Original article

Quantitative microbial risk assessment and seasonality of hepatitis A virus in a river, Southwest Nigeria

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Background: The pollution of water bodies is a serious concern in most rural areas in low- and middle-income countries due to anthropogenic activities which pose the greatest risk of human hepatitis viruses to public health. The aim of the study was to determine the probability of hepatitis A virus (HAV) infection associated with human consumption of water from River Owena, Nigeria.

Methods: Water samples were collected from points with intense anthropogenic activities during dry and wet periods. Loads of enteric bacteria and HAV were determined using culture-based method and molecular technique. Risks of HAV infection was estimated using dose-response model, and probabilities of clinical illness and mortality were also determined.

Results: Results revealed that the levels E. coli and faecal coliforms were greater during the wet period than the dry period (p < 0.05). Concentration of HAV was greater during the dry period than the wet period (p < 0.05). Risks of HAV infection were higher during the dry period than the wet period, and were all above the US EPA acceptable risk limit. Risk of clinical illness and mortality due to HAV were higher during the dry period than the wet period.

Conclusions: Ingestion of water from the river may result into liver inflammation, morbidity or death. Development of active water management plans to reduce pollutant fluxes and address contamination threats must be established.

Introduction

The Enteric viruses are found in the gastrointestinal tract (GIT) of warm-blooded animals, including humans. Common routes of transmission are through ingestion of water from rivers prone to faecal contamination from indiscriminate discharge of wastewater, solid wastes or agricultural land runoff. The groups of enteric viruses that cause inflammation of the liver are referred to as hepatitis virus [1]. Hepatitis A virus (HAV) is one of the most crucial human waterborne viruses and is widely prevalent in the world as millions of new cases of HAV infections occur worldwide every year [2,3]. It affects all age groups aided by its resistance to treatment, ability to survive in water, and faecal-oral transmission route [4]. Its incubation period is usually between 15 and 45 days [5]. Studies have shown that areas with inadequate water supply and poor wastewater facilities and hygienic conditions generally have very high HAV prevalence [6,7]. The geographic distribution pattern of HAV is highly correlated to socioeconomic level and sanitary conditions [8,9,10]. Outbreaks of infection may occur when contaminated water is used for irrigation purposes in agriculture, as sources of drinking water supply, recreational activities (swimming, bathing, surfing, canoeing, etc.) and shellfish harvesting [7,11,12].

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The World Health Organization (WHO) estimated varying levels of endemicity based on HAV seroprevalence in different countries. In high endemicity situations where HAV is widely circulating, most children are infected before the age of 10 and outbreaks are rarely reported as most children have asymptomatic infections and the majority of adults are immune [13]. In areas of intermediate endemicity a large proportion of children are not infected early in life, leading to higher susceptibility in older age groups and recurrent large outbreaks. Oftentimes, these outbreaks may be seasonal and to a large extent may depend on climate and weather patterns. The climatic condition in the southern part of Nigeria may be described as a tropical monsoon climate, with high temperature and high humidity for the most part of the year. The annual average temperature is about 32°C during the day and approximately 23 °C at night. In addition, the annual rainfall ranges from 1300 to 1650 mm, with two distinct rainfall peaks within a year. The wet (rainy) season normally begins in March and lasts till the end of July with a peak in June. This first wet period is followed by a short dry break in August. The rain then returns early September and lasts till mid-October with a peak period at the end of September. This second wet (rainy) period is followed by a long dry season from late October until early March.

River Owena situated in Owena, a Southwestern town in Nigeria, is prone to faecal contamination from humans, domestic sewage and from various anthropogenic activities taking place in and around the river. This pollution is of great significance to public health because human and around the river. This pollution is of great significance to public health because human population residing around river depends on it for bathing, drinking, irrigation and recreation purposes. Hepatitis has been under reported and only few cases are presented in health care facilities in this region. Faecal coliforms and Escherichia coli have been used as indicator organisms to detect faecal contamination of water in order to protect the public from waterborne pathogens [14]. The extent to which environmental and atmospheric conditions facilitate or constrain the survival, persistence and dispersal of waterborne pathogens and indicator organisms vary [15]. The unclear associations caused by climate change between environmental exposures such as temperature, rainfall and the prevalence and transmission of enteric pathogens has hindered efforts to produce projections of future disease burden trends [13].

