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ORIGINAL PAPER

Assessment of the Risks for Human Health of Adenoviruses, Hepatitis A Virus, Rotaviruses and Enteroviruses in the Buffalo River and Three Source Water Dams in the Eastern Cape

Vincent N. Chigor · Timothy Sibanda · Anthony I. Okoh

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Abstract Buffalo River is an important water resource in the Eastern Cape Province of South Africa. The potential risks of infection constituted by exposure to human enteric viruses in the Buffalo River and three source water dams along its course were assessed using mean values and static quantitative microbial risk assessment (QMRA). The daily risks of infection determined by the exponential model [for human adenovirus (HAdV) and enterovirus (EnV)] and the beta-Poisson model (for hepatitis A virus (HAV) and rotavirus (RoV)) varied with sites and exposure scenario. The estimated daily risks of infection values at the sites where the respective viruses were detected, ranged from 7.31×10^{-3} to 1 (for HAdV), 4.23×10^{-2} to 6.54×10^{-1} (RoV), 2.32×10^{-4} to 1.73×10^{-1} (HAV) and 1.32×10^{-4} to 5.70×10^{-2} (EnV). The yearly risks of infection in individuals exposed to the river/dam water via drinking, recreational, domestic or irrigational activities were unacceptably high, exceeding the acceptable risk of 0.01 % (10^{-4} infection/person/year), and the guideline value used as by several nations for drinking water. The risks of illness and death from infection ranged from 6.58×10^{-5} to 5.0×10^{-1} and 6.58×10^{-9} to 5.0×10^{-5}, respectively. The threats here are heightened by the high mortality rates for HAV, and its endemicity in South Africa. Therefore, we conclude that the Buffalo River and its source water dams are a public health hazard. The QMRA presented here is the first of its kinds in the Eastern Cape Province and provides the building block for a quantitatively oriented local guideline for water quality management in the Province.

Keywords Quantitative microbial risk assessment · Human enteric viruses · Risk of infection · Morbidity · Adenoviruses · Hepatitis A virus · Rotaviruses · Enteroviruses

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Introduction

Microbial risk assessment (MRA) is a process that evaluates the probability of adverse human health effects upon exposure to a medium in which pathogens are present (Soller and Eisenberg 2008). The estimation of risk or illness can be achieved directly using epidemiologic data or by indirect estimates, which employ exposure data as input to numerical models to compute estimates of illnesses (Soller 2006). The process entails four steps: hazard identification, exposure assessment, dose–response assessment and risk characterization (WHO 2001; Toze et al. 2010). Despite limitations such as difficulty in characterization of exposure due to uncertainty and variability, limited availability of dose–response relations and subjectivity in the selection of model and parameters (Soller 2006; Soller and Eisenberg 2008), quantitative methods for characterization of human health risks associated with exposure to pathogens have been reported in the last four decades (Fuhs 1975; Haas 1983; Haas et al. 1993; Ottoson and Stenstrom 2003; Teunis et al. 2009; Ahmed et al. 2010). And guidelines have, accordingly, been established (WHO 2001; FAO/WHO 2003; USEPA/USDA/FSIS 2012).

The two prevailing perspectives in quantitative microbial risk assessment (QMRA) are the individual level approaches (static models) and the population level or dynamic models (Soller 2006; Soller and Eisenberg 2008). Assessments using a static model for evaluating microbial risk typically focus on estimating the likelihood of infection or disease to an individual from a single exposure event, and assume that multiple or recurring exposures constitute independent events with identical distributions of contamination (Regli et al. 1991). In static MRA models, it is assumed that the population may be categorized into two epidemiological states: a susceptible state and an infected or diseased state. Susceptible individuals are exposed to the pathogen of interest and move into the infected/diseased state with a probability that is governed by the dose and infectivity of the pathogen to which they are exposed (Soller 2006). Several studies (Haas et al. 1993; Gerba et al. 1996; Mena et al. 2003; Toze et al. 2010) have applied the static model in estimating the risk of infection from enteric viruses. Crabtree et al. (1997) used a static risk assessment model to evaluate the potential health effects associated with adenovirus from drinking water exposures. The MRA method used was similar to that employed for rotavirus by Gerba et al. (1996). Mena et al. (2003) also employed static MRA methods to assess the public health risk associated with drinking waters contaminated with coxsackieviruses. In all, the probability of clinical illness was determined by multiplying the resulting probabilities of infection by 0.5. The probability of mortality was determined by multiplying the probability of illness by 0.01 %. More recently, Toze et al. (2010) used static QMRA to determine the risks in recovered water associated with pathogens, including rotavirus detected by real-time quantitative reverse transcriptase-polymerase chain reaction technique (RT-qPCR).

In QMRA, dose–response data are fit into mathematical models that relate the probability of infection to the mean ingested dose. The exponential and β-Poisson are the prevalent models (Haas et al. 1999). According to Haas et al. (1999), an exponential model is based on the following assumptions: microorganisms are distributed in water randomly and thus follow the Poisson distribution for infection to occur; at least one pathogen must survive within the host; and the probability of infection per ingested or inhaled organism is constant. In the exponential model, each microorganism has the same fixed probability (r) of surviving and reaching a host site at which infection may result. The β-Poisson model is based on comparable assumptions to the exponential model except that the probability of infection per ingested or inhaled pathogen varies with the population. In this model, the probability of surviving and reaching a host site ("r" in the exponential model) is beta distributed, and thus the model contains the two parameters (α and β) of the beta distribution (Soller 2006).

