Genomic copy concentrations of selected waterborne viruses in a slum environment in Kampala, Uganda

A. Y. Katukiza, H. Temanu, J. W. Chung, J. W. A. Foppen and P. N. L. Lens

ABSTRACT

The presence of viruses in a slum environment where sanitation is poor is a major concern. However, little is known of their occurrence and genomic copy concentration in the slum environment. The main objective of this study was to determine the genomic copy concentrations of human adenoviruses F and G, Rotavirus (RV), Hepatitis A virus (HAV), Hepatitis E virus (HEV) and human adenovirus species A,C,D,E, and F (HAdV-ACDEF) in Bwaise III, a typical slum in Kampala, Uganda. Forty-one samples from surface water, grey water and ground water were collected from 30 sampling locations. The virus particles were recovered by glass wool filtration with elution using beef extract. DNA and RNA viruses were detected by the real time quantitative polymerase chain reaction (qPCR) and the reverse transcription-qPCR (RT-qPCR), respectively. HAdV-F and G were detected in 70.7% of the samples with concentrations up to 2.65×10^1 genomic copies per mL (gc mL^{-1}). RV and HAV were detected in 60.9% and 17.1% of the samples, respectively. The maximum concentration of RV was 1.87×10^2 gc mL^{-1}. In addition, 78% of the samples tested positive for the HAdV-ACDEF, but all samples tested negative for HEV. These new data are essential for quantitative microbial risk assessment, and for understanding the effects of environmental pollution in slums.

Key words | glass wool filtration, polymerase chain reaction (PCR), sanitation, slums, viruses

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INTRODUCTION

The health risks caused by viruses present in water and wastewater streams to which slum dwellers are exposed are a major concern. Waterborne disease outbreaks in slums of developing countries are caused by enteric viruses, bacteria and protozoa (Jaykus 1997; Carr 2001; Ashbolt 2004; Montgomery & Elimelech 2007; Bloomfield et al. 2009) and the situation is made worse by poor sanitation and hygiene practices (Howard et al. 2003; Jenkins & Sugden 2006; Konteh 2009; Mara et al. 2010). Outbreaks of typhoid fever, cholera, dysentery and diarrhoea are associated with the low level of sanitation and are the major causes of mortality of children under the age of 5 in slum areas (Awasthi & Pande 1998; Legros et al. 2000; de Melo et al. 2008). Viruses, unlike bacteria, are very infective with one particle capable of causing an infection (Haas et al. 1993; Crabtree et al. 1997; Fong & Lipp 2005). They are persistent in the environment and are resistant to conventional water and

wastewater treatment technologies (Gantzer et al. 1997; Baggi et al. 2001; Fong et al. 2010). Enteric viruses can stay in the water matrix for months while remaining infective (Grabow 1996; Jiang 2006; Espinosa et al. 2008; Templeton et al. 2008).

The survival of virus particles in the environment is enhanced by the presence of faecal matter, colloidal clays, and soils, as well as biological and chemical floc particles (Templeton et al. 2008; Vasickova et al. 2010). Non-enveloped viruses are protected by a protein coat that interacts with the host cell surface and infectivity is dependent on whether the viral capsid is damaged or not (Templeton et al. 2008; Rodríguez et al. 2009). Infectivity of virus particles reduces with chlorination, exposure to sunlight, ultraviolet (UV) radiation and high temperature (Noble & Fuhrman 1997; Carter 2005; Espinosa et al. 2008; Rodríguez et al. 2009). Viruses are of public health concern due to their

low infectious dose. The risk of infection from viruses is at least tenfold greater than that for pathogenic bacteria, and 1 PFU (plaque forming unit) is capable of causing infection in 1% of healthy adults (Haas *et al.* 1993; Crabtree *et al.* 1997; Bosch 1998; Fong & Lipp 2005). In slums, the potential public health risk from pathogenic viruses may even be higher. The risk of infection from hepatitis A virus (HAV) in surface water was found to be higher in communities with low socio-economic status (Venter *et al.* 2007).

Human adenovirus (HAdV), rotavirus (RV), HAV, noroviruses (NoV) and enteroviruses (EV) are some of the most prevalent viruses in wastewater and contaminated surface water (Schvoerer *et al.* 2000; van Zyl *et al.* 2006; Xagoraraki *et al.* 2007; Wyn-Jones *et al.* 2011). The sources of viruses include untreated or treated sewage or runoff that has been in contact with animal manure or human excreta. Enteric viruses are transmitted via the faecal-oral route (Brugha *et al.* 1999; van Zyl *et al.* 2006; Bloomfield *et al.* 2009). Gastro-enteritis, conjunctivitis, diarrhoea and hepatitis are examples of diseases caused by viruses with morbidity and mortality occurring among children, elderly persons and patients with low immunity (Schvoerer *et al.* 2000; van Zyl *et al.* 2006; Xagoraraki *et al.* 2007). HAdV species F and G and RV are responsible for mortality in children and the elderly (Crabtree *et al.* 1997; Kocwa-Haluch & Zalewska 2002; Rigotto *et al.* 2011). HAdV contain a linear, double-stranded deoxyribonucleic acid (dsDNA) genome and are sometimes selected as a viral indicator of human faecal pollution because they are highly prevalent and stable (Pina *et al.* 1998; Wyn-Jones & Sellwood 2001; Muscillo *et al.* 2008; Rigotto *et al.* 2011). RV are double-stranded ribonucleic acid (dsRNA) viruses, with eleven genome segments covered within a three-layer capsid and are resistant to chloramines and UV light inactivation (Gerba *et al.* 1996). NoV, HAV and hepatitis E virus (HEV) are RNA viruses prevalent in Asia, China, Africa and South America, which pose a high risk of illness. In addition, the morbidity and mortality associated with HEV is significant for pregnant women (Wyn-Jones & Sellwood 2001; van Cuyck *et al.* 2005; Caron & Kazanji 2008; Dalton *et al.* 2008).

