# Viruses in the environment – presence and diversity of bacteriophage and enteric virus populations in the Umhlangane River, Durban, South Africa

Veronna Marie and Johnson Lin

## ABSTRACT

Due to the continued persistence of waterborne viral-associated infections, the presence of enteric viruses is a concern. Notwithstanding the health implications, viral diversity and abundance is an indicator of water quality declination in the environment. The aim of this study was to evaluate the presence of viruses (bacteriophage and enteric viruses) in a highly polluted, anthropogenic-influenced river system over a 6-month period at five sampling points. Cytopathic-based tissue culture assays revealed that the isolated viruses were infectious when tested on Hep-G2, HEK293 and Vero cells. While transmission electron microscopy (TEM) revealed that the majority of the viruses were bacteriophages, a number of presumptive enteric virus families were visualized, some of which include *Picornaviridae*, *Adenoviridae*, *Polyomaviridae* and *Reoviridae*. Finally, primer specific nested polymerase chain reaction (nested-PCR)/reverse transcription-polymerase chain reaction (RT-PCR) coupled with BLAST analysis identified human adenovirus, polyomavirus and hepatitis A and C virus genomes in river water samples. Taken together, the complexity of both bacteriophage and enteric virus populations in the river has potential health implications. Finally, a systematic integrated risk assessment and management plan to identify and minimize sources of faecal contamination is the most effective way of ensuring water safety and should be established in all future guidelines.

**Key words** | bacteriophages, cytopathic effect, enteric viruses, nested-PCR/RT-PCR, tangential flow filtration, waterborne viral infections

**Veronna Marie**
**Johnson Lin** (corresponding author)
Discipline of Microbiology, School of Life Sciences,
University of KwaZulu-Natal (Westville),
Private Bag X54001, Durban,
South Africa
E-mail: *linj@ukzn.ac.za*

## INTRODUCTION

The contamination of water bodies with diverse microbial communities is a major concern to public health (Pandey *et al.* 2014). Diarrhoeal outbreaks caused by waterborne pathogens is alarming, with most cases reported in developing countries due to the lack of proper drinking water and sanitation infrastructure (Hofstra 2011). The World Health Organization (WHO) approximates 1.8 billion people worldwide currently utilizing drinking water resources that are contaminated by faecal pollution (WHO 2017). Among these pathogens, the greatest cause for concern comes from the prevalence of enteric viruses in water (Leifels *et al.* 2016). Enteric viruses are excreted from the faeces of infected individuals at very high numbers ($10^{11}$/g faeces) while concomitantly exceeding other existing microbial communities by 10–100-fold in water environments (Ganesh & Lin 2013). Apart from the socio-economic implications of waterborne virus-related sicknesses in both developing and developed nations, the extent of the burden and impact of viral disease is far concentrated in regions with enormous environmental contaminations (Rodríguez-Díaz *et al.* 2009). Over 140 different types of viruses are known to infect humans, resulting in a variety

doi: 10.2166/wh.2017.066

of illnesses (Chigor & Okoh 2012). While gastroenteritis is the most common outcome, several acute illnesses such as meningitis, hepatitis, conjunctivitis and muscular syndromes (myocarditis, fibromyalgia) are associated with enteric viral infections. Waterborne viral infections have also been implicated in some chronic diseases such as chronic fatigue syndrome and diabetes (La Rosa *et al.* 2012). Enteric viruses enter water systems through agricultural and urban runoffs, septic systems, sewage outfalls and wastewater discharge which are then transmitted through the faecal–oral route to finally replicate in the gastrointestinal tract of humans and animals (Coudray-Meunier *et al.* 2013).

Among the many types of enteric viruses reported in aquatic environments, the most common include hepatitis A viruses (Dongdem *et al.* 2009), adenoviruses (Chigor & Okoh 2012), polyomaviruses (Haramoto *et al.* 2010) and noroviruses (Lopman *et al.* 2016). Hepatitis A viruses cause approximately 40% of severe hepatitis annually (Redwan & Abdullah 2012) with numerous outbreaks reported on a global scale (Frank *et al.* 2007; Robesyna *et al.* 2009; Chen *et al.* 2017). Furthermore, in comparison to hepatitis B, infection with hepatitis A viruses are three-fold greater in travellers (Redwan & Abdullah 2012). Although the incidence of hepatitis A infections varies across countries, sporadic hepatitis A outbreaks are mostly observed in non-industrialized regions with poor hygiene conditions (La Rosa *et al.* 2014). Adenoviruses cause a variety of clinical symptoms with many outbreaks related to recreational water exposure (Vieira *et al.* 2012). Adenovirus detection is simple and their numbers far exceed hepatitis A and enteroviruses in many aquatic habitats. Furthermore, adenoviruses show greater stability to chlorination and UV irradiation than enteroviruses (Hundesa *et al.* 2006), allowing them to persist in the environment for much longer time periods. Human BK and JC polyomaviruses cause chronic infections in humans and are frequently defecated in municipal sewage and urine. These viruses have been linked to other important diseases in immune-compromised individuals as well as some types of human cancer such as colorectal cancer (Hundesa *et al.* 2006). The ubiquitous noroviruses are the most common cause of gastroenteritis, associated with 18% of global diarrhoea cases and responsible for 212,000 deaths yearly on a global scale (Lopman *et al.* 2016). Even though hepatitis C viruses are not waterborne but fluid related (blood-borne), they are important aetiological agents of non-A, non-B hepatitis in people (Aslanzadeh *et al.* 1996). East and Central Asia as well as northern Africa are regions mostly affected by hepatitis C infections (WHO 2016). Beld *et al.* (2000) frequently detected hepatitis C viruses in the faeces of chronically infected patients. Therefore, speculation of their possible presence in water through surface runoffs could be made. Moreover, studies involving the transmission of hepatitis C viruses in injection drug users have demonstrated the presence of these viruses in water containers (Doerrbecker *et al.* 2013) and rinse water (Thorpe *et al.* 2002).

Current water quality guidelines mostly rely on bacterial indicators of faecal pollution. However, bacterial pathogens have been poorly correlated with the presence of enteric viruses (Lee *et al.* 2014) and are more susceptible than viruses to some water treatment processes (Rodríguez *et al.* 2008). Bacteriophages (or phages) are viruses that infect bacteria and are recommended as alternate indicators of faecal pollution (Ganesh *et al.* 2014). Apart from morphological and internal chemical similarities to enteric viruses, bacteriophage detection is usually cost-effective and easier to apply than enteric virus detection (Leclerc *et al.* 2000). However, the recommended plaque assays require long experimental time frames, are dependent on bacterial host viability and suffer from errors during enumeration (Edelman & Barletta 2003). Importantly, Wu *et al.* (2011) demonstrated via a logistic regression analysis that the correlation between coliphages and pathogens in water was poor ($p = 0.186$) where only 40 out of the 85 cases were correlated.

To investigate the presence and diversity of viruses (bacteriophages and enteric viruses) in the Umhlangane River, a tangential flow filtration (TFF) process was adopted to concentrate the virus particles. This method has been reported to effectively concentrate virus particles by reducing filter clogging via parallel fluid movement across the membrane filters. Moreover, TFF ensures that pore sizes and cross-flow prevents bacterial contamination of the viral concentrate (Cai *et al.* 2015). Together with TFF, various comparative detection methods including transmission electron microscopy (TEM) and tissue culture were used to assess the nature of the virus particles. Finally, a nested polymerase chain reaction (nested-PCR)/reverse

transcription-polymerase chain reaction (RT-PCR) was used to evaluate the presence of human adenovirus, polyomavirus and hepatitis A and C virus genomes in the Umhlangane River.

## METHODS

### Water sample collection

The Umhlangane River, located in the heart of Durban is surrounded by a plethora of both developed and undeveloped societies. The river spans approximately 15 km in length and drains into KwaZulu-Natal's main drinking water catchments, the Umgeni River (Hadlow 2011). In addition to being a conduit for domestic and industrial wastes, the Umhlangane River receives animal waste from nearby farms. Moreover, the surrounding informal settlement or squatter camp communities directly utilize the river water for drinking as well as other domestic practices (Hadlow 2011).

Twenty litres (20 L) of river water was collected at five different sampling points (designated P1 to P5), described in Table 1. Each sampling site was selected based on different land use zones to assess the extent of its influence on the catchment. Sampling was conducted in the second week of every month over a 6-month period, commencing in April 2014 and concluding in September 2014, for virus concentration and analysis. Water samples were collected in 25 L plastic drums (previously disinfected with 70% (v/v) alcohol and rinsed with deionized water). At each sampling point, the drums were rinsed with river water prior to being plunged approximately 0.3–0.5 m below the water surface to circumvent the disinfectant effect of UV light (Jurzik *et al.* 2010). Samples were transported on ice to the Discipline of Microbiology, University of KwaZulu-Natal (Westville campus) for virus concentration.

