Real-time PCR quantitative assessment of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River located in the Eastern Cape Province, South Africa

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ABSTRACT

We applied real-time RT-PCR (reverse transcription-polymerase chain reaction) to assess the incidence of hepatitis A virus, rotaviruses and enteroviruses in the Tyume River, an important water resource in the impoverished Eastern Cape Province of South Africa. Detection of noroviruses was done using conventional semi-nested RT-PCR. Water samples were collected once monthly from 6 sampling sites over a 12-month period starting in August 2010 and ending in July 2011. Hepatitis A virus was detected in 13% of the samples in concentrations ranging between 1.67×10^3 genome copies/ℓ and 1.64×10^4 genome copies/ℓ while rotaviruses were detected in 4% of the samples with concentrations ranging from 9×10^2 genome copies/ℓ to 5.64×10^4 genome copies/ℓ. Enterviruses were not detected in any of the samples, while noroviruses were detected in 4% of the samples. All hepatitis A and rotaviruses positive samples were from the upstream sections of Tyume River while noroviruses were detected in samples from downstream sections only. Statistical analysis showed that occurrence of the viruses in Tyume River was sporadic. Risk analysis showed that hepatitis A virus posed greater risk than rotaviruses for both recreational and domestic water uses. Because of the low infectious dose of enteric viruses, the detection of even low concentrations of hepatitis A virus, rotaviruses and noroviruses in surface water poses a significant risk to public health.

Keywords: Hepatitis A virus, rotaviruses, noroviruses, TaqMan real-time PCR, reverse transcription, Tyume River, public health

INTRODUCTION

Ground and surface water sources may be subjected to faecal contamination from a variety of sources, including sewage treatment plant effluents, on-site septic waste treatment discharge, land runoff from urban, agricultural and natural areas, and leachates from sanitary landfills (Abbaszadegan, 2001). Consequently, millions of people throughout the world do not have access to microbiologically safe water for domestic, recreational and other general purpose uses (Gibson et al., 2011). Health risks associated with surface water use, either raw or treated, include infectious diseases predominantly caused by human and animal enteric pathogens, most notably viruses. RNA viruses constitute the most abundant group of pathogens in man, animals and plants (Bustin and Mueller, 2005). Surveillance of source waters for viral pathogens is therefore necessary to protect public health. The culture-propagation procedure is still the best method to enumerate viruses and demonstrate their infectivity. However, for the detection of noroviruses (NoVs), hepatitis A Virus (HAV) and other enteric viruses like enteroviruses (EVs) for which appropriate cell cultures are not available, slow or limited (Hong et al., 2011), molecular techniques become the most viable option. Molecular techniques have been successfully applied on environmental samples, allowing a rapid and specific detection of human enteric viruses (Bosh et al., 2008; De Paula et al., 2007; Costafreda et al., 2006). The ability of real-time reverse transcription polymerase chain reaction (qRT-PCR) to generate accurate quantitative data has had a huge impact on the study of viral agents of infectious disease (Schutten and Niesters, 2001).

Hepatitis A virus has a worldwide distribution and is the aetiological agent of hepatitis, an acute, usually self-limiting infection of the liver. On average, about 1.5 million cases of clinical hepatitis are recorded world-wide each year (WHO, 2003). Hepatitis A virus belongs to the genus Hepatovirus (Feinstone, 1996) and has a positive-sense, single-stranded RNA genome which is 7.5 kb in length (Murray et al., 2005). The virus is shed in the faeces of persons with both asymptomatic and symptomatic infection and under favourable conditions HAV may survive in the environment for months (CDC, 1999). Hepatitis A disease is rarely fatal but may represent a substantial economic burden, particularly in countries with low and intermediate incidence rates where a larger portion of the adult population do not have immunity against the disease (Grabow, 1997). Infected persons are infective from 14 to 21 days before the onset of jaundice and up to 7 to 8 days after jaundice has resolved (Tong et al., 1995). Food and water have been identified as the main vehicles for HAV transmission (Koopmans et al., 2002). Possible routes of infection with HAV include close personal contact with infected persons (Adhami and Carey, 2010), recreational exposure to fecally polluted surface water (Hunter, 1997), as well as consumption of contaminated food and water (Koff, 1995).

