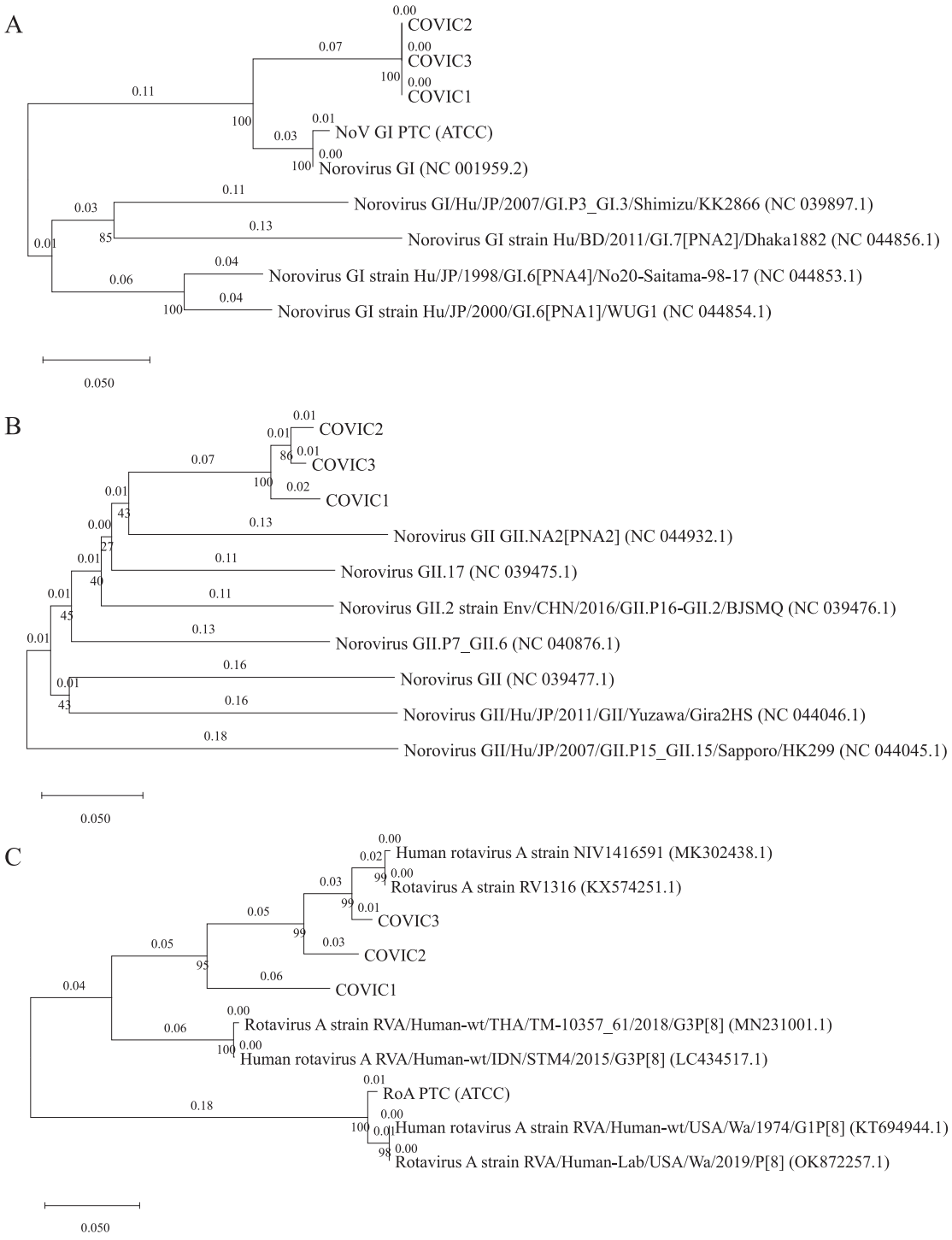

Fig. 4. Phylogenetic analysis of selected human viruses identified in wastewater in Trujillo-Peru. A: Norovirus GI. B: Norovirus GII. C: Rotavirus A. Reference genomes they are affiliated with, positive controls used in this study and human viral related sequences identified in the samples are included. The tree was constructed using the neighbor-joining method with the replicates of bootstrap test as 1000. Percentage of replicate trees in which the associated sequences clustered together are shown below the branches. The evolutionary distances were computed with the Kimura 2-parameter method for Norovirus GI and GII related sequences. Tamura 3-parameter method was used for Rotavirus A related sequences. Gaps and missing data were eliminated (complete deletion option). The scale bar represents the estimated number of base substitutions per site. MEGA 11 was used to perform the phylogenetic analysis. Accession numbers of the reference sequences in NCBI are shown in the brackets after the names.

mastadenovirus have been detected in Brazil [87], Argentina [88], Ecuador [65], and Venezuela [61]. Other examples include Saffold virus that belongs to *Cardiovirus* genus and has been detected in wastewater of Argentina [70] and Ecuador [65]. Human parechovirus, belonging to genus *Parechovirus*, has been detected in wastewater in Ecuador [65]. Human polyomaviruses belonging to genus *Betapolyomavirus* have been detected in raw sewage in Argentina [89], Brazil [90], and Chile [91].