**Table 1** | Coordinates and description of the five sampling points

| Sampling points | GPS coordinates | | Site description |
|---|---|---|---|
| | Latitude | Longitude | |
| P1 | 29° 42′47″S | 30° 59′33″E | Phoenix industrial |
| P2 | 29° 43′35″S | 31° 00′21″E | Upstream KwaMashu wastewater treatment plant |
| P3 | 29° 43′35″S | 30° 00′21″E | Natural wetlands |
| P4 | 29° 45′39″S | 30° 01′11″E | Riverhorse Valley business estate |
| P5 | 29° 46′10″S | 30° 00′24″E | Springfield industrial |

### Primary virus concentration

A modified TFF process described by Ganesh *et al.* (2014) was used to concentrate viruses from the collected water samples (Figure 1). Briefly, 20 L of river water was passed through a 0.45 μm sediment filter (Merck-Millipore Corp.) at 130 mL/min to remove large debris and solids. Virus concentration then involved two separate steps. First, the water was filtered through a 142 mm diameter, 0.22 μm membrane filter (Merck-Millipore Corp.) at 330 mL/min to remove all bacteria. The second step further concentrated the viruses from the 0.22 micron concentrate through a 100 kDa (molecular weight cut-off) cartridge filter. The resulting retentate was then re-circulated through the system until approximately 500 mL of sample remained.

### Secondary virus concentration

Re-concentration of the TFF samples was carried out using ultracentrifugation according to a procedure described by Colombet *et al.* (2007) with some modifications. Six tubes of 28 mL retentate were ultracentrifuged for 2.5 hours at 130,000 × *g* (i.e., 29,000 revolutions per minute (rpm); 4 °C) in a SW-32 *Ti* rotor (Optima L-100 XP, Beckman Coulter). The viral pellets were re-suspended in 500 μL phosphate buffered saline (PBS; pH 7.2), pooled together (final volume of 3 mL) and stored at −20 °C until further analyses.

### Tissue culture experiments

Prior to presumptive identification, cell lines were used to determine the infectious nature of the viruses in the river water. Viruses concentrated on the first (April) and fourth (July) months were tested for infectivity. Three different cell lines were used: (i) human hepatocellular carcinoma

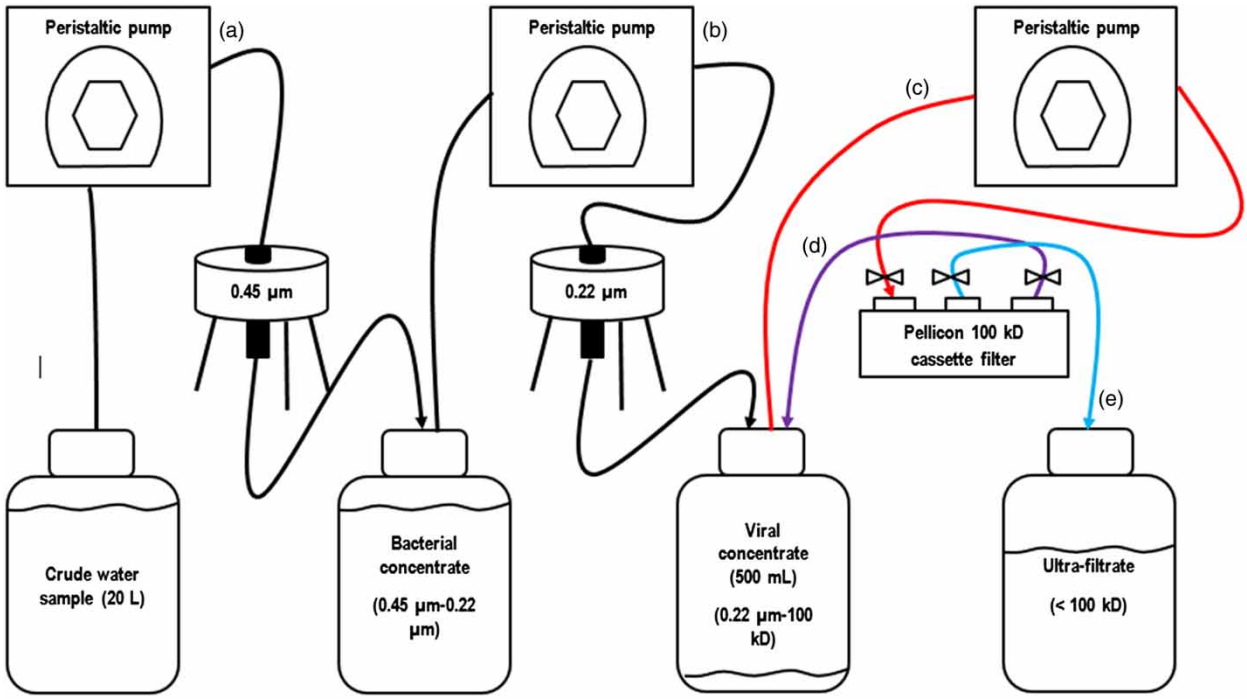


**Figure 1** | Schematic diagram of TFF procedure to concentrate viral particles from the river water samples (adapted from Ganesh *et al.* (2014) with some modifications). Debris removal and bacterial concentrate (a), removal of bacteria (b), feed (c), retentate (d) and permeate (e).

(Hep-G2), (ii) African green monkey kidney cells (Vero) and (iii) human embryonic kidney cells (HEK293). The cells were monitored daily using an inverted microscope (Olympus) at 400 × magnification for the production of a cytopathic effect (CPE) indicating positive virus infectivity. Adenovirus 9 VR-1086 and hepatitis A VR-1402 controls were tested on the three cell lines during both experiments. Detailed experimental procedures are described in Ganesh *et al.* (2014).

## TEM for viral diversity

Viral morphology and diversity was examined using TEM. After secondary concentration, one drop of each water sample was placed onto a carbon-coated grid (Electron Microscopy Sciences, Fort Washington, PA) for 2 minutes, stained with 4% uranyl acetate solution for 30 s, rinsed with deionized water for 10 s and air dried prior to visualization with a high resolution TEM (JEOL 2100). Electron micrographs of the virus particles were taken between 250,000 and 500,000 × magnification. Bacteriophage and virus particles were measured for size and compared to known viruses previously described in the literature for presumptive identification.

## Molecular detection of human enteric viruses in the Umhlangane River

Viral nucleic acids (total RNA and DNA) were extracted separately from 1 mL samples each using the High Pure Viral RNA and DNA Kits (Roche Diagnostics, Germany), respectively. The quantity and quality of the extracts were measured using the NanoDrop 2200 spectrophotometer (Thermo Scientific, Finland). For RNA samples, first strand cDNA synthesis was carried out using the DyNamo™ cDNA Synthesis Kit (Finnzymes, Thermo Fischer Scientific, Finland). Thereafter, primer specific (Table 2) nested-PCR was performed for four viral populations as described below. Five microlitres of the PCR product from the first round was used as a template for the second round for all reactions.

Following nested-PCR, random positive bands were selected with their primer sets and sequenced (Inqaba Biotech, South Africa). The sequences were analysed by

**Table 2** | Primer sequences for the PCR amplification of the four viral groups

| Primer | Primer sequence (5′ – 3′) | Amplicon size | Reference |
|---|---|---|---|
| Adenoviruses (A-F) | | | |
| AV-A1 | GCCGCAGTGGTCTTACATGCACATC | 300 bp | Allard *et al.* (1992) |
| AV-A2 | CAGCACGCCGCGGATGTCAAAGT | | |
| AV-B1[a] | GCCACCGAGACGTACTTCAGCCTG | 143 bp | |
| AV-B2[a] | TTGTACGAGTACGCGGTATCCTCGCGGTC | | |
| Polyomaviruses | | | |
| P1 | GTATACACAGCAAAGGAAGC | 630 bp | McQuaig *et al.* (2006) |
| P2 | GCTCATCAGCCTGATTTTGG | | |
| P3[a] | AGTCTTTAGGGTCTTCTACC | 173 bp | |
| P4[a] | GGTGCCAACCTATGGAACAG | | |
| Hepatitis A viruses | | | |
| HHA1 | TGCAAATTAYAAYCAYTCTGATGA | 532 bp | Pina *et al.* (2001) |
| HHA2 | TTTCTGTCCATTTYTCATCATTC | | |
| HHA3[a] | TTYAGTTGYTAYTTGTCTGT | 436 bp | |
| HHA4[a] | TCAAGAGTCCACACACTTC | | |
| Hepatitis C viruses | | | |
| HCV1 | ACTGTCTTCACGCAGAAAGCGTCTAGCCAT | 271 bp | Hu *et al.* (2003) |
| HCV2 | CGAGACCTCCCGGGGCACTCGCAAGCACCC | | |
| HCV3[a] | ACGCAGAAAGCGTCTAGCCATGGCGTTAGT | 255 bp | |
| HCV4[a] | TCCCGGGGCACTCGCAAGCACCCTATCAGG | | |

bp, base pair.
[a]Nested primers.

BLAST (http://www.ncbi.nlm.nih.gov/BLAST) to confirm the identities of the presumptive positive PCR products.