Quantitative microbial risk assessment (QMRA) is composed of four steps namely: hazard identification, dose-response determination, exposure assessment and risk characterization. It is useful for estimating the risks that may result from exposure to certain pathogens. In management of water safety, this tool is highly important for human health protection [16,17]. The microbial risks of HAV have been quantitatively assessed in river [18], and drinking water [19]. Studies have also demonstrated the use of QMRA for evaluating potential human health impacts associated with exposure to enteric pathogens in water systems [20,21,22].

The aim of the study was to determine the probability of hepatitis A virus (HAV) infection associated with human consumption of water from River Owena, Nigeria. The objectives of the study were to determine the level of faecal indicator bacteria in water samples from the river in wet and dry periods, investigate the concentration of hepatitis A virus in the water samples, and quantitatively assess the microbial risks of the pathogen in the water samples in wet and dry periods. This will enhance the development of medium- and long-term plans of action to reduce pollutant fluxes and active management plans that will address contamination threats and prevent the occurrence of hepatitis A viral infection in communities around the river.

Methods

Sampling area and collection of samples

River Owena is situated at Igbara-Oke in Ifedore Local government area of Ondo State, Nigeria. It covers an appropriate surface area of 7.8 km2 and located at latitude 7 °24’19.7” North of the equator and longitudes 5 °00’42.1” East. The human population in Owena community is approximately 100,000 and activities such as swimming, bathing, irrigation are popular in and around the river while some residents rely on the water as their drinking water source. Water samples were collected weekly from two representative monitoring points with intense anthropogenic activities on River Owena (n = 48) during dry (December, January, February) and wet (April, May, June) months following the standard methods for the examination of water and wastewater [23]. On each sampling occasion, a grab sample of approximately one liter of water from the river was collected in duplicates at about 20 – 30 cm depth in a clean, pre-sterilized polypropylene plastic
Magnesium chloride (5 mol MgCl₂) was added to 100 ml of water samples by the addition of (RT-qPCR) technique. HAV was concentrated from the water samples from the river using the membrane filtration method [24]. Faecal coliforms had purple methylene blue (EMB) agar and Membrane faecal coliform agar (m-FC). Agar plates were incubated at 37 °C for 24 hours (MLSA, EMB) and 44 °C for 24 hours (m-FC) following the standard membrane filtration method [24]. Faecal coliforms had purple colonies and E. coli had greenish metallic sheen in the completed test for the presumptive test. All colonies were counted, calculated and expressed as colony-forming units (CFU) per 100 ml of water.

**Molecular quantification of hepatitis A virus in the water samples from the river**

The water samples were prepared for analysis by concentration, nucleic acid extraction, and molecular quantification via the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) technique. HAV was concentrated from 100 ml of water samples by the addition of magnesium chloride (5 mol MgCl₂) to increase viral recovery by facilitating and enhancing virus attachment to the filters [25] and filtered through 0.45 µm membrane cellulose nitrate filters, which was used during the concentration step to filter out all bacterial cells from the water samples in order to prevent inhibition of the RT-qPCR. Viruses are smaller than this pore size, therefore, only viral particles will remain in the water samples for detection and quantification. Excess magnesium chloride was removed by passing 0.005 N of sodium chloride through the filter. The virus was then eluted by passing 6% glycine through the filter. The eluted virus was stored in –20 °C freezer. The viral nucleic acid was extracted using a commercial RNA purification kit, Quick-RNATM MiniPrep (Zymo Research, USA) following manufacturer’s protocol. HAV quantification was determined by real-time RT-PCR using a StepOnePlus PCR System (Applied Biosystems, USA) and following a two-step protocol involving a reverse-transcription step and complementary DNA (cDNA)-based qPCR step. HAV was quantified using the forward primer HAV 68 (5′-TCACCGCCGTTTGCTTAG-3′), reverse primer HAV 240 (5′-GGAGAGCCCTGGAAAGAAG-3′) and the FAM-MGBNFQ probe HAV150 (5′-CCTGAAACCTGGAGAATTA-3′) [26]. The real-time procedures for HAV were based on the amplification of a fragment of the highly conserved 5′ non-coding region (5′ NCR).