This proposed screening method has the potential to identify non-reportable (not required to be tested for at clinical settings) human and zoonotic viruses that may be emerging in the community. For some

Table 3
Clinical cases of viral diseases for the year 2022 in Peru.

Location/ Viral disease	Peru	La Libertad	Trujillo
Acute respiratory infections	622,259	448	148
Acute diarrheal disease	306,201	288	151
Dengue	38,877	162	21
Monkey pox	3367	20	5
Hepatitis B	811	8	0
Chickenpox	376	46	2
Chikungunya	164	0	0
Zika	10	0	0
Yellow fever	3	0	0

Note: All clinical cases were obtained from references [37–40].

of those emerging viruses there may not be validated clinical tests available. While this study demonstrates the advantages of wastewater surveillance with molecular methods, high-throughput sequencing, and bioinformatics to identify endemic and emerging diseases in the contributing population, there are several limitations. Particularly in resource-limited nations, obtaining all the requisite resources for such endeavors can pose challenges. Another limitation of this study is the sample size, as only three pooled samples were tested with high-throughput sequencing and bioinformatics methods. This restricted number of samples may limit the generalizability of the findings that can only serve as a pilot study. The results of this study only indicate the possibility of occurrence of the detected viral genera in the community. To develop a tool that can predict viral disease fluctuations over space a time multiple more samples have to be collected, analyzed, normalized, and compared with clinical data in the catchment area. This will require a long-term full-scale investigation that needs to involve local public health officials and environmental scientists and engineers.

5. Conclusions

Wastewater, or other environmental media surveillance, combined with bioinformatics has a vast potential to serve as a systematic screening tool for the identification of a myriad of human or zoonotic viruses that may cause disease. One common problem in Peru and other Latin America countries is that health agencies do not have the resources and tools to anticipate and systematically monitor disease outbreaks. The methodology presented here provides a practical method to assist with clinical surveillance and prediction.

The viral species detected with bioinformatics methods (Fig. 2) are at the genus level and need to be confirmed by additional PCR based methods for species identification. The results highlight the importance of the method as an initial screening tool. The results may be used by local professionals to guide further clinical monitoring and develop health care policies. Incorporation of this bioinformatic-based screening tool by public health officials in Latin America will help identify endemic and emerging diseases that could save human lives and resources.

Credit authorship contribution statement

Brijen Miyani: Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Yabing Li**: Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Heidy Peidro Guzman**: Validation, Formal analysis, Data curation, Writing – review & editing.

Ruben Kenny Briceno: Formal analysis, Writing – review & editing. **Sabrina Vieyra**: Validation, Formal analysis, Data curation, Funding acquisition, Writing – review & editing. **Rene Hinojosa**: Conceptualization, Writing – review & editing. **Irene Xagoraraki**: Conceptualization, Project administration, Funding acquisition, Supervision, Writing – review & editing.

CRediT authorship contribution statement

Brijen Miyani: Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Yabing Li:** Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Heidy Peidro Guzman:** Data curation, Formal analysis, Validation, Writing – review & editing. **Ruben Kenny Briceno:** Formal analysis, Writing – review & editing. **Sabrina Vieyra:** Data curation, Funding acquisition, Validation, Writing – review & editing. **Rene Hinojosa:** Investigation, Writing – review & editing. **Irene Xagoraraki:** Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interest.

Data availability

Data for this study are available upon request to the corresponding author.

Acknowledgements

We thank the Institute for Global Health at Michigan State University for funding this work.