### Human adenoviruses

The hexon gene of 47 different adenovirus serotype genomes were amplified (Allard *et al.* 1992). Both rounds contained an additional 0.4 mM $MgCl_2$. The PCR conditions were as follows: 4 min at 94 °C, 40 cycles of 92 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min. Final elongation was at 72 °C for 5 min. A positive control (cell cultured adenovirus) was included for all reactions.

### Human polyomaviruses

The human-specific BK and JC polyomavirus genomes were amplified according to McQuaig *et al.* (2006) with some modifications. Amplification conditions were carried out accordingly: 94 °C for 2 min, 45 cycles of 94 °C for 20 s, 55 °C for 20 s, 72 °C for 20 s. Final elongation was 72 °C for 2 min. Both rounds had an additional 0.5 mM $MgCl_2$.

### Hepatitis A viruses

The VP1/VP2 region of the hepatitis A virus genomes were amplified (Pina *et al.* 2001). The PCR conditions included: 95 °C for 3 min, 30 cycles of 95 °C for 60 s, 42 °C for 60 s and 72 °C for 60 s. Final extension was 72 °C for 5 min. Both rounds contained an additional 0.5 mM $MgCl_2$.

### Hepatitis C viruses

The 5′ untranslated region (5′ UTR) of the hepatitis C virus genomes were amplified according to Hu *et al.* (2003) with some modifications. The PCR were run under the following conditions: 95 °C for 3 min, 30 cycles of 95 °C for 60 s, 50 °C for 60 s and 72 °C for 60 s. Final extension was 72 °C for 5 min.

### Quality control

The probability of sample contamination due to DNA amplicons or cross-contamination was reduced through the practice of standard molecular preparation protocols. Separate areas were used to prepare the reagents and manipulate the amplified samples. Negative controls were included in all reactions and a positive control was also used where available. All RNA samples were manipulated in a separate RNA room (DNase/RNase-free zone) that contained PCR pipettes, filter tips, centrifuges, etc., specifically designated for RNA work only. Furthermore, master-mixes for the PCR reactions were prepared in the bio-safety cabinet to prevent contamination.

## RESULTS

### Virus infectivity

The CPE of the viral concentrate was based on morphological changes of the cells, the visibility of granulated, elongated cells, vacuole production and the loss of cell-to-cell adherence and the wall of the flask. The viral concentrate was capable of causing infectivity to all three cell lines with the exception of P3 (Vero) and P4 (HEK293; Vero) in April as well as P4 (Hep-G2) and P5 (Hep-G2; Vero) in July. Interestingly, while some morphological changes were much more apparent than others, some cells produced a much later CPE. In addition to clearer, more apparent morphological changes, the control-infected cells produced a CPE quicker than the concentrated viruses. However, in comparison to the adenovirus 9 VR-1086 control which was CPE positive on all cell lines, the hepatitis A VR-1402 control was CPE positive on the Hep-G2 and Vero cells only during both experiments.

### Visualization and presumptive identification of bacteriophage populations

TEM revealed a number of phage morphotypes at all sampling locations which are depicted in Figure 2. Bacteriophages were identified according to the International Committee on the Taxonomy of Viruses (ICTV) classification scheme described in Ackermann & Eisenstark (1974). Bacteriophages belonging to the *Caudovirales* order consisting of morphotypes A1 (*Myoviridae* – contractile tails), B1 (*Siphoviridae* – short capsid, non-contractile and long tail), B2 (*Siphoviridae* – long capsid, non-contractile and long tail) and C1 (*Podoviridae* – short tail) were identified in the Umhlangane River. *Siphoviridae* members B1 and B2 closely resembled the phages seen in Figure 2(a) and 2(g)), respectively while Figure 2(c) and 2(d)) closely resemble members of the *Podoviridae* and *Myoviridae* families.

While most bacteriophages comprised regular hexagonal outlined heads (Figure 2(e) and 2(f)), some were irregular (Figure 2(b)). The discrimination between octahedral, icosahedral and dodecahedral shapes could not be properly determined. Furthermore, the tail fibres, neck and baseplate were only visualized in Figure 2(d) (tail fibres and neck) and Figure 2(e) (neck and baseplate). *Myoviridae* tail contraction was observed in Figure 2(h).

Figure 2(i)–2(n)) represent phages identified in the Umhlangane River at the five sampling points that resemble some known bacteriophages. These phages include the T4-like *Vibrio parahaemolyticus* phage (Ackermann & Heldal 2010), the environmentally isolated *Mycobacterium* 40AC phage (Stella *et al.* 2013) and the VvAWI *Vibrio vulnificus* phage (Nigro *et al.* 2012).

### TEM for presumptive enteric virus identification

Figures 3 and 4 illustrate presumptive enteric viruses that were observed during the sampling period. Classification was performed according to size measurements and comparative structural similarities to known viruses found in the literature. Presumptive naked enterovirus-like particles (*Picornaviridae*) with sizes ranging from 25.92 to 27.46 nm (Figure 3(a)–3(c)) were visualized and compared to known coxsackieviruses (Figure 3(d)). Figure 3(e)–3(g)) depict the TEM images of presumptive naked *Adenoviridae*-like particles and (Figure 3(h)) known adenoviruses (70–90 nm). The viral particle sizes ranged from 67.29 to 78.11 nm. Although less commonly observed, TEM also revealed presumptive *Polyomaviridae*-like particles (Figure 3(i) and 3(j)), *Reoviridae*-like particles (Figure 3(l)–3(n)) and *Coronaviridae*-like particles (Figure 4(a)–4(c)) when compared

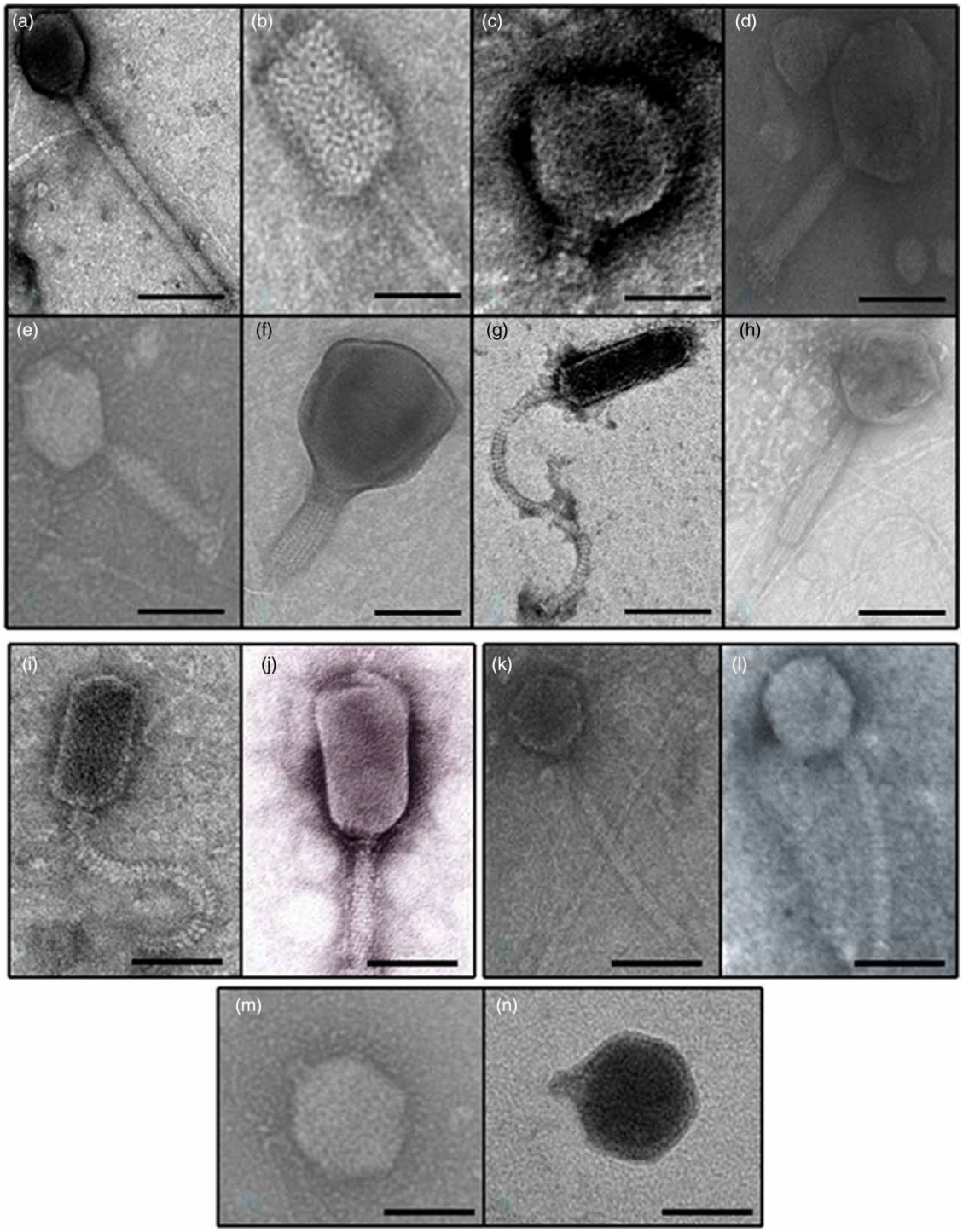


**Figure 2** | TEM images of diverse phage morphotypes visualized in the Umhlangane River (a)–(h). Bacteriophages (i), (k), (m) resemble the (j) known T4-like *Vibrio parahaemolyticus* phage (Ackermann & Heldal 2010), (l) known *Mycobacterium* 40AC phage (Stella *et al.* 2013) and known (n) VvAWI *Vibrio vulnificus* phage (Nigro *et al.* 2012). Images captured at 250,000 to 500,000 × magnification. Scale bar 100 nm.