Waterborne outbreaks of infections caused by human enteric viruses (HEntVs) have been reported worldwide (Ramachandran et al. 1998; Adah et al. 2001; Fong and Lipp 2005; Pinto and Saiz 2007; Tallon et al. 2008), South Africa inclusive (Taylor et al. 1993; Rinaldi et al. 2009; Mans et al. 2010). Viral contamination of source waters may cause considerable risk of waterborne infections to consumers or users. The presence of HEntVs in source water, therefore, constitutes a public health hazard. The term hazard represents the pathogen's potential to cause adverse effects in normally healthy humans (USEPA/USDA/FSIS 2012). This potential presents a threat that is dependent on host factors including age/life stage, pregnancy, immune status, natural microbiota, nutrition, social and behavioural traits, etc. (USEPA/USDA/FSIS 2012). In South Africa, a semi-arid, water-stressed country, surface reservoirs are the main water sources for the production of drinking water, as well as for agricultural and recreational purposes (Muller et al. 2009). Such surface waters are vulnerable to pollution and are continuously contaminated with HEntVs originating from sewage and other faecal waste sources (Shuval 1990; RHP 2004; Igbinosa and Okoh 2009; Okoh et al. 2010). Some studies carried out in South Africa have assessed the microbial risks associated with exposure to enteric viruses in raw and/or treated water (Rodda et al. 1993; Steyn et al. 2004; van Heerden et al. 2005a, b; Venter et al. 2007; le Roux et al. 2012). However, there are no reports on risk assessment for waterborne viruses in the Eastern Cape Province.

Earlier, we reported the quantitative PCR (qPCR) and RT-qPCR detection of HEntVs in Buffalo River and three source water dams along its course (Chigor and Okoh 2012a, b). Hepatitis A virus (HAV), human adenoviruses (HAdV), rotaviruses (RoV) and enteroviruses (EnV) were detected in 43.1, 34.7, 13.9 and 9.7 %, respectively, of a total of 72 water samples tested. Although Noroviruses (NoV) are a very important cause of gastroenteritis worldwide (Mans et al. 2010), and an outbreak of NoV infections has been previously reported in South Africa (Taylor et al. 1993), they were neither detected nor quantified in our earlier study for lack of a control strain. This paper reports the results of our studies on the static QMRA analysis, based on exposure and dose–response models for the same study period (August 2010—July 2011) carried out in order to estimate the human health risks due to exposure to HAdV, HAV, RoV and EnV in these surface waters. Located in the Eastern Cape Province, the Buffalo River is important as the major water source in one of the most populous areas on the East coast of southern Africa. Rising at an altitude of 1,200 m in the Amathola Mountains, the Buffalo River flows south-eastwards for about

126 km and drains a catchment of 1,287 km^2 before emptying into the Indian Ocean at East London harbour (RHP 2004). With four dams along its course, the river serves as a source of raw water for drinking water production, as well as for recreational purposes, especially at the estuary and fresh produce irrigation. The river and dam waters also find domestic applications amongst the rural communities in its catchment (Chigor and Okoh 2012a, Chigor et al. 2013b).

Materials and Methods

A four-step static QMRA was applied in this study and it involved: (i) hazard identification, (ii) exposure assessment, (iii) dose–response assessment and (iv) risk characterization (WHO 2001; Toze et al. 2010; USEPA/USDA/FSIS 2012).

Hazard Identification

HEntVs are excreted in high concentrations (10^5–10^{13}/g faeces) (Miagostovich et al. 2008; Bosch et al. 2008). They are transmitted mainly by the faecal-oral route, either directly from person-to-person or via consumption of contaminated food or water (Haramoto et al. 2008; WHO 2011). HEntVs have low infective doses (Ward et al. 1986) ranging from 10 to 100 virus particles (Taylor 2011), although a high infective dose of 1,500 virus particles has been reported for Echovirus 12 (Schiff et al. 1984). While RoV and HAV are the leading causes of epidemic gastroenteritis and acute hepatitis, respectively, in developing countries, HAdV is the second, after rotavirus, as the most important viral pathogen of infantile gastroenteritis (Fong et al. 2010). Additionally, HAdV is associated with respiratory, urinary tract and eye infections (Fong and Lipp 2005; WHO 2011).

HAdV is a 70–100 nm icosahedral, non-enveloped, double-stranded DNA virus belonging to the *Adenoviridae* family. At present, there are 51 serotypes of adenoviruses classified into six species, designated species A to F (Metzgar et al. 2005; Fong and Lipp 2005). Species F contains two fastidious enteric serotypes, 40 and 41, which constitute the majority of waterborne isolates and are amongst the leading causes of childhood diarrhoea (Tiemessen and Nel 1996; WHO 2011), although older children and adults may also be infected (Logan et al. 2006). HAV is a 27-32 nm icosahedral, non-enveloped, single-stranded, positive-sense RNA virus, belonging to the family *Picornaviridae* and the only member of the *Hepatovirus* genus (Kittigul et al. 2006). It is the aetiological agent of hepatitis A which is hyper-endemic in South Africa (Taylor et al. 2001; Venter et al. 2007).