Briefly, 1 µl of 100 µM Random Hexamer primer was added to 10 µl from the 60 µl of the extracted RNA, 1 µl dNTP mix (10 mM each of the four deoxynucleoside triphosphate stocks), 2.5 µl DEPC-treated water, 4 µl 5 × RT buffer, 0.5 µl Ribolock RNase inhibitor and 1 µl of 200 U/µl Revert AidTM Premium reverse transcriptase (Fermentas, Canada) into a 0.5 ml PCR tube on ice. The reaction mixture (25 µl) was mixed thoroughly using a vortex mixer (Heathrow Scientific, UK) and then centrifuged. The tubes were then incubated at 25 °C for 10 minutes followed by 30 minutes at 60 °C. The reaction was terminated by heating at 85 °C for 5 minutes. The resulting 20 µl of cDNA was kept at -20 °C until use for qPCR. About 5 µl out of 20 µl of the cDNA was mixed with 20 µl of a reaction buffer (containing 12.5 µl of 2 × TaqMan universal PCR master mix (Applied Biosystems), 400 nM sense primer, 400 nM antisense primer, and 250 nM TaqMan probe and PCR grade water to give a 25 µl total reaction mixture. Thereafter, the mixture was added to a 96-well micro-plate and loaded into the StepOnePlus PCR System. Fluorescence data were collected at the end of annealing step. The thermal cycling protocols included Taq activation at 95 °C for 10 mins, followed by 45 cycles of denaturation at 95 °C for 15 seconds, 45 cycles of annealing at 60 °C for 1 min, and 45 cycles of extension at 70 °C for 1 min. PCR amplification, data acquisition and analysis were performed by the real-time PCR machine SDS software (Applied Biosystems). RNase/DNase-free molecular grade water was used as the negative control. The genome copy numbers of HAV were determined by comparison with a standard curve generated with tenfold serially diluted positive control (ATCC VR-1357 Strain PA21) with a slope of -3.71 (i.e., amplification efficiency of 86%), Y-intercept (32.34) and R² value (0.993). A sample with threshold cycle (CT) value of ≤ 31 was defined as positive. The concentrations of the virus in the water samples were equivalent to the target genome copies per 100 ml.
HAV infectious concentration and microbial risk assessment

HAV in the water samples from the river was concentrated using adsorption-elution method described by Junter and Lebrun, [27] that showed a mean viral recovery efficiency of 82 ± 7%. The concentration of HAV detected in the water samples from the river may contain viable, infectious viral particles and non-viable, non-infectious or damaged virus fragments. To estimate the proportion of HAV that were infectious, the fraction (1/60) described by Pinto et al. [11] and Chigor et al. [18] was adopted.

The dose (d) was determined using the equation (1):

\[ d = C \times \frac{1}{R} \times I \times V \]  

(1)

Where: \( C \) = mean concentration of HAV in the water sample; \( R \) = viral recovery efficiency (82%); \( I \) = fraction of infectious HAV (1/60); and \( V \) = volume of water ingested intentionally or accidentally.

The exponential model (equation 2) was utilized to determine the probability of infection associated with exposure to HAV in the water from the river.

\[ P_i = 1 - \exp(-k \times d) \]  

(2)

Where: \( P_i \) = Probability of infection; and \( k \) = probability that the pathogen survives to initiate an infection (0.0001581) as described by Weir, [29].

The annual probability of infection (equation 3) as a result of consuming water from the river was also evaluated.

\[ P_A = 1 - (1 - P_i)^{365} \]  

(3)

Where: \( P_A \) = Annual probability of infection; and \( P_i \) = probability of infection

For exposure assessment, the minimum human health risks from ingestion of the water from the river were estimated. A single exposure to HAV in the water can result in some risk for humans. In this study, the concentration used for the single exposure was ‘1 genome copy and intake of 100 ml was assumed for intentional consumption while intake of 1 ml and 10 ml were assumed for accidental consumption as the goal for estimating the minimum human health risks from ingestion of water from the river.

Morbidity and mortality

The probability of infection was multiplied by 0.5 to determine morbidity (i.e., the probability of clinical illness), while the probability of mortality was determined by multiplying the probability of clinical illness by 0.01% for the population [29].

Statistical analysis

Data was transformed to log10 then examined using general descriptive statistics. One way analysis of variance was carried out and means were separated by Duncan’s New Multiple Range test using SPSS version 23.0. The exponential model was used to estimate the risk of HAV infection, thereafter, the probabilities of clinical illness and mortalities following exposure to infectious HAV were estimated.