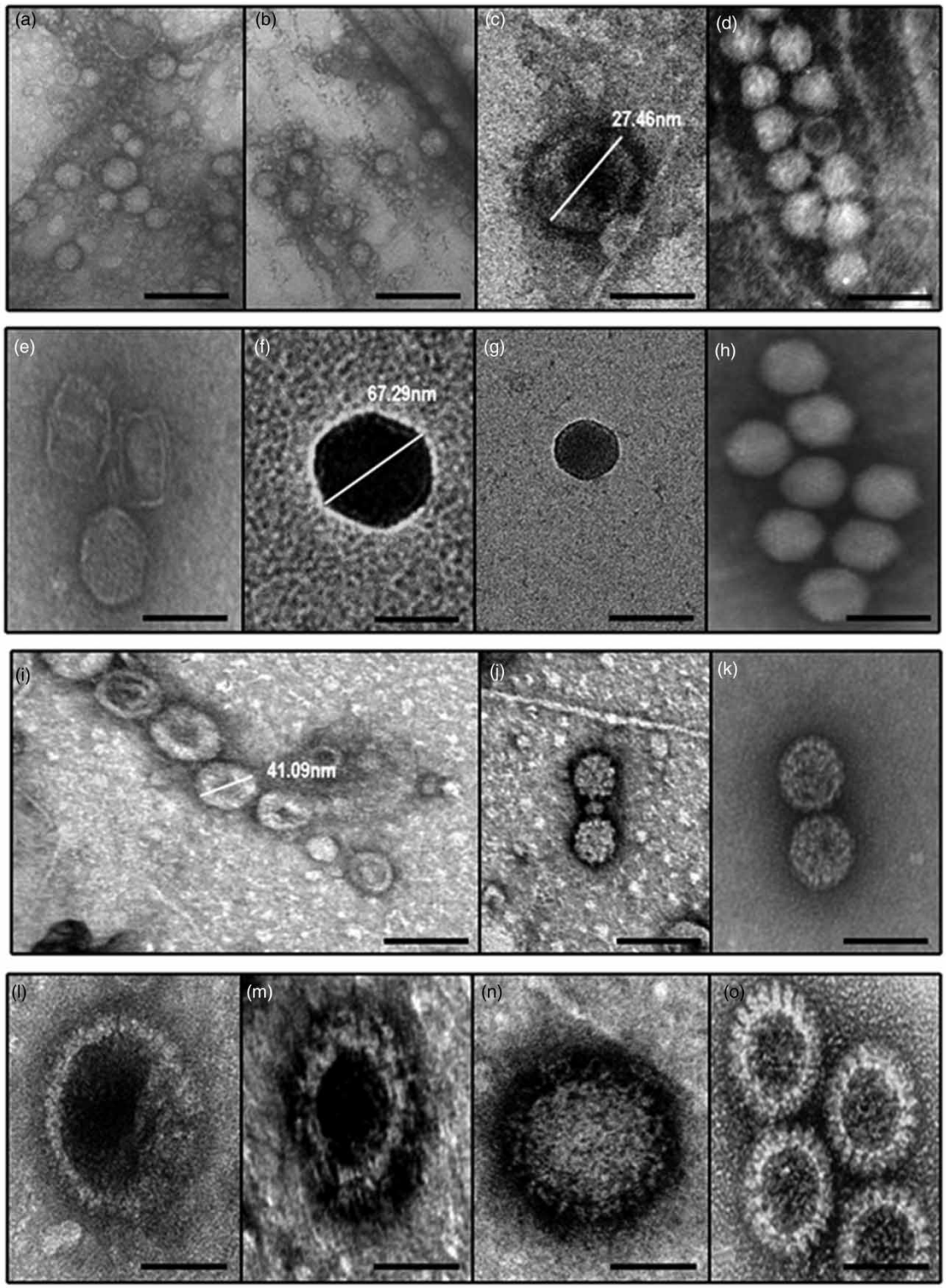


**Figure 3** | TEM images of presumptive enteric viruses. *Picornaviridae*-like particles (a)–(c), (d) known coxsackievirus (Schramlová *et al.* 2010), *Adenoviridae*-like particles (e)–(g), (h) known adenoviruses (Li *et al.* 2013), *Polyomaviridae*-like particles (i) and (j), (k) known polyomaviruses (Broekema & Imperiale 2012), *Reoviridae*-like particles (l)–(n) and (o) known rotaviruses (Zeng *et al.* 1996). Images captured at 250,000 to 500,000 × magnification. Scale bar 100 nm.

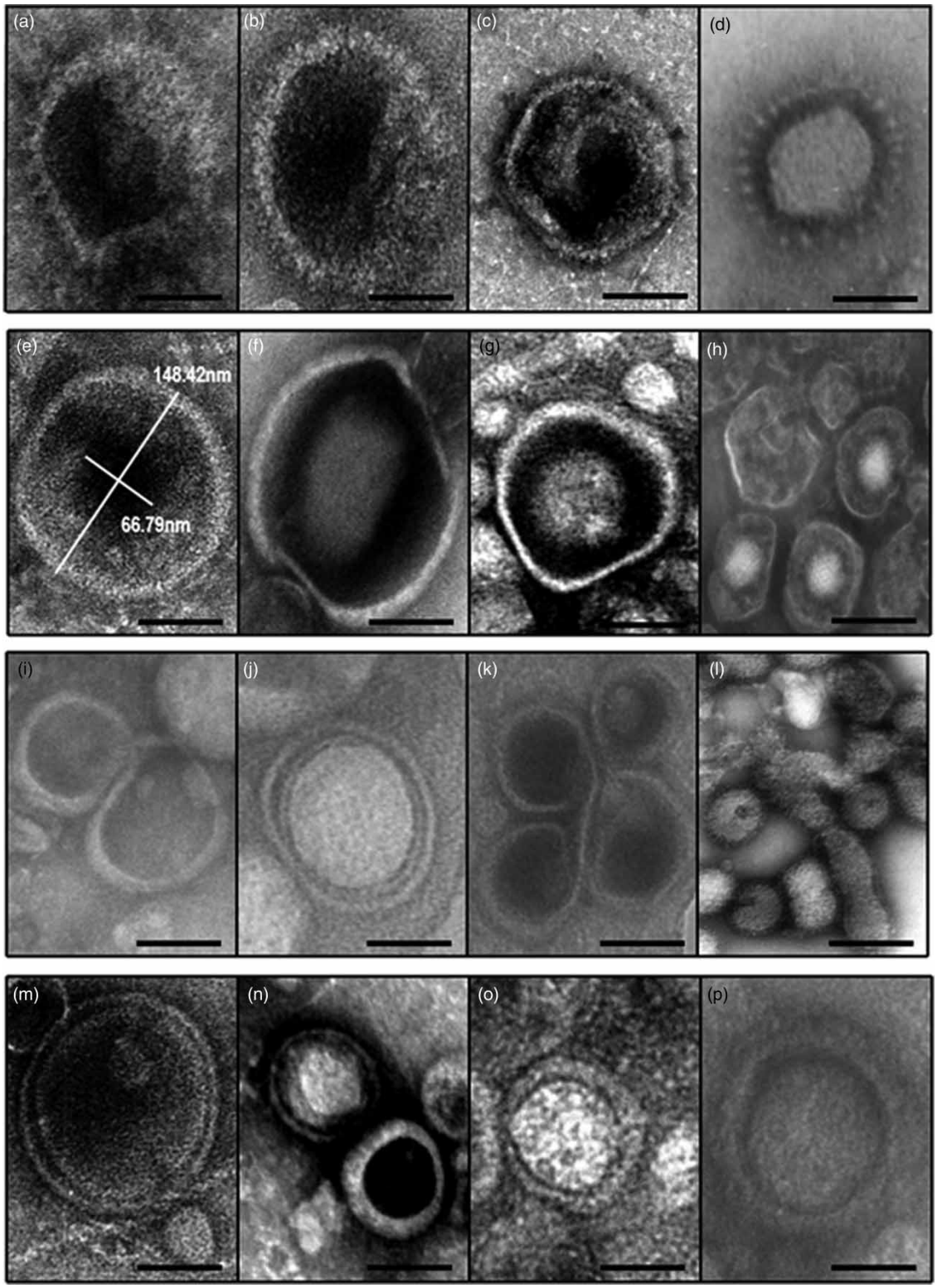


**Figure 4** | TEM images of presumptive enteric viruses. *Coronaviridae*-like particles (a)–(c), (d) known coronavirus (Schramlová *et al.* 2010), *Herpesviridae*-like particles (e)–(g), (h) known herpes viruses (Goldsmith & Miller 2009), *Orthomyxoviridae*-like particles (i)–(k), (l) known influenza viruses (Schramlová *et al.* 2010) and enveloped virus-like particles (m)–(p). Images captured at 250,000 to 500,000 × magnification. Scale bar 100 nm.

against known polyomaviruses (40–50 nm; Figure 3(k)), known rotaviruses (Figure 3(o)) and a known coronavirus (Figure 4(d)). *Herpesviridae*-like particles (Figure 4(e)–4(g)) and *Orthomyxoviridae*-like particles (Figure 4(i)–4(k)) were compared to known herpes (Figure 4(h)) and influenza viruses (Figure 4(l)), respectively. Lastly, many presumptive enveloped viruses (Figure 4(m)–4(p)) were seen in the Umhlangane River. However, due to inherently similar structures, comparisons between enveloped viruses and known viruses could not be made. Interestingly, TEM revealed some important morphological characteristics, such as the double-layered rotavirus (Figure 3(n)), the *Coronaviridae* peplomers or 'setting-sun' appearance (Figure 4(a)), the rough polyomavirus capsids (Figure 3(j)) and the envelopes and nucleocapsids of many viruses (Figure 4(f), 4(j) and 4(m)).