On the other hand rotaviruses (RVs) are responsible for the majority of acute gastroenteritis infections occurring in young children worldwide (Jothikumar et al., 2009). The genus Rotavirus belongs to the family Reoviridae and can be divided into 7 groups, A–G, with the majority of human infections being caused by viruses of Group A (Adlhoch et al., 2011). Rotaviruses are non-encapsulated RNA viruses (Fritzinger et al.,...
2011) with a segmented double-stranded RNA genome (Bustin and Mueller, 2005). Parashar et al. (2006) estimated that infection by Group A rotaviruses results in about 600 000 fatalities annually, most of them in developing countries, while Freeman et al. (2008) postulated that by the age of 5 years, nearly all children in both industrialised and developing countries would have been infected with Group A rotaviruses. While RV infections in young children can be severe, with life-threatening diarrhoea, Kang et al. (2004) report that infections in older individuals may be asymptomatic or be associated with mild enteric symptoms, which the authors speculated to be due to increasing cross-protective immunity as a result of repeated infections.

Noroviruses (NoVs) belong to the genus Norovirus. They comprise of non-enveloped, single-stranded RNA viruses which have been found in humans, pigs, cattle, sheep and mice (Wolf et al., 2010). Human NoVs (HuNoVs) are the most common etiological agent for gastroenteritis outbreaks as well as the leading cause of non-bacterial gastroenteritis in all age groups (Gentry et al., 2009; Siebenga et al., 2009). Human noroviruses GI and GII have been detected in both freshwaters and estuarine waters worldwide. Aw and Gin (2010) reported the detection of both NoV genogroups GI and GII in 100% of sewage and secondary effluent samples in Singapore while Lee and Kim (2008) reported genetic diversity of HuNoVs detected in river water in Korea. Numerous studies have also detected NoVs in environmental waters in Europe (Lysen et al., 2009; Lodder and de Roda Husman, 2005), in the United States of America (Gentry et al., 2009) and in South America (Victoria et al., 2010). A recent report on the detection of enteric viruses in selected urban and rural river water and sewage in Kenya (Kiulila et al., 2010) revealed that HuNoVs GI and GII were detected in 90% of samples collected from urban rivers and streams. Though the first documented NoV outbreaks in South Africa were described as early as 1993, the current NoV prevalence and circulating genotypes are unknown due to lack of NoV outbreak reporting systems (Mans et al., 2010). However, work done by Mans et al. (2013) shows that NoVs are still widely distributed in sewage-polluted river water in South Africa. Enteroviruses (EVs) are RNA viruses belonging to the Picornaviridae family and are classified into 5 human pathogenic species including poliovirus and human enteroviruses (HEVs) A, B, C and D (Dierssen et al., 2008). Echoviruses and coxsackieviruses are the most commonly identified causes of viral meningitis in the paediatric population (Archimbaud et al., 2004) and in adults (Hong et al., 2010). Enteroviruses are associated with diverse clinical syndromes, ranging from mild febrile illness to severe central nervous system diseases, such as aseptic meningitis and encephalitis, potentially leading to paralysis (Hong et al., 2011). These viruses are faecally shed in extremely high numbers from infected individuals, and stable in the environment for extended time periods (Connell et al., 2012). They have a worldwide distribution and have previously been detected in surface water in the USA (Donaldson et al., 2002), Switzerland (Gilgen et al., 1995), Japan (Haramoto et al., 2005) and in treated drinking water in South Africa (Vivier et al., 2004).

Contamination of surface waters with enteric viruses is a concern for public health, especially if these surface waters are used for recreational purposes and production of drinking water (Mans et al., 2013; Rutjes et al., 2005). Pathogen monitoring should be a part of the future characterisation of microbiological hazards in water in order to address risk and effect prevention strategies (Rose and Molloy, 2007). Many areas in developing countries lack access to safe drinking water with around 70% of the global population without improved drinking water sources residing in rural areas (WHO and UNICEF, 2010). Settlements in rural areas are situated far apart and divided by rough terrain, dramatically reducing the capacity to provide a centralised drinking water system. Thus, rural populations commonly obtain water on an individual or household basis from nearby surface and groundwater sources where the microbial quality is often unknown (Peter-Varbanets et al., 2009). Tyume River water is used for the production of drinking water, irrigation and recreational purposes. Faecal contamination of Tyume River has previously been linked to the presence of bacterial pathogens (Momba et al., 2006a). However, traditional bacterial indicators and/or pathogens often fail to predict or correlate with the occurrence of pathogenic waterborne viruses of public health concern (Brooks et al., 2005). To the best of our knowledge, no research has previously been done to detect the presence of viral pathogens in Tyume River. There is therefore a need to cover this knowledge gap. In this paper we report on incidences of some HAV, RVs, EVs and NoVs in Tyume River as well as the risk associated with accidental consumption of water contaminated with these viruses.

