ABUNDANCE OF VIRUSES IN WATERS IN SOUTHWEST KAMPALA, UGANDA

By

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ABSTRACT

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The prevalence of waterborne viral diseases is still a major problem in developing countries like Uganda. However, little is known of the viral pathogens that cause diseases. This research aimed to study the abundance of waterborne adenovirus, rotavirus, enterovirus and hepatitis A virus in the southwest region of Kampala, Uganda. Fifteen samples were collected during the summer of 2016 from five sampling locations that include: Wastewater Treatment Plant (WWTP) both influent and effluent, Nakivubo channel (upstream and downstream of the WWTP) and Nakivubo swamp. The sequence of analytical methods used include: collection and concentration of viruses using Argonide cartridge filters, elution of viruses with buffered beef extract, RNA and DNA extraction, quantification of prevalent viruses using quantitative Polymerase Chain Reaction method (qPCR). The concentrations of the viruses ranged from 5.79×10^{1} to 3.91×10^{7} copies/L. Adenovirus concentrations were relatively high, 5.45×10^{5} to 1.17×10^{7} copies/L at all the sampling locations. High concentrations of adenovirus in these waters may be due to the high persistence of these viruses in the environment. Enteroviruses predominated with a maximum concentration of 3.91×10^{7}copies/L over other viruses of interest. Human exposure to such pathogens through consumption of contaminated water and food can be harmful. These data are paramount for estimating the risk from exposure to microorganisms.

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CHAPTER 1 - MICROBIAL WATER POLLUTION IN KAMPALA, UGANDA (LITERATURE REVIEW)

1.1 Introduction

Kampala, the capital city of Uganda is located 8km north of Lake Victoria and approximately 42 km north of the Equator. The capital city originally situated on seven hills, has now expanded onto 24 hills surrounded by wetland valleys. Kampala has five administrative and political divisions: Central, Makindye, Rubaga, Kawempe and Nakawa (Figure 1-1) and serves as a political, administrative, commercial, and educational center. Due to the functions that the city serves, the population has increased 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 and this has resulted into settlements (slums) which accommodate more than 50% of the city's population (UN Habitat, 2007). The settlements face challenges of poor sanitation and hygiene that have exacerbated over the years as more people move into the city (UN Habitat 2007, Kulabako et al. 2007, Isunju et al. 2011 & Tumwebaze et al.2013). This has resulted into contamination of water sources and consequent outbreaks of diseases such as cholera and typhoid including diarrheal cases among children below the age of 5 years in Kampala (Odiit et al. 2014).

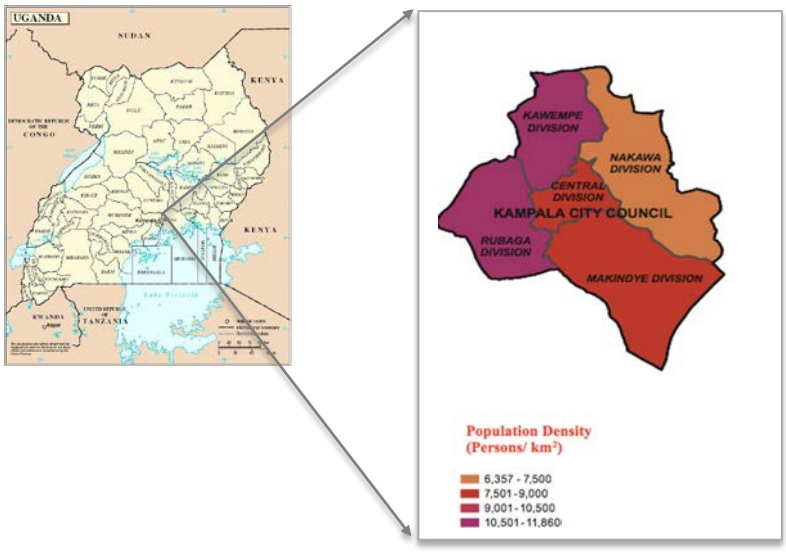

Figure 1-1: Map of Kampala divisions

1.2 Water supply and Sanitation Practices in Kampala

1.2.1 Millennium Development Goals

The World Health Organization (WHO) reports that from 1990 to 2012, 2.3 billion people worldwide had access to improved drinking water sources while 2 billion people gained access to improved sanitation facilities (WHO&UNICEF, 2015). The statistics showed progress on meeting the Millennium Development Goals (MDGs) on drinking water and sanitation facilities targets, however, access to improved sanitation facilities is still lacking especially in countries of Sub-Saharan Africa, South East Asia and South and Central America.

Uganda, like any other developing country still faces challenges in meeting MDGs on improved sanitation. By 2015, Uganda had not met the MDG 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 (VIP) (WHO& UNICEF 2015). However, concerning the MDG on drinking water, Uganda by 2015 had met its target whereby 96% of the urban population had access to improved drinking water sources like protected springs and wells,

piped water.

1.2.2 Drinking Water Sources

In Kampala, approximately 60% of the population access water from standpipes and 30% through protected springs and wells (Kulabako et al., 2010). A small percentage of 10% has private connections due to the high costs of installation and maintenance of such connections. Protected springs and wells are some of the main water sources for urban centers like Kampala, however, studies have shown that more than 80% of the protected springs and wells are contaminated with fecal matter (Byamukama et al., 2000, Haruna et al., 2005, Fuhrimann et al., 2015). This poses a health risk to city dwellers.

1.2.3 Human Excreta Disposal

Traditional pit latrines are the major disposal method of human excreta in Kampala (75%) , 10% use ventilated improved pit latrines (VIP) 10% use flush toilets and 5% practice open defecation (NWSC 2004 ,Okot-Okumu & Oosterveer, 2010). The lack of space to construct the latrines and low-income levels forces people to share pit latrines (Kwiringira, Atekyereza, Niwagaba, & Gunther, 2014). This presents a challenge and puts the population at risk of experiencing diseases due to poor hygiene and sanitation since the owners undertake the maintenance of the shared toilets.

1.2.4 Wastewater Management

With approximately 10% of the urban population connected to the sewer system (NWSC, 2004), most of the residents in Kampala dispose of the 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 60% of the city's population that resides in

slum 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 most of the residents dispose the greywater in open drains (37%) whereas 23% use both the open drains and open space. The rest of the population in the area also disposes the greywater in open space (23%), soak pits (3%). Poor disposal of wastewater affects the aesthetics of the surrounding. The wastewater consequently finds its way into surface water bodies and hence comprising the water quality of such water sources.

1.3 Water Quality Studies in Kampala

Studies conducted in Kampala have revealed high contamination of water sources like Lake Victoria, wells and protected springs (Table 1-1), with bacteriological characteristics being the major focus while the viral characteristics of the water less concentrated on. The concentrations of bacteria in water exceed both Uganda National Bureau of Standards (UNBS) levels and WHO standards (0 CFU /100 ml).

Lake Victoria, the main water supply to Kampala and surrounding areas has been subjected to high levels of pollution arising from poor sanitation practices at its shores. A study conducted by Muyodi et al., 2009 revealed high levels of water contamination at 10 sampling sites that include lakeshores, boreholes and shallow wells around Lake Victoria. The concentration of total coliforms ranged from 110-20.05×10^3 CFU/100mL and for fecal coliforms ranged from 3-2.88×10^3 CFU/100mL. The water quality parameters were correlated with prevalence of waterborne diseases such as cholera, dysentery and typhoid. The occurrence of the water borne diseases was higher in the wet season than in the dry season due to the deposition of fecal matter into the shallow wells and lakeshores.

