



Molecular Detection of Human Enteric Adenoviruses in Water Samples Collected from Lake Victoria Waters Along Homa Bay Town, Homa Bay County, Kenya

Wasonga Michael Opere¹ · Maingi John¹ · Omwoyo Ombori²

Received: 27 May 2020 / Accepted: 15 September 2020 / Published online: 3 November 2020
© Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Lake Victoria is the primary source of water for millions of people in the Sub-Saharan Africa region. In recent years, population development around the lake has resulted in compromised sanitation standards resulting in increased faecal pollution of the lake. Consequently, this condition has increased the chances of waterborne enteric viruses, such as adenoviruses' circulation in the community. Adenoviruses can affect health in both humans and animals by causing a myriad of diseases including the gastrointestinal infections. The study aimed to detect contamination of the lake water with pathogenic human adenoviruses along Homa Bay town, Homa Bay County, Kenya. To examine the presence of adenoviral genome, we collected a total of 216 (monthly $n=36$) water samples from six different locations marked by high levels of anthropogenic activities along the shoreline. Molecular amplification technique using the nested PCR procedure was used to detect the genomes from the water samples. Human adenoviruses were detected in 11 samples (5.09%). Statistical analyses indicated a significant correlation between adenovirus presence and the approximate distance from pit latrines and sewage treatment works at the area. The findings indicate that faecal contamination of the lake waters originated from the point sources. The findings also suggest a possibility of elevated levels of faecal pollution in different surface waters within the lake basin. The findings indicate that some of the enteric viruses circulating in the local community are human adenovirus type 40, and 41. The data may provide a basis for recognizing the need to prioritize environmental monitoring for enteric virus contamination on an on-going basis.

Keywords Adenoviruses · Lake Victoria · Faecal pollution · Environmental surveillance

Introduction

According to the Lake Victoria Environmental Management Project (LVEMP 2002), Lake Victoria (LV), the world's second-largest freshwater lake, is a source of livelihood for millions of people in sub-Saharan Africa. From the Kenyan side of the lake, over 5 million people (Kenya Population and Housing Census, KPHC 2019) rely on the lake for recreational, agricultural, industrial, and as a source of drinking

water among other economic activities like fishing (Baker and Eric 2008). The LV ecosystem and its water quality have declined considerably over the last five decades (Mwirigi et al. 2005). The alarming trends in the deterioration of the ecosystem have been attributed to the pollution of the lake waters as a result of a rise in the local population (Odada et al. 2004). Because there would be a corresponding rise in the usage of faecal disposal facilities needed to sustain the growing population, the population growth is implicated in this pollution equation. There is a risk that the said faecal disposal facilities will not be adequate in some areas to sustain the ever-increasing population (UN-HABITAT 2010b). Such a situation will inevitably result in a direct contribution of the lake's faecal load from open defecation activities (UN-HABITAT 2010a). Consequently, the increased contamination has exposed the lake waters and the local population to poor sanitation (Onyuka et al. 2011). Thus, the local population is predisposed to pathogenic microbes

✉ Wasonga Michael Opere
wasongamichaell@gmail.com

¹ Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

² Department of Plant Sciences, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

including faecal bacteria and enteric viruses that have been detected around the world from such poor sanitation settings (van Zyl et al. 2019).

Enteric viruses have been associated with a wide range of human infections ranging from gastrointestinal to respiratory syndromes especially in infants (Meqdam and Thwiny 2007). Their potential to cause a multitude of infections is because they can survive longer in the environment and are less affected by conventional wastewater treatment compared to faecal bacteria and other microbes (Ogorzaly et al. 2010; Carter 2005; Baggi and Peduzzi 2000). They are a community of viruses that may be found in the gastrointestinal tract but are typically released from human waste. They are therefore generally present in faecally infected areas such as sewage effluent (Gibson 2014). They have also been recovered from surface waters (Nascimento et al. 2015), and groundwater (Gibson and Schwab 2011). Some common families of enteric viruses include *picornaviridae* that include genera enteroviruses (Symonds et al. 2009), rotaviruses (Chigor and Okoh 2012) and noroviruses (Kabue et al. 2016). These viruses represent diverse groups of non-enveloped RNA viruses (Harrach et al. 2012). Another widely studied group of enteric viruses are adenoviruses. These are double-stranded DNA viruses of the family *Adenoviridae* comprising of over 80 human serotypes (Harrach et al. 2012; Dhingra et al. 2019). They have been reported as effective indicators for environmental faecal contamination and the general viral water quality (Donaldson et al. 2002). This suitability is because they are more stable in the environment compared to enteric RNA viruses, as they have a double-stranded genome (Kishida et al. 2014). They are more likely to tolerate adverse conditions like UV exposure (Bofill-Mas et al. 2006). Adenoviruses can be inactivated by heat but their heat sensitivity varies by the genera (Maheshwari et al. 2004). Formaldehyde or bleach (sodium hypochlorite) are substances known to inactivate the virus (Dong et al. 2009). Adenoviruses are transmitted via the faecal-oral route (Meqdam et al. 2007) or respiratory route (Cheng et al. 2016). They have been associated with several conditions such as gastroenteritis (Shimizua et al. 2007; Akihara et al. 2005); diarrhoea (Jalal et al. 2005), keratoconjunctivitis (Chaberny et al. 2003) and respiratory diseases (Chmielewicz et al. 2005).

With reported research on the circulation of these enteric viruses in various types of surface waters around the world, it is important to be vigilant and to track the emergence and presence of adenoviruses on a routine basis. The surveillance would be particularly important in areas with a high percentage of immunocompromised individuals (WHO 2015). One of the regions along the LV basin with a large percentage of the immunocompromised population is the Homa Bay area in western Kenya (UN-HABITAT 2010a). Contamination of LV waters with faecal contaminants is a

significant general concern both locally and regionally, considering the possible impact on public health to the dependent communities (UN-HABITAT 2010b). According to UN-HABITAT (2010a), some of the most prevalent diseases around Homa Bay town are both respiratory and diarrhoeal in nature. These syndromes have been previously associated with adenoviruses (Magwalivha et al. 2010; Chmielewicz et al. 2005) even though they may also be linked to other causative agents including several bacterial species. The risks of adenoviral infections have been exacerbated by the fact that the surrounding community directly rely on the lake waters for drinking (UN-HABITAT 2010a).

Research concerning the contamination of LV waters with adenoviruses has not been reported in Kenya. Byamukama et al. (2005) conducted a study on the lake water's microbial contamination in Kenya and Uganda with a focus on faecal coliforms. However, the use of bacterial indicators for viral contamination is not appropriate, as viruses are frequently detected without bacterial indication (Haramoto et al. 2018). Also, enteric viruses are immune to environmental conditions and can spread further than the bacteria (Prevost et al. 2016). Studies on enteric viruses in Kenya have recently been conducted from different environmental settings (van Zyl et al. 2019; Kiulia et al. 2014; Magwalivha et al. 2010). However, these studies were carried out from water sources in other parts of the country and did not consider studying the situation of adenoviruses in LV waters in Homa Bay County. Therefore, this study was conducted to examine the possible contamination of the LV waters around Homa Bay town with potentially pathogenic adenoviruses. The data from this study may be useful as a platform for Enteric Virus environmental monitoring. It may be useful in augmenting clinical surveillance of respiratory and infantile diarrhoeal infections for proper community remediation of disease burden.