MATERIALS AND METHODS

Description of study site

The Tyume River is located in the Nkonkobe Local Municipality, under the Amathole District Municipality, in the Eastern Cape Province of South Africa. It flows from the upper part of the Amathole Mountains in Hogsback, passing through the lower coastal escarpment down to Alice, through several rural settlements, and finally joins the Keiskamma River at Manqulweni community. Close proximity of the river to its host communities makes it an ideal water source for domestic activities where piped potable water is not available. The Tyume River also feeds the Binfeld Park Dam which serves as a source of raw water for a drinking water treatment plant in the area. The selected sampling sites, for this study were, from upstream to downstream: Hala, Khayaletlu, Sinakanaka, Alice, Drayini and Manqulweni.

Hala is a community immediately downstream from the source of the Tyume River in Hogsback. With the river source at Hogsback considered ‘pristine’ and inaccessible, a sampling point in this community located at 32°36'39"S 26°54'34"E was chosen as the first sampling site. Khayaletlu is a rural community upstream of the Binfeld Park Dam. The Khayaletlu sampling site is located at 32°38'22"S 26°56'10"E. The inhabitants of this settlement use the river water for irrigation, recreation, stock watering and domestic purposes. Sinakanaka is also a rural settlement further downstream from Khayaletlu. The sampling site in this community is located at 32°45'37"S 26°51'27"E. The Tyume River is very important to the inhabitants of this settlement as it is used for drinking, fishing, irrigation, livestock watering, recreation and other domestic purposes.

Alice is a small town which has several suburbs. The sampling site is located close to a bridge of a commercial road (R63) linking Alice Town to East London at 32°47'17"S 26°50'31"E. The river is extensively used for irrigation, fishing and domestic purposes, as well as a source of drinking water for livestock. Drayini is a rural town downstream of Alice on the banks of the Tyume River. The sampling site is located at 32°48'37"S 26°52'20"E just downstream of the University of Fort Hare.
farmlands and wastewater treatment plant effluent discharge point. Here the river water is mainly used for livestock watering. Manqulweni is located further downstream from Drayini near the confluence of Tyume River and the Keiskamma River, at 32°54′50″S 26°56′13″E.

**Sampling**

Water samples were collected once monthly at each sampling site over a 12 month period from August 2010 to July 2011, giving a total of 72 samples for the duration of the study. Samples were transported in portable ice chests (cooler boxes) to the Applied and Environmental Microbiology Research Group (AEMREG) Laboratory at the University of Fort Hare, Alice, for analysis within 6 h of sample collection, following the procedure recommended by APHA (2005).

**Concentration of viruses in water**

The adsorption-elution method of Haramoto et al. (2005), with a recovery efficiency of 56%, was used to concentrate viruses in water samples, with some modifications. Under neutral pH conditions viruses are negatively charged and are positively charged under acidic conditions. Multivalent cations (Mg\(^{2+}\), Al\(^{3+}\)) can change the surface charge of viruses thereby allowing adsorption to negatively charged membranes. Aliquots of 5 ml of 250 mM AlCl\(_3\), were passed through HA filters (0.45 μm pore size and 47 mm diameter, Millipore) to form cation (Al\(^{3+}\))-coated filters. Subsequently, 1 l of the water samples was passed through the filters. The cations were then washed by passing 200 ml volumes of 0.5 mM H\(_2\)SO\(_4\) through the membranes. Viral particles were eluted with 10 ml of 1 mM NaOH. Eluates were placed in tubes containing 0.1 ml of 50 mM H\(_2\)SO\(_4\) and 0.1 ml of 100X Tris-EDTA (TE) buffer for neutralisation before ultraconcentration. Ultraconcentration was done using Centriprep YM-50 ultrafiltration devices (Millipore) to obtain final volumes of approximately 700 μl. The sample concentrates were transported in portable ice chests (cooler boxes) to the laboratory over a 12 month period from August 2010 to July 2011, giving a total of 72 samples for the duration of the study. Samples were stored at −80°C until ready for use. Storage of viruses at temperatures below −60°C has been shown to result in insignificant loss of both titre and infectivity for periods longer than a decade (Merrill et al., 2012; Gould, 1999).