## Molecular detection of four enteric viral groups

Human adenovirus, polyomavirus, hepatitis A and C virus genomes detected using a nested PCR/RT-PCR are depicted in Table 3. All sampling points during the 6-month sampling period produced a positive PCR product of 143 bp corresponding to the adenovirus control. Positive BK and JC human polyomavirus genomes were detected in 60% ($n = 30$) of the tested samples, yielding the 173 bp expected product size. All samples tested during June were positive for polyomaviruses while only P1 (Phoenix industrial) during September depicted a positive PCR product (Table 3). The VP1/VP2 regions of hepatitis A viruses were detected in 70% ($n = 30$) of the samples yielding 436 bp products. The expected product size of 255 bp amplifying the 5′ UTR for positive hepatitis C viruses was only seen in three samples, including P2 in May (upstream Kwa-Mashu WWT), P4 in June (Riverhorse Valley business estate) and P5 in July (Springfield industrial). Finally, correlations between seasonality and the presence of the viral groups were not observed.

Comparisons between random positive PCR products and reference sequences in GenBank confirmed the presence of adenovirus, polyomavirus and hepatitis A and C virus genomes in the Umhlangane River. The percentage maximum identity and E-values obtained during the BLAST analysis are depicted in Table 4. The sequence

**Table 3** | Nested-PCR/RT-PCR amplification of the four viral groups

| | | Location | | | | |
|---|---|---|---|---|---|---|
| **Viruses** | **Month** | **P1** | **P2** | **P3** | **P4** | **P5** |
| Adenovirus | *April* | + | + | + | + | + |
| | *May* | + | + | + | + | + |
| | June | + | + | + | + | + |
| | July | + | + | + | + | + |
| | August | + | + | + | + | + |
| | September | + | + | + | + | + |
| Polyomavirus | *April* | + | + | − | + | − |
| | *May* | + | + | + | − | − |
| | June | + | + | + | + | + |
| | July | + | − | − | + | − |
| | August | + | − | + | + | + |
| | September | + | − | − | − | − |
| Hepatitis A virus | *April* | − | + | + | + | + |
| | *May* | − | + | − | − | + |
| | June | + | + | − | + | + |
| | July | − | + | + | + | + |
| | August | + | + | + | + | + |
| | September | + | − | + | − | − |
| Hepatitis C virus | *April* | − | − | − | − | − |
| | *May* | − | + | − | − | − |
| | June | − | − | − | + | − |
| | July | − | − | − | − | + |
| | August | − | − | − | − | − |
| | September | − | − | − | − | − |

+, positive PCR product; −, negative PCR product. *Note:* italicized font, autumn; normal font, winter; underlined font, spring.

**Table 4** | BLAST analysis showing the maximum identity and E-values for the tested samples

| **Accession** | **Description** | **E-value** | **Maximum identity (%)** |
|---|---|---|---|
| KF268310.1 | Human adenovirus c strain | 2e-28 | 89 |
| KM205587.1 | Adenovirus 2 isolate AAU4 | 1e-26 | 86 |
| KM225765.1 | JC polyomavirus isolate GCN8, complete genome | 9e-53 | 98 |
| AB081021.1 | JC virus DNA, isolate ME-5 | 4e-61 | 99 |
| HQ246217.1 | Hepatitis A virus CFH-HAV, complete genome | 3e-177 | 95 |
| FJ687513.1 | Hepatitis A isolate 9 polyprotein | 4e-166 | 96 |
| EF473252.1 | Hepatitis C isolate Ind-MN19 5′ UTR | 8e-05 | 100 |

identities ranged between 86% and 100% to their known complements on the GenBank database. Among the sequenced data, human adenovirus C strain, JC polyomavirus isolate GCN8, hepatitis A virus strain CFH and hepatitis C virus isolate Ind-MN19 were identified.

## DISCUSSION

Extensive concentration methods and/or the analysis of large volumes of water are usually required to obtain a sufficient amount of viruses for experimental testing (Symonds & Breitbart 2015). In this study, TFF coupled with ultracentrifugation efficiently concentrated viruses from the collected water samples. In addition to clear, bacterial-free concentrates, this two-step TFF procedure ensured that most viruses remained structurally intact.

The detection of infectious enteric viruses in water has long employed cell culture methods (Calgua *et al.* 2011). Previously, this was the only method approved by the Environmental Protection Agency (EPA) for the detection of enteric viruses (Jiang 2006). In 2012, the EPA modified Method 1615 to incorporate culture and quantitative RT-PCR (RT-qPCR) for the detection of norovirus and enterovirus (Cashdollar *et al.* 2013). Positive CPE produced by the viral concentrates indicates the presence of infectious enteric viruses in the Umhlangane River. Sampling points P1 (Phoenix industrial) and P2 (upstream KwaMashu WWTP) depicted positive CPE on all cell lines. This may be due to the influx of effluents containing human or animal faecal matter harbouring some enteric viruses (La Rosa *et al.* 2012). Cytopathic-based tissue culture assays (or quantal assays) are sensitive rather than quantitative (EPA 2001). Quantal assays are simplistic since the effect is either present or not (Zivin & Waud 1992) and in tissue culture only one infectious particle is enough to produce a successful CPE (EPA 2001). However, the efficacy of viral replication on various cell lines depends on the serotypes of the viruses present (Jiang 2006). Therefore, not all viruses or serotypes are susceptible to all cell lines (Lee *et al.* 2004). Furthermore, some enteric viruses are slow growing (Jiang *et al.* 2009) or produce unclear or no CPE (Calgua *et al.* 2011). Consequently, the negative CPE produced by some of the samples may be due to viral diversity or their slow growing nature, indicating negative, little or no CPE on the cell monolayers. Moreover, diverse viral concentrates can inhibit the growth of some viruses due to the interference by other groups of viruses (Carducci *et al.* 2002). In the present study, Hep-G2, HEK293 and Vero cell lines were used. The Hep-G2 and HEK293 carcinoma cell lines are common and can support the growth of many viruses (Leland & Ginocchio 2007). In particular, Hep-G2 cells are highly sensitive to hepatitis A, B and C viruses (WHO 2008) while HEK293 cells show great sensitivity to human adenovirus types 40 and 41 (Jiang *et al.* 2009). Since cell lines have an affinity to propagate certain viral groups the Vero cell line was used to increase the sensitivity of viruses in this study. Vero cells can support the growth of hepatitis viruses (Konduru & Kaplan 2006), measles viruses, rubella viruses and arboviruses (Osada *et al.* 2014).

TEM was used to visualize the types of viruses present in the Umhlangane River. Bacteriophages belonging to the *Myoviridae*, *Siphoviridae* and *Podoviridae* families were morphologically diverse showing great abundance. Bacteriophage diversity corresponds with the colossal dynamics and diversity observed in their bacterial counterparts and vice versa (Demuth *et al.* 1993; Beckett & Williams 2013; Williams 2013). The complexity between phage and bacterial populations were reiterated by the presumptive identification of phages described to those confirmed in the literature (Figure 2(i)–2(n)). For example, non-tuberculosis-causing, environmental *Mycobacteria* may be found in water and soil (Pedulla *et al.* 2003). However, bacteriophages could originate from allochthonous sources (Hewson *et al.* 2012), such as wild animals, waterfowl or anthropogenic practices near the river (Demuth *et al.* 1993).

A number of diverse enteric viruses observed in the Umhlangane River indicates a significant amount of faecal contamination is entering the catchment (Cantalupo *et al.* 2011). The source of pollution and consequent viral contamination may be from surface runoff, recreational activities, storm water discharge, sewage discharge and overflows as well as other humanized practices (Olaniran *et al.* 2009). Many of the detected viruses were found upstream in P1 and P2 (Phoenix residential/industrial; upstream KwaMashu WWTP) and downstream in P4 and P5 (Riverhorse Valley business estate; Springfield industrial). These areas are densely populated, comprising a variety of

anthropogenic practices with the additional input of animal faecal matter from the surrounding farms (Phoenix areas) and effluent from informal settlements (Riverhorse Valley). This may have contributed to the viruses observed in the river water samples.