Previous studies in peri-urban areas in developing countries concluded that a large part of the pollution was sanitation related (Chaggu *et al.* 2002; Howard *et al.* 2003; Kulabako *et al.* 2007; Oswald *et al.* 2007; Genser *et al.* 2008;

Katukiza *et al.* 2010; Mara *et al.* 2010). Boreholes, shallow wells, springs and surface water in slums and densely populated areas where the sanitary infrastructure is poor have been found to be contaminated with *Escherichia coli*, faecal coliforms and thermotolerant coliforms (Kimani-Murage & Ngindu 2007; Katukiza *et al.* 2010; van Geen *et al.* 2011). Inadequate onsite and offsite sanitation systems are the principal causes of high pathogen concentrations in ground water and surface water of peri-urban areas (Karn & Harada 2001; Paterson *et al.* 2007; van Geen *et al.* 2011). In developing countries, the major focus in solving sanitation related problems has been to reduce health risks rather than reduction of environmental impacts (Langergraber & Muellegger 2005; Moe & Rheingans 2006; Mara *et al.* 2010). The pollution load from slum areas, however, has the potential to result in both negative environmental and public health impacts as excreta disposal using onsite sanitation is inadequate.

Very limited studies have been carried out on the occurrence of enteric viruses in the environment in developing countries (Miagostovich *et al.* 2008; Verheyen *et al.* 2009; Rigotto *et al.* 2010), and even less in slum environments (Kiulia *et al.* 2010). In addition, public health and environmental specialists in developing countries generally lack information on the occurrence and concentrations of viruses in slum environments. This is likely hindering achievement of Millennium Development Goal 7: ensuring environmental sustainability (WHO & UNICEF 2010). This study was conducted in Bwaise III, a typical slum in Kampala (Uganda) with very poor sanitation infrastructure. Our main objective was to determine the genomic copy concentrations (GC) of selected enteric viruses in various water and wastewater streams present in Bwaise III.

MATERIALS AND METHODS

Sample collection

A total of 41 samples from 30 sampling locations were collected between January 11 and February 3, 2011. Samples were collected from 25 locations in Bwaise III shown in Figure 1 and also from five surface water locations (B1, B2, B3, C2 and C3) in the neighbouring slums of Mulago and Kyebando. Samples from locations P1, P2, P3, P4, P5,
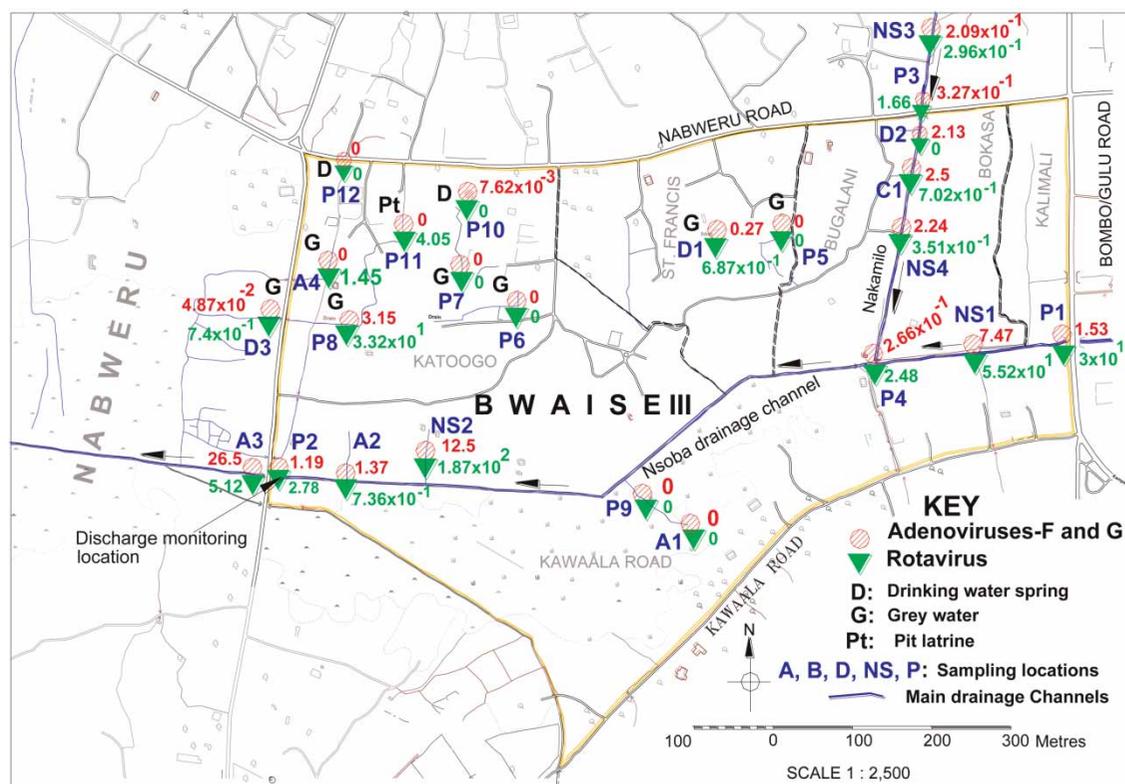
Figure 1 | Genomic copy concentrations of human adenoviruses F and G (HAdV-F and G) and rotavirus (RV) (genomic copies mL^{-1}) in the Bwaise III slum environment.

