Quantitative microbial risk assessment to estimate the health risk from exposure to noroviruses in polluted surface water in South Africa

Nicole Van Abel, Janet Mans and Maureen B. Taylor

ABSTRACT

This study assessed the risks posed by noroviruses (NoVs) in surface water used for drinking, domestic, and recreational purposes in South Africa (SA), using a quantitative microbial risk assessment (QMRA) methodology that took a probabilistic approach coupling an exposure assessment with four dose-response models to account for uncertainty. Water samples from three rivers were found to be contaminated with NoV GI (80–1,900 gc/L) and GII (420–9,760 gc/L) leading to risk estimates that were lower for GI than GII. The volume of water consumed and the probabilities of infection were lower for domestic (2.91×10^{-8} to 5.19×10^{-1}) than drinking water exposures (1.04×10^{-5} to 7.24×10^{-1}). The annual probabilities of illness varied depending on the type of recreational water exposure with boating (3.91×10^{-6} to 5.43×10^{-1}) and swimming (6.20×10^{-6} to 6.42×10^{-1}) being slightly greater than playing next to/in the river (5.30×10^{-7} to 5.48×10^{-1}). The QMRA was sensitive to the choice of dose-response model. The risk of NoV infection or illness from contaminated surface water is extremely high in SA, especially for lower socioeconomic individuals, but is similar to reported risks from limited international studies.

Key words | norovirus, QMRA, quantification, recreational water, river water, surface water

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INTRODUCTION

Exposure to contaminated water is an important route for the transmission of diarrhoeal pathogens (Enger *et al.* 2012) and the waterborne transmission of viruses is of public health concern (Gibson 2014). Noroviruses (NoVs) have been identified as the major cause of waterborne disease outbreaks in many regions of the world (Gibson 2014) and are recognised as the predominant cause of sporadic and epidemic gastroenteritis accounting for greater than 90% of viral gastroenteritis and approximately 50% of all-cause outbreaks (Atmar 2010). NoVs are members of the *Caliciviridae* family and are small non-enveloped viruses with a single stranded, positive-sense RNA genome. NoVs contain seven genogroups (GI–VII) with GI, GII and GIV causing infections in humans. Following a mean incubation time of 24 hours, NoV illness, characterised by diarrhoea, vomiting, fever, headache, and muscle aches, is self-limiting with symptoms usually lasting between 24 and 48 hours (Vinje 2015).

In South Africa (SA), outbreaks of NoV-associated gastrointestinal illness were first described in 1993 (Taylor *et al.* 1993) and NoVs have been shown to be the second most important viral agent causing gastroenteritis in children after rotavirus (Mans *et al.* 2010). Norovirus was detected in 21–95% of river samples from selected rivers in the Gauteng Province, SA (Mans *et al.* 2013) and a high diversity of NoV GI and GII genotypes has been identified in wastewater from several provinces of SA (Mans *et al.* 2013; Murray *et al.* 2013a). To estimate the consequences from exposure to NoV, quantitative microbial risk assessment (QMRA) with the four key steps of hazard

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identification, exposure assessment, dose-response assessment, and risk characterisation can be applied (Hunter *et al.* 2003; Mena 2007; Haas *et al.* 2014). In SA to date, only one small study using simple deterministic risk assessment of NoVs in water has been carried out (Genthe *et al.* 2013). The aim of the present study was to quantify NoV in selected river water samples in Gauteng, SA and to assess the risks posed by these NoVs from exposure via drinking, domestic use and recreation.