Results

Levels of E. coli and faecal coliforms in the water samples from the river

During the dry period, the mean total viable count of E. coli in the water samples from the river was 3.23 log10 CFU 100 ml⁻¹, whereas that of faecal coliforms was 3.33 log10 CFU 100 ml⁻¹. During the wet period, the mean total viable count of E. coli in the water samples from the river was 3.45 log10 CFU 100 ml⁻¹, whereas that of faecal coliforms was 3.60 log10 CFU 100 ml⁻¹. The levels E. coli and faecal coliforms in the water from the river were greater during the wet period than the dry period (Fig. 2).

Levels of HAV in the water samples from the river

The highest and the least mean concentrations of HAV in the water samples from the river were observed the months of December and February, respectively (Fig. 3). Approximately 42% of the water samples from the river presented positive for HAV. All cases of values below detection limit of 1 genome copy per 100 ml were treated as the detection limit. During the dry period, the mean concentration of HAV in the water samples from the river was 4.65 × 10⁵ genome copies 100 ml⁻¹ before taking the efficiency of the virus recovery method into account and it increased to 5.67 × 10⁵ genome copies 100 ml⁻¹ after correction. During the wet period, the mean concentration of HAV in the water samples from the river was 1.82 × 10⁵ genome copies 100 ml⁻¹ without correction and it increased to 2.22 × 10⁵ genome copies 100 ml⁻¹ after correction. The mean concentration of infectious HAV during the dry and wet periods were estimated to be 9.45 × 10⁴ genome copies 100 ml⁻¹ and 3.70 × 10⁴ genome copies 100 ml⁻¹, respectively. Although, the number of the
water samples from the river that presented positive for HAV were greater during the wet period (25%) than the dry period (17%), the mean concentration of infectious HAV were greater during the dry period than the wet period (Table 1).

**Probability of HAV infection from consumption of water from the river**

During the dry period, the mean probability of HAV infection from ingestion of 1 ml of water from the river was $3.03 \times 10^{-5}$, whereas consumption of 10 ml of water from the river revealed mean probability of infection of $3.03 \times 10^{-4}$. The mean probability of HAV infection from ingestion of 100 ml of water from the river was $3.03 \times 10^{-3}$. During the wet period, the mean probability of HAV infection from ingestion of 1 ml of water from the river was $1.19 \times 10^{-5}$, whereas consumption of 10 ml of water from the river revealed mean probability of infection of $1.19 \times 10^{-4}$. The mean probability of HAV infection from ingestion of 100 ml of water from the river was $1.19 \times 10^{-3}$. The risk of infection associated with HAV from the consumption of water from the river was greater during the dry period than the wet period (Fig. 4).

The mean annual probability of infection associated with HAV as a result of consuming water from the river during dry and wet periods was estimated. During the dry period, the mean annual probability of HAV infection due to ingestion of 1 ml of water from the river was $1.10 \times 10^{-2}$; 10 ml of water from the river was $1.05 \times 10^{-1}$; and 100 ml of water from the river was $6.70 \times 10^{-1}$. During the wet period, the mean annual probability of HAV infection due to ingestion of 1 ml of water from the river was $4.30 \times 10^{-3}$; 10 ml of water from the river was $4.30 \times 10^{-2}$; and 100 ml of water from the river was $3.52 \times 10^{-1}$. Again, the mean annual probability of HAV infection from ingestion of water from the river was greater during the dry period than the wet period (Fig. 5).

**Probability of clinical illness and mortality from HAV infection from consumption of water from the river**

During the dry period, the probability of clinical illness from HAV infection was $1.52 \times 10^{-3}$, and the probability of mortality from HAV infection was $3.04 \times 10^{-5}$. Again, the risks of clinical illness and mortality due to HAV infection were higher during the dry period than the wet period (Table 2).

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**Table 1.** Concentration of HAV in water samples from the river during dry (n=24) and wet (n=24) periods

<table>
<thead>
<tr>
<th>Period (% of positive samples)</th>
<th>Mean concentration (genome copies/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without correction</td>
</tr>
<tr>
<td>Dry (17%)</td>
<td>$4.65 \times 10^5$</td>
</tr>
<tr>
<td>Wet (25%)</td>
<td>$1.82 \times 10^5$</td>
</tr>
<tr>
<td>Total (42%)</td>
<td>$6.47 \times 10^5$</td>
</tr>
</tbody>
</table>

