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Viral diversity and abundance in polluted waters in Kampala, Uganda

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ABSTRACT

Waterborne viruses are a significant cause of human disease, especially in developing countries such as Uganda. A total of 15 virus-selective samples were collected at five sites (Bugolobi Wastewater Treatment Plant (WWTP) influent and effluent, Nakivubo Channel upstream and downstream of the WWTP, and Nakivubo Swamp) in July and August 2016. Quantitative PCR and quantitative RT-PCR was performed to determine the concentrations of four human viruses (adenovirus, enterovirus, rotavirus, and hepatitis A virus) in the samples. Adenovirus ($1.53*10^5$-$1.98*10^7$ copies/L) and enterovirus ($3.17*10^5$-$8.13*10^7$ copies/L) were found to have the highest concentrations in the samples compared to rotavirus ($5.79*10^1$-$3.77*10^3$ copies/L) and hepatitis A virus ($9.93*10^2$-$1.11*10^4$ copies/L). In addition, next-generation sequencing and metagenomic analyses were performed to assess viral diversity, and several human and vertebrate viruses were detected, including *Herpesvirales*, *Iridoviridae*, *Poxviridae*, *Circoviridae*, *Parvoviridae*, *Bunyaviridae* and others. Effluent from the wastewater treatment plant appears to impact surface water, as samples taken from surface water downstream of the treatment plant had higher viral concentrations than samples taken upstream. Temporal fluctuations in viral abundance and diversity were also observed. Continuous monitoring of wastewater may contribute to assessing viral disease patterns at a population level and provide early warning of potential outbreaks using wastewater-based epidemiology methods.

1. Introduction

It has been reported that between 1.5 and 12 million people die each year from waterborne diseases (Gleick, 2002; WHO, 2004) and diarrheal diseases are listed within the top 15 leading causes of death worldwide (Mathers and Loncar, 2006). Rapid population growth, climate change, natural disasters, immigration, globalization, urbanization, and the corresponding sanitation and waste management challenges are expected to intensify the problem in the years to come. In the vast majority of cases, all of the infectious agents have not been identified. However, most outbreaks of unidentified etiology are suggested to be caused by viruses (US EPA, 2006). Viruses have been cited as potentially the most important and hazardous pathogens found in wastewater (Toze, 1997). Viruses can be responsible for serious health outcomes, especially for children, the elderly and immunocompromised individuals, and are of great concern because of their low infectious dose, ability to mutate, inability to be treated by antibiotics, resistance to disinfection, small size that facilitates environmental transport, and high survivability in water and solids.

This study focuses on Kampala, the capital city of Uganda. Uganda, like any other developing country, still faces challenges in meeting Sustainable Development Goals (SDGs) on improved sanitation as outlined by the United Nations. By 2015, Uganda had not met the SDG target on sanitation with only 29% of the urban population having access to improved sanitation facilities such as flush/pour toilets and ventilated improved pit latrines (WHO and UNICEF, 2015). Kampala has undergone a 27% increase in population from approximately 1,189,150 in 2002 to 1,516,210 in 2014 (UBOS, 2014). The rapid increase in population is mainly attributed to rural-urban migration in search of better living standards resulting in rapid expansion of impoverished settlements which

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accommodate more than 50% of the city's population (UN-Habitat, 2007). The settlements face challenges of poor sanitation and hygiene that have exacerbated as more people move into the city (Isunju et al., 2011; Kulabako et al., 2007; Tumwebaze et al., 2013; UN-Habitat, 2007).

The increase in population has also heightened the need for sufficient treatment of wastewater. With approximately 10% of the urban population connected to the sewer system (MacDonald, 2004), most of the residents in Kampala dispose of wastewater in open channels and space. This is mainly caused by a lack of financial resources and little space for construction of sewer systems (Kulabako et al., 2010), especially among the more impoverished areas of the city (UN-Habitat, 2007). A case study by Kulabako et al. (2010) conducted in Bwaise III (an impoverished area in Kampala) revealed that 37% of the residents dispose of wastewater in open drains whereas 23% use both open drains and open space. The wastewater consequently finds its way into surface water bodies, compromising the quality of such water sources.

Kampala has experienced diarrheal disease outbreaks from cholera, dysentery, and cryptosporidiosis (Kulabako et al., 2010; Ssengooba et al., 1997; Tumwine et al., 2003). The outbreak or occurrence of such diseases is mainly attributed to unsafe water supplies, poor hygiene and sanitation practices. Numerous prior studies have investigated the quality of water sources in and around Kampala, determining that many of these water sources were contaminated (Fuhrimann et al., 2015; Haruna et al., 2005; Howard et al., 2003; Kulabako et al., 2007; Muyodi et al., 2009; Nsubuga et al., 2004). In these studies, water quality parameters were correlated with the prevalence of waterborne diseases such as cholera and dysentery (Muyodi et al., 2009). Contamination of drinking water sources posed a health risk to a majority of the city's population (Howard et al., 2003), and contamination of the water source was primarily due to poor waste disposal (Kulabako et al., 2007).

However, these studies focused on investigating bacterial contamination indicators. Waterborne viruses, meanwhile, have been shown in three studies to be responsible for diarrheal disease outbreaks in Kampala, particularly in children, with each study attributing recent diarrheal outbreaks to the presence of rotavirus in stool specimens (Bwogi et al., 2016; Mwenda et al., 2010; Odiit et al., 2014). Diarrhea has been determined to be one of the top causes of death in young children worldwide (Liu et al., 2015), and rotavirus has been shown to be responsible for approximately 45% of diarrheal cases in young children in Uganda (Nakawesi et al., 2010). Recently, studies have been performed investigating viral contamination of water sources in Kampala (Chung et al., 2013; Katukiza et al., 2013), but these studies focused primarily on surface water and did not investigate wastewater or its impact on the surrounding environment.

Rotavirus (RV), adenovirus (AdV), enterovirus (EV), and hepatitis A virus (HAV) were the human viruses chosen for investigation in this study as they are the most common viruses detected in wastewater (Katukiza et al., 2013; Kiulia et al., 2010; Rigotto et al., 2010; Schvoerer et al., 2000; Xagoraraki et al., 2014) and are all linked to disease outbreaks around the world (Maunula et al., 2008; Papapetropoulou and Vantarakis, 1998; Sjogren et al., 1987). Additionally, it has been concluded that adenovirus can serve as a reliable indicator of human pollution (La Rosa et al., 2010; Okoh et al., 2010; Rames et al., 2016). Quantitative polymerase chain reaction (qPCR) was used to detect and quantify these viruses as it is rapid, sensitive, reliable, and effective at low viral concentrations (La Rosa et al., 2010; Martin-Latil et al., 2012).

In addition, next-generation sequencing and metagenomic analysis has been used to assess viral diversity. Several studies have used these methods to investigate the detection and diversity of viruses in wastewater (Aw et al., 2014; Bibby and Peccia, 2013; Cantalupo et al., 2011; O'Brien et al., 2017; Tamaki et al., 2012). While metagenomic analyses are presently only able to identify a fraction of viruses present in the environment (Aw et al., 2014; Cantalupo et al., 2011), these methods still offer comprehensive characterization of the viruses in a sample, allowing for a wide range of detection and the possibility of identifying viruses previously unknown to be present in a sample.