METHODS

Study area, samples, and qualitative NoV detection

From January 2011 to December 2014, grab water samples (10 L) were collected at sampling points located on the Klip (KR), Suikerbosrant (SR), and Rietspruit (RV) rivers in the Gauteng province, SA. These rivers are all tributaries of the Vaal River, which forms one of the largest river systems in SA. Collected samples were transported cooled on ice blocks to the laboratory. On arrival the temperature and pH of the samples were measured and the samples were stored at 4 °C until processing – usually within 24 h of receipt in the laboratory. Viruses were recovered from the samples by glass wool adsorption-elution followed by secondary concentration using polyethylene glycol 6,000/sodium chloride precipitation as described previously (Mans *et al.* 2013; Kiulia *et al.* 2014). The recovered virus concentrates were resuspended in a final volume of 10 mL phosphate buffered saline (pH 7.4) (Sigma Aldrich Co., St Louis, MO). Nucleic acid was extracted from 1 mL virus concentrate using the MagNa Pure LC Total Nucleic Acid Isolation Kit (large volume) (Roche Diagnostics GmbH, Mannheim, Germany) on an automated MagNa Pure LC platform (Roche) according to the manufacturer's recommendations. The nucleic acid was eluted in a final volume of 100 μL. Norovirus GI and GII (using 5 μL RNA) were detected qualitatively by real-time reverse transcription polymerase-chain reaction (RT-PCR) using commercial NoV GI (norovirusGI@ceeramTools™ Kit, Ceeram S.A.S, La Chappelle-Sur-Erdre, France) and NoV GII (norovirusGII@ceeramTools™ Kit, Ceeram S.A.S) kits which had internal amplification controls. After NoV detection the remaining recovered virus concentrates were stored at −20 °C for between 1 and 4 years before NoV quantification in 2015.

Nov quantification

Norovirus-positive GI and GII water samples, with cycle threshold (C_t – the number of cycles required for the fluorescent signal to cross the background threshold level) values <40 and of sufficient volume, were selected for viral quantification. Samples were thawed and nucleic acid was extracted from 1 mL virus concentrate using using the semi-automated Nucli-SENS® EasyMAG® instrument (BioMérieux, Marcy l'Etoile, France) with the nucleic acid being eluted in a final volume of 100 μL. Prior to nucleic acid extraction, mengovirus (1.97×10^5 genome copies (gc)) was added to each sample as an extraction control. Standard curves for NoV GI and GII were prepared using commercial plasmid DNA quantification standards (Norovirus GI Q Standard & Norovirus GII Q Standard: Ceeram S.A.S). Mengovirus (5 μL RNA) was detected using a qualitative commercial assay mengo@ceeramTools™ Kit, Ceeram S.A.S). NoV GI and GII (5 μL RNA per assay) were quantified separately in each sample using commercial real-time RT-PCR assays (norovirusGI@ceeramTools™ Kit and norovirusGII@ceeramTools™ Kit, Ceeram S.A.S) with the inclusion of a DNA standard in each assay. The extraction efficiency (%) was calculated by comparing the mengovirus concentration after extraction with the input mengovirus concentration (% = final/input × 100). If the extraction efficiency was greater than or equal to 100%, then full extraction of NoV was assumed.

QMRA model

Statistical fitting was programmed in R software (R Foundation for Statistical Computing, Vienna, Austria) as was the QMRA model, which took a probabilistic approach (10,000 iterations) to account for variability and uncertainty. The exposure pathways considered were for both the lower and upper socioeconomic status populations (LSES and USES). For the LSES, the exposure pathways of: (a) consumption of contaminated river water for drinking; (b) incidental exposure by ingestion during domestic activities

such as laundry or body washing were used; and (c) incidental exposure by ingestion during recreational activities (playing by river). For the USES, the exposure pathway considered was incidental exposure by ingestion during recreational activities such as swimming or boating.

Exposure assessment

The exposure assessment quantifies the mean dose (gc per day or event) of NoV (D_{NoV}, Equation (1)) in water for each exposure based on the NoV concentration in water (C_{NoV}), the recovery efficiency of the detection method (R), the proportion of viruses that are infectious (Inf), sunlight inactivation (Inact), and the volume of water consumed (V_{cons}). All exposure assessment parameters are detailed in Table 1.

$$D_{NoV} = C_{NoV} \times \frac{1}{R} \times 10^{-Inact} \times Inf \times V_{cons} \quad (1)$$

The concentration of NoV (gc/L), estimated by laboratory methods, was fit to a lognormal distribution by maximum likelihood estimation. The recovery of NoVs by the glass wool adsorption-elution method has been reported, but with differing recovery values. One study observed low recovery values ranging from 0.01 to 3.80% when recovering NoV GII (Ruhanya 2013) while a second study found recovery efficiencies ranging from 7 to 60% for GI and GII (Lambertini *et al.* 2008). This QMRA model randomly determined which recovery efficiency (high or low) to use in each iteration. Sunlight inactivation was assumed to occur and two studies on the inactivation of murine NoV in water estimated sunlight inactivation to range from 0.1 to 4 logs at temperatures of 10 and 17 °C (Flannery *et al.* 2013; Lee & Ko 2013). This QMRA assumed inactivation to range from 0.1 to 3 logs because the temperature ranged from 8 to 18 °C, but the water was not totally clear (40–80 nephelometric turbidity units (NTU) turbidity) so full inactivation (4 logs) would not be expected. The drinking water was assumed to be consumed without treatment because of the lack of data on the likelihood of individuals to treat surface water before drinking. No dilution was assumed because the exposure pathways were assumed to occur directly in the river where the samples were obtained. All NoVs were assumed to be infectious.