A study conducted by Howard et al., 2003 to determine the variation of water quality in shallow protected springs in Kampala revealed a strong correlation between short-term rainfall events and degree of contamination of the springs. 25 shallow protected springs in both high and low density settlements were monitored for thermotolerant coliforms and fecal streptococci over a period of 12 months, April 1998 to April 1999. Thermotolerant coliforms and fecal streptococci were enumerated using membrane filtration, with growth on lauryl sulphate broth and enterococcus agar at 44^{o}C respectively. Results showed that most of the protected springs were contaminated with fecal matter, since the values for the indicator coliforms varied between < 1 to 23×10^{3} CFU/100ml, values that are above the WHO standards of 0 CFU/100ml. The median number of coliforms displayed a strong relationship with rainfall events of 24 hour and 48 hour periods compared to the monthly rainfall events. This could be attributed to the "first flush effect ", where rapid changes in water quality occurred after early rains. The contamination of the protected springs poses a health risk to the 60% of the population in the city that depend on springs for domestic water.

Kulabako et al., 2007 studied the quality of the shallow groundwater in Bwaise III, a slum area in Kampala. Samples from 9 monitoring wells and the protected spring were analyzed for thermotolerant coliforms and fecal streptococci for 19 months during the wet and dry seasons of Kampala in 2003. The membrane filtration technique with lauryl sulphate broth and enterococcus agar analyzed thermotolerant and fecal streptococci respectively. High levels of fecal contamination were observed in the groundwater with median values of 126×10^{3} CFU /100ml and 154×10^{3} CFU/100ml obtained for thermotolerant coliforms and fecal streptococci respectively. The microbial contamination of the groundwater was due to poor waste disposal in the area, poorly maintained and protected springs and hydrogeological characteristics of Bwaise

III such as high permeability of the soil and high water table.

Contamination of protected springs remains a challenge in both low and high-density settlements of Kampala City as studied by Nsubuga et al., 2004. Eight protected springs in Makindye and Kawempe divisions were observed for fecal coliforms and fecal streptococci, once for five successive weeks in the dry and wet seasons. Fecal coliforms and fecal streptococci were isolated and counted using membrane filtration, with lauryl sulphate broth and enterococcus agar as test media at 44^{o}C respectively. Protected springs in both the low and high density areas had high numbers of fecal coliforms and fecal streptococci bacteria, with values ranging from 3-18.3×10^3 CFU/100ml and 2-2.76×10^3 CFU/100ml respectively. The protected springs in high-density settlements were more polluted than those in the low-density settlements both in the dry and wet season. The highest number of coliforms recorded was 18.3×10^3 CFU/100ml in high-density settlements and 382 CFU/100ml for the low-density settlements. The levels of coliforms in the water were above both the Uganda National Bureau of Standards (UNBS) levels and WHO standard of 0 coliforms in every 100ml water sample. The presence of coliforms in water serves as indicator of fecal contamination.

Slum areas like Kisenyi and Katwe face similar water quality issues like areas such as Bwaise, in Kampala Uganda. Haruna et al., 2005 observed high levels of water contamination in 10 springs of Kisenyi and Katwe areas. Sampling was carried out biweekly from December 2001-March 2002 and the water samples were analyzed for total coliforms, fecal coliforms and fecal streptococci using the membrane filtration method. The median values were as high as 7.9×10^3 CFU/100ml for total coliforms, 4.050×10^3 CFU/100ml for fecal coliforms and 1.130×10^3 CFU/100 ml for fecal streptococci. Water contamination poses health risks to populations in such areas that depend on springs for domestic water.

Fuhrimann et al., 2015 studied the microbial contamination of water in the Nakivubo wetland area in Kampala. Twenty-three sites at Nakivubo channel, Nakivubo wetland, community areas and at the shore of Lake Victoria were sampled for thermotolerant coliforms (TTC), *Escherichia coli* (E.coli) and *Salmonella* spp. Water samples were taken from the sites for 8 weeks during the rainy season of October- December 2013. TTC and E.coli were detected using the membrane filtration, with membranes incubated on lauryl sulphate broth at 44 ^{o}C and 37^{o}C respectively. *Salmonella spp* were analyzed using the membrane filtration method, with membranes incubated on xylose lysine deoxycholate agar at 37^{o}C . The analyses showed that TTC, E.coli and *Salmonella spp* were present in the water at all the sampling locations. The mean concentrations of TTC, E.coli and *Salmonella spp* ranged from 3.7 to 4.3×10^{6}, 1.3 to 9.9×10^{4} and 1.3 to 3.8×10^{2} CFU/100ml respectively. High concentrations of the indicator bacteria were generally detected in Nakivubo channel and wetland and the lowest concentrations were detected at the shores of Lake Victoria. The water at all sampling sites was contaminated with fecal matter as indicated by the concentrations of the bacteria in the water.

A study conducted by Katukiza et al., 2013 in the slum areas of Bwaise III concluded that viral water contamination poses too a health risk to the surrounding communities. The viruses were concentrated using the glass wool method and detected using Real time quantitative polymerase chain reaction (qPCR) for DNA viruses. Rotavirus concentration ranged from 7.36×10^{-1} to 1.87×10^{2} copies/mL with the maximum concentration obtained from surface water. Human Adenovirus F and G were found to be prevalent in the water with the maximum concentration of 7.62×10^{-3} copies/mL. The water samples tested negative for Hepatitis E virus while few samples (15.4-27.2%) tested positive for Hepatitis A (7.4×10^{-1}-6.87×10^{-1} copies/mL). The surface water was more polluted than the grey and ground water. This could be attributed to

the poor solid waste, human excreta and wastewater management practices in slums such as Bwaise III. Diarrheal diseases caused by rotavirus are the major killers of children below the age of five.

Similarly, Chung et al., 2013 observed relatively high concentrations of rotavirus in grey water and surface water in Bwaise III. The rotavirus concentrations varied between 2.22 to 2.91 $TCID_{50}$/ml.

Table 1-1: Water quality studies in Kampala

Study no.	Water Source	Overall Results	Reference
1	Lake Victoria	TTC 110- 20.05× 10^3 CFU/100ml FC 3-2.88× 10^3 CFU/100ml	Muyodi et al.,2009
2	Protected Springs	TTC & FS <1-23× 10^3 CFU/100ml	Howard et al., 2003
3	Protected Springs	TTC <0-10× 10^3 CFU/ 100ml FS <0-8.3× 10^3 CFU/100ml	Kulabako et al., 2007
4	Wells	TTC <0-162× 10^6 CFU/ 100ml FS <0-176×10^6 CFU/100ml	
5	Protected Springs	FC 3-18.3× 10^3 CFU/100ml FS 2-2.76× 10^3 CFU/100ml	Nsubuga et al., 2004
6	Protected Springs	FC 1-60.8× 10^3 CFU/100ml FS 0- 3×10^3 CFU/100ml	Haruna et al., 2005
7	Wetland (wastewater)	TTC 4.0× 10^2 - 2.2× 10^8CFU/100ml, EC 1.0× 10^2 -7.9× 10^3 CFU/100ml	Fuhrimann et al.,2014
8	Drainage Channel (wastewater)	TTC 1.2× 10^3-1.8× 10^8 CFU/100ml, EC 8.4× 10^2 -9.0× 10^7CFU/100ml	Fuhrimann et al., 2014
9	Unprotected spring	7.62× 10^{-3} copies/mL	Katukiza et al.2014
	Open grey water tertiary drains	HAdV 1.35× 10^{-1} copies/mL , RV 1.45-3.32× 10^{-1} copies/mL , HAV 7.4×10^{-1} - 6.87× 10^{-1} copies/mL	
	Open storm water drainage channel	HAdV 1.53-5.32× 10^{-2} copies /mL , RV 7.36× 10^{-1} -1.87× 10^2 copies /mL	
10	Grey water & surface water	RV 2.22- 2.91 $TCID_{50}$/ml.	Chung et al.2013