There is potential to employ wastewater as an epidemiological tool to better identify and predict viral disease outbreaks. This approach has been used to track illicit drug use in various locations around the world, but so far has not been applied to track viral disease outbreaks. The approach was first theorized in 2001 (Daughton and Jones-Lepp, 2001) and first implemented and reported in the monitoring of cocaine use in 2005 where the method was termed sewage epidemiology (Zuccato et al., 2005). The methodology considers raw untreated wastewater as a reservoir of human excretion products that can serve as a sampling point for assessing population health. Environmental surveillance has already proven useful in the efforts to eradicate polio (Bosch et al., 2008; Lago et al., 2003; Tebbens et al., 2017), and wastewater surveillance could be a tool in the efforts to improve public health. Viruses notably do not replicate outside a host, are commonly excreted in human waste, and waterborne viruses are stable in wastewater (Xagoraraki et al., 2014). Therefore, viruses could be an ideal candidate for the wastewater epidemiology methodology.

The purpose of applying wastewater epidemiology to viruses is to more rapidly determine whether an outbreak is imminent or already in progress within a given population. Such an approach should include frequent sampling and analysis for viral concentrations and biomarkers, for population adjustment. Viral shedding rates and survival in wastewater should also be taken in to account. Attaining baseline concentrations of viruses in wastewater would be a necessary step in the wastewater epidemiology process, as it would establish levels with which sudden large rises in viral concentration could be compared. Continued monitoring of viral abundance could provide useful information for the development of wastewater-based epidemiology methods.

This study seeks to quantify the abundance of four human viruses in surface water and wastewater in Kampala, Uganda, characterize the viral diversity of these water samples, and to establish preliminary data that could indicate the possibility of using these methods in future wastewater-based epidemiology studies to identify early signals of and predict future viral disease outbreaks.

2. Materials and methods

2.1. Sample collection

A total number of 15 samples from five sampling locations were collected in the summer 2016. Samples were collected every other week at a depth of less than 1 m from the five locations in southwest Kampala: Bugolobi Wastewater Treatment Plant (WWTP) influent and effluent, Nakivubo Channel upstream and downstream of the WWTP, and Nakivubo Swamp, as shown in Fig. 1. The Bugolobi WWTP utilizes conventional activated sludge methods to treat wastewater. For each sampling event, water was pumped through a NanoCeram Virus Sampler filter (Argonide Corporation) at a rate of 11–12 L/min using a previously described method (Kuo et al., 2010; O'Brien et al., 2017; Simmons et al., 2011) shown to be effective in viral recovery from water samples (Ikner et al., 2012). Water was collected until the membrane fouled beyond the point at which water would no longer flow through the filter. Table 1 summarizes the locations, dates, and volumes for each sampling event. Filters were immediately kept on dry ice and transported to

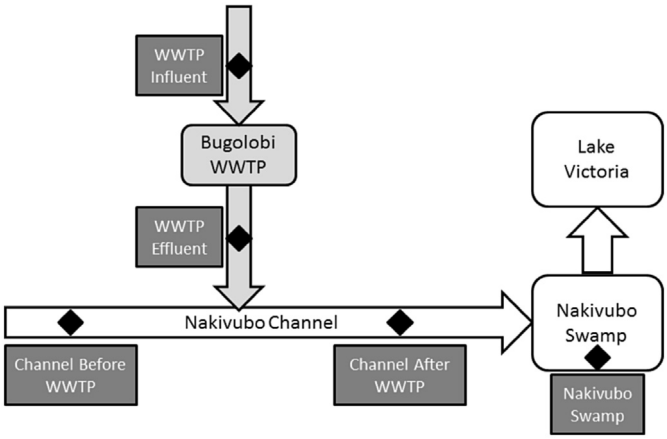

Fig. 1. Flowchart of sampling locations and surrounding surface waters. Note: Diamond symbols indicate sampling locations.

Michigan State University (MSU) in East Lansing, MI for further processing. The filters arrived at the MSU laboratory within 48–72 h of each sampling event.

2.2. Sample processing

All NanoCeram filters used to concentrate the samples were eluted immediately upon receipt according to the standard method (US EPA, 2001) which has been shown to be effective (Ikner et al., 2012). Briefly, a 1.5% w/v beef extract (0.05 M glycine, pH 9.0–9.5) solution was used as the eluent. The filters were submerged for a total of 2 min (two separate 1 min elutions) in filter housings with 1 L of beef extract added to the pressure vessel. After the beef extract was passed through each filter, the pH of the eluate was adjusted to 3.5 ± 0.1 using 1 M HCl and flocculated for 30 min. Further concentration of the solution was performed by two stages of centrifugations for 15 min at 2500*g* and 4 °C. The supernatant was then decanted and the process was repeated until all the beef extract solution was centrifuged. The accumulated pellets were resuspended using 30 mL of 0.15 M sodium phosphate (pH 9.0–9.5) and mixed until the pellet was mostly dissolved. The pH was then adjusted to 9.0–9.5 using 1 M NaOH. The solution was placed into a 50 mL centrifuge tube and centrifuged for 10 min at 4 °C at 7000*g*. The supernatant was poured off into a separate 50 mL centrifuge tube, the pH was adjusted to 7.0–7.5 for stabilization of the virus particles, and the pellet was discarded. The supernatant was loaded into a 60 mL syringe and passed through a 0.22 μm sterilized filter for removal of bacteria, fungi and other contaminants. Samples were completely mixed, placed into 2 mL cryogenic tubes, and stored at −80 °C until further analysis.

2.3. Nucleic acid extraction

Nucleic acids were extracted from the viral-concentrated samples using the QIAamp Viral RNA Mini Kit (Qiagen) following manufacturer's instructions. This kit allows for the recovery of both viral DNA and RNA. Extracted nucleic acid samples were stored at −20 °C until further analysis. The viral-concentrated eluate samples were also kept −80 °C for future analyses.

2.4. qPCR analyses

Real-time quantitative PCR and real-time quantitative reverse-transcriptase PCR (qRT-PCR) was performed on each sample using a Roche LightCycler 1.5 instrument (Roche Applied Sciences) for the detection of human adenovirus 40/41 (AdV), human enterovirus (EV), human rotavirus (RV), and hepatitis A virus (HAV) using previously described assays (Dierssen et al., 2008; Jothikumar et al., 2005; Pang et al., 2004; Xagoraraki et al., 2007) and previously prepared standard curves. Table 2 displays primers and probes used for virus quantification. For each of the four assays, the amplification efficiency was >98.0% with a detection limit of 10^1 copies per reaction.

For each sample, a 20-μL PCR mixture was created in triplicate containing 4 μL of 5× LightCycler TaqMan Master Mix, 0.8 μL of 10 μM forward primer (final concentration, 400 nM), 0.4 μL of each

Table 1
Summary of sampling volumes (L) for each sampling date and location in the study.

Sampling Date	Sampling Location				
	Channel Before WWTP	WWTP Influent	WWTP Effluent	Channel After WWTP	Swamp
12 July 2016	30.66	18.17	5.68	26.88	39.37
25 July 2016	10.98	2.65	19.31	9.46	24.23
8 August 2016	23.47	9.08	5.68	18.55	53.37

Note: Sampling volumes and elution volumes were taken into account when calculating qPCR concentrations for viruses.

Table 2
Primer and probe sequences for the qPCR assays used in the study.