P8, and P10 were collected 2–3 times to assess the temporal variability of2 virus presence and/or virus concentration.

Three types of samples were collected: 26 surface water samples, 11 grey water samples and four ground water samples (spring water and sample from beneath a pit latrine). The majority of the samples were collected from Bwaise III, which is a typical slum in sub-Saharan Africa. The criteria for study area selection included: informal settlement, poor sanitation infrastructure, low lying and with a high water table to be able to obtain ground water samples and the presence of main surface water drains originating from upstream of the slum, enabling the determination of effects of the slum on virus concentrations. Usually, six samples were collected from the area between 8 and 10 a.m. in new 10 L plastic containers that were reused after being washed with sodium hypochlorite (3.85% m/v) three times, and rinsed with distilled water. The containers were also washed with water of which the sample was finally taken. The sampling locations were inlets, outlets and junctions of primary, secondary and tertiary drains, unprotected springs used as drinking water sources,

and one ground water observation piezometer (5 m deep and unplasticised polyvinyl chloride with maximum pressure 6 bar and nominal diameter 50 mm (uPVC PN6 ND 50 mm), water table 1.5 m below ground level at the time of sampling). Primary and secondary drains convey a mixture of surface water and grey water while tertiary drains convey grey water from the bathrooms and kitchen verandas.

In addition to taking a sample in the field, temperature (°C), conductivity (EC) (µS cm^{-1}), dissolved oxygen (DO) (mg L^{-1}), and pH were measured immediately using a portable pH meter (pH 3310 SET 2, incl. a SenTix®41 probe). After collection, the samples were stored at +4 °C using ice blocks and transported to the Public Health Engineering Laboratory at Makerere University for processing within 1 hour.

Virus concentration by glass wool filtration

To concentrate virus particles from the samples, we used a glass wool filtration protocol described by Wyn-Jones

et al. (2011) which is a modification of the method of Vilaginès *et al.* (1993). Briefly, 10 L water samples were concentrated by adsorption at pH 3.5 to glass wool with a density of 0.25 g cm^{-3} and a total filter column height of 11.7 cm, and eluted with beef-extract/glycine buffer at pH 9.5 followed by organic flocculation at pH 4.5. The flocs were pelleted by centrifugation at $4,200 \times g$ for 30 min and the pellet was resuspended in 10 mL of phosphate buffered saline (PBS). For our experiments, we used oiled white glass wool (Insulsafe 12 Isover Saint-Gobain, The Netherlands) and unplasticised PVC columns of 40 cm length and 30 mm internal diameter. Since this type of glass wool and the dimensions of the columns were similar, but not identical to the described protocol by Wyn-Jones *et al.* (2011), we first tested the virus recovery from our glass wool columns using known concentrations of the bacteriophages PRD1 and φX174. The stock solutions were kindly provided by the Microbiological Laboratory for Health Protection of the National Institute of Public Health and the Environment, where bacteriophage concentrations in the effluent samples were determined following standard protocols ISO 10705-1 (Anonymous 1995) and ISO 10705-2 (Anonymous 2000).