Based on US data the volume of water consumed for drinking water ($V_{cons,DW}$) was estimated to be approximately 1.3 L per person per day (US Environmental Protection Agency (USEPA) 2006; Schijven *et al.* 2011). For domestic activities, the volume of water consumed ($V_{cons,\ dom}$) was estimated to be in the range of 1–10 mL per person per day for Africa (Steyn *et al.* 2004; Katukiza *et al.* 2013a; Yapo *et al.* 2014). The mean volume of water consumed during recreational activities ($V_{cons,\ RW,\ x}$) depended on the activity (s = swimming, p = playing, b = boating) ranging from approximately 2–55 mL per person per event while the range for the exposure events was from 1 to 108 exposure events (days) per year ($n_{RW,\ x}$) (Donovan *et al.* 2008; Dorevitch *et al.* 2011; Schets *et al.* 2011; Sales-Ortells & Medema 2014; US Environmental Protection Agency (USEPA) 2014).

Dose-response assessment

A variety of NoV dose-response models estimating the probability of infection have been proposed for the secretor positive fraction of the population, i.e. those that are capable of binding NoV and becoming infected (Lindesmith *et al.* 2003; Hutson *et al.* 2005). In addition, these models make a variety of assumptions about the aggregation of the dose (Teunis *et al.* 2008; Messner *et al.* 2014; Schmidt 2015). In this QMRA, to account for uncertainty in the predicted outcomes, multiple NoV dose-response models were used because they represent the extreme predicted values (Van Abel *et al.* 2017). The four models used (Table 2) include two disaggregated models, the ${}_1F_1$ hypergeometric (1F1) and the ${}_2F_1$ hypergeometric with immunity (2F1i) with an 'a' approaching 0, and 2 aggregated dose-response models, the ${}_2F_1$ hypergeometric (2F1) and the fractional Poisson (FP). Aggregated and disaggregated models were selected to take both a mechanistic view, the published models describe the mechanisms of norovirus dose-response and can be decomposed so $a \to 0$ can be assumed, and an empirical view, where similarity between the environmental conditions and the dose-response conditions is assumed so $a = 0.9997$ or $\mu_a = 1{,}106$. These two views, as well as the dose-response parameters, have been described in depth previously (Van Abel *et al.* 2017) and are briefly defined as follows: (α, β) are shape parameters of the beta distribution that describe variation in host susceptibility, 'a' is the aggregation parameter ($0 \leq a < 1$) where $a \to 0$ assumes disaggregation, μ_a is the mean