TTC- Thermotolerant Coliforms, FC-Fecal Coliforms, FS- Fecal Streptococci, EC- *Escherichia coli*, HAdV- Human Adenovirus, RV- Rotavirus, HAV- Hepatitis A

It is evident that water supplies in Kampala are highly contaminated with fecal matter. Consumption of contaminated water poses a health risk to the city population. Therefore, preventive measures like improved sanitation practices and adequate treatment of drinking water are paramount in preventing the outbreak of waterborne diseases.

1.4 Waterborne Disease Burden

Kampala city has experienced diseases such cholera, typhoid, dysentery, rotavirus diarrhea and cryptosporidiosis over the years (Table 1-2). The outbreak or occurrence of such diseases is mainly attributed to unsafe water supplies, poor hygiene and sanitation practices.

1.4.1 Rotavirus Diarrhea

Children under the age of 5 mainly suffer from diarrhea which accounts for 1 in 9 child deaths (CDC). Rotavirus is the leading cause of acute diarrhea and causes about 40% of hospitalizations in children under five. Most diarrheal associated diseases are attributed to unsafe drinking water, insufficient hygiene and inadequate sanitation (Bwogi et al., 2016). In Kampala, Rotavirus diarrheal cases have been reported at hospitals such as Mulago Hospital in periods between July 2006-June 2008 where 39% of the stool samples of children under the age of 5 tested positive for rotavirus (Mwenda et al., 2010). Odiit et al., 2014 conducted a study at Mulago hospital between July 2008-December 2012 which also revealed a high prevalence of acute rotavirus diarrhea among children <5 years. Also, 37% of children under the age of five at four hospitals in central Uganda had severe diarrhea (Bwogi et al., 2016). The three studies attributed the diarrhea outbreaks to the presence of rotavirus in stool specimens.

1.4.2 Cholera

Cholera, another common waterborne disease caused by bacteria *Vibrio cholera*e affected residents of Namuwongo, an informal settlement located in Makindye division in 1995. Six deaths were reported during the outbreak though other cases went unreported (Sengooba et.al 1995). The outbreak was attributed to poor sanitation practices like open defecation and consumption of unboiled spring water. Other slum areas like Bwaise III in Kawempe Division have also been affected by cholera. 23 cases were reported at Mulago hospital between October

2007-March 2008 (Kulabako et al., 2010).

1.4.3 Dysentery

Dysentery is caused by a number of parasites such as viruses, bacteria and protozoa. Symptoms such as bloody diarrheal stools, abdominal pains manifest in the patients and if left untreated may lead to death. Dysentery also remains a major threat in slums of Bwaise III, 42 and 109 cases were reported at Kawempe Health Centre and Mulago hospital respectively between October 2007-March 2008 (Kulabako et al., 2010).

1.4.4 Cryptosporidiosis

Cryptosporidiosis is a parasitic disease of the mammalian intestinal tract caused by the protozoa, *Cryptosporidium parvum* and is mainly transmitted through fecal-oral route in humans. Studies conducted by Tumwine et al., 2003 at Mulago hospital between November 1999-Januray 2001 revealed that 2,446 children below the age of five tested positive for *Cryptosporidium parvum*, of whom 72.7% had acute diarrhea and 191 deaths were also reported during the study. The prevalence of such a disease in children often leads to death if left untreated.

Table 1-2: Waterborne disease burden in Kampala

Disease	Period of prevalence	No.of cases/ Deaths reported	References
Rotavirus Diarrhea	Jul 2006-June 2008	615 cases	Mwenda et al.2010
	Jul 2006- Dec2012	1844 cases	Odiit et al.2014
	Sept 2012- 2013	263 cases	Bwogi et al. 2016
Cholera	Oct 1995	6 deaths 512 (12% diarrhea)	Ssengooba et al. 1995
	Oct 2007-Mar 2008	23 cases	Kulabako et al. 2010
Dysentery	Oct 2007-Mar 2008	151 cases	Kulabako et al. 2010
Cryptosporidiosis	Nov 1999-Jan2001	191 deaths 2,446 cases (72.7% diarrhea)	Tumwine et al. 2003

Prevention of waterborne diseases can be achieved through improved sanitation and hygiene practices, improved access to safe drinking water supplies and proper storage of drinking water. This calls for behavioral change among the people and enforcement of policies/ law by the Government of Uganda.

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CHAPTER 2 - ABUNDANCE OF VIRUSES IN WATERS IN SOUTHWEST KAMPALA, UGANDA

2.1 Introduction

Adenoviruses, rotaviruses and hepatitis A have been discovered to be prevalent both in drinking water and raw sewage in slum areas of Kampala (Chung et al., 2013, Katukiza et al., 2013). The presence of such waterborne viruses has led to the outbreak of illnesses such as diarrhea and gastro-enteritis with the most vulnerable groups being the elderly and children under the age of five (Odiit et al., 2014, Mwenda et al., 2010). Though the outbreaks of viral waterborne related illnesses are not well documented, a few studies conducted in Kampala have established a correlation between the diarrheal illnesses and occurrence of the viruses in children. A study conducted by Odiit et al. 2014 revealed 32.3% of children under the age of five admitted to Mulago Referral Hospital in Kampala, from July 2006 to December 2012 suffered from diarrhea and vomiting. Mwenda et al., 2010 discovered 39% of the stool samples of children under the age of five tested positive for rotavirus at Mulago Hospital from July 2006 to June 2008. In addition, 37% of children under the age of five at four hospitals in central Uganda had severe diarrhea (Bwogi et al.2016). All three studies attributed the diarrhea outbreaks to the presence of rotavirus in stool specimens.

Many studies conducted in Kampala have focused on the bacteriological characteristics of water (Byamukama et al., 2000, Fuhrimann et al., 2015, Haruna et al., 2005, Howard et al., 2003, Muyodi et al., 2009, Kulabako et al., 2007). A few studies have focused on the viral loading of both water and wastewater (Katukiza et al., 2013, Chung et al., 2013). There was a need to establish more data on the abundance and distribution of viruses in waters in Kampala. This was the goal for this study.

Rotavirus (RV), hepatitis A (HAV), adenovirus (HAdV) and enteroviruses (EV) were chosen for this study as they are the most common viruses in wastewater (Katukiza et al., 2013, Kiulia et al., 2010, Rigotto et al., 2010, Schvoerer et al., 2000, Xagoraraki et al., 2014). Secondly, the waterborne viruses aforementioned cause various diseases in population groups such as the elderly and children with low immunity and there is need for virus quantification to establish the risk associated with their exposure. Rotavirus causes severe diarrhea and is the main cause of mortality in children under the age of 5 not only in Kampala but also throughout the world (WHO 2009, Mwenda et al., 2010, Odiit et al., 2014, O'Ryan et al., 2001). Diseases such as conjunctivitis, gastroenteritis and respiratory diseases are attributed to consumption of food and water contaminated with HAdV B, C and F (Zou et al., 2012, van Heerden et al., 2005).