Nucleic acid extraction

Viral nucleic acids were extracted by a procedure described by Boom (Boom *et al.* 1990, 1999). The method is based on binding of nucleic acid to silica particles in the presence of a high molarity solution of guanidinium isothiocyanate (GuSCN). Thereto, 500 μL of Boom buffer L7A (5.25 M GuSCN, 50 mM Tris-HCl [pH 6.4], 20 mM ethylene diamine tetraacetic acid (EDTA), 1.3% [wt/vol] Triton X-100, and 1 mg/mL alpha-casein) was added together with 10 μL micron sized silica particles suspension to 100 μL of sample concentrate in PBS (obtained from the previous step). After 30 min incubation at room temperature and centrifuging (at $13,000 \times g$), the resulting silica pellet to which nucleic acids were adsorbed, was washed twice with Boom buffer L2 (5.25 M GuSCN, 50 mM Tris-HCl [pH 6.4]), twice with 70% ethanol and once with acetone. After centrifuging the silica pellet, disposal of the acetone, and drying of the pellet at 56 °C, 55 μL of Tris-EDTA (TE) buffer was added in order to desorb the nucleic acids from

the silica particles. Finally, the vessel containing the silica pellet and TE buffer was vortexed 3 min, and incubated for 10 min at 56 °C. Then, the vessel was centrifuged for 2 min at $13,000 \times g$, and 50 μL of the supernatant was pipetted into a new Eppendorf. This fluid containing DNA and RNA was stored at -80 °C, and used for further experiments.

Real time quantitative polymerase chain reaction (qPCR) and reverse transcription-qPCR (RT-qPCR)

The detection of DNA viruses was carried out using USB HotStart-IT Probe qPCR Master Mix (USB Cooperation, Cleveland, OH, USA), while the detection of RNA viruses was carried out using the Platinum Quantitative RT-PCR ThermoScript One-Step System (Invitrogen Life Technologies Cooperation, Grand Island, NY, USA). Primers and probe for HAdV-F and G were manufactured by Primerdesign Ltd (Southampton, UK), and were purchased as part of a kit ('Human Adenovirus Type F and G Standard Kit'). For HAdV-F and G, and HAdV-ACDEF, we used the thermocycler protocol as recommended by the USB HotStart-IT Probe qPCR Master Mix. Other primer and probe sequences including the thermocycler protocol were taken from literature (Table 1). All probes used consisted of a FAM fluorophore and a BHQ1 quencher and were manufactured by Biolegio (Nijmegen, The Netherlands). To each PCR well, 4 μL of sample was added to a total well volume of 25 μL. All qPCR and RT-qPCR analyses were carried out on a Mini-Opticon (Bio-Rad, Hercules, CA, USA).

Determining virus concentrations

Genomic copy concentrations of HAdV-F and G were determined with a standard curve (Figure 2), which was constructed in triplicate from a dilution series of a concentrated genomic copy number standard (2×10^5 copies μL^{-1}), that was included in the standard kit for quantification of human adenovirus type F and G (from Primerdesign). RV concentrations were determined with a standard curve (Figure 2), which was constructed in duplicate from a dilution series of concentrated genomic copy synthetic cDNA stock (2.41×10^7 copies μL^{-1}) with a length of 87 base pairs (position 963-1049; Pang

Table 1 | Overview of primers, probes and thermocycler protocol used for the detection of targeted viruses

Target virus[a]	Forward primer, reverse primer (and final concentrations)	Probe (and final concentration)	Thermocycler protocol	Reference
HAdV-ACDEF	CWTACATGCACATCKCSGG (900 nM), CRCGGGCRAAYTGCACCAG (900 nM)	CCGGGCTCAGGTACTCC GAGGCGTCCT (225 nM)	10 min at 95 °C, 40 cycles of [15 s at 95 °C and 1 min at 60 °C]	Hernroth *et al.* (2002)
HAdV-F and G	Details of primers and probe sequences are with manufacturer		10 min at 95 °C, 40 cycles of [15 s at 95 °C and 1 min at 60 °C]	Primerdesign Ltd (Southampton, UK)
RV	ACCATCTACACTGACCCTC (200 nM), GGTCACATAACGCCCC (200 nM)	ATGAGCACAATAGTTAAAA GCTAACACTGTCAA (150 nM)	30 min at 60 °C, 5 min at 95 °C, 45 cycles of [20 s at 94 °C and 1 min at 60 °C]	Pang *et al.* (2004)
HAV	GGAGAGCCCTGGAAGAAAG (500 nM), TCACCGCCGTTTGCCTAG (900 nM)	CCTGAACCTGCAGGAATTAA (250 nM)	60 min at 55 °C, 5 min at 95 °C, 45 cycles of [15 s at 95 °C, 1 min at 60 °C and 1 min at 65 °C]	Costafreda *et al.* (2006)
HEV	GGTGGTTTCTGGGGTGAC (250 nM), AGGGGTTGGTTGGATGAA (250 nM)	TGATTCTCAGCCCTTCGC (100 nM)	30 min at 50 °C, 15 min at 95 °C, 45 cycles of [10 s at 95 °C, 20 s at 55 °C, 30 s at 72 °C and 1 min at 65 °C]	Bouwknegt *et al.* (2009)