Presumptive *Polyomaviridae*, *Coronaviridae*, *Adenoviridae*, *Picornaviridae*, *Herpesviridae*, *Reoviridae* (rotavirus), *Orthomyxoviridae* and other unassigned enveloped viruses were visualized. Viruses with intact nucleocapsids are protected by an envelope while naked viruses comprise inflexible capsids that can withstand harsh conditions (Ackermann & Heldal 2010). These morphological characteristics allow enteric viruses to persist in the environment for long periods of time and, most often than not, survive many treatment processes (Steyer *et al.* 2011). However, since these viruses were concentrated from water, their morphological structures may have faulted through degradation effects (Eifan 2013) or via the concentration method (Karim *et al.* 2004). Finally, morphological characteristics coupled with seasonal periods and environmental factors are important points to consider when assessing viral persistence (Espinosa *et al.* 2009).

The visualization of a diverse array of enteric viruses in the Umhlangane River drove the motivation for the molecular detection of some enteric viruses that may be present in the river water samples. To the best of our knowledge, this is the first study conducted to evaluate the presence of enteric viruses in the Umhlangane River of Durban, South Africa. The application of PCR-based methods to study the presence of enteric viruses in aquatic environments has advanced over the years (Jiang 2006). Although conventional PCR has proved to be effective, specific and sensitive to detect low virus concentrations (Girones *et al.* 2010), nested-PCR/RT-PCR can further increase the sensitivity of detection by employing two rounds of PCR using two sets of primers (van Heerden *et al.* 2005). Nested-PCR/RT-PCR was used to detect the presence of human adenovirus, polyomavirus and hepatitis A and C virus populations in this study. First round PCR/RT-PCR was not able to amplify the viral genomes for some (adenovirus), many (polyomavirus) or all of the tested samples (hepatitis A and C viruses). However, the second round of PCR/RT-PCR using the inner primers and primer templates from the first rounds was sensitive enough to detect the viral genomes.

Human adenoviruses were observed in 100% ($n = 30$) of the tested water samples (Table 3). Currently, there are over 60 types of adenoviruses present within the human adenovirus A–G species (Robinson *et al.* 2013). Serotypes 40 and 41 are the second leading cause of gastroenteritis in children next to rotaviruses (Jiang *et al.* 2001).

Human polyomaviruses (BK and JC) were identified in 18 ($n = 30$) of river water samples (Table 3). The BK and JC polyomaviruses are exclusive to humans and cause asymptomatic viruria (Polo *et al.* 2004). Although these viruses can be excreted from human faeces (Hundesa *et al.* 2006), the abundance of human polyomaviruses may arise from urine since JC viruses have been found in 20–80% of adult urine samples (Kitamura *et al.* 1990). Moreover, these viruses are protected when ingested by food particles and are stable at acidic pH (Bofill-Mas *et al.* 2001).

The hepatitis A virus is the number one cause for gastroenteritis worldwide (Redwan & Abdullah 2012). These viruses have been successfully isolated from various water sources including dams (Taylor *et al.* 2001), rivers (Pina *et al.* 2001) and groundwater (Borchardt *et al.* 2003). Not only can hepatitis A viruses persist in groundwater for months (La Rosa *et al.* 2012), they also show resistance to common disinfectants (Bigliardi & Sansebastiano 2006). Furthermore, these viruses can survive exposure to 20% ether, acidity (pH 1.0 for 2 hours) and heating to 60 °C for 1 hour (Kocwa-Haluch 2001).

Although hepatitis C viruses were only detected in 10% ($n = 30$) of the collected water samples (Table 3), their presence is a cause for concern. These viruses infect approximately 150 to 200 million people worldwide (Maier & Wu 2002). Hepatitis C viruses cause chronic and acute hepatic diseases and, due to high viral mutation rates, no licensed therapeutic or prophylactic vaccines have been made available (Abdelwahab & Said 2016).

Although this study could not identify patterns of viral seasonal occurrence (Table 3), it is important to note that some viruses, such as rotaviruses, hepatitis A (Chigor & Okoh 2012) and astroviruses (Ganesh & Lin 2013), to name a few, are affected by seasonal conditions.

The nucleotide sequences of the adenovirus, polyomavirus and hepatitis A and C virus genomes confirmed using BLAST analysis revealed different virus strains. Not only does the BLAST analysis confirm the presence of

these four viral groups, it also indicates the diversity of these enteric viruses residing in the Umhlangane River.

## CONCLUSIONS

In conclusion, this study investigated the presence of viral populations in the Umhlangane River using TFF and subsequent molecular methods. The TFF concentration of viruses from 20 L of river water was effective and did not require sample manipulation such as pH adjustment or PCR inhibitors (beef extract) for elution (Jiang *et al.* 2001). When assessed for infectivity, the viral concentrates were found to be infectious to Hep-G2, HEK293 and Vero cell lines. Furthermore, the use of TEM allowed the visualization of diverse bacterial and eukaryotic viruses present in the catchment. Molecular detection further identified specific viral groups, namely, human adenovirus, polyomavirus and hepatitis A and C virus genomes present in the Umhlangane River. These viruses may impose a great health risk to individuals who may directly or indirectly utilize this water source. The findings presented in this study reiterate the potential danger of enteric viruses and bacterial-dependent phage persistence in water environments. Furthermore, land use activities and poor management in effluent disposal is a lesson not just for organizations dealing with the Umhlangane River but for all organizations managing any water resource, globally. Finally, a systematic integrated risk assessment and management plan to identify and minimize sources of faecal contamination is the most effective way of ensuring water safety and should be established in all future guidelines.

## STUDY LIMITATIONS

This study identified a diverse array of virus particles in the Umhlangane River. However, some limitations were noted. Although seasonal effects were not observed, the short sampling period of 6 months did not cover a seasonal cycle. Therefore, changes that may have occurred at a later stage would not have been detected. Additionally, to fully assess the extent of viral infectivity and the multiplicity of infection tissue culture assays are required.

## ACKNOWLEDGEMENTS

The authors would like to thank the National Research Foundation, South Africa for providing the Masters scholarship. The authors declare that they have no competing interests.

## REFERENCES

Abdelwahab, K. S. & Said, Z. N. A. 2016 Status of hepatitis C virus vaccination: recent update. *World Journal of Gastroenterology* **14**, 862–873.

Ackermann, H. W. & Eisenstark, A. 1974 The present state of phage taxonomy. *Intervirology* **3**, 201–219.

Ackermann, H.-W. & Heldal, M. 2010 Basic electron microscopy of aquatic viruses. In: *Manual of Aquatic Virology* (S. W. Wilhelm, M. G. Weinbauer & C. A. Suttle, eds). ASLO, Waco, TX, pp. 182–192.

Allard, A., Albinsson, A. & Wadell, G. 1992 Detection of adenoviruses in stools from healthy-persons and patients with diarrhea by 2-step polymerase chain-reaction. *Journal of Medical Virology* **37**, 149–157.

Aslanzadeh, J., Padilla, B. B. & Shanley, J. D. 1996 Evaluation of PCR and nested PCR for laboratory diagnosis of hepatitis C virus infection. *Molecular and Cellular Probe* **10**, 173–178.

Beckett, S. J. & Williams, H. T. P. 2013 Coevolutionary diversification creates nested-modular structure in phage–bacteria interaction networks. *Interface Focus* **3**, 20130033. doi:10.1098/rsfs.2013.0033.

Beld, M., Sentjens, R., Rebers, S., Weel, J., van Dillen, P. W., Sol, C. & Boom, R. 2000 Detection and quantitation of hepatitis C virus RNA in faeces of chronically infected individuals. *Journal of Clinical Microbiology* **38**, 3442–3444.

Bigliardi, L. & Sansebastiano, G. 2006 Study on inactivation kinetics of hepatitis A virus and enteroviruses with peracetic acid and chlorine. New ICC/PCR method to assess disinfection effectiveness. *Journal of Preventive Medicine and Hygiene* **47**, 56–63.

Bofill-Mas, S., Formiga-Cruz, M., Clemente-Casares, P., Calafell, F. & Girones, R. 2001 Potential transmission of human polyomaviruses through the gastrointestinal tract after exposure to virions or viral DNA. *Journal of Virology* **75**, 10290–10299.

Borchardt, M. A., Bertz, P. D., Spencer, S. K. & Battigelli, D. A. 2003 Incidence of enteric viruses in groundwater from household wells in Wisconsin. *Applied and Environmental Microbiology* **69**, 1172–1180.

Broekema, N. M. & Imperiale, M. J. 2012 Efficient propagation of archetype BK and JC polyomaviruses. *Virology* **20**, 235–241.

Cai, L., Yang, Y., Jiao, N. & Zhang, R. 2015 Evaluation of tangential flow filtration for the concentration and separation of bacteria and viruses in contrasting marine environments.