Several techniques have been used to detect viruses in environmental samples; cell culture method, plaque assays and qPCR (Xagoraraki et al., 2014). However, the cell culture method traditionally used to detect viruses lacks sensitivity, inability to characterize different viruses and takes a longer time to produce desired results (Lee-Montiel et al., 2011, Moore and Margolin, 1993). QPCR is a rapid, sensitive and reliable technique for quantifying viruses and it is efficient at analyzing low concentrations (Martin-Latil et al., 2012, La Rosa et al., 2010). The amplification process produces results in real time. In this study, we used qPCR method to quantify waterborne viruses via the DNA and RNA extracted from the environmental samples obtained from the Bugolobi wastewater treatment plant (WWTP), Nakivubo channel and Nakivubo swamp. The goal of this study was to establish the abundance of waterborne viruses at the sampling sites.

2.2 Materials and Methods

2.2.1 Sample Collection

A total number of 15 samples from five sampling locations were collected between July 7 and August 8, 2016. Samples were collected biweekly from five locations in Southwest Kampala at a depth of < 1m; Bugolobi Wastewater Treatment Plant (WWTP) both influent and effluent, Nakivubo channel (upstream and downstream of the WWTP) and Nakivubo swamp (Figure 2-1). Virus samples were collected with Argonide cartridges based on EPA's virus adsorption-elution (VIRADEL) method. The filter apparatus was sterilized with sodium hypochlorite and dechlorinated with sodium thiosulphate (2%) to avoid cross contamination and keep the viruses alive at sampling locations. Argonide cartridges were stored on dry ice and transported overnight to MSU laboratory for further processing.

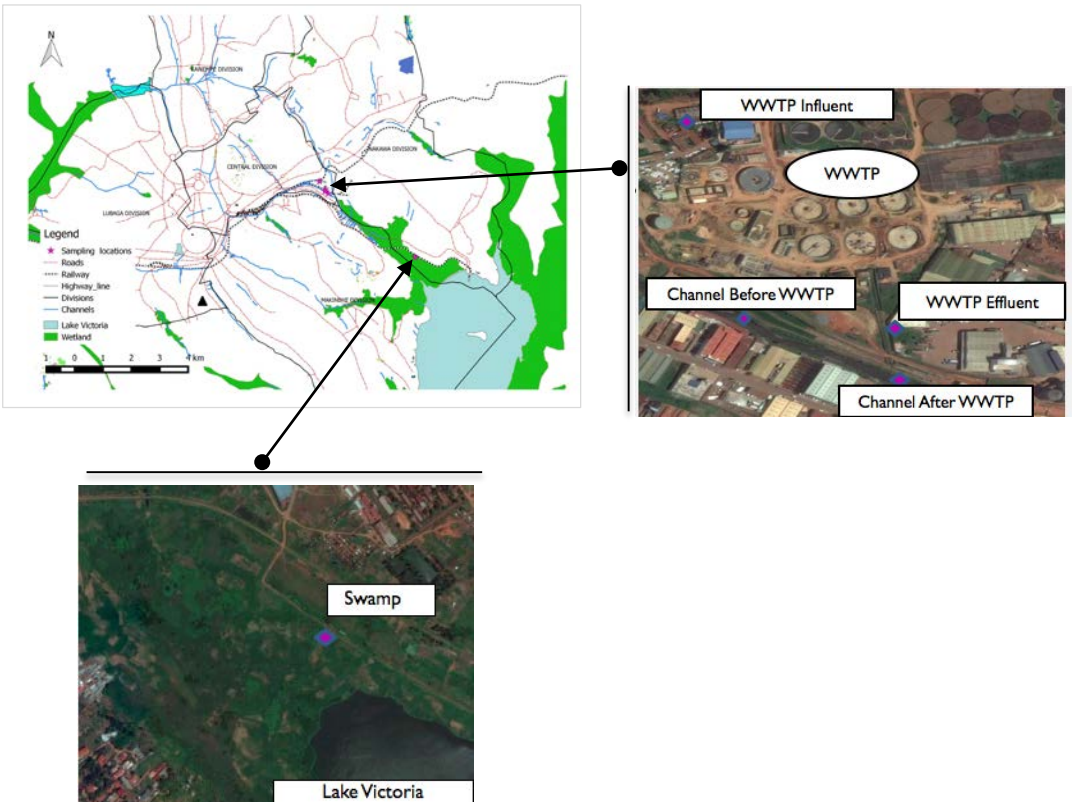


Figure 2-1: Sampling locations in Kampala

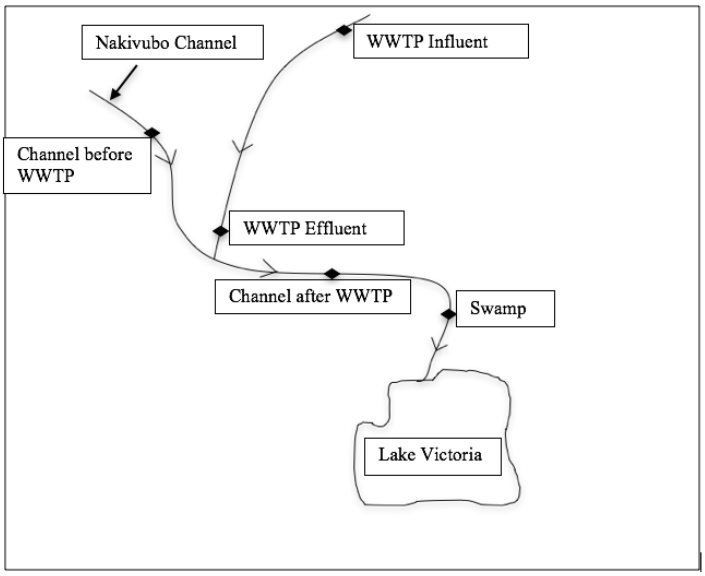


Figure 2-2: Water flow direction across sampling locations

2.2.2 Sample Processing

Sample processing entailed three steps: elution of Argonide cartridge filters, flocculation and microbial removal.

2.2.2.1 Elution of Argonide Cartridge Filters

Sections of braided tubing from the pressure vessel were attached to the inlet of the cartridge housing and the inlet port of the pressure vessel was connected to a positive air pressure source. 1000 mL of prepared beef extract was added into the pressure vessel. The Argonide filter was placed into the cartridge house, and closed tightly and a 2 L beaker was placed under spout of cartridge house to collect the filtrate. The air pressure was turned on and off as soon as the beef extract solution begun to flow until the beef extract filled the cartridge house. The beef extract made contact with the Argonide filter for 1 minute and the air pressure was turned on again to let beef extract exit the cartridge house into the 2L beaker. The filter was then removed

from the cartridge house and residual beef extract was poured into the properly labeled 2 L beaker. This was followed by flocculation.