Materials and Methods

Study Design

Over a cumulative sampling period of six months (October 2011 to April 2012), a total of 2160 L of water samples were collected from six points along the lake shoreline. Ten litres of water samples were collected monthly from each of six sampling sites using sterilized polyethylene containers along approximately 5 kms of shoreline stretch in Homa Bay town. The samples collected were packaged and transported for immediate processing in iceboxes to the Enteric Viruses Research Group (Institute of Primate Research Laboratory) in Nairobi. A total of 36 samples per site were collected per month for the entire cumulatively 6 months of sampling, totalling 216 samples.

The Study Site

The study site was situated on a shoreline strip in the town of Homa Bay, under Homa Bay County in Kenya's Nyanza region between latitudes $0^{\circ} 31'0''$ S and longitudes $34^{\circ} 27'0''$ E (Fig. 1). The town has a sewage system with inadequate coverage as it is only connected to a very small percentage of the municipal area (UN-HABITAT 2010a). The sewage treatment plant (STP) is located at the lake's nearshore (< 100 m). It discharges directly into the lake thereby increasing the potential for the lake's faecal pollution. The treatment system's stabilization ponds and treatment facilities are in dilapidated condition. Many households and units could not be connected to the sewer lines because of high connection charges (UN-HABITAT 2010a). Thus, most of the town's inhabitants dispose of faecal waste using Pit latrines. The pit latrines are cheap to build and maintain as they do not rely on the use of water. Fresh ones are normally dug to replace the filled ones though in some cases especially in institutions, they are treated and exhausted. Despite being the most common disposal method, the latrine

coverage in the area is still inadequate, particularly in the surrounding community, leading to open defecation around the lake as an alternative (UN-HABITAT 2010a). The sampling points were located in a section of the shore that is heavily in contact with the host community. The section is hosts to the principal source of faecal pollution; the dilapidated municipal sewage treatment facility serving the town's population of approximately 45,000 (KPHC 2019). It is also lined with multiple pit latrines and toilets for the public and private use. The segment is ideal for anthropogenic activities and is characterized by extensive use of the lake waters for domestic, agricultural, commercial, recreational, and industrial activities. The strip also acts as a source of raw water for water treatment plants in the town and the rural host community.

The sampling sites were located at the following areas: Boat landing area/Jetty, open-air fish market area, Capital fish processing plant area, sewage treatment area, residential section and open grassland/Livestock feeding ground area (Fig. 2). The town is located on a gently sloping topography ranging from about 1143 to 1220 m above sea level that

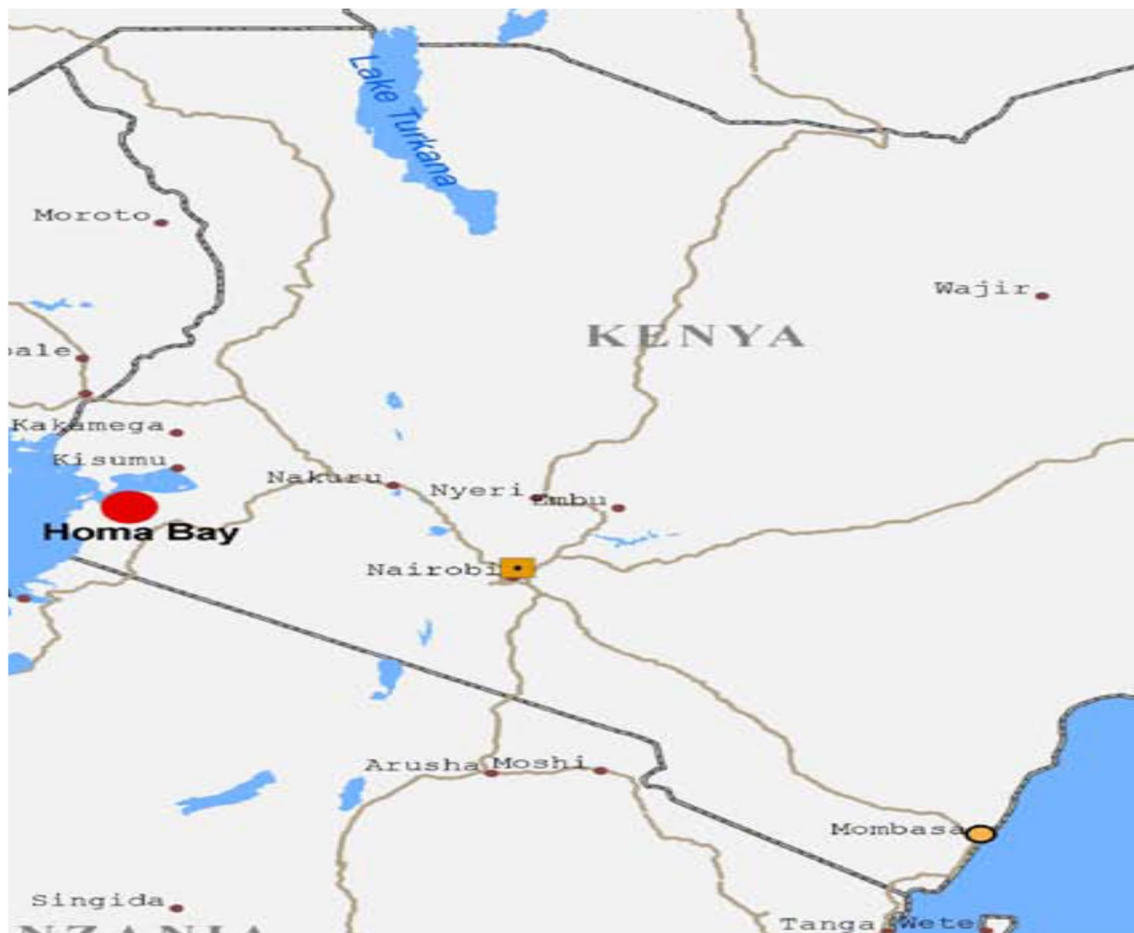


Fig. 1 Map of Kenya showing the location of Homa Bay town (UN-Habitat 2010a)