RoV is a 50–65 nm icosahedral, non-enveloped, segmented, double-stranded RNA virus, belonging to the family *Reoviridae* and is the leading cause of severe diarrhoea amongst infants and young children, with an estimated 611,000 deaths from RoV infection per year worldwide (MacIntyre and de Villiers 2010). Almost half of all RoV-induced deaths worldwide are estimated to occur in Africa (Mnwenda et al. 2010; Patel et al. 2011). The genus EnV belongs to the family, *Picornaviridae* and contains 28–30 nm icosahedral, non-enveloped, single-stranded RNA viruses that include poliovirus, coxsackieviruses, echoviruses and the numbered EnVs (Colbere-Garapin et al. 2007). On the current taxonomy, the genus *Enterovirus* consists of 4 species pathogenic to humans and these include: *Enterovirus A, Enterovirus B*, *Enterovirus C* and *Enterovirus D* (www.picornaviridae.com). Poliovirus is known to cause paralysis and meningitis, and about 70 % (62 serotypes) of non-poliovirus enteroviruses have been associated with human infections including meningitis, respiratory disease, hand-foot-and-mouth disease, myocarditis, heart anomalies, diabetes and gastroenteritis (Fong and Lipp 2005; Bosch et al. 2008).

Exposure Assessment

In this study, the exposure analysis was based on four principles: (1) the average concentration of each HEntV in the water samples from each of the six sampling sites on the river, (2) the efficiency of the virus recovery procedure, (3) the viability of the viruses and (4) the average volume of water consumed per individual during recreational activities, drinking and accidental ingestion during other domestic uses or irrigation. The concentrations of each HEntV in the water samples from the studied sites are equivalent to the target gene copies per litre amplified with StepOnePlus PCR System (OPTIPLEX 755; Applied Biosystems) as previously reported (Chigor and Okoh 2012a, b). The daily exposure (d) to each virus was determined using the equation that follows:

$$d = C \times 1/R \times I \times 10^{-\mathrm{DR}} \times V \quad (1)$$

where C = mean concentration of each HEntV in the water sample; R = recovery efficiency of the virus concentration method; I = infectivity (fraction of detected particles capable of infection); DR = removal or inactivation efficiency of the treatment process (DR = 0 for untreated river water); V = volume of river water consumed by an individual (Table 1).

The Efficiency of the Virus Recovery Procedure

As previously reported (Chigor and Okoh 2012a, b), viruses in the water samples studied were concentrated using

Table 1 Summary of parameters used in the estimation of daily and annual risks of infection of human enteric viruses

Parameter	Description	Values			
		HAdV	HAV	RoV	EnV
R	Virus recovery efficiency (%)	56	56	56	56
I	Infectivity (fraction of detected particles capable of infection)	1/2	1/60	1/10	1/100
V	Water consumed per day				
$P_{\text{infection/day}}$	Probability of infection				
d	Dose of infectious viral particles ingested				
r	Exponential model dose–response parameter	0.4172			0.0145
α	Beta-Poisson model dose–response parameter		0.200	0.2531	
β	Dose–response parameter of the beta distribution			0.4265	
N_{50}	Median infectious dose		1,000		
$P_{\text{infection/year}}$	Annual risk of infection				

an adsorption–elution method that was based on cation (Al^{3+})-coated negatively charged membrane filter as described by Haramoto et al. (2005). The method showed a mean recovery efficiency of 56 ± 32 % (Haramoto et al. 2005).

Viability and Estimation of Infectious Concentrations

A major drawback of the RT-PCR assay used in the detection of these HEntVs is its inability to determine the viability and infectivity of viruses detected, as the presence of viral nucleic acid does not necessarily indicate the presence of infectious viruses (Hamza et al. 2009; Bofill-Mas et al. 2010). To circumvent this limitation, previously estimated ratios of infectious viruses to total virus particles were used to estimate the proportion of infectious viruses in this work. For HAdV, the ratio was 1:2 (van Heerden et al. 2005a, b); while for HAV, the ratio was 1:60 (Pinto et al. 2009). In the case of RoV, the ratio of infectious virus particles to total detected virus particles was 1:10 (Rigotto et al. 2010), while for EnV the ratio was 1:100 (de Roda Husman et al. 2009). Nonetheless, the ratio between viable/infectious viruses and genome copies likely varies with the water matrix from which a sample was obtained, specific organism and primer/probe combinations (Ward et al. 1984; Rodríguez et al. 2009; Rutjes et al. 2009). Use of these ratios, therefore, brought with it an unknown level of uncertainty to our analysis. The loss of viruses during purification and viral nucleic acid extraction step was neglected.

Consumption

Although there are default volumes (2,000 mL/person/day for drinking water and 100 mL/day for contact recreational activities) for estimating exposure (WHO 2001; Venter et al. 2007), studies in South Africa on surface waters have used a conservative 100 mL (le Roux et al. 2012) and 30 mL (van Heerden et al. 2005a, b) for estimation of risk via drinking and recreation-based exposures, respectively. We, therefore, in this study assumed the same values. For domestic applications like use of the untreated river/dam water in laundry, dish washing and washing of fruits and vegetables eaten raw, we assumed an ingestion of 10 mL of untreated water (Steyn et al. 2004). We also assumed for persons using the river or dam water for fresh produce irrigation, the water ingested accidentally is a conservative 1 mL/person-event (Ottoson and Stenstrom 2003).