Target Virus	Primer and Probe	Sequence (5′-3′)	Amplicon Size	Reference
Human adenovirus (40,41)	HAdV-F4041-hex157f	ACCCACGATGTAACCACAGAC	88	Xagoraraki et al., 2007
	HAdV-F40-hex245r	ACTTTGTAAGAGTAGGCGGTTTC		
	HAdV-F41-hex246r	CACTTTGTAAGAATAAGCGGTGTC		
	HAdV-F4041-hex214rprobe	FAM-CGACKGGCACGAAKCGCAGCGT-TAMRA		
Hepatitis A virus	Forward Primer	GGTAGGCTACGGGTGAAAC	89	Jothikumar et al., 2005
	Reverse Primer	AACAACTCACCAATATCCGC		
	Probe	FAM-CTTAGGCTAATACTTCTATGAAGAGATGC-BBQ1		
Enteroviruses	EQ-1	ACATGGTGTGAAGAGTCTATTGAGCT	141	Dierssen et al., 2008
	EQ-2	CCAAAGTAGTCGGTTCCGC		
	EP	FAM-TCCGGCCCCTGAATGCGGCTAAT-TAMRA		
Rotavirus	Rota NVP3-F	ACCATCTACACATGACCCTC	86	Pang et al., 2004
	Rota NVP3-R	GGTCACATAACGCCCC		
	Probe	ATGAGCACAATAGTTAAAAGCTAACACTGTCAA		

Table 3
Average concentrations of viruses at each sampling location (copies/L). Ranges of minimum and maximum detected concentrations are listed in parentheses. ^Only one sample with positive signal.

Human Virus	Location				
	Channel Before WWTP	WWTP Influent	WWTP Effluent	Channel After WWTP	Swamp
Adenovirus	$5.45*10^5$ ($1.53*10^5$–$1.25*10^6$)	$1.17*10^7$ ($3.33*10^6$–$1.8*10^7$)	$9.43*10^6$ ($1.67*10^6$–$1.98*10^7$)	$2.08*10^6$ ($7.02*10^5$–$2.95*10^6$)	$5.72*10^5$ ($1.60*10^5$–$1.08*10^6$)
Enterovirus	$3.09*10^6$ ($1.73*10^6$–$4.71*10^6$)	$3.91*10^7$ ($4.73*10^6$–$8.13*10^7$)	$1.42*10^7$ ($1.25*10^6$–$2.12*10^7$)	$4.15*10^6$ ($1.10*10^6$–$7.22*10^6$)	$4.69*10^6$ ($3.17*10^5$–$1.48*10^7$)
Rotavirus	$1.16*10^2$ ($1.08*10^2$–$1.25*10^2$)	$1.81*10^3$ ($4.22*10^2$–$3.77*10^3$)	$5.79*10^{1}$^	$1.31*10^3$ ($1.87*10^2$–$3.72*10^3$)	$1.66*10^2$ ($6.49*10^1$–$2.99*10^2$)
Hepatitis A	$7.74*10^3$ ($5.88*10^3$–$1.03*10^4$)	$4.26*10^3$ ($2.01*10^3$–$8.39*10^3$)	$5.79*10^3$ ($1.93*10^3$–$8.70*10^3$)	$6.12*10^3$ ($1.41*10^3$–$1.11*10^4$)	$2.73*10^3$ ($9.93*10^2$–$4.48*10^3$)

10 μM reverse primer (final concentration, 200 nM), 0.6 μL of 10 μM TaqMan probe (final concentration, 300 nM), 8.8 μL of PCR-grade water, and 5 μL of DNA extract. The real-time PCR program used a denaturation step for 15 min at 95 °C, followed by an amplification step of 45 cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 10 s, concluding with a cooling step at 40 °C for 30 s.

2.5. Metagenomic analyses

2.5.1. Next-generation sequencing

To allow for sequencing of both DNA and RNA viruses, cDNA synthesis was performed to convert viral genomic RNA into cDNA using previously described methods (Bibby and Peccia, 2013; Cantalupo et al., 2011). Samples from the WWTP influent and the Nakivubo Swamp for each of the three sampling events were selected for sequencing, for a total of six samples. Viral nucleic acids were sequenced on an Illumina platform (Illumina HiSeq, Roche Technologies) at the Research Technology Support Facility (RTSF) at MSU. Libraries were prepared using the Illumina TruSeq Nano DNA Library Preparation Kit on a Perkin Elmer Sciclone robot following manufacturer's protocols. Completed libraries underwent quality control and were quantified using a combination of Qubit dsDNA HS and Caliper LabChipGX HS DNA assays and all samples passed quality control. Libraries were pooled in equimolar amounts for multiplexed sequencing. This pool was quantified using the Kapa Biosystems Illumina Library Quantification qPCR kit. The pool was loaded on one lane of an Illumina HiSeq 4000 flow cell and sequencing performed in a 2 × 150bp paired end format using

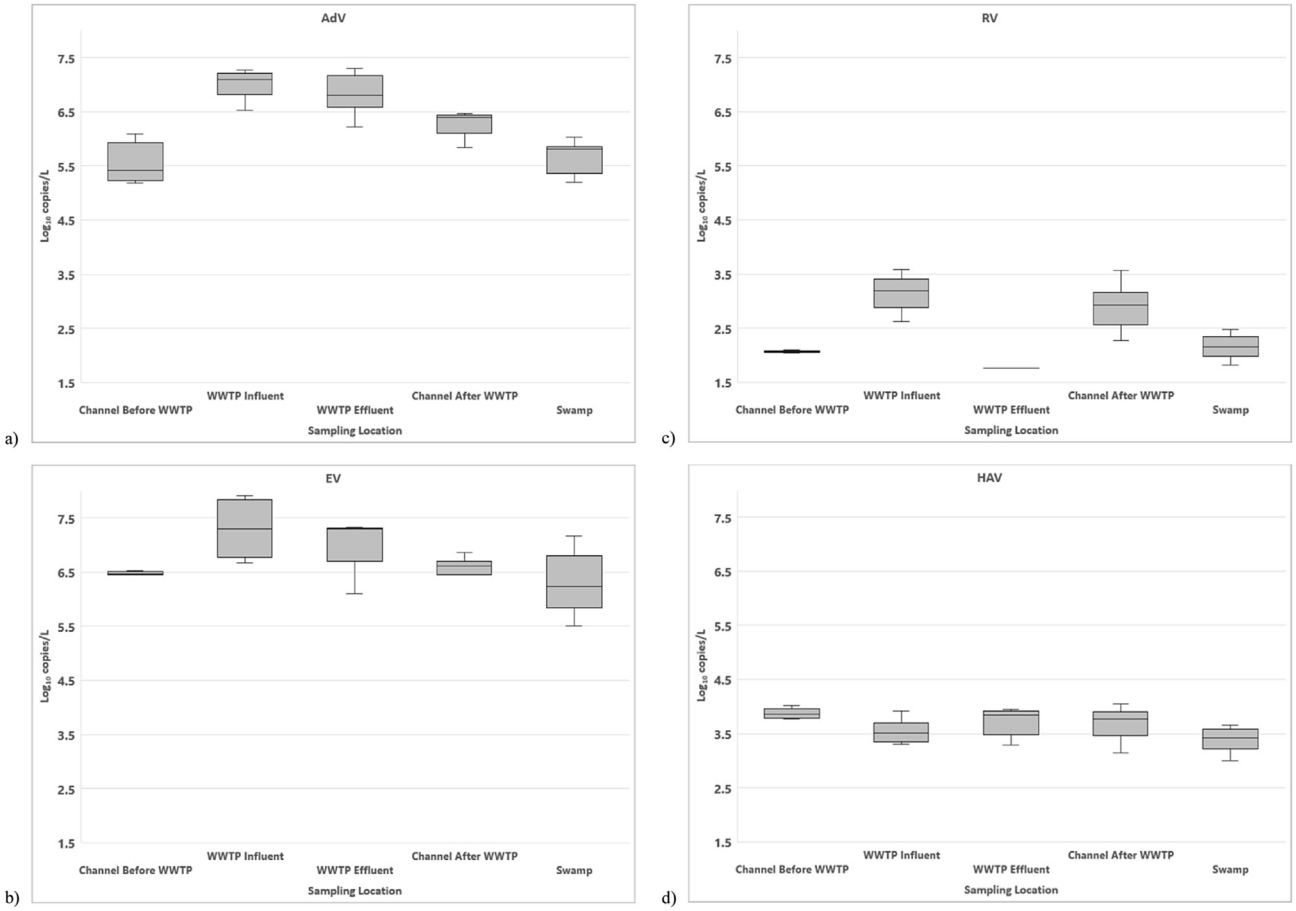

Fig. 2. Boxplots for detected concentrations of a) adenovirus, b) enterovirus, c) rotavirus and d) hepatitis A virus at each sampling location.