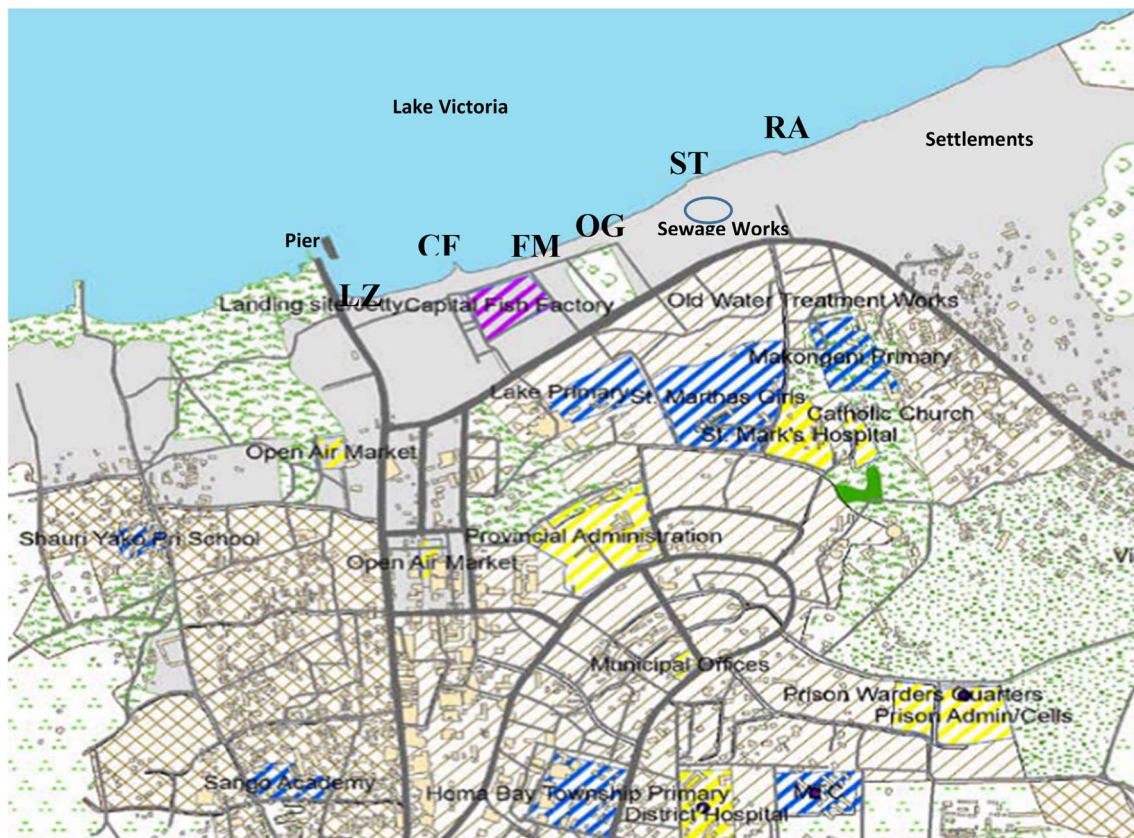


Fig. 2 Map of Homa Bay town showing the sampling area. *RA-Residential Area (decimal -0.51918 and 34.46404), ST—Sewage treatment area, (decimal -0.52001 and 34.46152), OG-Open grassland/Livestock feeding ground (decimal -0.52079 and 34.46058), FM-

Open-air fish market area (decimal -0.52115 and 34.45975), CF-Capital fish processing plant area (decimal -0.52201 and 34.45705) and LZ-Landing area/Jetty (decimal -0.52299 and 34.45524)

eventually flattens at the shoreline skirting of approximately 5 km (UN-HABITAT 2010a). Due to the rolling nature of the topography of the town towards the strip, both the natural and the county drainage systems both drains to the shoreline strip thereby increasing chances of faecal contamination from the stormwater and runoffs. The lake strip stretches approximately 16.5 km across the broader town's surroundings that encompass parts of the peri-urban Rangwe and Asego. The nearby Arujo stream is an affluent to the lake and is a major stream that flows through many rural surroundings marked by open defecation before eventually emptying into the lake. The town generally lacks proper stormwater drainage system, which has also been exacerbated by the blockage by solid wastes. Open and broken manhole cover slabs and sewer lines are a common sight along the streets. These conditions compound faecal contamination to the lake during runoffs (UN-HABITAT 2010a).

Virus Recovery

Viruses were recovered using the glass wool adsorption-elution technique as described in various past reports including

Vilaginès et al. (1993), Wolfaardt et al. (1995), Vilaginès et al. (1997) and Haramoto et al. (2005). The principle of the adsorption-elution method is based on the action of the negatively charged virus particles electrostatically getting adsorbed to the positively charged filter membranes (Lambertini et al. 2008). Briefly, by applying negative pressure, ten litres of the water samples were drained through a Perspex glass wool column. The glass wool column was prepared by plunging an oiled sodocalcic glass wool (Bourre 725 QN, Ouest Isol, Alizay, France) into a Perspex glass tube measuring 20 cm long with an internal diameter 3 cm. The adsorbed viruses on the glass wool were eluted from the column by washing using 100 ml of 9.5 pH glycine beef extract buffer (GBEB) containing a mixture of Glycine (Merck GmbH, Darmstadt, Germany) and Beef extract powder (beef extract (BBL Becton Dickinson and Company, Sparks, MD) for 15 min. For secondary concentration, 100 ml of the viral concentrates were mixed with polyethylene glycol (8% PEG) (Merck) and 0.2 M NaCl solution (Merck). The mixture was then centrifuged at $6000\times g$ at $4\text{ }^{\circ}\text{C}$ for 30 min in Eppendorf centrifugation device (Eppendorf 5402 microcentrifuge; Eppendorf Geraˆtebau, Netheler and Hinz GmbH, Hamburg

Germany). The resulting pellets were dissolved in a 20 ml phosphate-buffered saline (PBS) solution (Sigma-Aldrich (St Louis, MO) and then centrifuged at 4200 rpm for 45 min at 4 °C. The resulting pellets were resuspended in 10 ml of the resulting supernatant and centrifuged at 4200 rpm for 10 min. One ml of the final supernatant was immediately used for nucleic acid extraction, while the remainder was resuspended in 100 µl PBS and stored at –70 °C until later use.

Nucleic acid extraction

The nucleic acid was extracted from the recovered virus concentrates in a MagNA Pure LC instrument (Roche Diagnostics) following the manufacturer's instructions. Commercially available MagNA Pure LC Total Nucleic Acid Isolation Kit (Large Volume) (Roche Diagnostics) was used to extract the DNA from 1 ml of the concentrates. In brief, Proteinase K was first added to the samples followed by incubation for about 5 min. The samples were then mixed with a mixture of Magnetic glass beads containing silica particles and a lysis buffer. The glass beads from whose surfaces the release DNA particles got bound to were subjected to several washing steps using wash buffers to remove unbound substances. The Elution buffer (50 µl) was used to elute the purified DNA from the beads. The eluate was aliquoted and temporarily stored in Tris–EDTA (pH 8.0) at a temperature of –20 °C until use.

PCR amplifications

Viral genomes were detected from the DNA extracts using nested PCR (nPCR) procedures adopted from the methods previously defined by Allard et al. (1990), and modified by Santos et al. (2004) and Rodríguez-Díaz et al. (2009). The primer pairs used were adapted from those published in various articles (Allard et al. 1992; Puig et al. 1994;

Rodríguez-Díaz et al. 2009) designed from the open reading frame of the DNA sequence targeting the hexon genes of adenovirus type d40 and Ad41 (Table 1).