[a]HAdV: Human adenovirus; RV: Rotavirus; HAV: Hepatitis A virus; HEV: Hepatitis E virus.
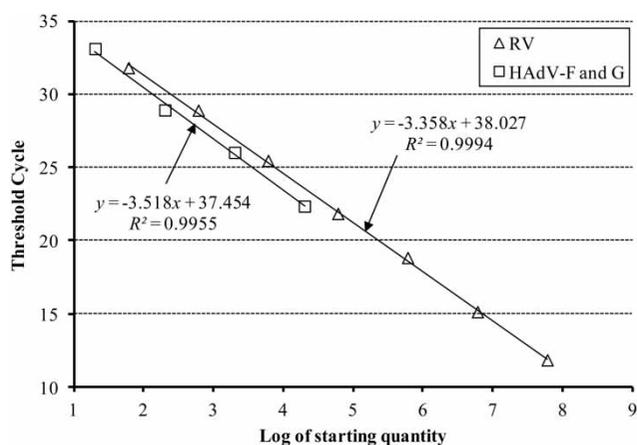
Figure 2 | Standard curves of human adenoviruses F and G (HAdV-F and G) and rotavirus (RV).

et al. 2004) of the NSP3 Region; GenBank Accession Number X81436).

The results obtained by (RT)-qPCR were multiplied by a constant factor of $(1,000 \times 0.38)^{-1}$ to account for the up-concentration of the virus particles due to the glass wool protocol (from 10 L to 10 mL), and to account for the glass wool recovery of virus particles in the samples, which was based on the average recovery of the bacteriophages PRD1 and φX174 of 38% (see also 'Recovery of the bacteriophages' in the Results section). The results were then multiplied by a factor of 0.5 to cater for the final recovered volume of RNA of 50 μL from the initial 100 μL of sample concentrate and by a dilution factor of either 10 or 100 for 16 samples that showed inhibition. The results of the target viruses HAdV-ACDEF, HAV, and HEV were reported in a qualitative way in terms of present or absent. Noroviruses were not investigated in this study, because RV is the leading cause of gastroenteritis and diarrhoea in children in developing countries (Clark & McKendrick 2004; Ramani & Kang 2009), who are among the vulnerable groups in slums.

Inhibition and false negative tests

To check for inhibition of the PCR reaction, prior to detection of viral genomic copies, 4 μL of sample together with 4 μL of a known concentration of an artificially manufactured 80 nucleotides long single stranded piece of DNA of

known composition (marker DNA3; Foppen et al. 2011) was added to a total volume of 25 μL, and then amplified according to the protocol described in Foppen et al. (2011).

Quality control

Separate equipment, pipettes, filter tips, vials and reagent tubes were used for each stage of the process to avoid contamination of samples and reagents. Viral concentrates were kept frozen during transportation from Uganda to the laboratories of UNESCO-IHE (Delft, The Netherlands), where they were immediately stored at $-80\,^{\circ}$C until further use. All qPCR and RT-qPCR assays included blank controls containing the same reaction mixture except for the nucleic acid template in addition to inhibition and false negative tests.

RESULTS

Recovery of the bacteriophages

In our column setup, the recovery of bacteriophage PRD1 ranged from 12.2 to 26.3% and from 17.6 to 35.5%, when flushed with 10 L of tap water spiked with 2.0×10^2 and 2.8×10^4 pfu mL^{-1} phages, respectively. The recovery of φX174 ranged from 36 to 46.3% and 40 to 57.8% for tap water spiked with concentrations of 9 and 3×10^3 pfu mL^{-1} respectively. From this, we concluded that our column setup and the protocol used worked satisfactorily. Two different concentrations were used to assess whether the glass wool protocol we applied yielded similar recovery rates for different concentrations. In addition, we did not know the genomic copy concentrations we could expect in the various surface water and ground water samples.

Sensitivity and efficiency of the HAdV-F and G and RV assays

For the HAdV-F and G assay, the coefficient of determination, R^2, was 0.995 (see Figure 2) and the efficiency of the qPCR reaction, determined as $E = [10^{-1/\text{slope}}] - 1$, was 0.92. For the RV assay, the coefficient of determination (R^2) was 0.999, while the efficiency of the RT-qPCR reaction was 1.23, which was high.

PCR inhibition tests

Out of 41 samples, 16 samples from 14 sampling locations showed inhibition of the PCR. This was 46% (12/26) of all surface water samples, 27% (3/11) of all grey water samples, and 25% (1/4) of all ground water samples. Out of four ground water samples, three spring water samples did not show PCR inhibition effects, but one sample from beneath the pit latrine did. To eliminate inhibition, 10 μL of the concentrated samples from the glass wool elution were diluted 10, 100, and 1,000 times, and, after nucleic acid extraction, were again checked for inhibition. The 10–100 times dilution was adequate to eliminate inhibition of the PCR.