Dose–Response Analysis and Risk Characterization

Dose–Response Models

The daily risks of infection with the enteric viruses were estimated using both the exponential model and the β-Poisson models (Haas 1996; WHO 2001) shown below. While Eq. 2 was used for HAdV and EnV, Eqs. 3 and 4 were used for HAV and RoV, respectively.

$$P_{\text{infection/day}} = 1-\exp(-rd) \tag{2}$$

$$P_{\text{infection/day}} = 1-\left[1+d/N_{50}\left(2^{1/\alpha}-1\right)\right]^{-\alpha} \tag{3}$$

$$P_{\text{infection/day}} = 1-\left[1+d/\beta\right]^{-\alpha} \tag{4}$$

Dose–response relations are needed in QMRA but are of limited availability. The models used in this study were chosen for each virus based on the availability of dose–response parameters. Subjectivity in model and parameter selection is a key drawback of QMRA (Soller 2006). The yearly risk of infection for each virus ($P_{i/\text{year}}$) was calculated as a function of daily risks using Eq. 5 (Haas et al. 1993; Venter et al. 2007). All the parameters are described in Table 1. A risk of 1 in 10,000 persons per year was considered acceptable risk of infection (Haas and Eisenberg 2001)

$$P_{\text{infection/year}} = 1 - \left(1 - P_{\text{infection/day}}\right)^{365} \quad (5)$$

Dose–Response Parameters

The dose–response parameter (r) in the exponential model used in estimating the risk of infection in this study equals 0.4172 for HAdV (van Heerden et al. 2005a, b; USEPA/USDA/FSIS 2012) and 0.0145 for EnV (Haas et al. 1999; Oesterholt et al. 2007). Using the beta-Poisson model to estimate the risk of infection due to HAV, the dose–response parameter, α was 0.200 (WHO 2001) while for RoV α and β assumed the values 0.2531 and 0.4265, respectively (Haas et al. 1999; USEPA/USDA/FSIS 2012). The N_{50} (median infectious dose) for HAV was assumed to have a conservative value of 1,000. Reported N_{50} values ranged from 5.6 to 10,000 (WHO 2001).

Morbidity and Mortality

The probability of clinical illness was determined by multiplying the daily risks of infection by 0.5, while the probability of mortality was determined by multiplying the probability of illness by 0.01 % for the general population (Soller 2006).

Sensitivity Analysis

Sensitivity analysis was performed, following a deterministic approach as described by USEPA/USDA/FSIS (2012), to investigate the contribution of dose–response parameter to the output of the risk models. The dose–response parameter values used are as given in Table 1, and this sensitivity analysis applies to the annual risk of infection, morbidity and mortality as they are all functions of the daily risk of infection. Two representative viruses and two sites were chosen to determine the influence of dose–response values on the daily risks of HEntV infection. HAdV (for the exponential model) and HAV (β-Poisson) were selected because both viruses were detected at both dam sites and non-dam sites, and at higher concentrations compared to the other two viral groups. Rooikrantz Dam was selected because it is located in a rural setting in the upper catchment of the Buffalo River, while Bridle Drift Dam has both urban and rural communities in its catchment.

Results

The detected and corrected mean concentrations as well as the estimated proportions of infectious viruses at each of the sites are shown in Table 2. The corrected mean concentrations ranged from 3.1×10^1 to 2.7×10^3, 2.3×10^3 to 9.8×10^4, 7.9×10^2 to 2.8×10^3 and 2.8×10^{-1} to 0.1×10^1 GC/L for HAdV, HAV, RoV and EnV, respectively. HAdV was detected at 5 of the 6 sites with the estimated mean concentrations of infectious particles ranging from 1.6×10^1 viruses/L (at Rooikrantz dam) to 1.4×10^3 viruses/L (at Parkside). The estimated concentrations of infectious HAV particles, at all the sites, ranged from 3.8×10^1 viruses/L (at Parkside) to 1.6×10^3 viruses/L (at Bridle Drift Dam). RoV and EnV were not detected at any of the dams. The mean concentrations of infectious particles ranged from 7.9×10^1 to 2.8×10^2 and 2.79×10^{-1} to 0.1×10^1 viruses/L for RoV and EnV, respectively.

Figure 1 shows the effect of variation in dose–response parameter on daily risk of enteric virus infection, while the recovery efficiency and volume of water ingested were kept constant at 56 % and 100 mL, respectively. While a similar trend was again observed for both dams, dissimilar trends were observed for both models. For the exponential model (Fig. 1a), the risk of HAdV infection increased with the dose–response parameter (r). Considering the β-Poisson model (Fig. 1b), an increase in the dose–response parameter (α) would decrease the risk of infection by HAV in the two dams.

Figure 2 shows variations in the daily risks of infection associated with the studied enteric viruses for individuals exposed to the river/dam at different sites, via different scenarios. It is clear that HAdV was responsible for the largest risk, with 52–100 % probability of infection resulting from drinking just 100 mL of untreated/unboiled river/dam water. This is closely followed by RoV with 53–65 % probability of infection resulting from consumption of the same amount of water. HAV and EnV represented a much lesser daily risks with 2–30 % and 1–6 % probability infection, respectively, in persons consuming 100 mL of the untreated river/dam water. Considering the sites, and for all exposure scenarios, HAdV represents the highest risks at the non-dam sites (range 10.4–100 %) compared to the dam sites (range 0.7–54 %). The reverse is the case with HAV with higher probabilities of infection at the dam sites (range 0.3–30 %) compared to the non-dam sites (range 0.02–4.2 %).