Table 4
Number of qPCR samples testing positive for each virus, date, and location. Adenovirus and enterovirus samples were run once in duplicate, rotavirus and hepatitis A virus were run twice in duplicate.

Virus	Sampling Date	Channel Before WWTP	WWTP Influent	WWTP Effluent	Channel After WWTP	Swamp
Adenovirus	12 July	2/2	2/2	2/2	2/2	2/2
	25 July	2/2	2/2	2/2	2/2	2/2
	8 August	2/2	2/2	2/2	2/2	2/2
Enterovirus	12 July	2/2	2/2	2/2	2/2	2/2
	25 July	2/2	0/2	1/2	2/2	2/2
	8 August	2/2	2/2	0/2	0/2	2/2
Rotavirus	12 July	1/4	0/4	0/4	1/4	0/4
	25 July	1/4	1/4	1/4	0/4	2/4
	8 August	0/4	4/4	0/4	4/4	2/4
Hepatitis A	12 July	4/4	2/4	1/4	2/4	3/4
	25 July	3/4	0/4	2/4	0/4	1/4
	8 August	0/4	2/4	3/4	1/4	4/4

HiSeq 4000 SBS reagents. Base calling was done by Illumina Real Time Analysis (RTA) v2.7.6 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.18.0.

2.5.2. Sequencing file processing

The raw sequencing files were assessed for quality control using FastQC (Andrews, 2010). The flexible read trimming tool for Illumina NGS data called Trimmomatic was used for trimming the paired-end raw reads from the Illumina sequencer and removing adapters using ILLUMINACLIP (Bolger et al., 2014). Trimmomatic was also used to trim the leading 26 base pairs representing the universal primer from cDNA synthesis. The trimmed reads were assembled into contig files in order to reduce the chances of false positive detection using an iterative de Bruijn graph de novo assembler for short reads sequencing data with highly uneven sequencing depth called IDBA-UD using a minimum k-mer length of 40, maximum k-mer length of 120, and an interval of 10 (Peng et al., 2012). The assembled contig files used in this study are available on the MG-RAST server under project accession ID mgp80872.

2.5.3. BLASTn analysis

The assembled contig files were BLASTed against the Complete RefSeq Release of Viral and Viroid Sequences (downloaded 16 Jan 2017) from NCBI using BLASTn and a maximum e-value of 10^{-3}, which has been used in prior studies and shown to minimize false positives (Bibby et al., 2011; Bibby and Peccia, 2013). BLAST output was parsed and annotated using MEGAN to allow for taxonomic classification of reads.

2.6. Statistical and principal component analysis

The Wilcoxon signed-rank test was used to test the significance difference in concentrations of the four tested viruses between the samples taken in the Nakivubo Channel before and after the Bugolobi WWTP as well as the samples taken in the WWTP influent and effluent ($p < 0.05$). This test was also used to assess significance of the differences in concentrations of AdV among the three sampling events ($p < 0.01$). Principal component analysis (PCA) was performed using the six metagenomic samples to assess sample similarity. Microsoft Excel was used to perform PCA with the Real Statistics Resource Pack software (Release 4.5) (Zaiontz, 2013). In order to perform PCA, the number of hits for each viral order as determined by MEGAN was converted into a relative abundance percentage for each individual sample. The relative abundances were then used in PCA to calculate the values of the first two principal components, PC1 and PC2. The two principal components were then charted on a scatter plot for the six samples analyzed.

3. Results

Average concentrations for the four tested viruses at each of the five sampling locations are summarized in Table 3 and boxplots for each virus are shown in Fig. 2. Across all locations, enterovirus (EV) was found to have the highest concentrations, followed by

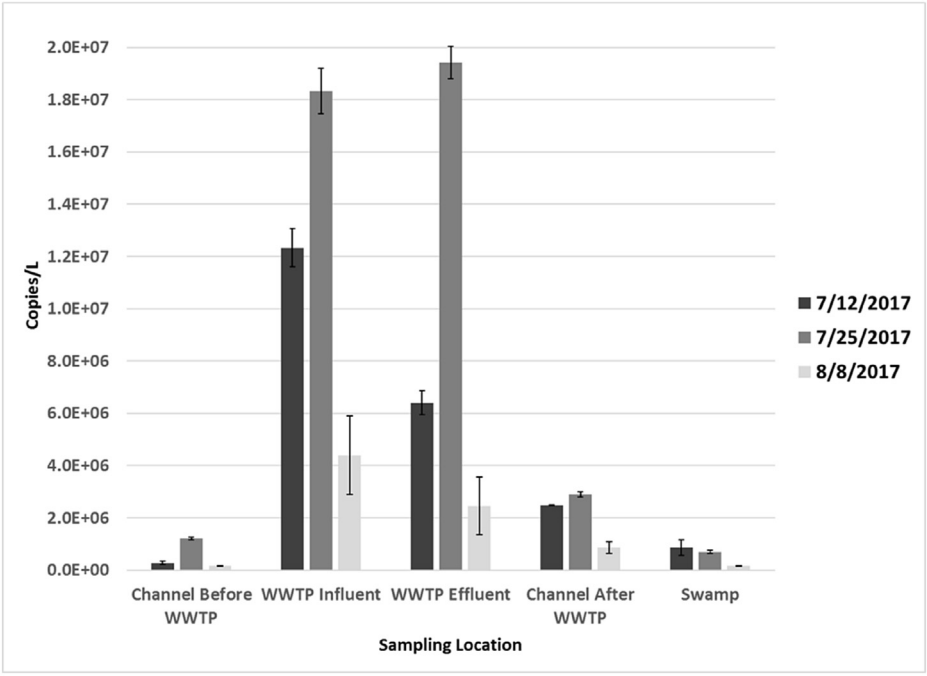

Fig. 3. Average adenovirus concentration (copies/L) at each sampling location on each sampling date. Error bars represent one standard deviation in each direction.

Table 5
Summary of metagenomic analysis statistics. Affiliated sequences refer to the number of sequences that registered a hit for a viral reference genome as determined by BLAST. Unaffiliated sequences did not register a hit for any viral reference genome during BLAST analysis. Affiliated ratio is the percentage of affiliated sequences relative to the number of contigs in the sample.

Metagenome Date	Number of contigs	Affiliated sequences	Unaffiliated sequences	Affilated ratio
WWTP Influent				
12 July	371,741	6203	365,538	1.67%
25 July	99,741	3063	96,678	3.07%
8 August	31,489	3479	28,010	11.05%
Nakivubo Swamp				
12 July	144,446	3685	140,761	2.55%
25 July	67,860	3821	64,039	5.63%
8 August	23,166	3731	19,435	16.11%

Table 6
Number of hits for vertebrate virus families for each sample.