References

[1] "Peru - National geographic kids." [Online]. Available, https://kids.nationalgeographic.com/geography/countries/article/peru [Accessed: 09-Dec-2023].
[2] M. Uhart, A. Pérez, M. Rostal, E.A. Robles, A.P. Mendoza, A. Nava, C.D. de Paula, F. Miranda, V. Iñiguez, C. Zambrana, E. Durigon, A 'One Health'approach to predict emerging zoonoses in the Amazon. Saúde Silvestre e Humana: Experiências e Perspectivas. Rio de Janeiro, FIOCRUZ (2013) 65–73.
[3] K.E. Jones, N.G. Patel, M.A. Levy, A. Storeygard, D. Balk, J.L. Gittleman, P. Daszak, Global trends in emerging infectious diseases, Nature. 451 (7181) (2008 Feb) 990–993.
[4] M. Woolhouse, E. Gaunt, Ecological origins of novel human pathogens, Crit. Rev. Microbiol. 33 (4) (2007 Jan 1) 231–242.
[5] W.B. Karesh, R.A. Cook, The human-animal link, Foreign Aff. 84 (2005) 38.
[6] M.L. Flanagan, C.R. Parrish, S. Cobey, G.E. Glass, R.M. Bush, T.J. Leighton, Anticipating the species jump: surveillance for emerging viral threats, Zoonoses Public Health 59 (3) (2012 May) 155–163.
[7] E. O'Brien, I. Xagoraraki, A water-focused one-health approach for early detection and prevention of viral outbreaks, One Health. 1 (7) (2019 Jun) 100094.
[8] I. Xagoraraki, E. O'Brien, Wastewater-based epidemiology for early detection of viral outbreaks, Women in water quality: Investigations by prominent female engineers. (2020) 75–97.
[9] I. Xagoraraki, Can we predict viral outbreaks using wastewater surveillance? J. Environ. Eng. 146 (11) (2020 Nov 1) 01820003.
[10] Y. Li, B. Miyani, L. Zhao, M. Spooner, Z. Gentry, Y. Zou, G. Rhodes, H. Li, A. Kaye, J. Norton, I. Xagoraraki, Surveillance of SARS-CoV-2 in nine neighborhood sewersheds in Detroit Tri-County area, United States: assessing per capita SARS-CoV-2 estimations and COVID-19 incidence, Sci. Total Environ. 10 (851) (2022 Dec) 158350.
[11] L. Zhao, Y. Zou, Y. Li, B. Miyani, M. Spooner, Z. Gentry, S. Jacobi, R.E. David, S. Withington, S. McFarlane, R. Faust, Five-week warning of COVID-19 peaks prior to the omicron surge in Detroit, Michigan using wastewater surveillance, Sci. Total Environ. 20 (844) (2022 Oct) 157040.
[12] B. Miyani, L. Zhao, M. Spooner, S. Buch, Z. Gentry, A. Mehrotra, J. Norton, I. Xagoraraki, Early warnings of COVID-19 second wave in Detroit, J. Environ. Eng. 147 (8) (2021 Aug) 06021004.
[13] C. McCall, H. Wu, B. Miyani, I. Xagoraraki, Identification of multiple potential viral diseases in a large urban center using wastewater surveillance, Water Res. 1 (184) (2020 Oct) 116160.
[14] B. Miyani, C. McCall, I. Xagoraraki, High abundance of human herpesvirus 8 in wastewater from a large urban area, J. Appl. Microbiol. 130 (5) (2021 May) 1402–1411.
[15] Y. Li, B. Miyani, K.L. Childs, S.H. Shiu, I. Xagoraraki, Effect of wastewater collection and concentration methods on assessment of viral diversity, Sci. Total Environ. 15 (908) (2024 Jan) 168128.
[16] Y. Li, B. Miyani, R.A. Faust, R.E. David, I. Xagoraraki, A broad wastewater screening and clinical data surveillance for virus-related diseases in the metropolitan Detroit area in Michigan, Hum. Genomics 18 (1) (2024 Dec) 1–8.
[17] Peru WHO Mortality Database Leading causes of death per year.
[18] Peru: WHO Coronavirus (COVID-19) Dashboard.