*PLoS ONE* **10**, e0136741. doi:10.1371/journal.pone.0136741.

Calgua, B., Barardi, C. R. M., Bofill-Mas, S., Rodriguez-Manzano, J. & Girones, R. 2011 Detection and quantitation of infectious human adenoviruses and JC polyomaviruses in water by immunofluorescence assay. *Journal of Virological Methods* **171**, 1–7.

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

Carducci, A., Cantiani, L., Moscatelli, R., Casini, B., Rovini, E., Mazzoni, F., Giuntini, A. & Verani, M. 2002 Interference between enterovirus and reovirus as a limiting factor in environmental virus detection. *Letters in Applied Microbiology* **34**, 110–113.

Cashdollar, J. L., Brinkman, N. E., Griffin, S. M., McMinn, B. R., Rhodes, E. R., Varughese, E. A., Grimm, A. C., Parshionikar, S. U., Wymer, L. & Fouta, G. S. 2013 Development and evaluation of EPA Method 1615 for detection of enterovirus and norovirus in water. *Applied and Environmental Microbiology* **79**, 215–223.

Chen, N.-Y., Liu, Z.-H., Shie, S.-S., Chen, T.-H. & Wu, T.-S. 2017 Clinical characteristics of acute hepatitis A outbreak in Taiwan, 2015–2016: observations from a tertiary medical center. *BMC Infectious Diseases* **17**, 441.

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 Environmental Research and Public Health* **9**, 4017–4032.

Colombet, J., Robin, A., Lavie, L., Bettarel, Y., Cauchie, H. M. & Sime-Ngando, T. 2007 Virioplankton 'pegylation': use of PEG (polyethylene glycol) to concentrate and purify viruses in pelagic ecosystems. *Journal of Microbiological Methods* **71**, 212–219.

Coudray-Meunier, C., Fraisse, A., Martin-Latil, S., Guillier, L. & Perelle, S. 2013 Discrimination of infectious hepatitis A virus and rotavirus by combining dyes and surfactants with RT-qPCR. *BMC Microbiology* **13**, 126. doi:10.1186/1471-2180-13-216.

Demuth, J., Neve, H. & Witzel, K. P. 1993 Direct electron microscopy study on the morphological diversity of bacteriophage populations in Lake Plußsee. *Applied and Environmental Microbiology* **59**, 3378–3384.

Doerrbecker, J., Behrendt, P., Mateu-Gelabert, P., Ciesek, S., Riebesehl, N., Wilhelm, C., Steinmann, J., Pietschmann, T. & Steinmann, E. 2013 Transmission of hepatitis C virus among people who inject drugs: viral stability and association with drug preparation equipment. *Journal of Infectious Diseases* **207**, 281–287.

Dongdem, J. T., Soyiri, I. & Ocloo, A. 2009 Public health significance of viral contamination of drinking water. *African Journal of Microbiology Research* **3**, 856–861.

Edelman, D. C. & Barletta, J. 2003 Real-time PCR provides improved detection and titer determination of bacteriophage. *BioTechniques* **35**, 368–375.

Eifan, S. A. 2013 Enteric viruses and aquatic environment. *The Internet Journal of Microbiology* **12** (1). Available from: http://ispub.com/IJMB/12/1/2926.

EPA 2001 *Methods for Virology: A Methods Manual.* United States Environmental Protection Agency Office of Water, Washington, DC. https://nepis.epa.gov/Exe/ZyNET.exe/P100EQ65.TXT?ZyActionD=ZyDocument&Client=EPA&Index=2000+Thru+2005&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5Czyfiles%5CIndex%20Data%5C00thru05%5CTxt%5C00000030%5CP100EQ65.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x&ZyPURL (accessed 13 February 2017).

Espinosa, A. C., Arias, C. F., Sánchez-Colón, S. & Mazari-Hiriart, M. 2009 Comparative study of enteric viruses, coliphages and indicator bacteria for evaluating water quality in a tropical high-altitude system. *Environmental Health* **8** (49). doi:10.1186/1476-069X-8-49.

Frank, C., Walter, J., Muehlen, M., Jansen, A., van Treeck, U., Hauri, A. M., Zoellner, I., Rakha, S. M., Hoehne, M., Hamouda, O., Schreier, E. & Stark, K. 2007 Major outbreak of hepatitis A associated with orange juice among tourists, Egypt, 2004. *Emerging Infectious Disease* **13**, 156–158.

Ganesh, A. & Lin, J. 2013 Waterborne human pathogenic viruses of public health concern. *International Journal of Environmental Health Research* **23**, 544–564.

Ganesh, A., Lin, J. & Singh, M. 2014 Detecting virus-like particles from the Umgeni River, South Africa. *Clean – Soil, Air, Water* **42**, 393–407.

Girones, R., Ferrus, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., Correa, A., Hundesa, A., Carratala, A. & Bofill-Mas, S. 2010 Molecular detection of pathogens in water – the pros and cons of molecular techniques. *Water Research* **44**, 4325–4339.

Goldsmith, C. S. & Miller, S. E. 2009 Modern uses of electron microscopy for detection of viruses. *Clinical Microbiology Reviews* **22**, 552–563.

Hadlow, D. 2011 *A River Quality Assessment of A Highly Impacted Tributary of the Mgeni River: the Umhlangane River, Durban, KwaZulu-Natal. Honours Dissertation*, Geography and Environmental Management, University of KwaZulu-Natal, South Africa.

Haramoto, E., Kitajima, M., Katayama, H. & Ohgaki, S. 2010 Real-time PCR detection of adenoviruses, polyomaviruses, and

torque teno viruses in river water in Japan. *Water Research* **44**, 1747–1752.

Hewson, I., Barbosa, J. G., Brown, J. M., Donelan, R. P., Eaglesham, J. B., Eggleston, E. M. & LaBarre, B. A. 2012 Temporal dynamics and decay of putatively allochthonous and autochthonous viral genotypes in contrasting freshwater lakes. *Applied and Environmental Microbiology* **78**, 6583–6591.

Hofstra, N. 2011 Quantifying the impact of climate change on enteric waterborne pathogen concentrations in surface water. *Current Opinion in Environmental Sustainability* **3**, 471–479.

Hu, Y., Shahidi, A., Park, S., Guilfoyle, D. & Hirshfield, I. 2003 Detection of extrahepatic hepatitis C virus replication by a novel, highly sensitive, single-tube nested polymerase chain reaction. *American Journal of Clinical Pathology* **119**, 95–100.

Hundesa, A., de Motes, C. M., Bofill-Mas, S., Albinana-Gimenez, N. & Girones, R. 2006 Identification of human and animal adenoviruses and polyomaviruses for determination of sources of faecal contamination in the environment. *Applied and Environmental Microbiology* **72**, 7886–7893.

Jiang, S. C. 2006 Human adenoviruses in water: occurrence and health implications: a critical review. *Environmental Science and Technology* **40**, 7132–7140.

Jiang, S. C., Noble, R. & Chu, W. 2001 Human adenoviruses and coliphages in urban runoff-impacted coastal waters of Southern California. *Applied and Environmental Microbiology* **67**, 179–184.

Jiang, S. C., Han, J., He, J.-W. & Chu, W. 2009 Evaluation of four cell lines for assay of infectious adenoviruses in water samples. *Journal of Water and Health* **7**, 650–656.

Jurzik, L., Hamza, I. A., Puchert, W., Uberla, K. & Wilhelm, M. 2010 Chemical and microbiological parameters as possible indicators for human enteric viruses in surface water. *International Journal of Hygiene and Environmental Health* **213**, 210–216.

Karim, M. R., Pontius, F. W. & LeChevallier, M. W. 2004 Detection of noroviruses in water – summary of an international workshop. *Journal of Infectious Diseases* **189**, 21–28.

Kitamura, T., Aso, Y., Kuniyoshi, N., Hara, K. & Yogo, Y. 1990 High incidence of urinary JC virus excretion in non-immunosuppressed older patients. *Journal of Infectious Diseases* **161**, 1128–1133.

Kocwa-Haluch, R. 2001 Waterborne enteroviruses as a hazard for human health. *Polish Journal of Environmental Studies* **10**, 485–487.

Konduru, K. & Kaplan, G. G. 2006 Stable growth of wild-type hepatitis A virus in cell culture. *Journal of Virology* **80**, 1352–1360.

La Rosa, G., Fratini, M., Libera, S. D., Iaconelli, M. & Muscillo, M. 2012 Emerging and potentially emerging viruses in water environments. *Ann Ist Super Sanita* **48**, 397–406.

La Rosa, G., Libera, S. D., Iaconelli, M., Ciccaglione, A. R., Bruni, R., Taffon, S., Equestre, M., Alfonsi, V., Rizzo, C., Tosti, M. E., Chironna, M., Romanò, L., Zanetti, A. R. & Muscillo, M. 2014 Surveillance of hepatitis A virus in urban sewages and comparison with cases notified in the course of an outbreak, Italy 2013. *BMC Infectious Diseases* **14** (419). Available from: https://doi.org/10.1186/1471-2334-14-419.