Key: Mean values were calculated during dry and wet periods (n = 48). $^a$: The virus recovery efficiency for the concentration of virus in this study was 82% [25]; $^b$: The fraction of detected HAV capable of causing infection estimated using ratio 1:60 [11]

**Table 2.** Probability of clinical illness and mortality from HAV infection during dry and wet periods

<table>
<thead>
<tr>
<th>Period</th>
<th>Probability of infection</th>
<th>Probability of clinical illness</th>
<th>Probability of mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>$3.03 \times 10^{-3}$</td>
<td>$1.52 \times 10^{-3}$</td>
<td>$3.04 \times 10^{-5}$</td>
</tr>
<tr>
<td>Wet</td>
<td>$1.19 \times 10^{-3}$</td>
<td>$5.95 \times 10^{-4}$</td>
<td>$1.19 \times 10^{-5}$</td>
</tr>
</tbody>
</table>
Figure 2. Mean concentration of *E. coli* and faecal coliforms in water from the river during dry (n=24) and wet (n=24) periods (*p* < 0.05)

![Graph showing mean concentration of E. coli and Faecal coliforms](image)

Figure 3. Mean concentration of hepatitis A virus in water from the river during dry (n=24) and wet (n=24) periods (*p* < 0.05)

![Graph showing mean concentration of HAV](image)
Discussion

This study investigated the level of faecal pollution in a river commonly used for bathing, drinking, domestic and irrigational activities in Owena, Nigeria and quantitatively assessed the microbial risks of hepatitis A virus due to ingestion of the water from the river in wet and dry periods. Pollution of surface waters with domestic sewage, agricultural and industrial wastes is common in low- and middle-income countries where wastewater treatment facilities are lacking or inadequate [31]. Although, water pollution is a global problem but the type of pollution varies according to the level of
development, hence communities or countries with high population growth rate and poor waste management systems tend to generate more waste that pollute the aquatic environment than those with low population growth rate and standard waste management systems [32].

In many parts of the world, *E. coli* has been used to indicate faecal pollution of the environment for over a century, since the origin of the bacteria is the faeces of warm-blooded animals; its presence is interpreted to suggest the potential presence of human enteric pathogens [3]. The high level of *E. coli* in water samples from River Owena suggests ‘critical’ faecal contamination exceeding the permissible threshold level of 3.0 log10 CFU 100 ml\(^{-1}\) for good recreational water. Olalemii, [30] had earlier reported that the level of faecal contamination in River Owena was strong, and higher than the levels observed in this present study. This variation may be due to reduction in the level anthropogenic activities contributing faecal pollutant fluxes into the river. The concentration of *E. coli* in the water from the river was greater during the wet period than the dry period. This may likely be due to pollutant influx through runoffs originating from municipal sewage, agricultural land, domestic and faecal waste into the river during rainfall. NNane et al., [33] reported that the concentration of *E. coli* in streams can vary greatly and rain events usually increase the levels of bacteria in the water. On the other hand, the concentration of *E. coli* in the water from River Owena was lower than those reported by Garba et al. [34] where the authors observed higher levels of *E. coli* in Gusau River, North-Western Nigeria. This may be as a result of the geographical location, anthropogenic activities and the level of influx of pollutants into the rivers. Similarly, the levels of faecal coliforms in the water from River Owena were greater during the wet period than the dry period and this may also be attributable to runoffs into the river after rainfall events.

Hepatitis A virus is one of the most important human waterborne viruses that has been observed to pose the greatest risk to public health and it is the most common type of hepatitis virus associated with human hepatitis [9,35,36]. Areas with inadequate water supply and poor wastewater facilities and hygienic conditions generally have very high HAV prevalence. The volume of the River Owena was greater during the wet months and the percentage of water samples positive for HAV were greater in the wet months (25%) than the dry months (17%), but the mean concentration of HAV were greater in the water samples collected in the dry months compared with the wet months. This observation was unlike the concentrations of *E. coli* and faecal coliforms that were higher during the wet months than the dry months. This observation may probably be due to the fact that enteric viruses persist longer than bacteria in environmental samples and during adverse conditions such as increased temperature, as a result of their simple structure and lack of membrane [12,37]. In addition, their non-enveloped structure, enhance their resistance to environmental degradation occasioned by pH changes or desiccation [38,39].