[19] M.M. Hasan, P.J. Hernández-Yépez, de los Angeles Rivera-Cabrera M, Sarkar A, dos Santos Costa AC, Essar MY., Concurrent epidemics of dengue and COVID-19 in Peru: which way forward? The Lancet Regional Health–Americas (2022 Aug) 12.
[20] Centro Nacional de Epidemiologia Prevencion y control de enfermedades, "Incremento de incidencia de casos y ocurrencia de brotes por Dengue y otras arbovirosis en el país,", Minist. Salud 19 (2) (2021) 6.
[21] "Instituto Nacional de Estadistica e Informatica, INEI, Republica del Peru (2017). La Libertad: Resultados Definitivos, Censo Nacional, 2017.
[22] Avila Calderón SL. Caracterización físico-química del efluente final de uso agrícola de la Planta de Tratamiento El Cortijo, Trujillo, Perú.
[23] I. D. E. C. Profesional, ca lio te de In g ie ría uí m ica ca lio te de Bi In g en ie ría m, 2006.
[24] M.T. Flood, N. D'Souza, J.B. Rose, T.G. Aw, Methods evaluation for rapid concentration and quantification of SARS-CoV-2 in raw wastewater using droplet digital and quantitative RT-PCR, Food Environ. Virol. 13 (2021 Sep) 303–315.
[25] D. Wang, L. Coscoy, M. Zylberberg, P.C. Avila, H.A. Boushey, D. Ganem, J. L. DeRisi, Microarray-based detection and genotyping of viral pathogens, Proc. Natl. Acad. Sci. 99 (24) (2002 Nov) 15687–15692.
[26] D. Wang, A. Urisman, Y.T. Liu, M. Springer, T.G. Ksiazek, D.D. Erdman, E. R. Mardis, M. Hickenbotham, V. Magrini, J. Eldred, J.P. Latreille, Viral discovery and sequence recovery using DNA microarrays, PLoS Biol. 1 (2) (2003 Nov) e2.
[27] Andrews S. FastQC: a quality control tool for high throughput sequence data.2010.
[28] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics. 30 (15) (2014 Aug) 2114–2120.
[29] P. Menzel, K.L. Ng, A. Krogh, Fast and sensitive taxonomic classification for metagenomics with kaiju, Nat. Commun. 7 (1) (2016 Apr) 11257.
[30] K. Bibby, E. Viau, J. Peccia, Viral metagenome analysis to guide human pathogen monitoring in environmental samples, Lett. Appl. Microbiol. 52 (4) (2011 Apr) 386–392.
[31] C. Bağcı, S. Patz, D.H. Huson, DIAMOND+ MEGAN: fast and easy taxonomic and functional analysis of short and long microbiome sequences, Current protocols. 1 (3) (2021 Mar) e59.
[32] D.H. Huson, S. Beier, I. Flade, A. Górska, M. El-Hadidi, S. Mitra, H.J. Ruscheweyh, R. Tappu, MEGAN community edition-interactive exploration and analysis of large-scale microbiome sequencing data, PLoS Comput. Biol. 12 (6) (2016 Jun) e1004957.
[33] Centers for disease control and prevention., "centers for disease control & Prevention Poxvirus & Rabies Branch (PRB)," 01 (01) (2022).
[34] Y. Li, H. Zhao, K. Wilkins, C. Hughes, I.K. Damon, Real-time PCR assays for the specific detection of monkeypox virus west African and Congo Basin strain DNA, J. Virol. Methods 169 (1) (2010 Oct) 223–227.
[35] A. Thongprachum, T. Fujimoto, S. Takanashi, H. Saito, S. Okitsu, H. Shimizu, P. Khamrin, N. Maneekarn, S. Hayakawa, H. Ushijima, Detection of nineteen enteric viruses in raw sewage in Japan, Infect. Genet. Evol. 1 (63) (2018 Sep) 17–23.
[36] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (7) (2021 Jul) 3022–3027.
[37] Hospital Regional Docente De Trujillo - Epidemiological Bulletins [Online]. Available: https://www.hrdt.gob.pe/site/index.php/servicios-linea/publicaciones/boletines-epidemiologicos. [Accessed: 01-Dec-2023].
[38] Peru Ministerio de Salud. National Center for Epidemiology, Disease Prevention and Control [Online]. Available: https://www.dge.gob.pe/portalnuevo/publicaciones/boletines-epidemiologicos/. [Accessed: 01-Dec-2023].
[39] Peru Ministerio de Salud, National Center for Epidemiology, Disease Prevention and Control - Cumulative Incident Cases. [Online], Available, https://www.dge.gob.pe/portalnuevo/informacion-publica/casos-de-incidencias-acumuladas/ [Accessed: 01-Dec-2023].
[40] "Unique digital platform of the Peruvian State - Ministry of Health." [Online]. Available: https://www.gob.pe/minsa. [Accessed: 01-Dec-2023].