**Extraction of viral nucleic acids**

RNA was extracted using 200 μl of the final concentrated sample using commercial RNA purification kits (Quick-RNA™ MiniPrep (Zymo Research, USA)) following the method of Boom et al. (1990). This method is based on the lysing and nuclease-inactivating properties of the chaotropic agent guanidinium thiocyanate (GSCN) together with the nucleic acid-binding properties of silica particles in the presence of this agent. To remove contaminating DNA, the eluates were treated with RNase-free DNase followed by heating at 70°C to inactivate the DNase. However, the RNA extraction efficiency of this method was not monitored but was assumed to be 100%. Purified viral RNA was eluted in 60 μl of RNase-free water.

**Quantification of HAV, RV and EV genomes by qRT-PCR**

Hepatitis A virus and RVs were quantified in a 2-step protocol where RNA was first reverse transcribed into cDNA in a separate reverse-transcription step. Briefly, 10 μl of template RNA, 1 μl of Random Hexamer Primer, 1 μl dNTP mix, 2.5 μl DEPC-treated water, 4 μl 5X RT buffer, 0.5 μl Ribolock RNase inhibitor and 1 μl RevertAid Premium Reverse Transcriptase (Fermentas Life Sciences) were added in the indicated order into a 0.5 ml PCR tube on ice. The mixture was briefly vortexed to ensure total mixing and thereafter centrifuged. The tubes were then incubated at 25°C for 10 min followed by 30 min at 60°C. The reaction was terminated by heating at 85°C for 5 min. For RVs, prior to the reverse transcription reaction the RNA was denatured by heating at 95°C for 5 min followed by incubation on ice for 2 min to separate its double-stranded RNA.

To quantify the viruses, the resultant cDNA was used as template in a quantitative TaqMan real-time PCR (StepOnePlus PCR system; OPTIPLEX 755, Applied Biosystems) with TaqMan probes using a 96-well plate. The wells were loaded with 20 μl of a reaction buffer containing 12.5 μl of 2× TaqMan universal PCR MasterMix [Applied Biosystems], 400 nM sense primer, 400 nM antisense primer, and 250 nM TaqMan probe and PCR grade water (Haramoto et al., 2008). Subsequently, 5 μl aliquots of sample cDNA were added with mixing to give 25-μl total reaction mixtures. The plate was sealed and loaded in the thermocycler. The assays were performed under the following cycling conditions: HAV (10 min at 95°C for Taq activation, and 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 70°C for 1 min); RVs (Taq activation at 95°C for 15 min; 45 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s) and EVs (Taq activation at 95°C for 10 min; 45 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 1 min, and extension at 72°C for 20 s). Fluorescence data were collected at the end of the annealing step. The probes and primer pairs used for quantification are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Enteric virus</th>
<th>Primers and labelled TaqMan Probe</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>HAV</td>
<td>HAV68 (F): 5'-TCA CCG CCG TTT GCC TAG-3' HAV240 (R): 5'-GGG GAG CCC TGG AAG AAA G-3' HAV150 (P): 5'-FAM-CCT GAA CCT GCA GGA ATT AA- MGBFQ-3'</td>
<td>Pinto et al., 2009</td>
</tr>
<tr>
<td>RVs</td>
<td>JVK (F): 5'-CATGTTGTTATGCTAAGATGGG-3' JVK (R): 5'-CTATTGTCAAATCATATTTGAATACCCA-3' JVK (P): 5'-FAM-ACAATGCGACTTCTAAAAGAAGWG-3'</td>
<td>Jothikumar et al., 2009</td>
</tr>
<tr>
<td>EVs</td>
<td>EV1 (F): 5'-GCCCTAGATGCGCTTAAT-3' EV1 (R): 5'-GTGCCAACCATA CAGGCCCA-3' EV (P): 5'-FAM-ACCGACACACAAAAAGTGTGTTCC--MGBFQ-3'</td>
<td>Gregory et al., 2006</td>
</tr>
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Abbreviations: F, forward/sense; R, reverse/antisense; P, probe; FAM, 6-carboxyfluorescein (reporter dye); MGBNFQ, minor groove binder/non-fluorescent quencher