For the one-step PCR process, 10 µl of the extracted adenovirus nucleic acid samples corresponding to 4 dm³ of the original lake water samples were mixed with 40 µl of the PCR mixture to make a final volume of 50 µl. The PCR mixture consisted of 5 µl of 1×PCR buffer, 8 µl of 2.5 mM MgCl₂, 2 µl of 200 µM of each dNTP, 20 µl of 1 µM concentration of each of the hexAA1885(F) and hexAA1913(R) primers, 1 U of thermostable Taq DNA polymerase (Promega Corp., (Madison, WI)) and 22 µl of nuclease-free water. The amplification condition settings were as follows: Initial denaturation at 95 °C for 15 min followed by 35 cycles that consisted of 95 °C for 0.5 min each for denaturation, 57 °C for 0.5 min annealing and 72 °C for 0.5 min elongation followed by a final elongation step at 72 °C for 5 min. For the nested PCR process, 1 µl of the first-round PCR amplification was subjected to further amplification by adding to 40 µl of the reaction mixture containing 5 µl of 1×PCR buffer, 8 µl of 2.5 mM MgCl₂, 2 µl of 200 µM of each dNTP, 20 µl of 1 µM concentration of each of the nested primers [nehexAA1893 (F) and nehexAA1905(R)], 1 U of thermostable Taq DNA polymerase (Promega) and 25 µl of nuclease-free water. The cycling conditions were maintained as in the initial amplification in the first-round process. The PCR products were confirmed by 2% agarose gel electrophoresis visualized by staining using 1 µg/ml ethidium bromide. The stained products were illuminated using UV transilluminator (Alpha Innotech Corporation). The amplicons size was compared to 100 bp molecular weight (MW) ladder (Promega) and the positive control.

Quality Control

In all the manipulations, standard measures were applied to minimize the risk of cross-contamination between

Table 1 Primers used in the study targeting adenovirus hexon gene

Virus	Function	Sequence(5'-3')	Product length	References
Human Adenovirus-F40/41	hexAA1885(F) hexAA1913(R)	GCCGCAGTG GTCTTACAT GCACATC CAGCACGCC GCGGATGTC AAAGT	301	Allard et al. (1992)
Human Adenovirus	nehexAA1893(F) nehexAA1905(R)	GCCACCGAG ACGTACTTC AGCCTG TTGTACGAG TACGCG GTATCCTCG CGGTC	143	Allard et al. (1992)

*F-Forward primer, R-Reverse primer

samples and between samples and DNA amplicons. Separate laboratories, each fitted with its instruments, pipettes, filter tips, and reagent tubes were used for preparing and processing of reagents and samples and in the handling of the amplified fragments. Negative control (nuclease-free water) and a positive control derived from a cell-cultured HAdV-C2 human adenovirus strain (ATCC VR-1079 AS/Rab) were included in each virus detection reaction to monitor for false-positive and false-negative reactions. Probability of amplifying contaminant DNA was reduced by treating the nucleic acid samples with uracil DNA glycosylase (UDG) (Kittigul et al. 2005). The treatment with UDG primarily removes carryover contaminants but do not normally interfere with the other components of the PCR mixture (Tetzner et al. 2007). Nested PCR inhibition was evaluated by spiking the sample aliquots with the specific virus control for another PCR process. A representative 18 samples, comprising of one positive and 2 negative samples from each of the six sampling sites were selected for the process. Inhibition was deemed absent when the PCR amplification results of all the 18 spiked samples produced as expected amplifications consistent with the 300 bp fragments.

Data Analysis

Differences in the recovery of the viruses among the sampling points were analysed using Analysis of Variance (ANOVA) and a post hoc Tukey's HSD test. Linear Regression analysis was used to evaluate the association between the recovery of the viruses as the dependent variable and the independent variables; the estimated distances from pit latrines and the sewage treatment site from the sampling points. The analyses were performed using SAS version 9.1 (SAS Inst., Inc., Cary, NC). *P*-values that were less than 0.05 were considered to be statistically significant.

Results

The water samples were dichotomised into either presence or absence of an adenovirus genome. Of the 216 samples that were collected during the entire study period, 11 were found to be positive for adenoviral genome representing 5.09%. Of the 11 positive samples, 72.72% (8/11) were from the sites located on the northern region of the sampling strip (ST and RA). No viral genomes were recorded from sites CF and OG which were both centrally located along the study strip. Table 2 shows the detection rates of the viruses among the six study sites as well as the mean and standard error of recoveries of the viruses from the six sites, while Fig. 3 shows the 2% gel electrophoresis showing PCR amplicons generated by the primers. The principal cause of faecal pollution at the site of samples collection was the municipal sewage treatment plant located at $-0.52149, 34.46193$. Table 2 shows the estimated distances from the six sampling points from the STP.

When the samples were first analysed in the first month of sampling (October 2011), out of the 36 water samples, the adenovirus genome was detected in 1 (2.78%). The adenovirus DNA that was detected was from a sample from site ST. From this very site, adenovirus DNA was further detected from four more samples at different sampling months. Overall 5 out of 36 samples from all six months of sampling from site ST tested positive with adenovirus. Three of the remaining 5 sites tested positive six times with adenoviruses during the observation period to bring the total number of adenoviruses detected to 11. There was a significant difference between the sampling points in adenovirus detection ($P=0.0373$), with mean detection values varying from 0.03 to 0.14 (Table 2). The furthest site (LZ) was situated at an estimated distance of 3000 m from the STP. Less than 500 m from the treatment plant was the nearest site (ST), whose fence extends up to the shoreline (Table 2).

A total of 16 pit latrines were counted within the study area's capture zone. Some were owned by private

Table 2 Adenovirus detection rates and the mean \pm standard error from the six sampling points

Site	Distance from sewage treatment works (m)	Vol. of samples filtered (l)	No. of samples (n)	Adenovirus (%)	Mean \pm standard error ($P=0.0373$)
LZ	3000	360	36	1 (2.78)	0.03 \pm 0.03a
CF	2000	360	36	0 (0)	0.00 \pm 0.00b
FM	1500	360	36	2 (5.56)	0.06 \pm 0.04a
OG	700	360	36	0 (0)	0.00 \pm 0.00b
ST	<500	360	36	5 (13.89)	0.14 \pm 0.06a
RA	<500	360	36	3 (8.33)	0.08 \pm 0.05a
Total		2160	216	11(5.09)	

*Values followed by the same letter along the column are not significantly different based on Tukey's HSD at $P \leq 0.05$

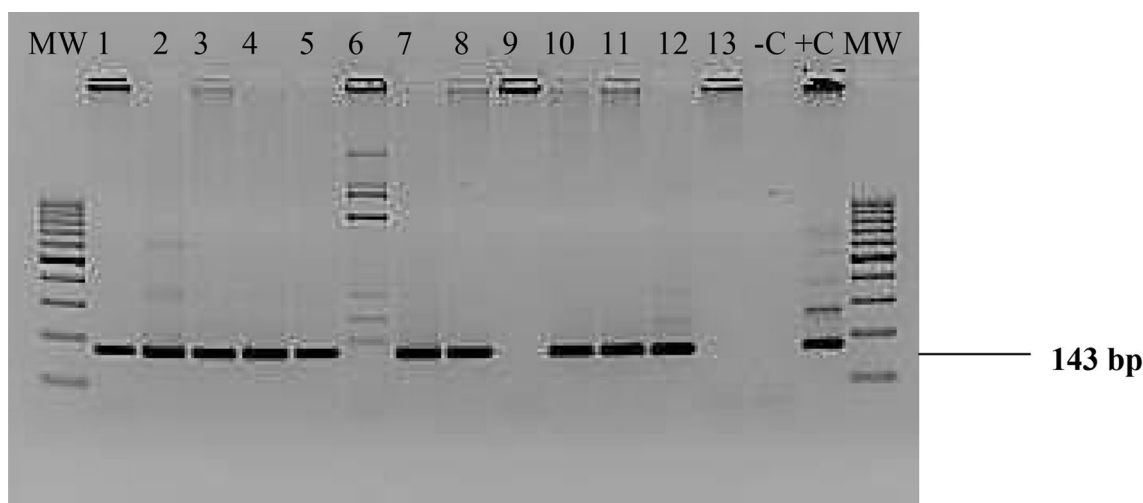


Fig. 3 Agarose gel electrophoresis showing PCR amplicons. *MW, 100 bp molecular weight marker (Promega); Lane 1, sample from LZ; Lanes 2–5, samples from site ST, Lanes 6–8, samples from site

FM, Lane 9 and 13 samples from sites CF & OG; Lanes 10 to 12, samples from site RA; +C, positive control and – C, negative control

households, while others were public, mainly located in public places such as the landing zone and open-air markets (Table 3). They were located at varying distances from the sampling sites with some in an abandoned condition.