[41] E.M. Symonds, K.H. Nguyen, V.J. Harwood, M. Breitbart, Pepper mild mottle virus: a plant pathogen with a greater purpose in (waste) water treatment development and public health management, Water Res. 1 (144) (2018 Nov) 1–2.
[42] B.E. Dutilh, N. Cassman, K. McNair, S.E. Sanchez, G.G. Silva, L. Boling, J.J. Barr, D. R. Speth, V. Seguritan, R.K. Aziz, B. Felts, A highly abundant bacteriophage discovered in the unknown sequences of human faecal metagenomes, Nat. Commun. 5 (1) (2014 Jul) 4498.
[43] E. Stachler, K. Bibby, Metagenomic evaluation of the highly abundant human gut bacteriophage CrAssphage for source tracking of human fecal pollution, Environ. Sci. Technol. Lett. 1 (10) (2014 Oct) 405–409.
[44] B. Malla, R. Ghaju Shrestha, S. Tandukar, J.B. Sherchand, E. Haramoto, Performance evaluation of human-specific viral markers and application of pepper mild mottle virus and crAssphage to environmental water samples as fecal pollution markers in the Kathmandu Valley, Nepal, Food and Environ. Virology. 1 (11) (2019 Sep) 274–287.
[45] S. Tandukar, S.P. Sherchan, E. Haramoto, Applicability of crAssphage, pepper mild mottle virus, and tobacco mosaic virus as indicators of reduction of enteric viruses during wastewater treatment, Sci. Rep. 10 (1) (2020 Feb) 3616.
[46] R.H. Holm, M. Nagarkar, R.A. Yeager, D. Talley, A.C. Chaney, J.P. Rai, A. Mukherjee, S.N. Rai, A. Bhatnagar, T. Smith, Surveillance of RNase P, PMMoV, and CrAssphage in wastewater as indicators of human fecal concentration across urban sewer neighborhoods, Kentucky, FEMS microbes. 3 (2022) xtac003.
[47] Y. Meuchi, M. Nakada, K. Kuroda, S. Hanamoto, A. Hata, Applicability of F-specific bacteriophage subgroups, PMMoV and crAssphage as indicators of source specific fecal contamination and viral inactivation in rivers in Japan, PLoS One 18 (7) (2023 Jul) e0288454.
[48] B. Miyani, X. Fonoll, J. Norton, A. Mehrotra, I. Xagoraraki, SARS-CoV-2 in Detroit wastewater, J. Environ. Eng. 146 (11) (2020 Nov) 06020004.
[49] W. Randazzo, P. Truchado, E. Cuevas-Ferrando, P. Simón, A. Allende, G. Sánchez, SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area, Water Res. 15 (181) (2020 Aug) 115942.
[50] I. Girón-Guzmán, A. Díaz-Reolid, P. Truchado, A. Carcereny, D. García-Pedemonte, B. Hernáez, A. Bosch, R.M. Pintó, S. Guix, A. Allende, A. Alcamí, Spanish wastewater reveals the current spread of Monkeypox virus, Water Res. 1 (231) (2023 Mar) 119621.
[51] E.F. de Jonge, C.M. Peterse, J.M. Koelewijn, A.M. van der Drift, R.F. van der Beek, E. Nagelkerke, W.J. Lodder, The detection of monkeypox virus DNA in wastewater samples in the Netherlands, Sci. Total Environ. 15 (852) (2022 Dec) 158265.
[52] S. Wurtzer, M. Levert, E. Dhenain, M. Boni, J.N. Tournier, N. Londinsky, A. Lefranc, OBEPINE SIG, Ferraris O, Moulin L., First detection of monkeypox virus genome in sewersheds in France: the potential of wastewater-based epidemiology for monitoring emerging disease, Environ. Sci. Technol. Lett. 9 (11) (2022 Oct) 991–996.
[53] C. McCall, H. Wu, E. O'Brien, I. Xagoraraki, Assessment of enteric viruses during a hepatitis outbreak in Detroit MI using wastewater surveillance and metagenomic analysis, J. Appl. Microbiol. 131 (3) (2021 Sep) 1539–1554.
[54] Pan American Health Organization (PAHO) World Health Organization (WHO) - Peru Responds to Monkeypox by Engaging Affected Communities [Online]. Available: https://www.paho.org/en/stories/peru-responds-monkeypox-engaging-affected-communities. [Accessed: 07-Feb-2024].
[55] M. Ampuero, C. Martínez-Valdebenito, M. Ferrés, R. Soto-Rifo, A. Gaggero, Monkeypox virus in wastewater samples from Santiago metropolitan region, Chile, Emerg. Infect. Dis. 29 (11) (2023 Nov) 2358.
[56] M.G. Peres, T.S. Bacchiega, C.M. Appolinário, A.F. Vicente, S.D. Allendorf, J. M. Antunes, S.A. Moreira, E. Legatti, C.R. Fonseca, E.M. Pituco, L.H. Okuda, Serological study of vaccinia virus reservoirs in areas with and without official reports of outbreaks in cattle and humans in São Paulo, Brazil, Archiv. Virol. 158 (2013 Dec) 2433–2441.