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To determine the qRT-PCR limit of detection, tenfold serial dilutions of cDNA (for each virus) with quantities ranging from 100 000 genomic equivalents to 0.1 genomic equivalents were run under the same conditions as those of the individual viruses. Primer specificity and possible cross-reactivity was determined by substituting target cDNA with non-target DNA. The standard curve for each virus was formulated as described by Brooks et al. (2005) and Haramoto et al. (2008). Briefly, RNA was extracted from positive ATCC strains (HAV: ATCC VR-1357; strain PA21 and RVs: ATCC VR-2274; strain 248) using commercially available extraction kits (Quick-RNA™ MiniPrep (Zymo Research, USA)). The RNA extracts were then reverse transcribed into cDNA using random primers. The resultant cDNA was subsequently quantified using a Qubit® fluorometer (probes.invitrogen.com/qubit) and diluted by serial tenfold dilution. The sample extracts and standards were then subjected to real-time PCR simultaneously, followed by analysis using SDS software (Applied Biosystems) to obtain quantitative data on the titre of viral cDNA in a well. Two wells were used for each of the standards, negative controls (no template controls) and samples, and the average used for subsequent calculations. The total number of viruses in the viral suspensions and eluted samples were estimated by multiplying the titre of viruses per millilitre by the volumes of the samples.

Detection of NoV genogroups by conventional semi-nested PCR

Norovirus genogroups GI and GII were detected by conventional semi-nested PCR as described by Victoria et al. (2010). NoV genogroup GI and GII specific primer sets which target the viral RNA-dependent RNA polymerase gene (Boxman et al., 2006) were used. These primer sets have been used in previous studies for detecting NoV GI and GII in environmental samples (Victoria et al., 2010). The primer sets are shown in Table 2.

PCR cycling conditions for both norovirus GI and GII were as follows: 1st round PCR; 3 min at 94°C to activate the Taq DNA polymerase followed by 40 cycles of 1 min at 94°C, 1.5 min at 37°C, 1 min at 72°C, and a final extension of 72°C for 7 min. The 2nd round PCR was run under the same conditions as the first round, except that initial Taq activation temperature time was increased from 3 min at 94°C to 5 min at 94°C. The composition of the reaction mixtures was as follows: 12.5 μl PCR Master Mix (Fermentas), 1 μl of each of the forward and reverse primers, 5 μl of cDNA (and for 2nd round PCR, 2.5 μl of 1st round PCR amplicon), and nuclease-free water to give a total reaction volume of 25 μl. Amplified products were analysed on ethidium bromide-stained 2% agarose gels.

Risk assessment

A major limitation of the real-time PCR assay used in this study 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 (Bofill-Mas et al., 2010; Hamza et al., 2009). To circumvent this limitation, ratios of infectious viruses to total virus particles based on outcomes of previous studies (Pinto et al., 2009; Grabow et al., 1992) were used to estimate the infectious virus doses for the viruses in this work. In the case of rotaviruses grown in the MA104 cell line, the ratio of infectious virus particles to total detected virus particles was 1:40 000 (Rodriguez et al., 2009; Ward et al., 1984) while for hepatitis A virus the ratio was 1:60 (Pinto et al., 2009; Deng et al., 1994). Calculations for the microbial risk assessment were done to assess the fitness-of-use of the water for domestic and recreational purposes based on assuming accidental consumption of 10 mℓ (Steyn et al., 2004) and 100 mℓ (Venter et al., 2007) of the river water, respectively. The total number of infectious viruses was estimated first by ‘correcting’ the virus concentrations obtained directly from the qRT-PCR assay. ‘Correcting’ was done by multiplying the virus concentrations by the inverse of the mean recovery efficiency of the filtration method, which in this case was 56% (Haramoto et al., 2005). The corrected virus concentrations were then multiplied by the ratio of infectious virus particles to total virus particles for each virus in order to obtain the infectious dose of each virus. Estimates of risks of daily infection for the enteric viruses were determined using the models (WHO, 2001; Haas, 1996) shown below:

\[
P_i = 1 - \left[1 + d / N_{50}(2^{\alpha i} - 1)\right]^{-\alpha}
\]

\[
P_i = 1 - \left[1 + d / \beta\right]^{-\alpha}
\]