2.2.2.2 Flocculation

The 2L beaker was placed on a magnetic stirrer and stirred at a sufficient speed to develop a vortex. 1M HCL was added to the beaker until the pH of the beef extract elutes reached 3.5 +/- 0.1. Beef extract elute was stirred slowly for 30 minutes and after 30 minutes, the beaker was taken off the magnetic stirrer and contents were poured into the 500 mL centrifuge bottle to the brim. Bottle was centrifuged at 3,800 rpm for 15 minutes at 4º C and then the supernatant was discarded by carefully and slowly pouring it out in order not to disrupt the line of precipitate. 30 mL of 0.15M Na_2HPO_4 (sodium phosphate) at pH 9.0-9.5 was added to the bottle and contents were swirled until the precipitate dissolved completely. The contents of 500 mL centrifuge tube were poured into each labeled 30mL centrifuge tube and pH readjusted to 9.0-9.5 with 1M NaOH. The centrifuge tube was centrifuged at 7,500 rpm for 10 minutes at 4º C. After 10 minutes, the tubes were removed from centrifuge and the supernatant poured carefully into a new 50 mL centrifuge tube, so as not to disrupt the pellet. The pellet was discarded and the pH of supernatant adjusted to ~ 7.25 with 1M HCL. The supernatant was further processed for microbial removal.

2.2.2.3 Microbial Removal

A 0.45 μm filter was placed onto the tip of a 50 mL syringe and the supernatant from 50 mL centrifuge tube was loaded into a 50 mL syringe. The supernatant was forced through the filter, back into the same 50 mL centrifuge tube. The filter was replaced continually until all the supernatant was forced through the filter and syringe. The used 0.45 μm filter was removed and replaced with a 0.22 μm filter on the syringe. The supernatant was loaded back into the syringe

and forced through the filter into a clean 50 mL centrifuge tube labeled with the proper number. This procedure was repeated until all supernatant was forced through. 2000 µL of sample was then aliquoted from the 50 mL centrifuge tubes, into the cryogenic tubes. The cryogenic tubes were stored into the -80ºC freezer along with the leftover supernatant. DNA extraction followed after the flocculation process.

2.2.3 DNA Extraction

Virus DNA was extracted using QIAamp viral RNA mini kit following the protocol in the manufacturer's manual. 140µL of the eluted viral samples were used in the extraction step and 80 µL of viral RNA was obtained. Reverse transcription was then performed to obtain cDNA using iscript reverse transcription super mix for RT-qPCR (Bio-Rad). Generally, 10 µL of viral RNA samples, 4 µL of iscript RT super mix and 6 µL of water were used to obtain 20µL reaction mix for each RT-qPCR reaction. The processed cDNA samples were stored in a freezer at -20ºC.

2.2.4 Virus Quantification

qPCR was used to quantify four viruses: adenovirus, rotavirus, enterovirus and hepatitis A virus. All qPCR analyses were performed in the light cycler with a different cycling program for each of the viruses (Table 2-1). The primer and probe sequences used during qPCR and RT-qPCR were taken from literature (Table 2-2).

Table 2-1: QPCR cycling conditions used for detection of targeted viruses

Assays	Program	Analysis Mode	Number of Cycle	Target (ºC)	Hold (hh:mm:ss)	Reference
HAdV (40,41)	Denaturation	None	1	95	00:15:00	Xagoraraki et al. 2007
	Amplification	Quantification	45	95	00:00:10	
				60	00:00:30	
				72	00:00:12	
	Cooling	None	1	40	00:00:30	
Hepatitis A						Jothikumar et al. 2005
	Denaturation	None	1	95	00:15:00	
	Amplification	Quantification	45	95	00:00:10	
				55	00:00:20	
				72	00:00:15	
Enteroviruses						Dierseen et al.2007
	Denaturation	Quantification	1	95	00:15:00	
	Amplification		45	95	00:00:15	
				60	00:01:00	
Rotavirus	Denaturation	Quantification	1	95	00:15:00	Pang et al. 2004
	Amplification		445	94	00:00:20	
				60	00:01:00	

Table 2-2: Primers and probes used for detection of targeted viruses

Target Virus	Primer Name	Sequence (5'-3')	Amplicon Size	Reference
HAdV (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	Forward Primer	GGTAGGCTACGGGTGAAAC	89	Jothikumar et al. 2005
	Reverse Primer	AACAACTCACCAATATCCGC		
	Probe	FAM-CTTAGGCTAATACTTCTATGAAGAGATGC-BBQ1		
Enteroviruses	EQ-1	ACATGGTGTGAAGAGTCTATTGAGCT	141	Dierseen et al. 2007
	EQ-2	CCAAAGTAGTCGGTTCCGC		
	EP	FAM-TCCGGCCCCTGAATGCGGCTAAT-TAMRA		
Target Virus	**Primer and Probe**	**Nucleotide sequence (50– 30)**	**Location**	
Rotavirus	Rota NVP3-F	ACCATCTACACATGACCCTC	963–982	Pang et al.2014
	Rota NVP3-R	GGTCACATAACGCCCC	1,034–1,049	
	TagMan probe	ATGAGCACAATAGTTAAAAGCTAACACTGTCAA	984–1016	

2.3 Results

The quantitative PCR revealed that all samples collected from the five sampling locations were polluted with viruses of interest. The concentrations varied across the sampling locations as shown in Table 2-3, Figure 2-3 and Figure 2-4. The number of samples that tested positive for viruses per qPCR runs is shown in Table 2-4.

Table 2-3: Mean genomic copy concentrations of viruses

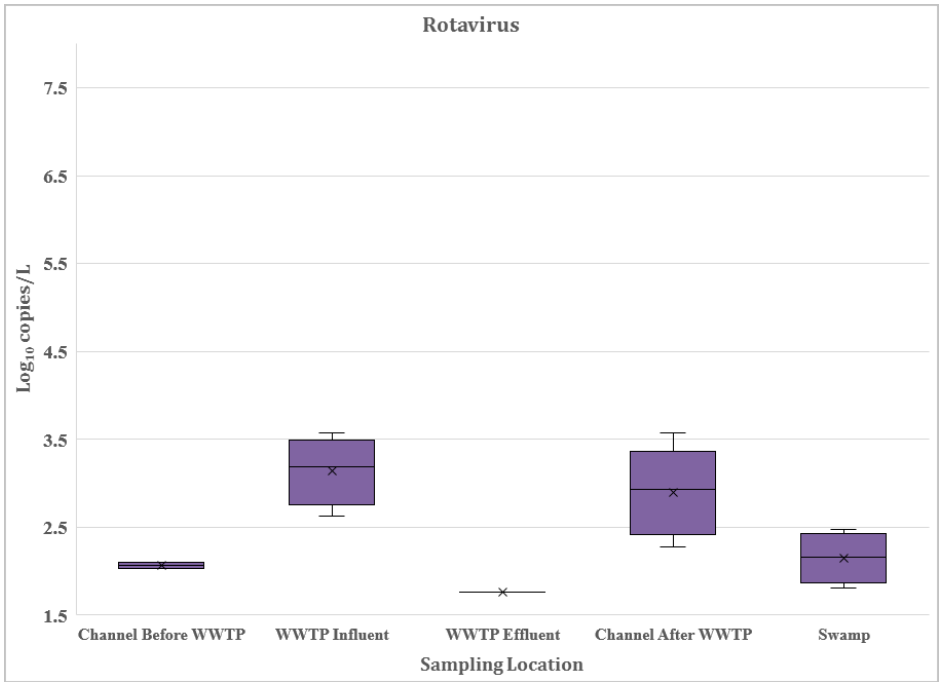
Sampling Locations	RV	HAV	EV	HAdV
Channel Before WWTP	*1.16×10^{2}	7.74×10^{3} ($\pm1.90\times10^{3}$)	3.09×10^{6} ($\pm9.63\times10^{5}$)	5.45×10^{5} ($\pm5.22\times10^{5}$)
WWTP Influent	1.81×10^{3} ($\pm1.37\times10^{3}$)	4.26×10^{3} ($\pm2.93\times10^{3}$)	3.91×10^{7} ($\pm3.94\times10^{7}$)	1.17×10^{7} ($\pm6.30\times10^{6}$)
WWTP Effluent	*5.79×10^{1}	5.79×10^{3} ($\pm3.09\times10^{3}$)	1.42×10^{7} ($\pm1.12\times10^{7}$)	9.43×10^{6} ($\pm7.96\times10^{6}$)
Channel After WWTP	1.31×10^{3} ($\pm1.43\times10^{3}$)	6.12×10^{3} ($\pm4.83\times10^{3}$)	4.15×10^{6} ($\pm2.51\times10^{6}$)	2.08×10^{6} ($\pm9.65\times10^{5}$)
Swamp	1.66×10^{2} ($\pm1.04\times10^{3}$)	2.73×10^{3} ($\pm1.44\times\times10^{3}$)	4.69×10^{6} ($\pm5.85\times10^{6}$)	5.72×10^{5} ($\pm3.56\times10^{5}$)