[57] J.S. Abrahão, R.K. Campos, Trindade G. de Souza, F.G. Da Fonseca, P.C. Ferreira, E. G. Kroon, Outbreak of severe zoonotic vaccinia virus infection, southeastern Brazil, Emerg. Infect. Dis. 21 (4) (2015 Apr) 695.
[58] E.G. Kroon, B.E. Mota, J.S. Abrahão, F.G. da Fonseca, Trindade G. de Souza, Zoonotic Brazilian vaccinia virus: from field to therapy, Antivir. Res. 92 (2) (2011 Nov) 150–163.
[59] A. Styczynski, J. Burgado, D. Walteros, J. Usme-Ciro, K. Laiton, A.P. Farias, Y. Nakazawa, C. Chapman, W. Davidson, M. Mauldin, C. Morgan, Seroprevalence and risk factors possibly associated with emerging zoonotic vaccinia virus in a farming community, Colombia, Emerg. Infect. Dis. 25 (12) (2019 Dec) 2169.
[60] T. Prado, T.M. Fumian, M.P. Miagostovich, A.M. Gaspar, Monitoring the hepatitis A virus in urban wastewater from Rio de Janeiro, Brazil, Trans. R. Soc. Trop. Med. Hyg. 106 (2) (2012 Feb) 104–109.
[61] J. Rodríguez-Díaz, L. Querales, L. Caraballo, E. Vizzi, F. Liprandi, H. Takiff, W. Q. Betancourt, Detection and characterization of waterborne gastroenteritis viruses in urban sewage and sewage-polluted river waters in Caracas, Venezuela, Appl. Environ. Microbiol. 75 (2) (2009 Jan) 387–394.
[62] L.A. Yanez, N.S. Lucero, P.A. Barril, M.D. Díaz, M.M. Tenaglia, L.I. Spinsanti, S. V. Nates, M.B. Isa, V.E. Ré, Evidence of hepatitis A virus circulation in Central Argentina: seroprevalence and environmental surveillance, J. Clin. Virol. 59 (1) (2014 Jan) 38–43.
[63] P.A. Baez, M.C. Lopez, A. Duque-Jaramillo, D. Pelaez, F. Molina, M.C. Navas, First evidence of the hepatitis E virus in environmental waters in Colombia, PLoS One 12 (5) (2017 May) e0177525.
[64] I.L. Castro, C. Espul, V.S. Paula, N.R. Altabert, J.E. Gonzalez, B.V. Lago, L.M. Villar, High prevalence of hepatitis A and E viruses in environmental and clinical samples from West Argentina, Braz. J. Infect. Dis. 19 (27) (2023 May) 102738.
[65] L. Guerrero-Latorre, B. Romero, E. Bonifaz, N. Timoneda, M. Rusiñol, R. Girones, B. Rios-Touma, Quito's virome: metagenomic analysis of viral diversity in urban streams of Ecuador's capital city, Sci. Total Environ. 15 (645) (2018 Dec) 1334–1343.
[66] B. Pardo-Figueroa, E. Mindreau-Ganoza, A. Reyes-Calderon, S.P. Yufra, I. M. Solorzano-Ortiz, A.J. Donayre-Torres, C. Antonini, J.M. Renom, A.M. Quispe, C. R. Mota, C.A. Chernicharo, Spatiotemporal surveillance of SARS-CoV-2 in the sewage of three major urban areas in Peru: generating valuable data where clinical testing is extremely limited, Acs Es&T Water. 2 (11) (2022 May) 2144–2157.
[67] A. Reyes-Calderón, E. Mindreau-Ganoza, B. Pardo-Figueroa, K.R. Garcia-Luquillas, S.P. Yufra, P.E. Romero, C. Antonini, J.M. Renom, C. Mota, M.C. Santa-Maria, Evaluation of Low-Cost SARS-CoV-2 Purification Methods for Viral Quantification and NGS Analysis: Implications for Wider Wastewater-Based Epidemiology Adoption, Available at SSRN 4308586, 2022 Dec 23.
[68] G. Fongaro, P.H. Stoco, D.S. Souza, E.C. Grisard, M.E. Magri, P. Rogovski, M. A. Schörner, F.H. Barazzetti, A.P. Christoff, L.F. de Oliveira, M.L. Bazzo, The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina, Brazil, November 2019, Sci. Total Environ. 15 (778) (2021 Jul) 146198.
[69] L. Guerrero-Latorre, I. Ballesteros, I. Villacrés-Granda, M.G. Granda, B. Freire-Paspuel, B. Ríos-Touma, SARS-CoV-2 in river water: implications in low sanitation countries, Sci. Total Environ. 15 (743) (2020 Nov) 140832.
[70] G.R. López, L.M. Martinez, L. Freyre, M.C. Freire, S. Vladimirsky, A. Rabossi, D. M. Cisterna, Persistent detection of cosavirus and saffold cardiovirus in Riachuelo River, Argentina, Food and Environ. Virol. 13 (2021 Mar) 64–73.
[71] A. Zamora-Figueroa, R.E. Rosales, R. Fernández, V. Ramírez, M. Bastardo, A. Farías, E. Vizzi, Detection and diversity of gastrointestinal viruses in wastewater from Caracas, Venezuela, 2021-2022, Virology. 1 (589) (2024 Jan) 109913.