Statistical analyses to assess if detection of the viruses was significantly different at distance from the potential sources of faecal contaminants revealed positive results. The regression analysis to compare the viral contamination rates with the pit latrines' average distances shows that there was a significant relationship with R^2 being 3.3 and a P -value of 0.007. The slope coefficient for distance from the pit latrines was -89.08 . The detection of adenoviruses increased with a decrease in distance from the pit latrines (Fig. 4).

Similarly, linear regression analysis to evaluate the detection of the relationship between the distance from the STP and the sampling points indicated some significant relationships ($R^2 = 1.9$, $P = 0.042$). Detection increased slightly with a decrease of every 500 m in distance from the sewage treatment plant (Fig. 5).

Discussion

Human adenoviruses have been reported to be some of the common contaminants of surface waters all over the world (Muscillo et al. 2008). The results of this study corroborate

Table 3 Location of the pit latrines and distance estimates from the nearest sampling point

Estimated distance from the nearest site (m)	No. of latrines	Global positioning system location	Ownership	Nearest sampling site
1–50	2	–0.51933, 34.46438	Private	RA
		–0.52114, 34.46456	Public	LZ
51–100	5	–0.52322, 34.45582	Public	LZ
		–0.52321, 34.45561	Public	LZ
		–0.51923, 34.46421	Private	RA
		–0.52153, 34.45940	Public	FM
		–0.52195, 34.46082	<i>Jua Kali</i> association	OG
100–150	5	–0.52258, 34.45877	Public	FM
		–0.52321, 34.45564	Public	FM
		–0.52188, 34.46111	<i>Jua Kali</i> association	OG
		–0.52301, 34.45596	Public	LZ
		–0.52296, 34.45599	Public	LZ
> 150	4	–0.52386, 34.45829	Industrial estate	CF
		–0.52222, 34.45942	Feeds Industry	FM
		–0.52255, 34.46316	Water Company	RA
		–0.52199, 34.46150	Vocational Institution	OG

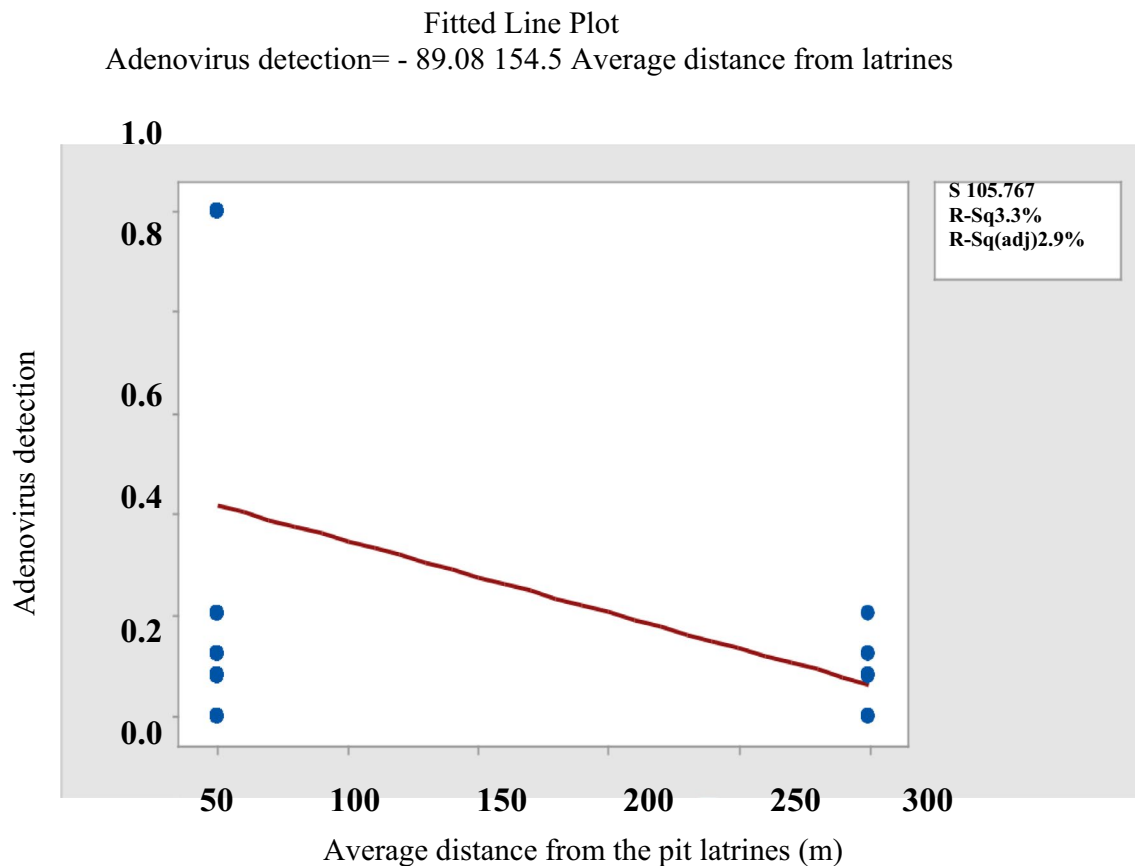


Fig. 4 Regression analysis between the estimated distance from pit latrines vs mean adenovirus detection

previous findings in other parts of the world including Asia (Lee et al. 2005), South America (Rigotto et al. 2010), and Europe (D’Ugo, et al. 2016), with HAdV being detected from 4 of the 6 study sites. The highest detection rate was from point ST (13.89%) a site that was characterized by lower human traffic. Contrastingly, a lower rate of human adenovirus detection was reported from site LZ (2.78%). In comparison to the rest of the sites, Site LZ was characterized by the highest level of human activity ranging from transport, fishing, recreation and trade. Site ST was situated in an area strongly impacted by wastewater discharge from the dilapidated sewage treatment works. The higher detection from this site could be due to the impact of the sewage effluent. Despite the sites being located in conventionally busy areas with activities such as industrialization and livestock farming respectively, no HAdV genomes were detected from points CF and OG.

The zero detection from sites CF and OG together with the low detection from site LZ may be due to lack of a direct source of faecal contamination in the vicinity of the said areas. A general potential reason for the poor detection at certain sites is that the concentrations of the viruses in environmental samples are often low and thus, conventional

molecular techniques may not easily confirm their presence (D’Ugo et al. 2016). Point RA had the second-highest number of positive samples for the HAdV genome at 8.33% of the total 36 samples analysed during the study period. This could be attributed directly to an informal urban settlement located on the site’s eastern side. The informal settlement is characterized by numerous latrines sank as close as about 50 m from the shore. Both ST and RA tend to be the sampling stretch’s most contaminated area, probably because they were situated close to the informal residential portion and sewage treatment facility. Site FM had a detection rate of 5.56%. The detection from this point could be attributed to increased human activities around the site as a result of fish trading as well as the use of the nearby pit latrines. However, it is noteworthy that the detection of the viral genomes from the different sites may not necessarily be as a result of contamination arising from the nearest possible sources of faecal pollutants. The contamination may also be due to possible transportation of the viruses from distant sites and sources resulting from the frequent changes in lake water flow and simulations.