Human Virus	WWTP Influent			Nakivubo Swamp		
	12 July	25 July	8 August	12 July	25 July	8 August
Adenoviridae	9	1	0	5	1	0
Herpesvirales	15	4	0	4	0	0
Iridoviridae	27	5	2	14	6	2
Papillomaviridae	1	0	0	1	0	0
Polyomaviridae	7	0	0	0	0	0
Poxviridae	62	11	3	47	25	3
Picobirnaviridae	0	4	2	0	0	0
Reoviridae	2	1	4	1	0	0
Retroviridae	0	0	0	1	0	0
Anelloviridae	1	0	0	0	0	0
Circoviridae	60	29	12	34	26	5
Parvoviridae	20	30	2	45	38	6
Bunyaviridae	3	18	32	15	26	24
Orthomyxoviridae	0	1	0	0	0	0
Mononegavirales	6	0	0	0	0	0
Astroviridae	6	1	2	1	1	1
Flaviviridae	1	0	0	1	0	0
Hepeviridae	1	0	0	1	0	0
Nidovirales	4	1	1	4	1	1
Nodaviridae	0	0	0	3	0	0
Picornaviridae	9	8	0	1	0	0
Total	**234**	**114**	**60**	**178**	**124**	**42**

adenovirus (AdV), then hepatitis A virus (HAV), and finally rotavirus (RV) which had the lowest concentrations as calculated by qPCR. Concentrations were highest at the WWTP influent and there was not a significant decrease from the influent to the effluent of the WWTP according to the Wilcoxon signed-rank test. The Wilcoxon signed-rank test also determined that the higher concentrations of AdV in Nakivubo Channel after the WWTP were significant compared to concentrations from before the WWTP ($p < 0.05$). Concentrations of EV and RV were also higher in Nakivubo Channel after the WWTP compared to before, but these differences were not found to be statistically significant ($p < 0.05$).

Quantitative PCR results were also analyzed for temporal changes among the three sampling events. Table 4 shows the occurrence of each virus at each location for each of the three dates on which sampling took place. AdV was detected at all locations on each sampling date, while the other three viruses were detected on certain dates but not others at some locations. For example, RV was most prevalent in the samples from August 8th, while EV was most common in the samples from July 12th. Since AdV was detected in all samples, changes in concentration across the three sampling events were also investigated. Fig. 3 displays the average concentration for AdV at each location separated by date. Temporal variations in AdV concentration are evident based on these qPCR results; concentrations of AdV were highest in four of the five locations on July 25th, and lowest in all five locations on August 8th. The Wilcoxon signed-rank test determined that the differences in concentrations of AdV among the three sampling dates were statistically significant ($p < 0.01$).

Sequencing data were analyzed using BLAST and MEGAN. A summary of the metagenomics analysis data is shown in Table 5. The vast majority of affiliated sequences were assigned to viruses, with viruses comprising within 89.94%–99.79% of assigned sequences for each of the six samples. As shown in Fig. 4, the majority of viral sequences correspond to bacteriophages and invertebrate viruses in each sample. Vertebrate viruses, including those infecting humans, comprise from 1.18% of viral sequences in the August

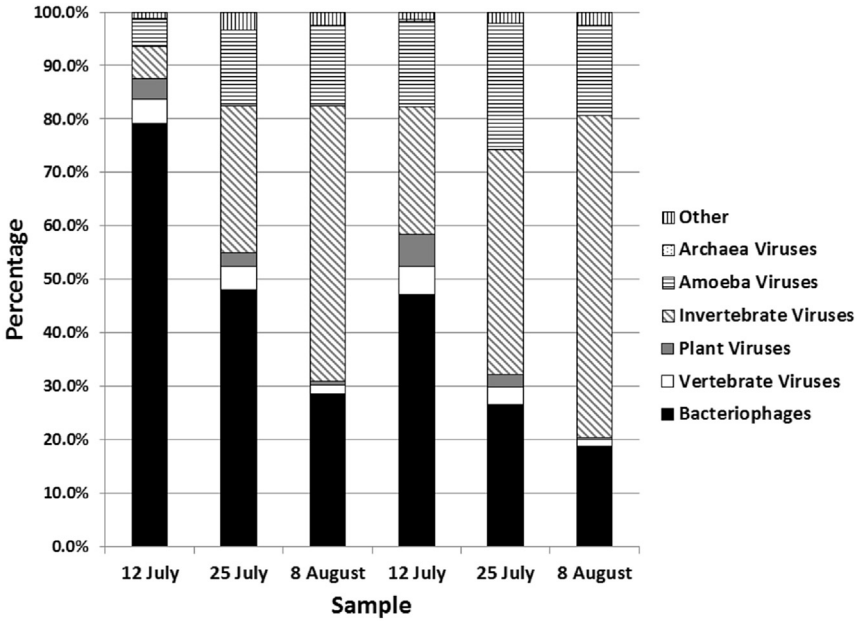

Fig. 4. Affiliated viral sequences by host type for each sample.

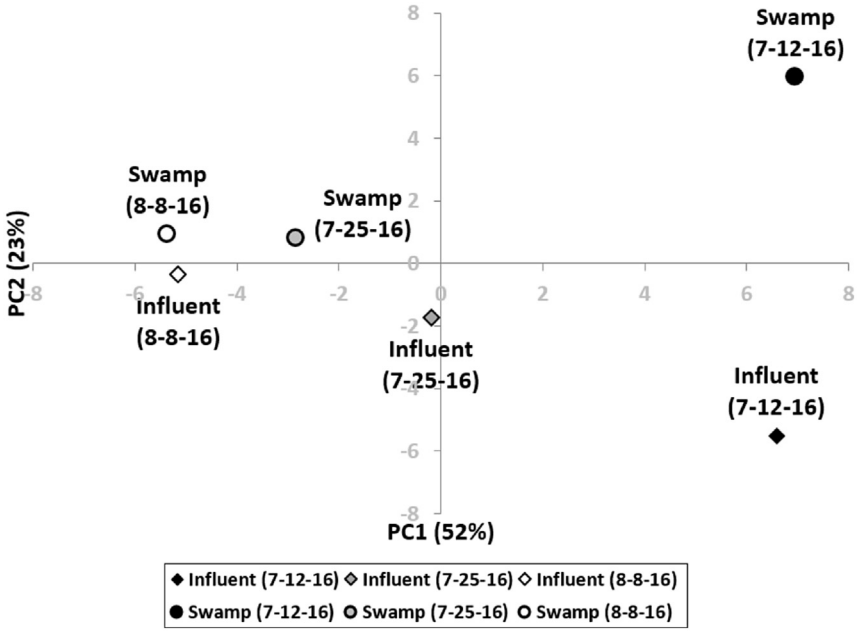

Fig. 5. Principal component analysis plot for the six metagenomic samples. Relative abundance percentages for each viral order of each metagenome were used to compute the principal components.

8th Swamp sample to 5.40% of viral sequences in the July 12th Swamp sample. Table 6 displays the number of hits for each vertebrate virus family.

Among the vertebrate viruses detected in the metagenomic samples, a number were human viruses. Three of the viruses analyzed via qPCR (AdV, EV, and RV) were detected with Illumina sequencing, with RV having positive hits in each of the three Influent samples. Human AdV was detected in both the Influent and Swamp samples from July 12th. Human papillomavirus (HPV) was also detected in the two samples from July 12th, as well as Cacipacore virus, a virus of genus Flavivirus, the genus of Zika virus. Other human viruses detected in the samples include astrovirus, picobirnavirus, circovirus, tanapox virus, Torque teno virus, and one hit for Ebola virus.

When comparing the six samples against one another, more similarity is observed between samples from each respective sampling date compared to samples from each respective location. Fig. 5 displays a PCA plot for the six metagenomic samples. As shown in the PCA plot, the first principal component separates the samples by date, and it is the second principal component that separates the samples by location.