Prevalence of viruses in Bwaise III slum

Physicochemical parameters for the four groups of samples taken from surface water, spring water, grey water, and ground water in the area differed from each other, while the standard deviations were relatively low (Table 2), indicating that the groups of waters we had sampled were relatively distinct and homogeneous from a physicochemical point of view. HAdV-ACDEF was detected in 78% of all samples (31/41; Table 3). All surface water samples

Table 2 | Physicochemical properties of tested samples

| Parameter | Types of samples and corresponding property values[a] | | | |
	Surface water (n = 26)	Spring water (n = 3)	Grey water (n = 11)	Ground water beneath a pit latrine (n = 1)
pH	7.1 (±0.2)	5.3 (±0.3)	7.5 (±0.4)	7.6
Temperature (°C)	22.9 (±1.4)	24.5 (±1.5)	23.5 (±1.2)	25.2
Electrical conductivity (μS cm^{-1})	575.4 (±110)	613.5 (±151)	1,755.1 (±1,443)	2,480
Dissolved oxygen (mg L^{-1})	0.98 (±0.44)	4.17 (±0.54)	0.47 (±0.34)	ND

[a]In brackets is the standard deviation of property values.

contained HAdV-ACDEF, and so did 45% of the grey waters sampled. In addition, HAdV-ACDEF was found in one protected spring water sample, used for drinking water purposes. HAdV-F and G were detected in 70.7% of all samples (29/41): 96% of all surface water samples, 27% of grey water samples, and one spring sample that also contained HAdV-ACDEF (Table 3). RV was detected in 61% of all samples (25/41). Most of the surface water samples were positive for RV, and so were 36% of the grey water samples. In addition, RV was found in an observation piezometer sampling ground water, almost beneath a pit latrine. HAV was found on a few occasions (7/41), while HEV was not found at all. Finally, 17.1% (7/41) of the samples tested negative for all viruses. These were two spring water samples and five grey water samples. The distribution of viruses in the tested surface water, grey water and ground water samples is shown in Table 4.

Genomic copy concentrations (GC) of HAdV-F and G, and RV

We determined the genomic copy concentrations of HAdV-F and G and RV in surface water, spring water and grey water (Figure 1 and Table 3). The lowest concentration of HAdV-F and G for samples that tested positive was 7.62×10^{-3} gc mL^{-1} in a spring water sample, while the maximum was 2.65×10^{1} gc mL^{-1} in surface water at the outlet of the slum (Figure 1). In the surrounding slums, the highest concentration was 4.8 gc mL^{-1} indicating that HAdV-F and G was practically omnipresent. The genomic copy concentration of HAdV-F and G increased downstream of the two main storm water drains in the area. In the Nakamilo drain, the genomic copy concentration of HAdV-F and G increased from 2.09×10^{-1} gc mL^{-1} immediately north of Bwaise III to 2.24 gc mL^{-1} while in the Nsooba drain, it increased from 0.153×10^{1} to 2.65×10^{1} gc mL^{-1}. In contrast, the genomic copy concentration of RV fluctuated along the drains without a clear pattern. The highest concentration of RV was 1.87×10^{2} gc mL^{-1} in surface water while the lowest concentration of RV was 2.96×10^{-1} gc mL^{-1} in a surface water. When comparing the 3 groups of waters, virus concentrations were the highest in surface water followed by grey water, and then ground water, either from springs or from the observation piezometer we sampled (Table 3).

DISCUSSION

Virus recovery and PCR accuracy

The recovery of the bacteriophages PRD1 and φX174 between 12.2 and 57.8% by glass wool adsorption in this study was comparable to the 8–28% recovery of adenovirus serotype 41 (Lambertini *et al.* 2008), 34.2–78.2% recovery of adenovirus spiked in fresh water concentrated by glass wool followed by elution with beef extract (Wyn-Jones *et al.* 2011), and 40% recovery of enteric viruses from wastewater, ground water and surface water (Wolfaardt *et al.* 1995; Grabow 1996; van Heerden *et al.* 2005; van Zyl *et al.* 2006; Venter *et al.* 2007). The recovery of φX174 with an average diameter of 27 nm and an iso-electric point between 4.6 and 7.8 was much higher than that of PRD1 with an average diameter of 62 nm and iso-electric point of 3–4. Apparently, bacteriophage φX174 attached more to glass wool than PRD1, as a result of the larger electrostatic repulsion of PRD1, compared to the less negatively charged φX174 (Vilaginès *et al.* 1993; Wyn-Jones *et al.* 2011).

RT-qPCR/qPCR was used because it was shown to be an accurate method with high sensitivity to detect viral RNA/DNA in a variety of environmental samples, despite the presence of inhibitors in samples and adsorption of viruses to particulate material (Jiang *et al.* 2005; Espinosa *et al.* 2008; Rodríguez *et al.* 2009; Wyn-Jones *et al.* 2011). Nucleic acid amplification inhibition effects exhibited by some samples during PCR were probably due to the presence of humic acids and other organic compounds and high bacterial concentrations. Most of the samples had no PCR inhibition probably because they were removed during the elution with PBS after glass wool filtration and nucleic acid adsorption on the silica colloids. In addition, the PCR inhibitors may not have adsorbed to glass wool during filtration (Van Heerden *et al.* 2005).