[72] L. Burutarán, A. Lizasoain, M. García, L.F. Tort, R. Colina, M. Victoria, Detection and molecular characterization of Aichivirus 1 in wastewater samples from Uruguay, Food Environ. Virol. 8 (2016 Mar) 13–17.
[73] F.R. Guimarães, F.F. Ferreira, C.B. Vieira, T.M. Fumian, T. Shubo, J.P. Leite, M. P. Miagostovich, Molecular detection of human astrovirus in an urban sewage treatment plant in Rio de Janeiro, Brazil, Memórias do Instituto Oswaldo Cruz. 103 (2008) 819–823.
[74] A. Lizasoain, L.F. Tort, M. García, M.M. Gómez, J. Cristina, J.P. Leite, M. P. Miagostovich, M. Victoria, R. Colina, Environmental assessment of classical human astrovirus in Uruguay, Food Environ. Virol. 7 (2015 Jun) 142–148.
[75] T.M. Fumian, J.M. Fioretti, J.H. Lun, I.A. Dos Santos, P.A. White, M. P. Miagostovich, Detection of norovirus epidemic genotypes in raw sewage using next generation sequencing, Environ. Int. 1 (123) (2019 Feb) 282–291.
[76] J. Díaz, V. Solari, O. Cáceres, J. Mena, S. Baeza, X. Muñoz, M. O'Ryan, H. Galeno, A. Maldonado, N. Mamani, Brote de gastroenteritis aguda en la Región de Antofagasta, Chile: 2010, Rev. Chil. Infectol. 29 (1) (2012 Feb) 19–25.
[77] M.D. Fernández, C. Torres, L.C. Martínez, M.O. Giordano, G. Masachessi, P. A. Barril, M.B. Isa, R.H. Campos, S.V. Nates, V.A. Mbayed, Genetic and evolutionary characterization of norovirus from sewage and surface waters in Cordoba City, Argentina, Infect. Genet. Evol. 11 (7) (2011 Oct) 1631–1637.
[78] F. Bucardo, P.E. Lindgren, L. Svensson, J. Nordgren, Low prevalence of rotavirus and high prevalence of norovirus in hospital and community wastewater after introduction of rotavirus vaccine in Nicaragua, PLoS One 6 (10) (2011 Oct) e25962.
[79] M. Victoria, L.L. Tort, M. García, A. Lizasoain, L. Maya, J.P. Leite, M. P. Miagostovich, J. Cristina, R. Colina, Assessment of gastroenteric viruses from wastewater directly discharged into Uruguay River, Uruguay. Food and Environmental Virology. 6 (2014 Jun) 116–124.
[80] L.F. Tort, M. Victoria, A. Lizasoain, M. García, M. Berois, J. Cristina, J.P. Leite, M. M. Gómez, M.P. Miagostovich, R. Colina, Detection of common, emerging and uncommon VP4, and VP7 human group A rotavirus genotypes from urban sewage samples in Uruguay, Food Environ. Virol. 7 (2015 Dec) 342–353.
[81] D.U. Mehnert, K.E. Stewien, Detection and distribution of rotavirus in raw sewage and creeks in São Paulo, Brazil, Appl. Environ. Microbiol. 59 (1) (1993 Jan) 140–143.
[82] P.A. Barril, M.O. Giordano, M.B. Isa, G. Masachessi, L.J. Ferreyra, A.A. Castello, G. Glikmann, S.V. Nates, Correlation between rotavirus A genotypes detected in hospitalized children and sewage samples in 2006, Córdoba, Argentina, J. Med. Virol. 82 (7) (2010 Jul) 1277–1281.
[83] B. Calgua, T. Fumian, M. Rusinol, J. Rodriguez-Manzano, V.A. Mbayed, S. Bofill-Mas, M. Miagostovich, R. Girones, Detection and quantification of classic and emerging viruses by skimmed-milk flocculation and PCR in river water from two geographical areas, Water Res. 47 (8) (2013 May) 2797–2810.
[84] J.M. Fioretti, M.S. Rocha, T.M. Fumian, A. Ginuino, T.P. da Silva, M.R. de Assis, J. D. Rodrigues, F.A. Carvalho-Costa, M.P. Miagostovich, Occurrence of human sapoviruses in wastewater and stool samples in Rio De Janeiro, Brazil, J. Appl. Microbiol. 121 (3) (2016 Sep) 855–862.