The findings generally indicate that adenoviruses within the study environment are steadily circulating and persistent,

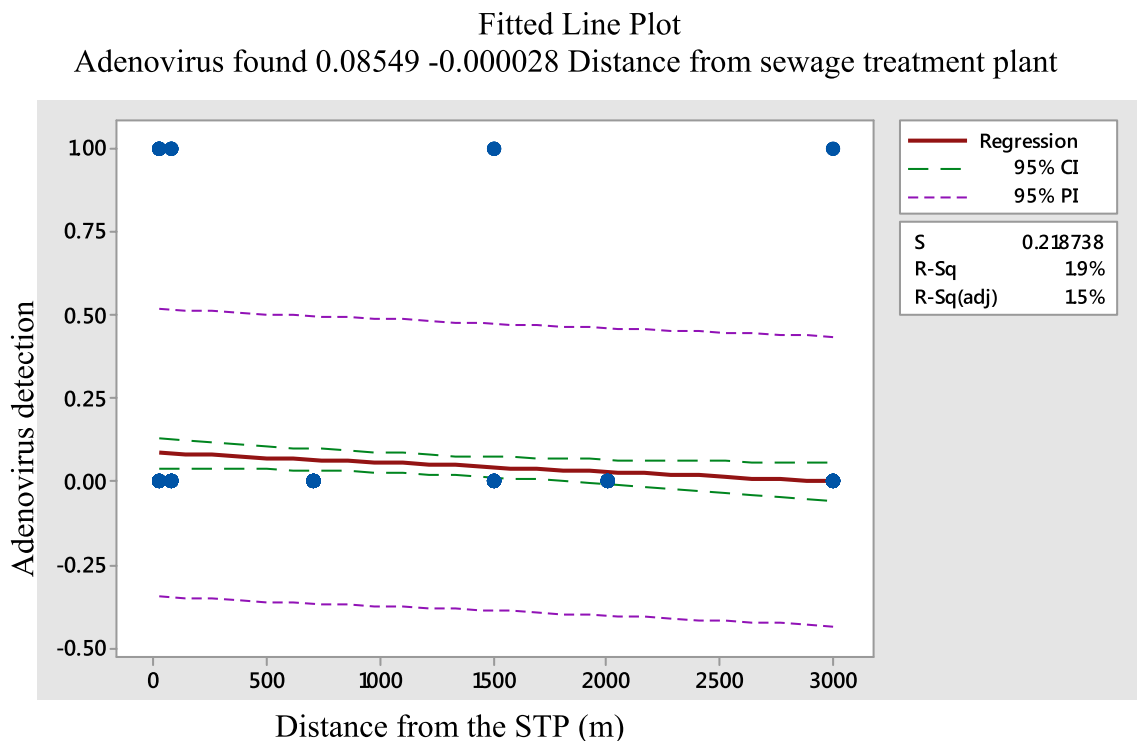


Fig. 5 Regression analysis between the estimated distance from STP vs mean adenovirus detection

an observation that is consistent with results published elsewhere (Fong et al. 2010). Even though all the adenoviruses detected might not necessarily be infectious, the findings suggest that the local community is at potential risk of adenovirus infection. In past studies, human adenovirus-related outbreaks of infection have been reported especially in recreational waters (van Heerden et al. 2005). Human adenoviruses are highly stable in the environment. They are less influenced by changes in physical environments such as temperature, pH, humidity and UV radiation, as well as the conventional wastewater treatment procedures such as sedimentation and chlorination. Their stability in the environment has been attributed to the double-stranded DNA structure (Bofill-Mas et al. 2006; Gerba et al. 2008; Fong et al. 2010). It has also been reported that they are easily detectable from the environmental samples compared to other potential viral indicators (Albinana-Gimenez et al. 2009).

The results of the statistical analysis on the correlation between the approximate distance from the key point sources of faecal pollution (Pit latrines and sewage treatment works) and the detection indicate that viruses may probably end up in the lake water through various pathways. One such pathway is the aquifer pathway which involves the movement of viruses to surface waters through the subsoil (Fong et al. 2007). Due to their minute size, the viruses can pass through porous sediments when infiltration of polluted sources (Fong et al. 2007). The findings indicate that

establishing such faecal disposal facilities nearshore to the surface waters and inadequate maintenance are likely to increase the rates of pollution through the localized pathway systems. The correlation results are quite in agreement with numerous studies published elsewhere relating to the identification of adenoviruses and sources of faecal pollutants (Magwalivha et al. 2010). The findings reaffirm the assumption that HAdVs can be regarded as a reliable predictor for viral contamination of the environment. This is critical for environmental surveillance in the study region where pathogen data are still limited.

Conclusion

The detection of human adenoviruses confirms that the LV waters were being impacted by faecal matter. Source tracking of the pollution markers suggests a correlation to the impact of the wastewater treatment works and the pit latrines along the shoreline. This thus demonstrates the value of environmental monitoring and the need to implement efficient ways of removing enteric viruses during wastewater treatment processes. Continuous monitoring of enteric viral contamination of surface waters such as LV is important not only as a strategy for mitigating future public health emergencies, but also to raise public awareness on safe methods of faecal waste disposal. For example, the unregulated

sinking of pit latrines along the shoreline undermines the goal of the much-desired continuous environmental monitoring and effective wastewater treatment processes. The data may provide a baseline for information and understanding of enteric viral contamination in the region for a proper design for environmental surveillance.

Acknowledgements Michael Wasonga acknowledges a Karachuonyo Constituency Development Fund bursary for postgraduate students. We thank the entire staff of the Enteric Virus research group at the Institute of Primate Research and the Kenyatta University's Department of Biochemistry, Microbiology and Biotechnology for the support.

Author Contributions This work was carried out in collaboration between all authors. WMO conceptualized and designed the study, carried out fieldwork, performed the experiments and the statistical data analysis and wrote the first draft of the manuscript. MJ, and OO were involved in formal analyses of the study. All authors read and approved the final manuscript.

Funding Not applicable.

Data Availability The Metadata used to support the findings of this study have been deposited in the Kenyatta University Institutional repository at <https://41.89.10.36>

Compliance with Ethical Standards

Conflict of interest The authors declare that there were no competing interests.