Waterborne viral contamination in the Bwaise III slum

The concentration of HAdV-F and G virus particles generally increased downstream of the two main drains (Nsooba and Nakamilo; Figure 1) in the Bwaise III slum. There was an increase from 1.53 gc mL^{-1} at the inlet of the Nsooba drain to 26.5 gc mL^{-1} at the outlet, and an increase from 2.09×10^{-1} gc mL^{-1} upstream to 2.24 gc mL^{-1} downstream of the Nakamilo drain. These values were lower than the

Table 3 | Occurrence of viruses in all tested surface water, ground water and grey water samples[a]

No	Sampling location ID	Sampling location	Surface water					Grey water					Ground water				
			HAdV-F and G	AdV-ACDEF	RV	HAV	HEV	HAdV-F and G	AdV-ACDEF	RV	HAV	HEV	HAdV-F and G	AdV-ACDEF	RV	HAV	HEV
1	A1	Secondary drain						0	Abst	0	Abst	Abst					
2	A2	Nsooba	1.37	Prst	7.36×10^{-1}	Abst	Abst										
3	A3	Nsooba	2.65×10^{1}	Prst	5.12	Abst	Abst										
4	A4	Tertiary drain						0	Prst	1.45	Abst		Abst				
5	A5	Nakamilo	2.64×10^{-1}	Prst	1.04	Abst	Abst										
6	B1	Kyebando slum	1.09	Prst	0	Abst	Abst										
7	B2	Kyebando slum	0	Prst	0	Abst	Abst										
8	B3	Kyebando slum	0.69	Prst	0	Abst	Abst										
9	C1	Nakamilo	2.50	Prst	7.02×10^{-1}	Abst	Abst										
10	C2	Kyebando slum	4.41	Prst	6.63	Abst	Abst										
11	C3	Mulago slum	4.80	Prst	5.87×10^{-1}	Prst	Abst										
12	D1	Tertiary drain						2.7×10^{-1}	Prst		6.87×10^{-1}	Abst	Abst				
13	D2	Nakamilo	2.13	Prst	0	Abst	Abst				7.4×10^{-1}	Prst	Abst				
14	D3	Tertiary drain						2.13	Prst								
15	NS1	Nsooba	7.47	Prst	5.52×10^{1}	Abst	Abst										
16	NS2	Nsooba	1.25×10^{1}	Prst	1.87×10^{2}	Abst	Abst										
17	NS3	Nakamilo	4.18×10^{-1}	Prst	2.96×10^{-1}	Abst	Abst										
18	NS4	Nakamilo	2.24	Prst	3.51×10^{-1}	Abst	Abst										
19	P1	Nsooba	1.53 (±1.1)	Prst	2.98×10^{1} (±3.66×10^{1})	Abst	Abst										
20	P2	Nsooba	1.19 (±0.2)	Prst	2.78 (±1.0^{7})	Prst	Abst										
21	P3	Nakamilo	3.27×10^{-1} (±4.8×10^{-2})	Prst	1.66 (±5.63×10^{-1})	Prst	Abst										
22	P4	Nsooba	5.32×10^{-1} (±4.0×10^{-2})	Prst	2.48 (±9.61×10^{-2})	Prst	Abst										
23	P5	Tertiary drain						0	Abst	0	Abst	Abst					

(*continued*)

Table 3 | continued

No	Sampling location ID	Sampling location	Surface water					Grey water					Ground water				
			HAdV-F and G	AdV-ACDEF	RV	HAV	HEV	HAdV-F and G	AdV-ACDEF	RV	HAV	HEV	HAdV-F and G	AdV-ACDEF	RV	HAV	HEV
24	P6	Tertiary drain						0	Abst	0	Abst	Abst					
25	P7	Tertiary drain						0	Abst	0	Abst	Abst					
26	P8	Tertiary drain						3.15 (±4.5)	Prst	3.32×10^1 $(\pm 4.7 \times 10^1)$	Prst	Abst					
27	P9	Secondary drain						0	Abst	0	Abst	Abst					
28	P10[b]	Spring water											7.62×10^{-3} $(\pm 1 \times 10^{-2})$	Prst	0	Abst	Abst
29	P11[c]	Piezometer											0	Abst	4.05	Abst	Abst
30	P12[b]	Spring water											0	Abst	0	Abst	Abst

[a]Genomic copies ml^{-1} for HAdV-F and G, and RV; Prst (present)/Abst (Absent) for AdV-ACDEF, HAV and HEV. In brackets is the standard deviation.
[b]Ground water samples from spring water.
[c]Sample from beneath the pit latrine.

Table 4 | Distribution of viruses in all tested surface water, ground water and grey water samples

Virus type	Number (%) of samples that tested positive for viruses			
	Surface water ($n = 26$)	Spring water ($n = 3$)[a]	Grey water ($n = 11$)[b]	Ground water beneath a pit latrine ($n = 1$)
Human adenoviruses- ACDEF	26 (100%)	1 (33%)	5 (45%)	0
Human adenoviruses- F and G	25 (96.2%)	1 (33%)	3 (27.2%)	0
Rotavirus	20 (76.9%)	0	4 (36.4%)	1 (100%)
Hepatitis A virus	4 (15.4%)	0	3 (27.2%)	0
Hepatitis E virus	0	0	0	0

[a]Two samples from two different springs, which act as a drinking water source tested negative for all viruses.
[b]Five grey water samples from tertiary drains tested negative for all viruses.