[85] M. Salvo, A. Lizasoain, M. Castells, V. Bortagaray, S. Castro, R. Colina, F.L. Tort, M. Victoria, Human bocavirus: detection, quantification and molecular characterization in sewage and surface waters in Uruguay, Food Environ. Virol. 10 (2018 Jun) 193–200.
[86] M. Salvo, D. Mir, L.F. Tort, A. Lizasoain, R. Colina, M. Victoria, Description of a human Bocavirus recombinant strain in the Americas, Mem. Inst. Oswaldo Cruz 21 (2019 Oct) 114.
[87] F.M. Santos, M.J. Vieira, P. Garrafa, T.A. Monezi, V.H. Pellizari, C.M. Hársi, D. U. Mehnert, Discrimination of adenovirus types circulating in urban sewage and surface polluted waters in São Paulo city, Brazil, Water Sci. Technol. Water Supply 4 (2) (2004 Apr) 79–85.
[88] L.J. Ferreyra, M.O. Giordano, L.C. Martinez, P.A. Barril, G. Masachessi, M.B. Isa, R. Poma, V. Rajal, P. Biganzoli, S.V. Nates, J.V. Pavan, Tracking novel adenovirus in environmental and human clinical samples: no evidence of endemic human adenovirus type 58 circulation in Cordoba city, Argentina, Epidemiol. Infect. 143 (7) (2015 May) 1427–1431.
[89] C. Torres, M.E. Barrios, R.V. Cammarata, D.M. Cisterna, T. Estrada, S.M. Novas, P. Cahn, M.D. Fernández, V.A. Mbayed, High diversity of human polyomaviruses in environmental and clinical samples in Argentina: detection of JC, BK, Merkel-cell, Malawi, and human 6 and 7 polyomaviruses, Sci. Total Environ. 15 (542) (2016 Jan) 192–202.
[90] T.M. Fumian, F.R. Guimarães, B.J. Pereira Vaz, M.T. da Silva, F.F. Muylaert, S. Bofill-Mas, R. Gironés, J.P. Leite, M.P. Miagostovich, Molecular detection, quantification and characterization of human polyomavirus JC from waste water in Rio De Janeiro, Brazil, J. Water and Health. 8 (3) (2010 Sep) 438–445.
[91] J. Levican, A. Levican, M. Ampuero, A. Gaggero, JC polyomavirus circulation in one-year surveillance in wastewater in Santiago, Chile, Infect. Genet. Evol. 1 (71) (2019 Jul) 151–158.
[92] Institute for Health Metrics and Evaluation (IHME) Covid-19 (https://covid19.healthdata.org/global?view=cumulative-deaths&tab=trend).
[93] Peru Covid-19 statistics worldometer (https://www.worldometers.info/coronavirus/country/peru/).
[94] Peru has the world's highest COVID death rate. Here's why NPR WAMU 88.5 (https://www.npr.org/sections/goatsandsoda/2021/11/27/1057387896/peru-has-the-worlds-highest-covid-death-rate-heres-why).
[95] Johns Hopkins University & Medicine CORONAVIRUS RESEARCH CENTER (https://coronavirus.jhu.edu/data/mortality).
[96] World Health Organization Disease Outbreak News; Multi-country monkeypox outbreak in non-endemic countries. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON390, 2022.
[97] A. Antinori, V. Mazzotta, S. Vita, F. Carletti, D. Tacconi, L.E. Lapini, A. D'Abramo, S. Cicalini, D. Lapa, S. Pittalis, V. Puro, Epidemiological, clinical and virological characteristics of four cases of monkeypox support transmission through sexual contact, Italy, may 2022, Eurosurveillance. 27 (22) (2022 Jun) 2200421.
[98] E.F. de Jonge, C.M. Peterse, J.M. Koelewijn, A.M. van der Drift, R.F. van der Beek, E. Nagelkerke, W.J. Lodder, The detection of monkeypox virus DNA in wastewater samples in the Netherlands, Sci. Total Environ. 15 (852) (2022 Dec) 158265.