WWTP - Wastewater Treatment Plant RV-Rotavirus, HAV- Hepatitis A virus, EV- Enterovirus, HAdV- Adenovirus. Mean values of concentrations of samples that tested positive measured in copies/L. In brackets is the standard deviation. * No standard deviation.

Table 2-4: Number of samples that tested positive per qPCR runs

Rotavirus					
Date of Sampling	Channel before WWTP	WWTP Influent	WWTP Effluent	Channel After WWTP	Swamp
07/12/2016	1/4	0/4	0/4	1/4	0/4
07/25/2016	0/4	1/4	1/4	0/6	2/4
08/08/2016	0/6	4/4	0/4	4/4	2/4
Hepatitis A					
07/12/2016	4/4	2/6	1/6	2/6	3/6
07/25/2016	3/6	0/6	2/6	0/4	1/4
08/08/2016	0/4	2/6	3/4	1/6	5/6
Enterovirus					
07/12/2016	2/2	2/2	2/2	2/2	2/2
07/25/2016	2/2	0/4	1/4	2/2	2/2
08/08/2016	2/2	2/2	0/4	0/4	2/2
Adenovirus					
07/12/2016	2/2	2/2	2/2	2/2	2/2
07/25/2016	2/2	2/2	2/2	2/2	2/2
08/08/2016	2/2	2/2	2/2	2/2	2/2

a)



b)

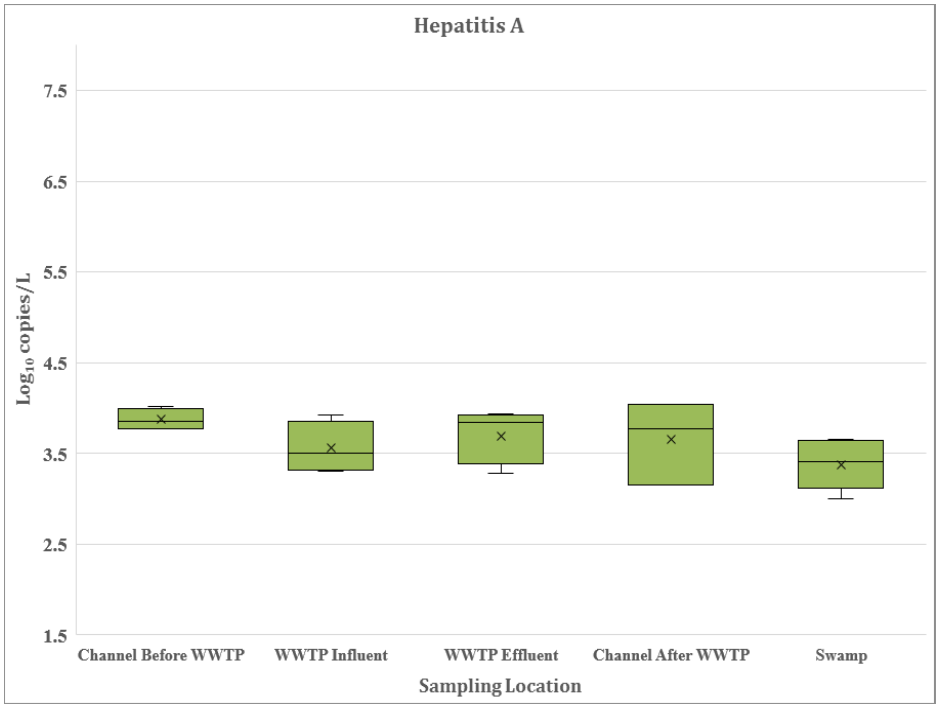
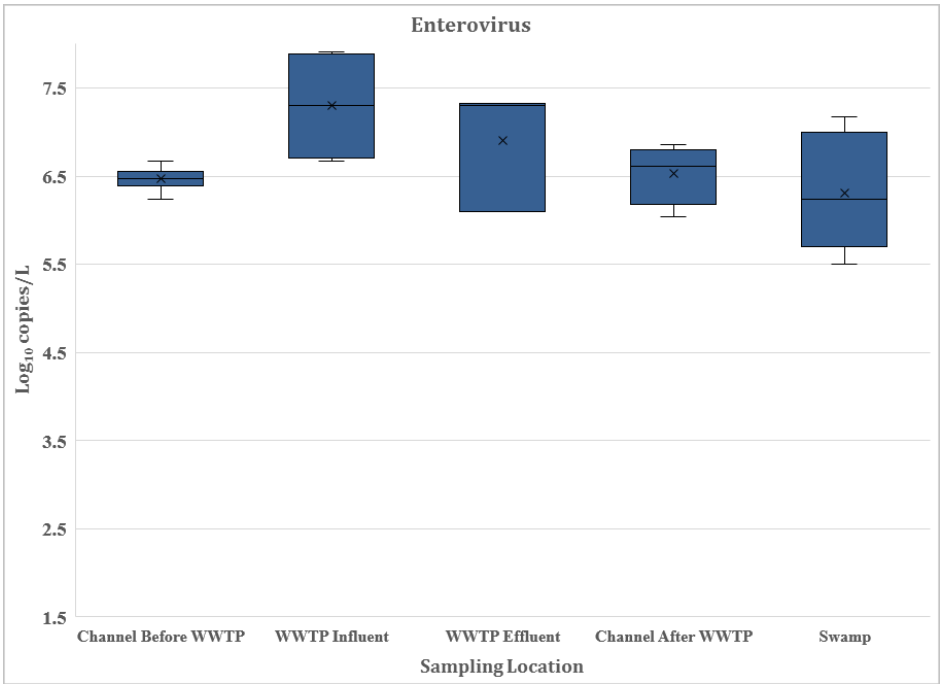


Figure 2-3: Distribution of viruses at sampling locations: a) Rotavirus b) Hepatitis A

c)



d)