Equations (1) and (2) were used for HAV and RVs, respectively. The parameters are described in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Reference</th>
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<tbody>
<tr>
<td>(P_i)</td>
<td>Probability (risk) of infection</td>
<td>Haas, 1996</td>
</tr>
<tr>
<td>(d)</td>
<td>Dose or exposure</td>
<td></td>
</tr>
<tr>
<td>(\alpha) and (\beta)</td>
<td>Parameter characterised by dose-response relationship</td>
<td>Haas et al., 1999</td>
</tr>
<tr>
<td>(N_{50})</td>
<td>Median infectious dose</td>
<td>WHO, 2001</td>
</tr>
</tbody>
</table>

Values of \(\alpha\) and \(\beta\) were 0.2531 and 0.4265, respectively, for rotaviruses (Haas et al., 1993), while for HAV, \(N_{50}\) and \(\alpha\) assumed values of 100 and 0.2, respectively.

Statistical analysis

Results were analysed using the Statistical Package for the Social Sciences (IBM SPSS Statistics release 19; IBM, USA). To test if viral detection depended on season or sampling site, a one-way
ANOVA and Tukey’s studentized range (HSD) Test were used. All tests were carried out at a 5% level of significance.

RESULTS

The detection limit of the real-time PCR assays was determined to be 10 genomic equivalents achieved, with a C<sub>t</sub> value ≤35. Samples with C<sub>t</sub> values ≥35 were therefore considered negative. When non-target cDNA was used, there was no amplification, confirming the specificity of the primer-probe combinations for the target cDNA. Hepatitis A virus was detected in 13% of the samples in concentrations ranging between 1.67×10<sup>3</sup> genome copies/ℓ and 1.64×10<sup>4</sup> genome copies/ℓ. Of these positive samples, 56% were collected between October 2010 and January 2011 while the other 44% were collected between June and July 2011. Hepatitis A virus detection was not affected by season (P>0.05). The amplification plot and standard curve and the log<sub>10</sub> genome copies of HAV per litre obtained after the real-time PCR assay are displayed in Figs. 1 and 2.

Rotavirus RNA was detected in 4% of the samples in concentrations ranging between 9×10<sup>1</sup> genome copies/ℓ and 5.64×10<sup>3</sup> genome copies/ℓ. Of the positive samples about 67% were collected during the winter months (June and July 2011) while 33% were collected in September 2010 (spring). Rotavirus detection did not follow any seasonal pattern (P<0.05). Enteroviruses were not detected in any of the samples, while noroviruses were detected in 4% of the samples (gel picture not shown). All NoV positive samples were collected from 2 downstream sites. Norovirus GI was detected in samples from Alice and Drayini sampling sites in August and September 2010 respectively while NoV GII was detected in a sample from the Alice sampling site in October 2010. Detection of NoVs was sporadic (P<0.05).

DISCUSSION

Hepatitis A infection is the leading cause of acute viral hepatitis throughout the world (Costafreda et al., 2006) while RV gastroenteritis continues to be the single most important cause of dehydration in young children (Kang et al., 2004). In this study, both viruses were detected only in samples collected from the upstream sampling sites where population pressure is less compared to the downstream stretch of Tyume River. This observation is in tandem with documented findings that the distribution patterns of HAV and RVs in different geographical areas of the world are closely related to socioeconomic development, with endemicity being high in less-developed

<table>
<thead>
<tr>
<th>Enteric virus</th>
<th>Infectious: Total number ratio</th>
<th>Reference</th>
<th>Uncorrected concentration (genome copies/ℓ)</th>
<th>Corrected concentration (genome copies/ℓ)</th>
<th>Calculated concentration of infectious viruses (genome copies/ℓ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAV</td>
<td>1:60</td>
<td>Deng et al., 1994; Pinto et al., 2009</td>
<td>8.05×10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.44×10&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.4×10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>RVs</td>
<td>1:40 000</td>
<td>Ward et al., 1984; Rodriguez et al., 2009</td>
<td>1.89×10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.37×10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>8.43×10&lt;sup&gt;-2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Risk assessment

The uncorrected and corrected mean concentrations of viruses as well as the calculated mean concentration of infectious viruses in Tyume River are shown in Table 4.