Estimates of the annual risks of enteric virus infection for individuals exposed to the Buffalo River/dam water via all the four exposure scenarios considered in this study are presented in Table 3. The estimated yearly risks of enteric virus infection were extremely high: always 100 % for RoV, and ranging from 93–100 % for HAdV. Even the risks arising from accidental ingestion of as little as 1 mL of river water (during irrigational use) ranged from 5–19 %. The range of values for yearly risks of infection was wide (8–100 % and 5–100 %) for HAV and EnV, respectively. The data presented show that yearly risks of

Table 2 Mean concentrations of human enteric viruses detected in water samples collected from six sites on the Buffalo River

Enteric virus	Mean concentration (GC/L)	Sampling sites					
		Maden Dam	Rooikrantz Dam	Bridle Drift Dam	King William's Town	Eluxolzweni	Parkside
Human adenoviruses	Uncorrected	VND	1.74×10^{1}	1.86×10^{1}	1.39×10^{3}	2.60×10^{2}	1.51×10^{3}
	Corrected[a]	VND	3.11×10^{1}	3.32×10^{1}	2.48×10^{3}	4.64×10^{2}	2.70×10^{3}
	Infectious[b]	VND	1.55×10^{1}	1.66×10^{1}	1.24×10^{3}	2.32×10^{2}	1.35×10^{3}
Hepatitis A virus	Uncorrected	1.72×10^{4}	1.49×10^{4}	5.42×10^{4}	2.57×10^{3}	1.42×10^{3}	1.26×10^{3}
	Corrected	3.06×10^{4}	2.66×10^{4}	9.68×10^{4}	4.59×10^{3}	2.54×10^{3}	2.25×10^{3}
	Infectious	5.11×10^{2}	4.43×10^{2}	1.61×10^{3}	7.64×10^{1}	4.23×10^{1}	3.75×10^{1}
Rotaviruses	Uncorrected	VND	VND	VND	6.03×10^{2}	1.56×10^{3}	4.44×10^{2}
	Corrected	VND	VND	VND	1.08×10^{3}	2.79×10^{3}	7.93×10^{2}
	Infectious	VND	VND	VND	1.08×10^{2}	2.79×10^{2}	7.93×10^{1}
Enteroviruses	Uncorrected	VND	VND	VND	2.03×10^{1}	1.57×10^{1}	6.98×10^{1}
	Corrected	VND	VND	VND	3.63×10^{1}	2.79×10^{1}	1.25×10^{2}
	Infectious	VND	VND	VND	3.63×10^{-1}	2.79×10^{-1}	1.25×10^{0}

GC genome copies, *VND* virus not detected

[a] The efficiency of recovery of the method of Haramoto et al. used for the concentration of viruses from water samples in this study was 56 % (Haramoto et al. (2005)

[b] The infectious concentrations were estimated based on ratios of infectious viruses to total virus particles based on outcomes of previous studies were used to estimate the proportion of infectious virus for the viruses: HAdV, 1:2 (van Heerden et al. 2005a, b), HAV 1:60 (Pinto et al. 2009), RoV 1:10 (Rigotto et al. 2010), EnV 1:100 (de Roda Husman et al. 2009)

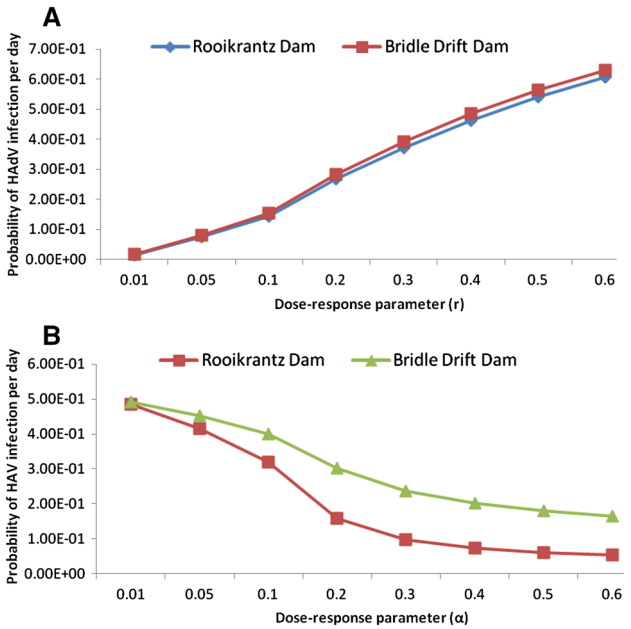

Fig. 1 The influence of dose–response parameter on daily risk of enteric virus infection to individuals using Rooikrantz Dam and Bridle Drift Dam waters for drinking, determined using the exponential model for human adenovirus (**a**) and the beta-Poisson model for hepatitis A virus (**b**)

enteric virus infection were highest if exposure was through drinking, recreational activities or domestic applications, and least when exposure was via irrigational practices. Morbidity and mortality in individuals exposed to the Buffalo River/dam water were each calculated as a function of daily risks. The results shown in Table 4 reveal a similar trend with daily risk of infection (Fig. 2) but lower probabilities.

Discussion

The lack of discernible correlation between enteric viruses and all the previously tested chemical and bacteriological parameters (Chigor et al. 2013a, b) validates previous findings. Jurzik et al. (2010) had earlier reported a lack of correlation between enteric viruses and chemical parameters. Muscillo et al. (2008) reported a prevalence of adenovirus in the environments, where bacterial indicators were absent. These findings call for the evaluation of enteric viruses as possible indicators of viral contamination of water.