4. Discussion

All four viruses tested were detected in each of the five sampling locations. With the exception of HAV (which had similar concentrations at each location), the tested viruses followed a similar trend across the five locations. The WWTP influent was found to have the highest concentration of any location, with a slight reduction (<2 log) in the WWTP effluent. The virus reduction of <2 logs at the WWTP was lower than the log reductions in prior studies from other locations around the world (Hewitt et al., 2011; Katayama et al., 2008). The low virus reduction may be attributed to virus adsorption onto particles that did not settle in the clarifier and not removed effectively during the clarification process. Furthermore, the samples from the Nakivubo Channel after the WWTP had statistically significant higher concentrations of AdV than the samples from the Nakivubo Channel before the WWTP. This suggests that the WWTP effluent is releasing viruses back into the surface waters surrounding the WWTP.

Concentrations were typically further reduced in the samples from the Nakivubo Swamp. Natural wetlands such as the Nakivubo Swamp are capable of reducing viruses in wastewater through exposure to sunlight, microbial interactions, and plant uptake (Bosch et al., 2006; Raphael et al., 1985). However, persistence of viruses in the wetland is a challenge causing the soil to behave as a reservoir for viruses (Dowd et al., 1998; Powell et al., 2000; Sobsey et al., 1980).

Effluent from the WWTP is released into the Nakivubo Channel, which empties into the Nakivubo Swamp, ultimately flowing into Lake Victoria, a drinking water source for the area. Therefore, pollution from the WWTP effluent could ultimately affect drinking water quality, hence the necessity for more robust monitoring and improved removal of human pathogenic viruses in the wastewater treatment process.

The large majority of sequences from the metagenomic samples were unaffiliated with any known viral genome, which is consistent with prior studies using these methods that also found significant proportions of unaffiliated sequences (Aw et al., 2014; Bibby and Peccia, 2013; Cantalupo et al., 2011; O'Brien et al., 2017; Tamaki et al., 2012). Among the sequences affiliated with viral genomes, higher proportions were affiliated with vertebrate hosts compared to prior studies from other locations (Bibby and Peccia, 2013; O'Brien et al., 2017; Tamaki et al., 2012). This could suggest that there is a higher viral disease burden to humans in Kampala compared to other more developed countries around the world, heightening the importance for implementation of effective wastewater treatment techniques.

AdV, EV, and RV were all among the human viruses detected in the metagenomic samples, in addition to several others that were not investigated via qPCR, including astrovirus, papillomavirus, and even Ebola virus. The fact that other human viruses were assigned approximately the same number of BLAST hits as those viruses investigated via qPCR indicates that the practice of metagenomic methods for diversity analysis can be useful to detect other viruses that may pose a health risk to humans. It is important to note,

however, that molecular detection methods such as qPCR and BLAST annotation do not offer information regarding viral infectivity.

Results from both qPCR and metagenomic analyses indicate that concentrations and diversity of viruses in wastewater have temporal variation. AdV was shown to have statistically significant differences in concentration from one week to the next at each sampling location, and the other three viruses tested via qPCR were detected in some sampling events but not in others. Results from metagenomic analyses also support the notion that the viral community varies temporally, as samples from different locations during the same sampling event were more similar to one another than samples from the same location during different sampling events as shown by PCA, though it should be acknowledged that PCA was performed with a small sample size of only six samples and therefore the conclusions we can draw from this analysis are limited.

These temporal changes indicate that wastewater surveillance can be used as an epidemiological tool to identify and predict disease outbreaks at a population level. The wastewater-based epidemiology methodology is founded upon the idea that concentrations of a human excretion product in wastewater can be hind-cast to an initial source concentration. The application of this methodology to viral disease outbreaks is therefore predicated on detecting significant fluctuations over a short timeframe in viral concentration in wastewater, as this could indicate a potential disease outbreak due to the detected virus. This study shows that such significant weekly fluctuations are indeed detectable in wastewater, establishing the viability of the practice of this methodology.

Several steps, though, must be taken in order to implement this methodology. First, accurate baseline concentrations in wastewater for viruses of interest must be established with replicated samples ($N > 3$). This requires regular sampling and quantification via qPCR performed throughout the year to account for seasonal variations. In order to control for variations in serviced population, biomarkers in wastewater should also be quantified. A study of several biomarkers determined 5-HIAA to be a viable biomarker in wastewater for population estimation (Chen et al., 2014). Once these data are obtained, the detected concentrations could be compared to clinical data from the surrounding area to determine whether there is a correlation between fluctuations in viral concentration in wastewater and an increase in reported cases of viral human disease. Were a correlation to be established, wastewater surveillance would then be an invaluable tool in predicting and identifying viral disease outbreaks.

5. Conclusions

This study established the prevalence and concentrations of four waterborne viruses, adenovirus, enterovirus, rotavirus, and hepatitis A virus, in wastewater and surrounding surface waters in Kampala, the capital of Uganda. Additionally, overall and vertebrate viral diversity was assessed. The study provided preliminary data showing that continuous monitoring of wastewater for viral concentration and diversity can indicate temporal variations that may correlate with changing levels of disease at a population level. These results may be useful in the application of wastewater surveillance as an epidemiological tool to better monitor the disease burden of the serviced population and provide indication of early detection of potential viral outbreaks. Further investigation is necessary to establish more statistically robust baseline viral concentrations in these water bodies and to correlate viral concentrations with clinical data to fully implement this methodology.

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References

Andrews, S., 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

Aw, T.G., Howe, A., Rose, J.B., 2014. Metagenomic approaches for direct and cell culture evaluation of the virological quality of wastewater. J. Virol. Meth. 210, 15–21. https://doi.org/10.1016/j.jviromet.2014.09.017.

Bibby, K., Peccia, J., 2013. Identification of viral pathogen diversity in sewage sludge by metagenome analysis. Environ. Sci. Technol. 47, 1945–1951. https://doi.org/10.1021/es305181x.

Bibby, K., Viau, E., Peccia, J., 2011. Viral metagenome analysis to guide human pathogen monitoring in environmental samples. Lett. Appl. Microbiol. 52, 386–392. https://doi.org/10.1111/j.1472-765X.2011.03014.x.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. https://doi.org/10.1093/bioinformatics/btu170 btu170.

Bosch, A., Guix, S., Sano, D., Pintó, R.M., 2008. New tools for the study and direct surveillance of viral pathogens in water. Curr. Opin. Biotechnol. Energy Biotechnol. Environ. Biotechnol. 19, 295–301. https://doi.org/10.1016/j.copbio.2008.04.006.

Bosch, A., Pinto, R.M., Abad, F.X., 2006. Survival and transport of enteric viruses in the environment. Viruses Foods 151–187.

Bwogi, J., Malamba, S., Kigozi, B., Namuwulya, P., Tushabe, P., Kiguli, S., Byarugaba, D.K., Desselberger, U., Iturriza-Gomara, M., Karamagi, C., 2016. The epidemiology of rotavirus disease in under-five-year-old children hospitalized with acute diarrhea in central Uganda, 2012-2013. Arch. Virol. 161, 999–1003.

Cantalupo, P.G., Calgua, B., Zhao, G., Hundesa, A., Wier, A.D., Katz, J.P., Grabe, M., Hendrix, R.W., Girones, R., Wang, D., Pipas, J.M., 2011. Raw sewage harbors diverse viral populations. mBio 2. https://doi.org/10.1128/mBio.00180-11 e00180–11.

Chen, C., Kostakis, C., Gerber, J.P., Tscharke, B.J., Irvine, R.J., White, J.M., 2014. Towards finding a population biomarker for wastewater epidemiology studies. Sci. Total Environ. 487, 621–628. https://doi.org/10.1016/j.scitotenv.2013.11.075.