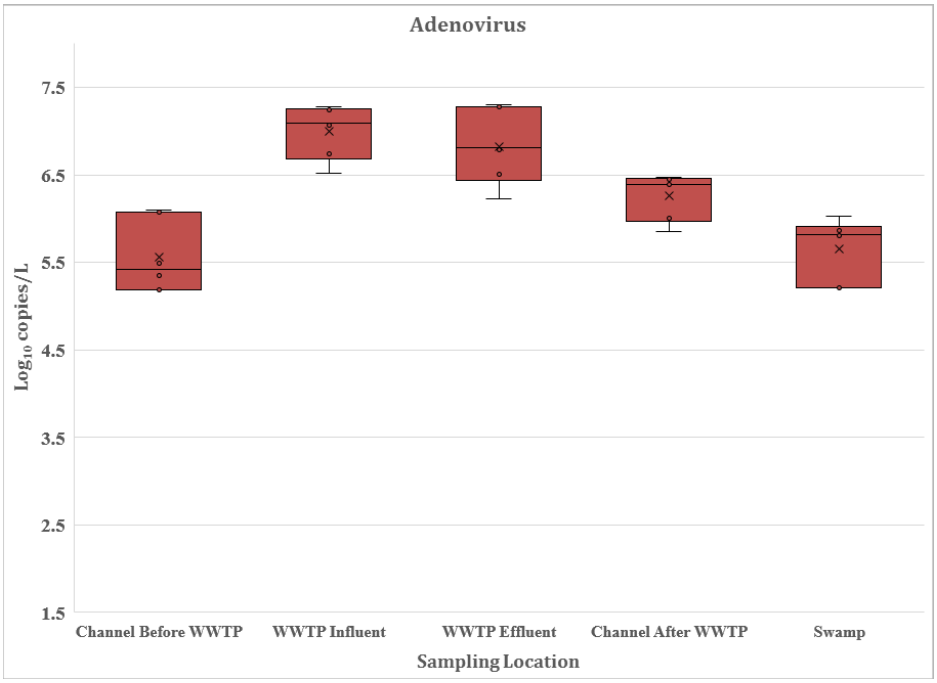


Figure 2-4: Distribution of viruses at sampling locations: c) Enterovirus d) Adenovirus

The concentrations of viruses for samples that tested positive ranged from 5.79×10^{1} to 3.91×10^{7} copies/L with RV having the lowest concentration and EV having the highest concentration. The one-way ANOVA was used to test the significant difference in mean concentrations of the viruses at the sampling locations. The lowest concentration of RV was 5.79×10^{1} copies/L in WWTP effluent samples while the maximum was 1.81×10^{3} copies/L in WWTP influent samples. The genomic copy concentration of RV at the WWTP decreased from 1.81×10^{3} copies/L to 5.79×10^{1} copies/L in influent and effluent samples respectively. There was no significant difference ($p>0.05$) observed in RV concentrations across the sampling locations. In contrast, the genomic copy concentration of HAV at the WWTP slightly increased from 4.26×10^{3} copies/L to 5.79×10^{3} copies/L in influent and effluent samples respectively. Water samples obtained from the swamp showed the least concentration of HAV of 2.73×10^{3} copies/L. Similar to RV, there was no significant difference ($p>0.05$) observed in HAV concentrations. Among the four viruses of interest, EV concentrations were the most predominant ranging from 3.09×10^{6} to 3.91×10^{7} copies/L. The lowest concentration of EV was 3.09×10^{6} copies/L in samples obtained downstream from the WWTP. EV concentrations decreased by 2.49 copies/L at the WWTP, a pattern dissimilar to that observed with the HAV concentrations at the same location. Similarly, the genomic copy concentrations of HAdV were high, 5.45×10^{5} to 1.17×10^{7} copies/L. The concentration of HAdV increased to 2.08×10^{6} copies/L downstream from the WWTP after mixing with effluent from the WWTP. This is different from the concentration of HAV that fluctuated at the same location. HAdV concentrations for WWTP influent and effluent samples were significantly different ($p<0.05$) from concentrations observed at the other three sampling locations. Generally, EV were the predominant enteric viruses followed by AdV, then HAV and lastly RV.

2.4 Discussion

The qPCR method quantified the abundance of enteric viruses in environmental samples. Enteric viruses such as rotavirus, hepatitis A virus, enterovirus and adenovirus were detected at the five sampling locations.

Rotavirus concentrations were the lowest compared to the concentrations of the other three viruses. In contrast, Fumian et al., 2010 evaluated the abundance of RV in raw sewage in Brazil and revealed that the raw sewage was enriched with RV, ranging from 2.5×10^{-1} to 1.6×10^{4} genomes per mL. The concentration of RV was higher in treated sewage than in a raw sewage at the WWTP. The RV trend at the WWTP is comparable to another study conducted at a WWTP in the Netherlands (Lodder and Husman, 2005). The concentration of RV downstream from WWTP was higher than the concentration upstream at the same location. The WWTP effluent, with relatively higher concentration of RV mixed with sewage in the channel hence the increase in concentration. In addition, industries located along the channel dispose untreated sewage into the channel. RV is a non enveloped virus, with double stranded RNA surrounded by an inner capsid outside its core, hence its persistence in the environment (Schwab, 2007) as shown by the results in this study. However, other factors like UV light, temperature and pH influence persistence of viruses in the environment. The low concentration of RV in the channel upstream from WWTP are in agreement with these facts. Temperature influences the denaturization rate of RNA and protein, hence the higher the temperature the higher the rate of viral capsid destruction (Gerba, 2007). RV is also less stable compared to HAdV due to its structure.

Hepatitis A virus (HAV) contains a single stranded RNA with capsid resistant to variability (Pinto and Saiz, 2007). This enables HAV to be resistant to acidic conditions and temperature changes in the environment (Gerba, 2007). However, the concentrations of HAV

were second lowest after concentrations of RV. A study conducted by Rodriguez-Manzano et al., 2010 revealed similar concentrations of HAV in raw sewage of Barcelona, Spain while no HAV was detected in raw sewage as revealed by Vantarakis et al., (1999). This could have been due to non-occurrence of HAV infections at the time of sampling. WWTP effluent samples exhibited concentrations slightly higher than concentrations of WWTP influent, a pattern dissimilar to that of AdV at the same location. The low virus reductions of HAdV reveal that the conventional activated sludge treatment methods at WWTP are ineffective to reduce virus concentrations to acceptable standards. HAV concentration in channel upstream from WWTP is greater than the RV concentration due to higher stability of HAV in the environment.

Similarly, enteroviruses (EV) can survive in high temperatures and saline conditions, making enteroviruses persistent in environmental samples (Gregory et al., 2006, Wetz et al.,2004). This is evident in the channel upstream from WWTP where the EV concentration, 3.09×10^{6} copies/L was higher than the concentrations of the other three viruses of interest at the same location. Enteroviruses were the most predominant among the viruses of interest. EV concentrations are observed to be in the order of magnitude of 10^{6}-10^{7}. The discharge of WWTP effluent into the channel increased EV concentration in channel downstream. The Nakivubo swamp, that acts as a natural filter was observed to have high EV concentrations. The prevalence of EV in the swamp may be as a result of surrounding industries and homes discharging untreated sewage into the swamp. Additionally, swamps may act as reservoirs for viruses.