Table 1 | Exposure assessment parameters

Variable	Definition	Units	Distribution	Value	Description	Reference
$C_{NoV\ GI}$	NoV GI Concentration	gc/L	Lognormal	$\mu = 5.650$, $\sigma = 0.7158$	Mean = 360 gc/L	Collected & analysed data (see Table 3 for raw data)
$C_{NoV\ GII}$	NoV GII Concentration			$\mu = 7.203$, $\sigma = 0.7412$	Mean = 1,780 gc/L	
R_{low}	% recovery	%	Uniform	Min = 0.01, max = 3.80	Tap & raw water	Ruhanya (2013)
R_{high}	% recovery	%	Uniform	Min = 7, max = 60	Range across 3 water sources	Lambertini *et al.* (2008)
Inact	Inactivation by sunlight	Log reduction	Uniform	Min = 1, max = 3	NTU = 40 to 80	Best estimate; based on Flannery *et al.* (2013) and Lee & Ko (2013)
Inf	% infectious (viable)	%	n/a	100	Conservative assumption	Best estimate
$V_{cons,\ DW}$	Consumption DW	L/day	Lognormal	$\mu = -0.036$, $\sigma = 0.772$	Mean = 1.3 L per person per day	US Environmental Protection Agency (USEPA) (2006) and Schijven *et al.* (2011)
$V_{cons,\ dom}$	Consumption of water during domestic activities	mL/day	Uniform	Min = 1, max = 10	Ranges of values reported from 1 to 10 mL per person per day for Africa	Steyn *et al.* (2004), Katukiza *et al.* (2013a) and Yapo *et al.* (2014)
$V_{cons,\ RW,\ s}$	Consumption RW, swimming	mL/event	Lognormal	$\mu = 2.92$, $\sigma = 1.43$	Mean = 55 mL/event	Dufour *et al.* (2006) and US Environmental Protection Agency (USEPA) (2014)
$n_{RW,\ s}$	Number of recreation (swimming) events per year	Events/yr	Negative binomial	$r = 1$, $\lambda = 0.04$	Mean = 24 events/yr	Schets *et al.* (2011)
$V_{cons,\ RW,\ p}$	Consumption RW, children playing	mL/hr	Triangle	Min = 0.1, mode = 2.5, max = 11.2	Mean = 2.1 mL/hr	Dorevitch *et al.* (2011) and Sales-Ortells & Medema (2014)
$t_{RW,\ p}$	Time spent playing in or near water	Min/day	Lognormal	$\mu = 4.1$, $\sigma = 0.80$	Mean = 82 min/day	Schets *et al.* (2011) and Sales-Ortells & Medema (2014)
$n_{RW,\ p}$	Number of days per year where child plays by water	Days/yr	Triangle	Min = 1, mode = 12, max = 95	Mean = 15 days/yr	Donovan *et al.* (2008) and Sales-Ortells & Medema (2014)
$V_{cons,\ RW,\ b}$	Consumption RW, boating	mL/hr	Triangle	Min = 0.1, mode = 3.9, max = 11.8	Mean = 1.9 ml/hr; based on canoeing	Dorevitch *et al.* (2011) and Sales-Ortells & Medema (2014)
$t_{RW,\ b}$	Time spent boating	Hr/day	Triangle	Min = 1, mode = 2, max = 4	Mean = 2.1 hr/day	Sales-Ortells & Medema (2014)
$n_{RW,\ b}$	Numbe of recreation (boating) days per year	Days/yr	Uniform	Min = 1, max = 108	Boating can occur from 1 to 108 days per year	Sales-Ortells & Medema (2014)

Legend: gc, genome copies; NTU, nephelometric turbidity unit; DW, drinking water; dom, domestic water use; RW, recreational water; s, swimming; p, playing by riverside; b, boating; min, minimum; max, maximum.

Table 2 | Dose-response models and parameters

DR model	Equation	Parameters	Reference
Disaggregated models			
1F1	$P_{inf} = 1 - {}_1F_1(\alpha, \alpha+\beta, -dose)$	$\alpha = 0.04$, $\beta = 0.055$	Teunis *et al.* (2008)
2F1i	$P_{inf} = (1-\varphi) \times \left[\left(1 - \theta \cdot {}_2F_1\left(\beta, \frac{dose(1-a)}{a}; \alpha+\beta; a\right)\right)\right]$ $\theta = e^{\frac{dose(1-a)*\ln(1-a)}{a}}$	$\alpha = 2.91$, $\beta = 2{,}734$ $\varphi = 0.2754$ $a \to 0$	Schmidt (2015)
Aggregated models			
2F1	$P_{inf} = 1 - {}_2F_1\left(\alpha, \frac{dose(1-a)}{a}, \alpha+\beta, \frac{-a}{1-a}\right)$	$\alpha = 0.04$, $\beta = 0.055$, $a = 0.9997$	Teunis *et al.* (2008)
FP	$P_{inf} = P \times \left(1 - e^{-dose/\mu_a}\right)$	$P = 0.72$, $\mu_a = 1{,}106$	Messner *et al.* (2014)

1F1, ${}_1F_1$ hypergeometric; 2F1i, ${}_2F_1$ hypergeometric with immunity; 2F1, ${}_2F_1$ hypergeometric; FP, fractional Poisson.
(α, β) are shape parameters of the beta distribution that describe variation in host susceptibility, φ is the fraction of secretor positive individuals who are immune to NoV, 'a' is the aggregation parameter ($0 \leq a < 1$), P is the fraction of individuals who are secretor positive that are fully susceptible to NoV infection, and μ_a is the mean number of genomes per aggregate.