Chung, J.W., Foppen, J.W., Lens, P.N.L., 2013. Development of low cost two-step reverse transcription-quantitative polymerase chain reaction assays for rotavirus detection in foul surface water drains. Food Environ. Virol. 5, 126–133.

Daughton, C.G., Jones-Lepp, T.L., 2001. Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues. American Chemical Society, Washington, DC.

Dierssen, U., Rehren, F., Henke-Gendo, C., Harste, G., Heim, A., 2008. Rapid routine detection of enterovirus RNA in cerebrospinal fluid by a one-step real-time RT-PCR assay. J. Clin. Virol. 42, 58–64. https://doi.org/10.1016/j.jcv.2007.11.016.

Dowd, S.E., Pillai, S.D., Wang, S., Corapcioglu, M.Y., 1998. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. Appl. Environ. Microbiol. 64, 405–410.

Fuhrimann, S., Stalder, M., Winkler, M.S., Niwagaba, C.B., Babu, M., Masaba, G., Kabatereine, N.B., Halage, A.A., Schneeberger, P.H., Utzinger, J., others, 2015. Microbial and chemical contamination of water, sediment and soil in the Nakivubo wetland area in Kampala, Uganda. Environ. Monit. Assess. 187, 475.

Gleick, P.H., 2002. Dirty-water: Estimated Deaths from Water-related Diseases 2000-2020 (Citeseer).

Haruna, R., Ejobi, F., Kabagambe, E.K., 2005. The quality of water from protected springs in Katwe and Kisenyi parishes, Kampala city, Uganda. Afr. Health Sci. 5, 14–20.

Hewitt, J., Leonard, M., Greening, G.E., Lewis, G.D., 2011. Influence of wastewater treatment process and the population size on human virus profiles in wastewater. Water Res. 45, 6267–6276. https://doi.org/10.1016/j.watres.2011.09.029.

Howard, G., Pedley, S., Barrett, M., Nalubega, M., Johal, K., 2003. Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda. Water Res. 37, 3421–3429. https://doi.org/10.1016/S0043-1354(03)00235-5.

Ikner, L.A., Gerba, C.P., Bright, K.R., 2012. Concentration and recovery of viruses from water: a comprehensive review. Food Environ. Virol. 4, 41–67. https://doi.org/10.1007/s12560-012-9080-2.

Isunju, J.B., Schwartz, K., Schouten, M.A., Johnson, W.P., van Dijk, M.P., 2011. Socio-economic aspects of improved sanitation in slums: a review. Public Health 125, 368–376. https://doi.org/10.1016/j.puhe.2011.03.008.

Jothikumar, N., Cromeans, T.L., Sobsey, M.D., Robertson, B.H., 2005. Development and evaluation of a broadly reactive TaqMan assay for rapid detection of hepatitis a virus. Appl. Environ. Microbiol. 71, 3359–3363. https://doi.org/10.1128/