concentration of HAdV-F and G reported in polluted surface water ranging from 48 to 4.24×10^4 gc mL^{-1} (Xagoraraki *et al.* 2007; Haramoto *et al.* 2010) and higher than the values obtained in other polluted environments (e.g. 1.03×10^{-5} to 3.23×10^{-3} gc mL^{-1}; Chapron *et al.* 2000; Jiang *et al.* 2005; van Heerden *et al.* 2005; Mena & Gerba 2009). In addition, the concentration of HAdV-F and G in drinking water of 7.62×10^{-3} gc mL^{-1} from the spring (sampling point P10; Figure 1) obtained under this study was higher than the 6.15×10^{-6} to 7×10^{-5} gc mL^{-1} reported in non-chlorinated municipal drinking water (Lambertini *et al.* 2008), but comparable to 1.4×10^{-4} to 3×10^4 gc mL^{-1} of HAdVs in untreated surface water for domestic consumption (Xagoraraki *et al.* 2007; Rigotto *et al.* 2010).

We attributed the increase in the concentration of HAdV-F and G downstream of the drains to the high spatial density of inhabitants using unlined and elevated pit latrines discharging untreated wastewater in the environment, especially along the Nakamilo drain. A dilution effect by the Nsooba channel is then responsible for the decrease in concentration of HAdV-F and G virus particles after the junction of two drains in comparison to that in the tributary Nakamilo drain. We observed that this pattern was not exhibited by RV, which could be due to the differences in stability of RV compared to HAdV. HAdV remain more infectious and stable in the environment than RV due to their double-stranded DNA (Enriquez *et al.* 1995; Mena & Gerba 2009; Rigotto *et al.* 2011; Wyn-Jones *et al.* 2011). This is also evident from reported decay rate coefficients of around 0.025 day^{-1} for HAdV-F and G compared to 0.36 day^{-1} for RV (Pedley *et al.* 2006). These values show that HAdV-F and G are more stable than RV although the survival rate also depends on intrinsic (chemical and biological) as well as extrinsic (temperature, light) factors (Espinosa *et al.* 2008; Rodríguez *et al.* 2009). Another reason for the observed RV patterns could be that the primary reaction step converting RV RNA into DNA with reverse transcriptase enzyme could have affected the accuracy among the various samples tested for RV. The low concentration of RV and HAdV-F and G at the same location may be attributed to higher bacterial contamination and reduced stability of virus particles by sunlight compared to other locations (Espinosa *et al.* 2008; Rodríguez *et al.* 2009). The high concentration for both HAdV-F and G, and RV in surface water at the slum outlet (sampling location A3) may be attributed to the resultant pollution load into the main drain from upstream anthropogenic activities.

The high genomic copy concentrations of enteric HAdV-F and G, and RV suggest the presence of infective viruses although PCR does not distinguish between infectious and non-infectious virus particles. In addition, virus particles remain stable and persistent in the environment while free nucleic acid is unstable in the environment (Enriquez *et al.* 1995; Bosch 1998; Meleg *et al.* 2006; Espinosa *et al.* 2008). The presence of virus particles in the environment is therefore a public health concern even though the correlation between infectivity and persistence of viruses is still a challenge (Hamza *et al.* 2009). Virus infectivity is important for quantifying the public health risks in the slum.

HAdV-ACDEF was detected in all surface water samples and 78% of all samples, which was high compared to the presence of other viruses. We think this was because it was more persistent in this faecally contaminated environment. HAV was detected in surface water and grey water, but not in ground water because it is associated with infections in the communities and is less stable in the environment compared to adenoviruses (Biziagos *et al.* 1988; Venter *et al.* 2007; Wyn-Jones *et al.* 2011). HEV is associated with sporadic infections and epidemics in areas with poor sanitation and weak public health infrastructure (van Cuyck *et al.* 2005; Dalton *et al.* 2008). HEV was not detected in any sample probably because there were no related disease outbreaks in the study area at the time of this investigation and hence no HEV particles were discharged in the environment.

CONCLUSIONS

This study found that 85.4% (35/41) of the samples tested positive for at least one of the investigated viruses, which indicated that the slum environment was polluted. Human adenoviruses F species (serotypes 40 and 41) and G species (serotype 52), and RV were dominantly detected in the samples throughout the area investigated. The concentration range of HAdV-F and G was 7.62×10^{-3} to 2.65×10^1 gc mL^{-1} while the concentration range of RV was 2.96×10^{-1} to 1.87×10^2 gc mL^{-1} for samples that tested positive. The presence of HAdV-F and G in the drinking water source constitutes a potential public health hazard that requires urgent attention by the relevant authorities. Detection of RV in a ground water sample from beneath a pit latrine was an indication of diffuse contamination of ground water

with viral pathogens. The potential public health risks from these viruses are higher in slums because of high population density and environmental pollution from unlined and elevated pit latrines, which are mostly used for excreta disposal. Further studies should be conducted on viral infectivity, viral loadings and their periodic variations in a slum environment.

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