The estimated concentrations of infectious particles reported in this study are unacceptably high considering that for many HEntVs, the number of infectious virus particles needed to cause an infection can be very low (Ward et al. 1986; Health Canada 2011). Theoretically, a single infectious virus particle is capable of causing infection, although more than one infectious virus particle is generally required (Health Canada 2011). For instance, the median infectious dose for RoV is 5.597 (Haas et al. 1999) while HAV and NoV have infectious doses that

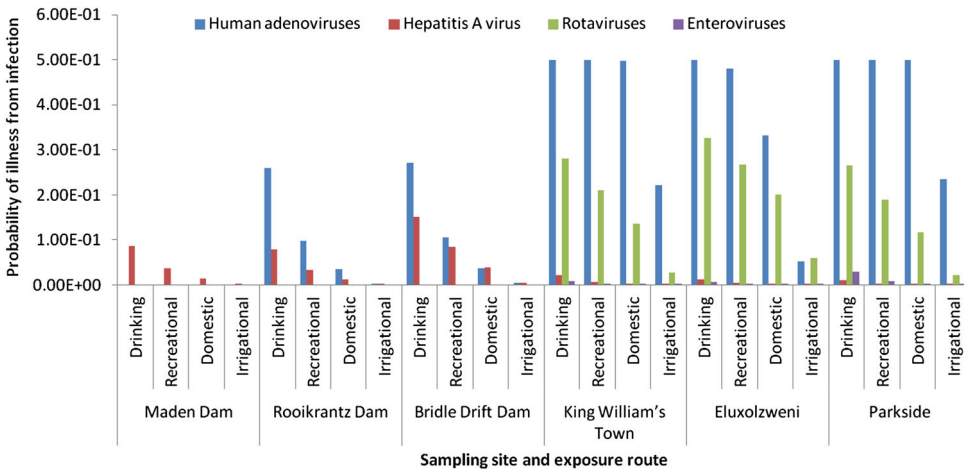

Fig. 2 Daily risk of infection associated with enteric viruses infections for individuals exposed to the Buffalo River/dam waters

Table 3 Calculated probabilities of enteric virus infection for individuals exposed to Buffalo River water

Exposure scenario	Probability of infection/year						
	Enteric viruses	Maden Dam	Rooikrantz Dam	Bridle Drift Dam	King William's Town	Eluxolzweni	Parkside
Drinking	HAdV	VND	1	1	1	1	1
	HAV	1	1	1	1	1	1
	RoV	0	0	0	1	1	1
	EnV	VND	VND	VND	1	9.91×10^{-1}	1
Recreational	HAdV	VND	1	1	1	1	1
	HAV	1	1	1	9.93×10^{-1}	9.40×10^{-1}	9.18×10^{-1}
	RoV	VND	VND	VND	1	1	1
	EnV	VND	VND	VND	8.46×10^{-1}	7.64×10^{-1}	1
Domestic	HAdV	VND	1	1	1	1	1
	HAV	1	1	1	8.19×10^{-1}	6.13×10^{-1}	5.70×10^{-1}
	RoV	VND	VND	VND	1	1	1
	EnV	VND	VND	VND	4.64×10^{-1}	3.82×10^{-1}	8.83×10^{-1}
Irrigational	HAdV	VND	9.31×10^{-1}	9.43×10^{-1}	1	1	1
	HAV	6.82×10^{-1}	6.31×10^{-1}	9.72×10^{-1}	1.59×10^{-1}	9.11×10^{-2}	8.13×10^{-2}
	RoV	VND	VND	VND	1	1	1
	EnV	VND	VND	VND	6.05×10^{-2}	4.69×10^{-2}	1.93×10^{-1}

HAdV human adenoviruses, *HAV* hepatitis A virus, *RoV* rotaviruses, *EnV* enteroviruses, *VND* virus not detected

range from 10 to 100 virus particles (Taylor 2011). However, a high infectious dose of 1,500 virus particles has been reported for Echovirus 12 (Schiff et al. 1984). While the default volume for estimating drinking water exposure is 2,000 mL/person/day (WHO 2001), the daily risks in this study were, however, estimated using volumes of ≤100 mL, thus lowering virus concentrations and hence the risks for the 4 viruses studied. If volumes larger than 100 mL were used, the estimated risks could only be higher.

Being that dose–response parameters do not exist for all pathogens, what is typically done in studies on viruses for which dose–response data have not been generated from clinical trials, for instance HAV, is to adapt previously reported dose–response parameters (Pinto et al. 2009). The implication is that different dose–response parameter values have been applied in QMRA for a single pathogen. While Venter et al. (2007) used the exponential model and used a value of 0.549 for the dose–response parameter (r) to assess the risk of HAV infection in surface water in South Africa, Pinto et al. (2009) used the dose–response parameters α (0.374) and β (186.69) previously reported for echovirus 12 to determine the likelihood of acquiring hepatitis A from consumption of contaminated shellfish in Spain. The present study presents sensitivity analysis data on the effect of dose–response parameter on both the exponential and β-Poisson models.