AEM.71.6.3359-3363.2005.
Katayama, H., Haramoto, E., Oguma, K., Yamashita, H., Tajima, A., Nakajima, H., Ohgaki, S., 2008. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. Water Res. 42, 1441–1448. https://doi.org/10.1016/j.watres.2007.10.029.
Katukiza, A.Y., Temanu, H., Chung, J.W., Foppen, J.W.A., Lens, P.N.L., 2013. Genomic copy concentrations of selected waterborne viruses in a slum environment in Kampala, Uganda. J. Water Health 11, 358–370.
Kiulia, N.M., Netshikweta, R., Page, N.A., Van Zyl, W.B., Kiraithe, M.M., Nyachieo, A., Mwenda, J.M., Taylor, M.B., 2010. The detection of enteric viruses in selected urban and rural river water and sewage in Kenya, with special reference to rotaviruses. J. Appl. Microbiol. 109, 818–828. https://doi.org/10.1111/j.1365-2672.2010.04710.x.
Kulabako, N.R., Nalubega, M., Thunvik, R., 2007. Study of the impact of land use and hydrogeological settings on the shallow groundwater quality in a peri-urban area of Kampala, Uganda. Sci. Total Environ. 381, 180–199. https://doi.org/10.1016/j.scitotenv.2007.03.035.
Kulabako, R.N., Nalubega, M., Wozei, E., Thunvik, R., 2010. Environmental health practices, constraints and possible interventions in peri-urban settlements in developing countries – A review of Kampala, Uganda. Int. J. Environ. Health Res. 20, 231–257. https://doi.org/10.1080/09603120903545745.
Kuo, D.H.-W., Simmons, F.J., Blair, S., Hart, E., Rose, J.B., Xagoraraki, I., 2010. Assessment of human adenovirus removal in a full-scale membrane bioreactor treating municipal wastewater. Water Res. 44, 1520–1530. https://doi.org/10.1016/j.watres.2009.10.039.
La Rosa, G., Pourshaban, M., Iaconelli, M., Muscillo, M., 2010. Quantitative real-time PCR of enteric viruses in influent and effluent samples from wastewater treatment plants in Italy. Ann. DellIstituto Super. Sanità 46, 266–273. https://doi.org/10.4415/ANN_10_03_07.
Lago, P.M., Gary, H.E., Pérez, L.S., Cáceres, V., Olivera, J.B., Puentes, R.P., Corredor, M.B., Jímenez, P., Pallansch, M.A., Cruz, R.G., 2003. Poliovirus detection in wastewater and stools following an immunization campaign in Havana, Cuba. Int. J. Epidemiol. 32, 772–777. https://doi.org/10.1093/ije/dyg185.
Liu, L., Oza, S., Hogan, D., Perin, J., Rudan, I., Lawn, J.E., Cousens, S., Mathers, C., Black, R.E., 2015. Global, regional, and national causes of child mortality in 2000–13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet 385, 430–440. https://doi.org/10.1016/S0140-6736(14)61698-6.
MacDonald, M., 2004. Sanitation Strategy & Master Plan for Kampala City. Volume.
Martin-Latil, S., Hennechart-Collette, C., Guillier, L., Perelle, S., 2012. Duplex RT-qPCR for the detection of hepatitis E virus in water, using a process control. Int. J. Food Microbiol. 157, 167–173. https://doi.org/10.1016/j.ijfoodmicro.2012.05.001.
Mathers, C.D., Loncar, D., 2006. Projections of global mortality and burden of disease from 2002 to 2030. PLOS Med. 3, e442. https://doi.org/10.1371/journal.pmed.0030442.
Maunula, L., Klemola, P., Kauppinen, A., Söderberg, K., Nguyen, T., Pitkänen, T., Kaijalainen, S., Simonen, M.L., Miettinen, I.T., Lappalainen, M., Laine, J., Vuento, R., Kuusi, M., Roivainen, M., 2008. Enteric viruses in a large waterborne outbreak of acute gastroenteritis in Finland. Food Environ. Virol. 1, 31–36. https://doi.org/10.1007/s12560-008-9004-3.
Muyodi, F.J., Hecky, R.E., Kitamirike, J.M., Odong, R., 2009. Trends in health risks from water-related diseases and cyanotoxins in Ugandan portion of Lake Victoria basin. Lakes Reserv. Res. Manag. 14, 247–257. https://doi.org/10.1111/j.1440-1770.2009.00407.x.
Mwenda, J.M., Ntoto, K.M., Abebe, A., Enweronu-Laryea, C., Amina, I., Mchomvu, J., Kisakye, A., Mpabalwani, E.M., Pazvakavambwa, I., Armah, G.E., Seheri, L.M., Kiulia, N.M., Page, N., Widdowson, M.-A., Duncan Steele, A., 2010. Burden and epidemiology of rotavirus diarrhea in selected african countries: preliminary results from the african rotavirus surveillance network. J. Infect. Dis. 202, S5–S11. https://doi.org/10.1086/653557.
Nakawesi, J.S., Wobudeya, E., Ndeezi, G., Mworozi, E.A., Tumwine, J.K., 2010. Prevalence and factors associated with rotavirus infection among children admitted with acute diarrhea in Uganda. BMC Pediatr. 10, 69. https://doi.org/10.1186/1471-2431-10-69.
Nsubuga, F.B., Kansiime, F., Okot-Okumu, J., 2004. Pollution of protected springs in relation to high and low density settlements in Kampala—Uganda. Phys. Chem. Earth Parts ABC Water Sci. Technol. 29, 1153–1159. https://doi.org/10.1016/j.pce.2004.09.001 and Policy Convergence and Action by All (A Meeting Point for Action leading to Sustainable Development).
O'Brien, E., Munir, M., Marsh, T., Heran, M., Lesage, G., Tarabara, V.V., Xagoraraki, I., 2017. Diversity of DNA viruses in effluents of membrane bioreactors in traverse city, MI (USA) and La grande motte (France). Water Res. 111, 338–345. https://doi.org/10.1016/j.watres.2017.01.014.
Odiit, A., Mulindwa, A., Nalumansi, E., Mphahlele, M.J., Seheri, L.M., Mwenda, J.M., Kisakye, A., 2014. Rotavirus prevalence and genotypes among children younger than 5 years with acute diarrhea at Mulago National Referral Hospital, Kampala, Uganda. Pediatr. Infect. Dis. J. 33, S41–S44.
Okoh, A.I., Sibanda, T., Gusha, S.S., 2010. Inadequately treated wastewater as a source of human enteric viruses in the environment. Int. J. Environ. Res. Public. Health 7, 2620–2637. https://doi.org/10.3390/ijerph7062620.
Pang, X.L., Lee, B., Boroumand, N., Leblanc, B., Preiksaitis, J.K., Yu Ip, C.C., 2004. Increased detection of rotavirus using a real time reverse transcription-polymerase chain reaction (RT-PCR) assay in stool specimens from children with diarrhea. J. Med. Virol. 72, 496–501. https://doi.org/10.1002/jmv.20009.
Papapetropoulou, M., Vantarakis, A.C., 1998. Detection of adenovirus outbreak at a municipal swimming pool by nested PCR amplification. J. Infect. 36, 101–103. https://doi.org/10.1016/S0163-4453(98)93414-4.
Peng, Y., Leung, H.C.M., Yiu, S.M., Chin, F.Y.L., 2012. IDBA-UD: a de novo assembler for single-cell and metagenomic sequencing data with highly uneven depth. Bioinformatics 28, 1420–1428. https://doi.org/10.1093/bioinformatics/bts174.
Powell, T., Brion, G.M., Jagtoyen, M., Derbyshire, F., 2000. Investigating the effect of carbon shape on virus adsorption. Environ. Sci. Technol. 34, 2779–2783. https://doi.org/10.1021/es991097w.
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.
Raphael, R.A., Sattar, S.A., Springthorpe, V.S., 1985. Long-term survival of human rotavirus in raw and treated river water. Can. J. Microbiol. 31, 124–128. https://doi.org/10.1139/m85-024.
Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C.K., Corrêa, A.A., Souza, D.S.M., Miagostovich, M.P., Simões, C.M.O., Barardi, C.R.M., 2010. Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianopolis, South Brazil. J. Appl. Microbiol. 109, 1979–1987. https://doi.org/10.1111/j.1365-2672.2010.04827.x.
Schvoerer, E., Bonnet, F., Dubois, V., Cazaux, G., Serceau, R., Fleury Hervé, J.A., Lafon, M.-E., 2000. PCR detection of human enteric viruses in bathing areas, waste waters and human stools in southwestern France. Res. Microbiol. 151, 693–701. https://doi.org/10.1016/S0923-2508(00)90132-3.
Simmons, F.J., Kuo, D.H.-W., Xagoraraki, I., 2011. Removal of human enteric viruses by a full-scale membrane bioreactor during municipal wastewater processing. Water Res. 45, 2739–2750. https://doi.org/10.1016/j.watres.2011.02.001.
Sjogren, M.H., Tanno, H., Fay, O., Sileoni, S., Cohen, B.D., Burke, D.S., Feighny, R.J., 1987. Hepatitis a virus in stool during clinical relapse. Ann. Intern. Med. 106, 221–226. https://doi.org/10.7326/0003-4819-106-2-221.
Sobsey, M.D., Dean, C.H., Knuckles, M.E., Wagner, R.A., 1980. Interactions and survival of enteric viruses in soil materials. Appl. Environ. Microbiol. 40, 92–101.
Ssengooba, F.P., White, M., others, 1997. Cholera outbreak kampala-city suburb, Uganda, october 1995. J. Clin. Epidemiol. 50, S19.
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.
Tebbens, R.J.D., Zimmermann, M., Pallansch, M.A., Thompson, K.M., 2017. Insights from a systematic search for information on designs, costs, and effectiveness of poliovirus environmental surveillance systems. Food Environ. Virol. 1–22. https://doi.org/10.1007/s12560-017-9314-4.
Toze, S., 1997. Microbial Pathogens in Wastewater: Literature Review for Urban Water Systems Multi-divisional Research Program. CSIRO Land and Water.
Tumwebaze, I.K., Orach, C.G., Niwagaba, C., Luthi, C., Mosler, H.-J., 2013. Sanitation facilities in Kampala slums, Uganda: users' satisfaction and determinant factors. Int. J. Environ. Health Res. 23, 191–204. https://doi.org/10.1080/09603123.2012.713095.
Tumwine, J.K., Kekitiinwa, A., Nabukeera, N., Akiyoshi, D.E., Rich, S.M., Widmer, G., Feng, X., Tzipori, S., 2003. Cryptosporidium parvum in children with diarrhea in mulago hospital, Kampala, Uganda. Am. J. Trop. Med. Hyg. 68, 710–715.
UBOS, 2014. Census 2014 Final Results | Uganda Bureau of Statistics [WWW Document]. URL. http://www.ubos.org/2016/03/24/census-2014-final-results/ (accessed 5.21.17).
UN-Habitat, 2007. Situation Analysis of Informal Settlements in Kampala.
US EPA, 2006. Prepublication of the Ground Water Rule Federal Register Notice.
US EPA, 2001. Manual of Methods for Virology (Chapter 14).
WHO, 2004. Water Sanitation and Health: Water Related Diseases: Diarrhea.
WHO, UNICEF, 2015. Progress on Sanitation and Drinking-water. World Health Organization.
Xagoraraki, I., Kuo, D.H.-W., Wong, K., Wong, M., Rose, J.B., 2007. Occurrence of human adenoviruses at two recreational beaches of the great lakes. Appl. Environ. Microbiol. 73, 7874–7881. https://doi.org/10.1128/AEM.01239-07.
Xagoraraki, I., Yin, Z., Svambayev, Z., 2014. Fate of viruses in water systems. J. Environ. Eng. 140 https://doi.org/10.1061/(ASCE)EE.1943-7870.0000827, 04014020.
Zaiontz, C., 2013. Principal Component Analysis (PCA) | Real Statistics Using Excel.
Zuccato, E., Chiabrando, C., Castiglioni, S., Calamari, D., Bagnati, R., Schiarea, S., Fanelli, R., 2005. Cocaine in surface waters: a new evidence-based tool to monitor community drug abuse. Environ. Health 4, 14. https://doi.org/10.1186/1476-069X-4-14.