Human adenovirus (HAdV) exhibited high concentrations at all the locations, ranging from 5.45×10^{5} to 1.17×10^{7} copies/L. These values were higher than 3.16 to 1.38×10^{2} copies/mL and 7.62×10^{-3} to 7.47 copies/mL detected in other polluted environmental samples (Katukiza et al., 2013, Haramoto et al., 2010). The detection of HAdV in the channel upstream from WWTP

is an indication of the presence of fecal matter. The concentration of HAdV at this location was the second highest due to the relative stability of virus particles from exposure to high temperatures. The activated sludge process at the WWTP supported the adsorption of HAdV onto particles that settled in the clarifier, hence a slight decrease in concentration in WWTP effluent. An increase in concentration of HAdV in channel downstream from WWTP may be attributed to mixing with WWTP effluent, which contained high HAdV concentrations. Low concentrations of HAdV were observed in the swamp. Swamps act as natural strainers (Raphael et al., 1985) to attenuate pollution in water. The linear double stranded DNA of HAdV surrounded by a capsid makes HAdV resistant to adverse environmental conditions such as pH and temperature hence its stability in the environment (Mena and Gerba, 2009, Rigotto et al., 2011). Furthermore, the intact strand can serve as a template during DNA replication process in case one strand is destroyed by adverse environmental conditions (Enriquez et al., 1995) .

Generally, the virus concentrations of the effluent samples were found to be comparable with the concentrations of the influent samples. This implies a low reduction of virus concentration at the WWTP. The virus reduction of $< \log 2$ at the WWTP was lower than the log reductions revealed by (Katayama et al., 2008, Hewitt et al., 2011) while comparable to those observed by (Petrinca et al., 2009). The low virus reduction may be attributed to virus adsorption onto particles that do not settle in the clarifier and not removed effectively during the clarification process.

Additionally, several factors influence virus survival in water. High temperatures denature nucleic acid and protein, consequently destroying the viral capsid (Bosch et al., 2006). Ultraviolet light causes nucleotides to cross link leading to virus inactivation. PH also influences virus survival in water. Laboratory processes such as conversion of RNA to cDNA affect

quantitative results due to RNA degradation (Fleige et al., 2006).

Bugolobi wastewater treatment plant effluent is discharged into Nakivubo channel, and then enters the Nakivubo wetland before discharge into Lake Victoria hence the high virus concentration of up to 4.69×10^6 copies/L. The high virus concentration can be also attributed to the untreated sewage released into the wetland from surrounding industries and homes. Natural wetlands such as 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 since viruses adsorb strongly onto soil hence the soil may act as a reservoir for viruses (Powell et al., 2000, Sobsey et al., 1980, Dowd et al., 1998). Nakivubo swamp discharges water into Lake Victoria, therefore water pumped from Lake Victoria at the Ggaba water treatment plant requires effective treatment before distribution and human consumption to prevent waterborne disease outbreaks.

2.5 Conclusion and Recommendations

Waterborne diseases caused by viral pathogens remain a major challenge to developing cities like Kampala. Limited studies have been conducted to establish the distribution and abundance of enteric viruses in the environment in Kampala. This study established the abundance of enteric viruses at five locations in Kampala (Nakivubo channel (before and after WWTP), Bugolobi wastewater treatment plant (Influent and Effluent) and Nakivubo swamp) during the dry season, from July 7 to August 8, 2016. These data can be used to monitor the prevalence of diseases such as rotavirus diarrhea, respiratory and gastro-enteritis illnesses among populations in Kampala. Further studies are recommended to establish the temporal distribution of viruses at the sampling sites and assess the risk of exposure to waterborne viruses in Kampala.

APPENDIX

Table A-1: Sample data sheet for fieldwork

SAMPLE DATA SHEET
SAMPLE NUMBER:
DATE OF SAMPLING:
UTILITY NAME:
UTILITY ADDRESS: COUNTRY: CITY: GPS COORDINATES:
SAMPLER'S NAME:
WEATHER CONDITIONS:
WATER pH:
ADJUSTED WATER pH:
FILTER APPARATUS DISINFECTED: (CHECK) _____ YES _____ NO
FILTER APPARATUS DECHLORINATED: (CHECK) _____ YES _____ NO
INITIAL METER READING: _____ Gallons
FINAL METER READING: _____ Gallons
TOTAL SAMPLE VOLUME: _____ L (Final –Initial Meter Readings* 3.7854)

Table A-2: Field data sheet for sampling carried out on 07/12/2016

Utility Name	Sample Number	GPS Coordinates	Water pH	Initial Meter Reading (Gallons)	Final Meter Reading (Gallons)	Total Sample Volume (L)
Nakivubo Swamp	NKS	459541 E, 32635 N	7.6	379	389.4	39.37
Bugolobi Sewage Plant Effluent	BSPE	456380 E, 35019 N	7.55	411.2	412.7	5.68
Bugolobi Sewage Plant Influent	BSPI	456168E, 35296 N	7.58	414.7	419.5	18.17
Nakivubo Channel (After Bugolobi)	NKCAB	456483 E, 34869N	7.54	391.3	398.4	26.88
Nakivubo Channel (Before Bugolobi)	NKCBB	456369E , 34939N	7.41	401.1	409.2	30.66

Table A-3: Field data sheet for sampling carried out on 07/25/2016

Utility Name	Sample Number	GPS Coordinates	Water pH	Adjusted Water pH	Initial Meter Reading (Gallons)	Final Meter Reading (Gallons)	Total Sample Volume(L)
Nakivubo Swamp	NKS	459541E, 32635 N	7.95	7.58	422	428.4	24.23
Bugolobi Sewage Plant Effluent	BSPE	456380E, 35019 N	8.32	6.53	436.2	441.3	19.31
Bugolobi Sewage Plant Influent	BSPI	456168E, 35296 N	8.3	7.07	444	444.7	2.65
Nakivubo Channel (After Bugolobi)	NKCAB	456483E, 34869N	8.38	7.49	429.9	432.4	9.46
Nakivubo Channel (Before Bugolobi)	NKCBB	456369E, 34939N	8.12	7.54	432.9	435.8	10.98

Table A-4: Field data sheet for sampling carried out on 08/08/2016

Utility Name	Sample Number	GPS Coordinates	Water pH	Adjusted Water pH	Initial Meter Reading (Gallons)	Final Meter Reading (Gallons)	Total Sample Volume (L)
Nakivubo Swamp	NKS	459541 E, 32635 N	7.63	-	446.3	460.4	53.37
Bugolobi Sewage Plant Effluent	BSPE	456380 E, 35019 N	7.94	7.56	475.7	477.2	5.68
Bugolobi Sewage Plant Influent	BSPI	456168 E, 35296 N	8.01	6.51	479	481.4	9.08
Nakivubo Channel (After Bugolobi)	NKCAB	456483 E, 34869N	8.07	6.55	461.7	466.6	18.55
Nakivubo Channel (Before Bugolobi)	NKCBB	456369 E, 34939N	7.96	7.42	467.7	473.9	23.47

Table A-5: Field photos for the research work carried out between 07/12-08/08/2016

	
Sampling along Nakivubo channel	Sampling at Bugolobi WWTP (Influent)
	
WWTP effluent drains into Nakivubo channel	Sampling point in Nakivubo swamp
	
Filter apparatus sterilization and dechlorination	Cartridge filter packed with dry ice ready for shipping

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