Table 4 Estimated risk of morbidity and mortality associated with enteric viruses infections for individuals exposed to the Buffalo River/dam waters

Sampling site	Probability of illness from infection				Probability of death from infection			
	HAdV	HAV	RoV	EnV	HAdV	HAV	RoV	EnV
Maden Dam								
Drinking	VND	8.64×10^{-2}	VND	VND	VND	8.64×10^{-6}	VND	VND
Recreational	VND	3.74×10^{-2}	VND	VND	VND	3.74×10^{-6}	VND	VND
Domestic	VND	1.45×10^{-2}	VND	VND	VND	1.45×10^{-6}	VND	VND
Irrigational	VND	1.57×10^{-3}	VND	VND	VND	1.57×10^{-7}	VND	VND
Rooikrantz Dam								
Drinking	2.60×10^{-1}	7.94×10^{-2}	VND	VND	2.60×10^{-5}	7.94×10^{-6}	VND	VND
Recreational	9.86×10^{-2}	3.33×10^{-2}	VND	VND	9.86×10^{-6}	3.33×10^{-6}	VND	VND
Domestic	3.53×10^{-2}	1.27×10^{-2}	VND	VND	3.53×10^{-6}	1.27×10^{-6}	VND	VND
Irrigational	3.65×10^{-3}	1.36×10^{-3}	VND	VND	3.65×10^{-7}	1.36×10^{-7}	VND	VND
Bridle Drift Dam								
Drinking	2.71×10^{-1}	1.51×10^{-1}	VND	VND	2.71×10^{-5}	1.51×10^{-5}	VND	VND
Recreational	1.05×10^{-1}	8.37×10^{-2}	VND	VND	1.05×10^{-5}	8.37×10^{-6}	VND	VND
Domestic	3.76×10^{-2}	3.90×10^{-2}	VND	VND	3.76×10^{-6}	3.90×10^{-6}	VND	VND
Irrigational	3.90×10^{-3}	4.86×10^{-3}	VND	VND	3.90×10^{-7}	4.86×10^{-7}	VND	VND
King William's Town								
Drinking	5.0×10^{-1}	2.08×10^{-2}	2.81×10^{-1}	8.47×10^{-3}	5.0×10^{-5}	2.08×10^{-6}	2.81×10^{-5}	8.47×10^{-7}
Recreational	5.0×10^{-1}	6.82×10^{-3}	2.10×10^{-1}	2.56×10^{-3}	5.0×10^{-5}	6.82×10^{-7}	2.10×10^{-5}	2.56×10^{-7}
Domestic	4.99×10^{-1}	1.30×10^{-3}	1.36×10^{-1}	8.54×10^{-4}	4.99×10^{-5}	2.34×10^{-7}	1.36×10^{-5}	8.54×10^{-8}
Irrigational	2.21×10^{-1}	2.37×10^{-4}	2.77×10^{-2}	8.55×10^{-5}	2.21×10^{-5}	2.37×10^{-8}	2.77×10^{-6}	8.55×10^{-9}
Eluxolzweni								
Drinking	5.0×10^{-1}	1.22×10^{-2}	3.27×10^{-1}	6.54×10^{-3}	5.0×10^{-5}	1.22×10^{-6}	3.27×10^{-5}	6.54×10^{-7}
Recreational	4.81×10^{-1}	3.84×10^{-3}	2.68×10^{-1}	1.97×10^{-3}	4.81×10^{-5}	3.84×10^{-7}	2.68×10^{-5}	1.97×10^{-7}
Domestic	3.33×10^{-1}	1.30×10^{-3}	2.0×10^{-1}	6.58×10^{-4}	3.33×10^{-5}	1.30×10^{-7}	2.0×10^{-5}	6.58×10^{-8}
Irrigational	5.18×10^{-2}	1.31×10^{-4}	5.98×10^{-2}	6.58×10^{-5}	5.18×10^{-6}	1.31×10^{-8}	5.98×10^{-6}	6.58×10^{-9}
Parkside								
Drinking	5.0×10^{-1}	1.09×10^{-2}	2.65×10^{-1}	2.85×10^{-2}	5.0×10^{-5}	1.09×10^{-6}	2.65×10^{-5}	2.85×10^{-6}
Recreational	5.0×10^{-1}	3.42×10^{-3}	1.90×10^{-1}	8.73×10^{-3}	5.0×10^{-5}	3.42×10^{-7}	1.90×10^{-5}	8.73×10^{-7}
Domestic	4.99×10^{-1}	1.15×10^{-3}	1.17×10^{-1}	2.93×10^{-3}	4.99×10^{-5}	1.15×10^{-7}	1.17×10^{-5}	2.93×10^{-7}
Irrigational	2.36×10^{-1}	1.16×10^{-4}	2.11×10^{-2}	2.93×10^{-4}	2.36×10^{-5}	1.16×10^{-8}	2.11×10^{-6}	2.93×10^{-8}

HAdV human adenoviruses, *HAV* hepatitis A virus, *RoV* rotaviruses, *EnV* enteroviruses, *VND* virus not detected

Figure 1a reveals that an increase in the dose–response parameter (r) would increase the risk of HAdV infection in the two dams determined by the exponential model. A similar pattern was reported in previous studies (van Heerden et al. 2005a, b; Venter et al. 2007). Conversely, for the β-Poisson model (Fig. 1b), an increase in the dose–response parameter (α) would decrease the risk of infection by HAV in the two dams. This could be attributable to the inverse functions in the beta-Poisson equations. As the fraction of $1/\alpha$ or d/β becomes smaller, the probability of infection becomes lower.