Leclerc, S., Edberg, H., Pierzo, V. & Delattre, J. M. 2000 Bacteriophages as indicators of enteric viruses and public health risk in groundwaters. *Journal of Applied Microbiology* **88**, 5–21.

Lee, C., Lee, S.-H., Han, E. & Kim, S.-J. 2004 Use of cell culture-PCR assay based on combination of a549 and BGMK cell lines and molecular identification as a tool to monitor infectious adenoviruses and enteroviruses in river water. *Applied and Environmental Microbiology* **70**, 6695–6705.

Lee, C. S., Lee, C., Marion, J., Wang, Q., Saif, L. & Lee, J. 2014 Occurrence of human enteric viruses at freshwater beaches during swimming season and its link to water inflow. *Science of the Total Environment* **472**, 757–766.

Leifels, M., Hamza, I. A., Krieger, M., Wilhelm, M., Mackowiak, M. & Jurzik, L. 2016 From lab to lake – evaluation of current molecular methods for the detection of infectious enteric viruses in complex water matrices in an urban area. *PLoS ONE* **11**, e0167105. doi:10.1371/journal.pone.0167105.

Leland, D. S. & Ginocchio, C. C. 2007 Role of cell culture for virus detection in the age of technology. *Clinical Microbiology Reviews* **20**, 49–78.

Li, L., Yan, W. G. Y., Lee, S. & Wang, T. 2013 Label-free super-resolution imaging of adenoviruses by submerged microsphere optical nanoscopy. *Light: Science and Applications* **2**, 104. doi:10.1038/lsa.2013.60.

Lopman, B. A., Steele, D., Kirkwood, C. D. & Parashar, U. D. 2016 The vast and varied global burden of norovirus: prospects for prevention and control. *PLoS Medicine* **13**, e1001999. doi:10.1371/ journal.pmed.1001999.

Maier, I. & Wu, G. Y. 2002 Hepatitis C and HIV co-infection: a review. *World Journal of Gastroenterology* **8**, 577–579.

McQuaig, S. M., Scott, T. M., Harwood, V. J., Farrah, S. R. & Lukasik, J. O. 2006 Detection of human-derived faecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Applied and Environmental Microbiology* **72**, 7567–7574.

Nigro, O. D., Culley, A. I. & Steward, C. F. 2012 Complete genome sequence of bacteriophage VvAW1, which infects *Vibrio vulnificus*. *Standards in Genomic Sciences* **6** (3), 415–426. doi:10.4056/sigs.2846206.

Olaniran, A. O., Naicker, K. & Pillay, B. 2009 Antibiotic resistance profiles of *Escherichia coli* isolates from river sources in Durban, South Africa. *World Journal of Microbial Biotechnology* **25**, 1743–1749.

Osada, N., Kohara, A., Yamaji, T., Hirayama, N., Kasai, F., Sekizuka, T., Kuroda, M. & Hanada, K. 2014 The genome landscape of the African green monkey kidney-derived Vero cell line. *DNA Research* **21**, 673–683.

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S. & Singh, V. J. 2014 Contamination of water resources by pathogenic bacteria. *AMB Express* **4**, 51. doi:10.1186/s13568-014-0051-x.

Pedulla, M. L., Ford, M. E., Houtz, J. M., Karthikeyan, T., Wadsworth, C., Lewis, J. A., Jacobs-Sera, D., Falbo, J., Gross, J., Pannunzio, N. R., Brucker, W., Kumar, V., Kandasamy, J., Keenan, L., Bardarov, S., Kriakov, J., Lawrence, J. G., Jacobs Jr, W. R., Hendrix, R. W. & Hatfull, G. F. 2003 Origins of highly mosaic mycobacteriophage genomes. *Cell* **113**, 117–182.

Pina, S., Buti, M., Jardi, R., Clemente-Casares, P., Jofre, J. & Girones, R. 2001 Genetic analysis of hepatitis A virus strains recovered from the environment and from patients with acute hepatitis. *Journal of General Virology* **82**, 2955–2963.

Polo, C., Perez, J. L., Mielnichuck, A., Fedele, C. G., Niuboand, J. & Tenorio, A. 2004 Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. *Clinical Microbiology Infection* **10**, 640–644.

Redwan, N. A. & Abdullah, A. A. 2012 Molecular detection of Hepatitis A virus in wastewater in Jeddah Province, Kingdom of Saudi Arabia. *Journal of Applied Sciences Research* **8**, 1–9.

Robesyna, E., Schrijvera, K. D., Elke Wollants, B., Topa, G., Verbeeck, J. & Rant, B. M. V. 2009 An outbreak of hepatitis A associated with the consumption of raw beef. *Journal of Clinical Virology* **44**, 207–210.

Robinson, C. M., Singh, G., Lee, J. Y., Dehghan, S., Rajaiya, J., Liu, E. B., Yousuf, M. A., Betensky, R. A., Jones, M. S., Dyer, D. W., Seto, D. & Chodosh, J. 2013 Molecular evolution of human adenoviruses. *Scientific Reports* **3**, 1812. doi:10.1038/srep01812.

Rodríguez, R. A., Gundy, P. M. & Gerba, C. P. 2008 Comparison of BGM and PLC/PRF/5 cell lines for total culturable viral assay of treated sewage. *Applied and Environmental Microbiology* **74**, 2583–2587.

Rodríguez-Díaz, J., Querales, L., Caraballo, L., Vizzi, E., Liprandi, F., Takiff, H. & Betancourt, W. Q. 2009 Detection and characterization of waterborne gastroenteritis viruses in urban sewage and sewage-polluted river waters in Caracas, Venezuela. *Applied and Environmental Microbiology* **75**, 387–394.

Schramlová, J., Arientová, S. & Hulínská, D. 2010 The role of electron microscopy in the rapid diagnosis of viral infections – review. *Folia Microbiology* **55**, 88–101.

Stella, E. J., Franceschelli, J. J., Tasselli, S. E. & Morbidoni, H. R. 2013 Analysis of novel mycobacteriophages indicates the existence of different strategies for phage inheritance in *Mycobacteria*. *PLoS ONE* **8**, e56384. doi:10.1371/journal.pone.0056384.

Steyer, A., Torkar, K. G., Gutierrez-Aguirre, I. & Poljsak-Prijatelj, M. 2011 High prevalence of enteric viruses in untreated individual drinking water sources and surface water in Solvenia. *International Journal of Hygiene and Environmental Health* **214**, 392–398.

Symonds, E. M. & Breitbart, M. 2015 Affordable enteric virus detection techniques are needed to support changing paradigms in water quality management. *Clean – Soil, Air, Water* **43**, 8–12.

Taylor, M. B., Cox, N., Very, M. A. & Grabow, W. O. K. 2001 The occurrence of hepatitis A and astroviruses in selected river and dam waters in South Africa. *Water Research* **35**, 2653–2660.

Thorpe, L. E., Ouellet, L. J., Hershow, R., Bailey, S. L., Williams, I. T., Williamson, J., Monterroso, E. R. & Garfein, R. S. 2002 Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *American Journal of Epidemiology* **155**, 645–653.

van Heerden, J., Ehlers, M. M., Heim, A. & Grabow, W. O. K. 2005 Prevalence, quantification and typing of adenoviruses detected in river and treated drinking water in South Africa. *Journal of Applied Microbiology* **99**, 234–242.

Vieira, C. B., de Oliveira Mendes, A. C., Guimaraes, F. R., Fumian, T. M., Leite, J. P. G., Gaspar, A. M. C. & Miagostovich, M. P. 2012 Detection of enteric viruses in recreational waters of an urban lagoon in the city of Rio de Janerio, Brazil. *Memórias do Instituto Oswaldo Cruz* **107**, 778–784.

WHO 2008 *Guidelines for Drinking-Water Quality*. WHO, Geneva. http://www.who.int/water_sanitation_health/dwq/fulltext.pdf (accessed 13 February 2017).

WHO 2016 *Fact Sheet: Hepatitis C [Online]*. World Health Organization. http://www.who.int/mediacentre/factsheets/fs164/en/ (accessed 13 February 2017).

WHO 2017 *Water, Sanitation and Hygiene Links to Health: Water Safety and Quality Facts [Online]*. World Health Organization. http://www.who.int/water_sanitation_health/water-quality/en/ (accessed 13 February 2017).

Williams, H. T. P. 2013 Phage-induced diversification improves host evolvability. *BMC Evolutionary Biology* **13**, 17. doi: 10.1186/1471-2148-13-17.

Wu, J., Long, S. C., Das, D. & Dorner, S. M. 2011 Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. *Journal of Water and Health* **9**, 265–278.

Zeng, C. Q.-Y., Wentz, M. J., Cohen, J., Estes, M. K. & Ramig, R. F. 1996 Characterization and replicase activity of double-layered and single-layered rotavirus-like particles expressed from baculovirus recombinants. *Journal of Virology* **70**, 2736–2742.

Zivin, J. A. & Waud, D. R. 1992 Quantal bioassay and stroke. *Stoke* **23**, 767–773.

First received 15 February 2017; accepted in revised form 15 September 2017. Available online 19 October 2017