Assuming accidental ingestion of 10 mℓ and 100 mℓ for domestic and recreational water usage, respectively, the calculated doses were 2.4×10<sup>0</sup> and 2.4×10<sup>1</sup> for HAV and 8.43×10<sup>-4</sup> and 8.43×10<sup>-3</sup> for RVs, respectively. The daily risk of infection results are displayed in Fig. 3.
This page discusses the prevalence and detection of rotavirus (RV) and hepatitis A virus (HAV) in rural areas, particularly in the Tyume River region of South Africa. The paper highlights the importance of adequate sanitation and water treatment to prevent the spread of enteric viruses.

**Detection Methods and Suitability**

- **PCR and EIA**: While PCR is highly sensitive, it can suffer from inhibitors present in water samples, reducing the accuracy of results. The suitability of different detection methods varies; for example, cell culture is more sensitive than conventional RT-PCR, but PCR methods are more sensitive to inhibitors in water.

**Public Health Implications**

- **Swimmer Risk**: Swimming in water sources contaminated with HAV or RVs is a potential health risk for the public, especially in areas with high prevalence of enteric pathogens.
- **Economic Burden**: Waterborne RV infections can lead to extended periods of work loss, estimated at 30 days per annum (Berge et al., 2000). RV infections can also result in long-term health issues, estimated to impose a burden of $20 billion annually in the United States.

**Conclusions**

- The detection of HAV in this study, along with other findings, underscores the importance of adequate sanitation and water treatment in preventing the spread of enteric viruses in rural areas. Further research is needed to better understand the environmental persistence, transmission, and infection risk associated with RVs.

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*Available on website http://www.wrc.org.za*
In general, seasonality of virus infections is difficult to detect in surface water samples because, whereas circulation within the human population is mainly during the summer season, their detection in environmental water samples is mainly during winter (Rutjes et al., 2009), chiefly because of the low temperatures which significantly reduce the deactivation rates. Previous findings note that, whereas RV infections are common all-year round in tropical climates (Cook et al., 1990), RV levels in the environment are generally higher during winter and spring (Heikal et al., 1984), corresponding to seasonal variations of rotoviral diarrhoea in the population (Mehnert and Stiewen, 1993).

Unlike other RNA viruses detected in this study, all NoV detections were in samples collected from the downstream stretch of Tyume River which is more impacted by wastewa- ter effluents than the upper stretch of the river. Noroviruses have previously been detected in wastewater and surface water (Haramoto et al., 2005; Lodder and De Roda Husman, 2005). It has also been noted that NoVs in the urban environment may be transported by stormwater runoff, combined and sanitary sewer overflows, and discharge of wastewater treatment plant effluents (Arnone and Walling, 2007). Norovirus detection in samples from the downstream sampling sites might have been influenced by their relatively close proximities to sewage outfall points. The Alice sampling site is immediately downstream of the effluent discharge points of the Victoria Hospital and Alice Town wastewater treatment plants. The Drayini sampling site, while located downstream of the Alice sampling site, is also located immediately downstream of the University of Fort Hare wastewater treatment plant effluent discharge point. Similar results have been reported by Aw et al. (2009), who detected human NoVs in downstream waters of urban rivers and the receiving estuarine bay, suggesting urban runoff as a source of viral contamination. Considering that viral RNA was concen- trated from only 1 ℓ of sample, it is possible that more positive samples would have been detected had larger volumes been used, since previous findings have shown that there are low concentrations of viruses in environmental waters (Aw et al., 2009), necessitating the concentration of NoVs from larger vol- umes of water. Noroviruses, like most other enteric viruses, are able to survive treatment processes if there is inadequate chlorination. While it is reported that NoVs can be destroyed by ‘adequate chlorination’ (Shin and Sobsey, 2008), their physico-chemical stability helps them to pass through sewage treatment without inactivation and reach many kinds of environmental waters (Victoria et al., 2010).