References

- Akihara, S., Phan, T. G., Nguyen, T. A., Hansman, G., Okitsu, S., & Ushijima, H. (2005). Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. *Archives of Virology*, *150*, 2061–2075.
- Albinana-Gimenez, N., Clemente-Casares, P., Calgua, B., Huguet, J. M., Courtois, S., & Girones, R. (2009). Comparison of methods for concentrating human adenoviruses, polyomavirus and noroviruses in source waters and drinking water using quantitative PCR. *Journal of Virological Methods*, *158*, 104–109.
- Allard, A., Albinsson, B., & Wadell, B. (1992). Detection of adenoviruses in stools from healthy persons and patients with diarrhoea by two-step polymerase chain reaction. *Journal of Medical Virology*, *37*, 149–157.
- Allard, A., Girones, R., Juto, P., & Wadell, G. (1990). Polymerase chain reaction for detection of adenoviruses in stool samples. *Journal of Clinical Microbiology*, *28*, 2659–2667.
- Baggi, F., & Peduzzi, R. (2000). Genotyping of rotaviruses in environmental water and stool samples in southern Switzerland by nucleotide sequence analysis of 189 base pairs at the 5' end of the VP7 gene. *Journal of Clinical Microbiology*, *38*, 3681–3685.
- Baker, N. J., & Eric, E. (2008). Integrating wildlife in natural resources management for tourism and community livelihoods in Lake Victoria basin: East Africa. *African Journal of Environmental Science Technology*, *2*, 287–295.
- Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., et al. (2006). Quantitation and stability of human adenoviruses and polyomavirus in wastewater matrices. *Applied and Environmental Microbiology*, *72*, 7894–7896.
- Byamukama, D., Kansime, F., Mach, R. L., & Farnleitner, A. H. (2005). Determination of *Escherichia coli* contamination with chromocult coliform agar showed a high level of discrimination efficiency for differing fecal pollution levels in tropical waters of Kampala, Uganda. *Applied and Environmental Microbiology*, *66*, 864–868.
- Carter, M. J. (2005). Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology*, *6*, 1354–1380.
- Chaberny, I. F., Schnitzler, P., Geiss, H. K., & Wendt, C. (2003). An outbreak of epidemic keratoconjunctivitis in a pediatric unit due to adenovirus type 8. *Infection Control and Hospital Epidemiology*, *24*, 514–519.
- Cheng, J., Qi, X., Chen, D., Xu, X., Wang, G., Dai, Y., et al. (2016). Epidemiology and transmission characteristics of human adenovirus type 7 caused acute respiratory disease outbreak in military trainees in East China. *American Journal of Translational Research*, *8*(5), 2331–2342.
- Chigor, V. N., & Okoh, A. I. (2012). Quantitative RT-PCR detection of hepatitis A virus, rotaviruses and enteroviruses in the Buffalo River and source water dams in the Eastern Cape Province of South Africa. *International Journal of Environment Research and Public Health*, *9*, 4017–4032.
- Chmielewicz, B., Benzler, J., Pauli, G., Krause, G., Bergmann, F., & Schweiger, B. (2005). Respiratory disease caused by a species B2 adenovirus in a military camp in Turkey. *Journal of Medical Virology*, *77*, 232–237.
- D'Ugo, E., Marcheggiani, S., Fioramonti, I., Giuseppetti, R., Spurio, R., Helmik, K., et al. (2016). Detection of enteric viruses in fresh water from European countries. *Food and Environmental Virology*, *8*, 206–2014.
- Dhingra, A., Hage, E., Ganzenmueller, T., et al. (2019). Molecular evolution of 533 human adenovirus (HAdV) species C. *Scientific Reports*, *9*, 1039.
- Donaldson, K. A., Griffin, D. W., & Paul, J. H. (2002). Detection, quantitation and identification of enteroviruses from surface waters and sponge tissue from the Florida Keys using RT-PCR. *Water Resources*, *36*, 2505–2514.
- Dong, Y., Kim, J., & Lewis, G. D. (2009). Evaluation of methodology for detection of human adenoviruses in wastewater, drinking water, stream water and recreational waters. *Journal of Applied Microbiology*, *108*, 800–809.
- Fong, T., Mansfield, D. L., Wilson, D. J., Schwab, L., Molloy, S., & Rose, J. B. (2007). Massive microbiological groundwater contamination associated with a waterborne outbreak in Lake Erie, South Bass Island. *Environmental Health Perspective*, *115*, 856–864.
- Fong, T. T., Phanikumar, M. S., Xagorarakis, I., & Rose, J. B. (2010). Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan River. *Applied and Environmental Microbiology*, *76*, 715–723.
- Gerba, C. P., Gramos, D. M., & Nwachuku, N. (2008). Comparative inactivation of adenoviruses and enteroviruses by UV light. *Applied and Environmental Microbiology*, *68*, 5167–5169.
- Gibson, K. E. (2014). Viral pathogens in water: occurrence, public health impact, and available control strategies. *Current Opinion in Virology*, *4*, 50–57.
- Gibson, K. E., & Schwab, K. J. (2011). Detection of bacterial indicators and human and bovine enteric viruses in surface water and groundwater sources potentially impacted by animal and human wastes in Lower Yakima Valley, Washington. *Applied and Environmental Microbiology*, *77*, 355–362.
- Haramoto, E., Katayama, H., Oguma, K., & Ohgaki, S. (2005). Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses

