



Viral diversity and abundance in polluted waters in Kampala, Uganda



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ARTICLE INFO

Article history:

Received 17 July 2017

Received in revised form

29 September 2017

Accepted 30 September 2017

Available online 6 October 2017

Keywords:

Viruses

Wastewater treatment

Wastewater epidemiology

Metagenomics

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.

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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; Ssegooba 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; Xagorarakis 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 (Xagorarakis 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 into 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

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