Naked RNA has been found to have limited survival in the environment (Tsai et al., 1995). Also, viral capsids and not naked RNA has been observed to bind to the membrane used for the selective recovery of viruses (Katayama et al., 2002). This may imply that the RNA found in the water is most likely accompanied by virus particles and would most probably cause infection. Viruses have also been reported to survive and remain infective for up to 130 days in seawater, and for up to 120 days in freshwater and sewage (Fong and Lipp, 2005). Rotaviruses in particular have been found to be stable in environ- mental conditions. Fischer et al. (2002) have reported that in tropical temperatures (30°C), RV particles can survive for more than 2 months and can maintain infectivity for more than 32 months at ≤10°C. While molecular detection methods cannot differentiate between infective and damaged viruses, both these classes of viruses are nevertheless detected by PCR and any such detections therefore could pose a public health risk to the consumer. In a rural setting with no potable water sources, the risk is made all the more real not only by the dependence (of rural people) on surface water for domestic and general purpose uses, but also by the possibility that all contaminating faecal matter, in which viruses could be present in large quanti- ties, would have undergone little or no treatment at all.

Risk assessment

For both HAV and RVs, the calculated risk of infection was higher for recreational water use compared to domestic water use, most likely because of the larger volume (100 mℓ) involved which has the consequence of also increasing the dosage. Even though EVs were not detected in this study, they are nevertheless very widespread in environmental waters and the risk they pose to public health can therefore not be underestimated. It is possible that the results could have been different had sample volumes larger than 1 ℓ been used, considering that these enteric viruses are found in low concentrations in natural water environments and also the possibility of PCR inhibition which could have led to false negative results being obtained.

Exposure to a minimal volume of 10 mℓ Tyume River water would lead to a 1:2 000 risk of infection from RVs which is 5 times more than the acceptable risk level of 1:10 000. Still, this assumes accidental consumption in a domestic set-up as opposed to complete reliance on such water for all domestic uses including drinking. Rotaviruses are the leading cause of gastrointestinal morbidity and mortality among young children and are of much greater public health concern to young children and immunocompromised persons and populations than the general population (USEPA, 2010). Hepatitis A virus presented significantly higher risk of infection figures com- pared to RVs in the case of ingestion of 10 mℓ of river water. The risk of infection with HAV in facecally-polluted water has been found to increase with increased immersion in contaminated water (Gammie and Wyn-Jones, 1997; Taylor et al., 1995). This implies that water from Tyume River may not be suitable for full-contact recreational activities. This risk is significantly higher in children under 10 years of age as well as in immu- nocompromised individuals (Venter et al., 2007). This risk of infection calculation presents a number of uncertainties: The volume of water that was used to assess the fitness-for-use of the water for domestic purposes is far below what an individual human being could consume per day. Even then, the amount of water that samples of individuals consume per day may differ between individuals depending on their levels of physical activity and the state of their health. In this regard, if a volume larger than 10 mℓ were used, the estimated risk could only be larger. However, since environmental samples usually yield a much higher ratio of infectious viruses to total PCR detectable viruses than those viruses that have been adapted to cell culture (Reynolds et al., 1996), the calculated risk of infection values in this study could still be an overestimation of the actual risk. A combination of characteristics cause human enteric viruses to be of great public health concern and these are: increased sta- bility in the environment when compared with faecal bacteria (Fujikawa and Yoneyama, 2002), resistance to some water treat- ment processes (WHO, 2008) and ability to cause infection at low doses (Fong and Lipp, 2005). Jiang et al. (2001) established a link between urban stormwater run-off and the virological contamination of receiving waters making it needful to study the virological water quality of streams and rivers, especially those relied upon as source waters for drinking and recreation. Since the detection of NoVs and HAV on the basis of infectivity is complicated by the absence of reliable cell culture methods.
(Blaise-Boisseau et al., 2010), this real-time PCR detection of these and other viruses in a river of strategic importance to its host community, as Tyme River is, is enough to constitute a public health risk. Because access to potable water in some pockets of the population within the Eastern Cape Province remains a pipe-dream (Momba et al., 2006b) the findings of this study point to a possible public health concern which needs urgent interventions from the responsible authorities.

CONCLUSION

Even though the proportion of infective viruses was only estimated in this study, the fact remains that there is considerable risk of infection posed by the use of raw surface water for either domestic or recreational purposes. The findings of this study also suggest that the presence of enteric viruses in the environmental surface waters is related less to general faecal pollution than it is to the presence of infected individuals in the host population. Future work in this field may include cell culture to verify the proportion of infectious viruses to total virus particles in environmental water samples. Questionnaire surveys may also be conducted in communities within river catchments in the wider Eastern Cape Province so that risk assessment profiling is aligned to water-use patterns specific for communities in those catchments.

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