This study represents the first investigation of probability of infection due to HAV in water from River Owena in Ondo State. The results revealed that the risks of infection associated with HAV were higher during the dry period than the wet period and were all above the US EPA acceptable risk limit (10\(^{-4}\)) [40]. Studies have suggested that climatic and behavioral factors such as summer travel to endemic areas and swimming habit of the population in hot months may play a significant role in the seasonality of HAV infection [41,42]. The risk of infection with HAV is greatly influenced to a large extent by poor hygiene, inadequate environmental sanitation and human faecal contamination and this is in agreement with Ouardani et al. [6] where the authors reported high HAV infection rates ranging from 46 % to 68.3 % as a result of ingestion of contaminated surface waters and treated wastewater samples, thereby suggesting the important role of the source of virus in the transmission of the disease. There is no wastewater treatment facility in the community around River Owena and the major sources of faecal pollution of the river are likely from direct defecation, failing septic systems, flooding during wet periods among others. The mean probability of HAV infection from ingestion of 100 ml of water from River Owena were lower than the estimated daily risk of infection observed by Chigor et al. [18] where the authors reported high risks of hepatitis A viral infection that ranged from 2.32 \(\times\) \(10^4\) to 1.73 \(\times\) \(10^4\) due to the ingestion of water from Maden Dam that is among the rivers receiving effluents from some wastewater treatment facilities in South Africa.

Rzezetka et al. [35] reported that the burden of ill health, morbidity and mortality as a result of viral hepatitis is a major public health
challenge. In this study, the risks of clinical illness and mortality due to HAV infection were higher during the dry period than the wet period. The human population in Owena community is approximately 100,000 and activities such as swimming, bathing, irrigation are popular in and around the river while some residents rely on the water as their drinking water source. Assuming that there was uniform risk and if each person in the population was exposed to infectious doses of HAV in the water from the river once a year, the annual cases of clinical illness associated with exposure to HAV would be predicted to be 152 cases during the dry period and 60 cases during the wet period, while the annual cases of mortality due to exposure to HAV would be predicted to be 3 cases during the dry period and 1 case during the wet period. It is important to note that more water samples were positive for HAV, but with lower concentrations and risks of infection, during the wet period than the dry period. The observed greater spatial distribution of HAV during the wet period may lead to the occurrence of higher cases of illness and mortality due to exposure to infectious doses of HAV. However, more individuals swim in rivers during the hot months, thus, exposing greater population to infectious doses of HAV and thereby leading to more cases of illness and mortality during the dry period than the wet period. Residents of the Owena community must be sensitized on the potential human health risks (liver inflammation, morbidity or mortality) that are associated with the usage of water from the river for activities such as swimming, bathing, irrigation or drinking. Provision of wastewater treatment facilities in Owena community, development of active water safety and management plans to reduce pollutant fluxes into the river must be established and contamination threats from point and non-point sources around the river must be addressed in order to protect human health.

Conclusions

Findings from this study revealed that the level of faecal contamination in River Owena may be classified as ‘critical’, and the load of E. coli and faecal coliforms in the water from the river was greater during the wet period than the dry period. The mean concentration of HAV were greater during the dry period than the wet period, the risks of infection associated with HAV were higher during the dry period than the wet period, and were all above the acceptable risk limit. The risks of clinical illness and mortality due to HAV were higher during the dry period than the wet period. Accidental or intentional ingestion of water from the river may result into liver inflammation, morbidity or death. Development of active water management plans to reduce pollutant fluxes and address contamination threats must be established for human health protection.

List of abbreviations

DNA: Deoxyribose nucleic acid
RNA: Ribose nucleic acid
MgCl₂: Magnesium chloride
MdNTPs: Modified dynamic needle tip positioning
Mm: Millimolar
μL: Microlitre
μM: Micromolar
PCR: Polymerase chain reaction
qPCR: Quantitative Polymerase chain reaction
cDNA: Complementary Deoxyribose nucleic acid
ATCC: American Type Culture Centre
US EPA: United States Environmental Protection Agency

Authors’ contribution

‘AOO’: Conceptualization, project administration, validation, visualization and supervision. ‘AEA’: Methodology, software, formal analysis, resources and data curation. ‘MTB’: Writing, original draft preparation, writing review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interests

Authors declare that no competing financial interests or personal relationship exist.

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References

1-Chigor VN, Okoh AI. 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


28- Soller JA. Use of microbial risk assessment to inform the national estimate of acute gastrointestinal illness attributable to microbes in drinking water. J Wat Health 2006;4:165–186.