- in the Tamagawa River in Japan. *Applied and Environmental Microbiology*, 71(5), 2403–2411.
- Haramoto, E., Kitajima, M., Hata, A., Torrey, J., Masago, Y., Sano, D., et al. (2018). A review on recent progress in the detection methods and prevalence of human enteric viruses in water. *Water Research*, 135, 168–186.
- Harrach, B., Benkő, M., Both, G. W., Brown, M., Davison, A. J., Echavarría, M., et al. (2012). Family Adenoviridae. In A. M. Q. King, M. J. Adams, E. B. Carstens, & E. J. Lefkowitz (Eds.), *Virus taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses* (pp. 125–141). New York: Elsevier.
- Jalal, H., Bibby, J. W., Tang, J., Bennett, C., Kyriakou, K., Peggs, D., et al. (2005). First reported outbreak of diarrhea due to adenovirus infection in a hematology unit for adults. *Journal of Clinical Microbiology*, 43, 2575–2580.
- Kabue, J. P., Meader, E., Hunter, P. R., & Potgieter, N. (2016). Human Norovirus prevalence in Africa: A review of studies from 1990 to 2013. *Tropical Medicine & International Health*, 21(1), 2–17.
- Kenya Population and Housing Census (KPHC). (2019). Distribution of Population by Administrative Units. Kenya National Bureau of Statistics. Volume II:
- Kishida, N., Noda, N., Haramoto, E., Kawaharasaki, M., Akiba, M., & Sekiguchi, Y. (2014). Quantitative detection of human enteric adenoviruses in river water by microfluidic digital polymerase chain reaction. *Water Science Technology*, 70, 555–560.
- Kittigul, L., Ekchaloemkiet, S., Utrarachkij, F., Siripanichgon, K., Sujirarat, D., Pungchitton, S., et al. (2005). An efficient virus concentration method and RT-nested PCR for detection of rotaviruses in environmental water samples. *Journal of Virological Methods*, 124, 117–122.
- Kiulia, N. M., Nyaga, M. M., Seheri, M. L., Wolfaardt, M., van Zyl, W. B., Esona, M. D., et al. (2014). Rotavirus G and P types circulating in the Eastern region of Kenya: Predominance of G9 and emergence of G12 genotypes. *Journal of Paediatric Infectious Diseases*, 33, S85–S88.
- Lambertini, E., Spencer, S. K., Bertz, P. D., Loge, F. J., Kieke, B. A., & Borchardt, M. A. (2008). Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. *Applied and Environmental Microbiology*, 74, 2990–2996.
- Lee, S. H., Lee, C., Lee, K. W., Cho, H. B., & Kim, S. J. (2005). The simultaneous detection of both enteroviruses and adenoviruses in environmental water samples including tap water with an integrated cell culturemultiplex-nested PCR procedure. *Journal of Applied Microbiology*, 98, 1020–1029.
- LVEMP. (2002). Lake Victoria Environmental Management Project, Integrated Water Quality / Limnology Study for Lake Victoria, Water Quality and Ecosystem Management Component, Part II Technical report. Retrieved May 15, 2017, from <https://repository.eac.int:8080/handle/11671/692/>.
- Magwaliha, M., Wolfaardt, M., Kiulia, M., van Zyl, W. B., Mwenda, J. M., & Taylor, M. B. (2010). High prevalence of species D human adenoviruses in fecal specimens from Kenyan children with diarrhoea. *Journal Medical Virology*, 82, 77–84.
- Maheshwari, G., Risat, J., Lisa, M., & David, H. (2004). Thermal inactivation of adenovirus type 5. *Journal of Virological Methods*, 118(2), 141–146. <https://doi.org/10.1016/j.jviromet.2004.02.003>.
- Meqdam, M. M., & Thwiny, I. R. (2007). Prevalence of group A rotavirus, enteric adenovirus, norovirus and astrovirus infections among children with acute gastroenteritis in Al-Qassim, Saudi Arabia. *Journal of Medical Science*, 23, 551–555.
- Muscillo, M., Pourshaban, M., Iaconelli, M., Fontana, S., Di Grazia, A., Manzara, S., et al. (2008). Detection and quantification of human adenoviruses in surface waters by nested PCR, TaqMan real-time PCR and cell culture assay. *Water Air & Soil Pollution*, 19, 83–93.
- Mwirigi, P.M., Gikuma-Njuru, P., Okungu, J.O., Abuodha J.O.Z., & Hecky, R.E. (2005). Lake Victoria monitoring of the pelagic, littoral, river mouths and near shore urban environments, Kenya. Lake Victoria environment Management project (LVEMP), Kenya national water quality synthesis report.
- Nascimento, M. A., Maria, E. M., Camila, D. S., & Célia, R. M. B. (2015). Recombinant adenovirus as a model to evaluate the efficiency of free chlorine disinfection in filtered water samples. *Virology Journal*. <https://doi.org/10.1186/s12985-015-0259-7>.
- Odada, E. O., Olago, D. O., Kulindwa, K., Ntiba, M., & Wandiga, S. (2004). Mitigation of environmental problems in Lake Victoria, East Africa: Casual Chain and Policy Options Analyses. *Ambio*, 33, 13–23.
- Ogorzaly, L., Bertrand, I., Paris, M., Maul, A., & Gantzer, C. (2010). Occurrence, survival, and persistence of human adenoviruses and specific RNA phages in raw groundwater. *Applied and Environmental Microbiology*, 76, 8019–8025.
- Onyuka, J., Kakai, R., Onyango, D., Arama, P., Gichuki, J., & Ofulla, O. (2011). Prevalence and antimicrobial susceptibility patterns of enteric bacteria isolated from water and fish in Lake Victoria Basin of Western Kenya. *World Academy of Science, Engineering and Technology*, 75, 1320–1350.
- Prevost, B., Goulet, M., Lucas, F. S., Joyeux, M., Moulin, L., & Wurtzer, S. (2016). Viral persistence in surface and drinking water: Suitability of PCR pre-treatment with intercalating dyes. *Water Research*, 91, 68–76.
- Puig, M., Jofre, J., Lucena, F., Allard, A., Wadell, G., & Girones, R. (1994). Detection of adenoviruses and enteroviruses in polluted waters by nested PCR amplification. *Applied and Environmental Microbiology*, 60, 2963–2970.
- Rigotto, C., Victoria, M., Moresco, V., Kolesnikovas, C. K., Corrêa, A. A., Souza, D. S., et al. (2010). Assessment of adenovirus, hepatitis A virus and rotavirus presence in environmental samples in Florianópolis, South Brazil. *Journal of Applied Microbiology*, 109, 1979–1987.
- Rodriguez, R. A., Pepper, I. A., & Gerba, C. P. (2009). Application of PCR based methods to assess the infectivity of enteric viruses in environmental samples. *Applied and Environmental Microbiology*, 75, 297–307.
- Santos, F. M., Vieira, M. J., Garrafa, P., Monezi, T. A., Pellizari, V. H., Hársi, C. M., et al. (2004). Discrimination of adenovirus types circulating in urban sewage and surface polluted waters in São Paulo city, Brazil. *Water Science and Technology*, 4, 79–85.
- Shimizu, H., Phana, G., Nishimuraa, S., Okitsua, S., Maneekarnb, N., & Ushijima, H. (2007). An outbreak of adenovirus serotype 41 infection in infants and children with acute gastroenteritis in Maizuru City, Japan. *Infectious Genotypic Evolution*, 7, 279–284.
- Symonds, E. M., Griffin, D. W., & Breitbart, M. (2009). Eukaryotic viruses in wastewater samples from the United States. *Applied and Environmental Microbiology*, 75(5), 1402–1409.
- Tetzner, R., Dietrich, D., & Distler, J. (2007). Control of carry-over contamination for PCR-based DNA methylation quantification using bisulfite treated DNA. *Nucleic Acids Research*, 35(1), e4. <https://doi.org/10.1093/nar/gkl955>.
- UN-HABITAT. (2010a). Strategic urban development plan for Homa Bay municipality (2008–2030). United Nations Human Settlement Programme (UN-HABITAT); Nairobi, Kenya.
- UN-HABITAT. (2010b). Lake Victoria water and sanitation initiative. United Nations Human Settlement Programme (UN-HABITAT); Nairobi, Kenya.
- Van Heerden, J., Ehlers, M. M., & Grabow, W. O. (2005). Detection and risk assessment of adenoviruses in swimming pool water. *Journal of Applied Microbiology*, 99, 1256–1264.
- Van Zyl, W. B., Zhou, N. A., Wolfaardt, M., Matsapola, P. N., Ngwana, F. B., Symonds, E. M., et al. (2019). Detection of potentially pathogenic enteric viruses in environmental samples from Kenya using

- the bag-mediated filtration system. *Water Supply*, 19, 1680–1676. <https://doi.org/10.2166/ws.2019.046>.
- Vilaginès, P., Sarrette, B., Husson, G., & Vilaginès, R. (1993). Glass wool for virus concentration at ambient water pH level. *Water Science Technology*, 27, 299–306.
- Vilaginès, Ph., Suarez, A., Sarrette, B., & Vilaginès, R. (1997). Optimisation of the PEG reconcentration procedure for virus detection by cell culture or genomic amplification. *Water Science Technology*, 35, 455–459.
- WHO. (2015). Guidelines on Environmental Surveillance for Detection of Polioviruses. Retrieved 10 April, 2018, from https://polioeradication.org/wpcontent/uploads/2016/07/GPLN_GuidelineES_April2015.pdf.
- Wolfaardt, M., Moe, C. L., & Grabow, W. O. K. (1995). Detection of small rounded structured viruses in clinical and environmental samples by polymerase chain reaction. *Water Science Technology*, 31, 375–438.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.