Almost, the estimated yearly risks of enteric virus infection were always about 100 % (range 93–100 %) for all exposure scenarios, for HAdV and RoV at the sites, where the respective viruses were detected (Fig. 2). However, with regard to the predicted risk (daily or annually) presented by HAdV, Borchardt et al. (2012) found that although infectious adenoviruses were often found in community drinking water, they posed no detectable health risk as measured by acute gastroenteritis during a community intervention/epidemiology study. Therefore, even though the models predict a high risk, the degree of risk is uncertain due to the uncertainty surrounding the assumptions used to run the model. The least probabilities for HAV infection (range 8–16 %) would arise from irrigational water use at the non-dam sites. These values are unacceptably high, given that the generally reported acceptable risk of 0.01 % (10^{-4} infection/person/year)

(Masago et al. 2006; Venter et al. 2007). In their report, on the risk assessment in shellfish-borne outbreaks of hepatitis A in Valencia, Spain, Pinto et al. (2009) assumed that risk of infection equalled risk of illness. In the present study, we estimated both the risk of illness (morbidity) and risk of death (mortality). Exposures to the Buffalo River water via drinking or using untreated water collected from King William's Town or Parkside for domestic purposes and swimming in the river at either site present a 50 % probability of illness from HAdV infection. The risk is lessened by more than half if exposure to the water was through accidental ingestion during irrigational activities. Although the probability of death from HAdV is less than that of illness by 4 logs, the pattern is the same when the sites are compared. The risks of morbidity due to HAdV infections are lower for irrigational workers than for individual that were exposed through drinking, recreation or domestic activities.

The Buffalo River and its dams constitute an important source of raw water for drinking water production. Water from these sources is treated and supplied to a total population of about 880,000 people in the Buffalo City Metropolitan Municipality (BCMM 2012). Although the level of enteric virus removal at the treatment plants in the study area was not known, there are numerous reports on detection of viruses in 5–18.7 % of treated drinking water elsewhere in South Africa (van Zyl et al. 2004; Ehlers et al. 2005; van Heerden et al. 2005a, b; van Zyl et al. 2006). Additionally, treatment can fail. This implies that even people using treated water sourced from the studied sites on the Buffalo River, and its dams may be exposed to unacceptable risk of infection. A recent report (Borchardt et al. 2012) showed that communities with the highest virus measures have correspondingly high acute gastrointestinal illness incidence. However, data regarding the burden of HEntV infections and diseases in South Africa are inadequate, and the current HEntV prevalence and circulating genotypes in South Africa remain largely unknown (Mans et al. 2010).

The use of contaminated river/dam water for irrigation represents potential health risks not only to the vegetable growers and farm workers that may accidentally ingest the water but also to the consumers (Bosch et al. 2011). Fresh produce irrigation is wide spread in the Buffalo River catchment. Studies have demonstrated very high inactivation rates for viruses on the surface of the lettuce leaf due to conditions that greatly promote virus inactivation including exposure to sunlight, high temperatures and desiccation. Therefore, if sufficient time is allowed between final irrigation and consumption (for example 14 days), then, exposure of the consumer to infectious viruses would be very low (Hamilton et al. 2006). Nevertheless, it could be assumed that there may be no reductions in enteric viruses in the fields given that farmers do not cease irrigation for some period before harvesting as they want their vegetables to look fresh at the point of harvest (Seidu et al. 2008).

Besides being based on assumptions made, QMRA has additional limitations. Although dose–response data from the results of clinical trials have been reported for some pathogens and can be used in analysis for other pathogens, it is known that these data were obtained from studies done with healthy adults (Soller 2006). The susceptibility of various individuals to waterborne viral infections may differ depending on various factors, including the immune status and age of an individual as well as the virulence, serotype and route of infection of the virus (Regli et al. 1991). Thus, certain portions of the population including children, the elderly, and individuals with compromised immune systems are not well represented by such data. Furthermore, the risk of infection is related to the viability of the viruses detected. As the viability of HEntVs detected in this study was not determined, the assumed viability of 50, 1.7, 10 and 1 % for HAdV, HAV, RoV and EnV, respectively, based on relevant earlier studies (van Heerden et al. 2005a, b; Pinto et al. 2009; Rigotto et al. 2010; de Roda Husman et al. 2009), may be too high or too low. The consequence of this is that the risk of infection represented by the HEntVs in the Buffalo River water might have been either under-estimated or over-estimated.

The only enteric virus detected at Maden Dam was HAV. However, none of the HAV detected in this study has been typed. It is, therefore, worth noting that the sequence alignment of target regions of the primers and probe used in this study allows for the quantification of all HAV genotypes (Costafreda et al. 2006), including human and simian strains. Should the HAV detected at Maden Dam (in whose catchment exists a large population of monkeys) be simian genotypes, the waters thereof could represent no risk to public health. Being that RoV and EnV were not detected at any of the dams, the dam waters appear to pose no health hazard associated with these two viral pathogens.

Conclusion

There are many assumptions in this risk calculation; however, what is indicated is that the annual risks are significantly higher than the recommended 1 in 10,000 probability of infection (the guideline value used as by several nations for drinking water). The threat here is heightened by the high mortality rates due to HAV and its endemicity in South Africa. The Buffalo River and the source water dams along its course, therefore, are a public health hazard. The QMRA presented here provides the

building block for a quantitatively oriented local guideline for water quality management in the Eastern Cape and the need for public awareness of the health implications of the use of the river water for various recreational and other purposes.

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