number of genomes per aggregate, P is the fraction of individuals who are secretor positive that are fully susceptible, and φ is the fraction of secretor positive individuals who are immune to norovirus (Teunis *et al.* 2008; Messner *et al.* 2014; Schmidt 2015). It should be noted that these dose-response models relate to the mean ingested dose to the outcome of probability of infection (Haas 2002; Haas *et al.* 2014). Also, harmonisation of the PCR procedures between the environmental and human challenge data (McBride *et al.* 2013) was not completed because the environmental data included two genogroups and the human challenge data was compiled from four studies with two genogroups; thus, estimating one adjustment factor to harmonise all the data was not judicious.

The probability of illness was estimated based on the conditional likelihood that the person will become ill if infected ($P_{ill|inf}$) and was calculated by $P_{ill} = P_{ill|inf} \times P_{inf}$. The probability of becoming ill once infected ($P_{ill|inf}$) was defined by a triangular distribution (minimum = 0.3, maximum = 1, mode = 0.6) based on the number of illnesses observed from the infected individuals in a human challenge study (Teunis *et al.* 2008).

Finally, annual illness exposures were estimated for recreational exposures. Annual exposures are based on the number of events per year (Table 1) a person is exposed (Equation (2)). The annual probability of illness ($P_{ill,yr}$) is calculated by the following equation:

$$P_{ill,yr} = 1 - \left(1 - P_{ill,daily}\right)^n \quad (2)$$

where $P_{ill,daily}$ is the daily probability of illness (equation above) and n is the number of exposure events per year (Venter *et al.* 2007; Sales-Ortells & Medema 2014).

Risk characterisation

The risk characterisation determined if any exposure scenarios exceeded the allowable health benchmarks. For drinking and domestic water exposures, the daily allowable risk of less than approximately one in 1,000,000 infections was used (Signor & Ashbolt 2009). For recreational (bathing) water, the annual illness benchmark of less than three illnesses per 100 events was used, which is the revised benchmark that excludes fever as a symptom of highly credible gastrointestinal illness (EU Directive 2006; Soller *et al.* 2010). The risk characterisation also described the impact of risk based on socioeconomic status.

RESULTS

Virus concentration

Norovirus concentrations could be estimated in 50% (17/34) NoV GI and 74% (25/34) NoV GII positive water samples, which had a detection $C_t < 40$. The concentration in the surface waters ranged from approximately 80 to 1,900 gc/L for GI and from 420 to 9,760 gc/L for GII (Table 3). The turbidity as reported by Rand Water ranged from 40 to 80 NTU while

Table 3 | Norovirus GI and GII concentrations in selected surface water in Gauteng, South Africa

Sample		NoV GI				NoV GII			
Date NoV detected	River	Ct value	Raw concentration (gc/L)	Extraction efficiency (%)	Final concentration (gc/L)	Ct value	Raw concentration (gc/L)	Extraction efficiency (%)	Final concentration (gc/L)
2011/02/28	RV	33.99	2.94×10^2	110	2.94×10^2				
2011/05/23	KR					35.95	6.92×10^2	54	1.28×10^3
2011/05/30	RV	34.41	2.30×10^2	43	5.31×10^2	35.73	7.50×10^2	43	1.73×10^3
2011/06/27	SR					37.94	4.20×10^{2a}	325	4.20×10^2
2011/07/04	KR					34.63	1.20×10^3	320	1.20×10^3
2011/07/25	RV	34.66	1.98×10^{2a}	126[b]	1.98×10^2	33.45	2.24×10^3	126	2.24×10^3
2011/08/22	RV	33.51	3.96×10^2	266	3.96×10^2	35.21	9.20×10^2	266	9.20×10^2
2011/08/29	KR	36.24	8.32×10^{1a}	563	8.32×10^1	35.52	8.10×10^2	563	8.10×10^2
2011/09/19	RV	33.98	2.96×10^2	119	2.96×10^2	34.15	1.52×10^3	119	1.52×10^3
2011/10/17	RV	34.68	1.96×10^{2a}	181	1.96×10^2	34.62	1.20×10^3	181	1.20×10^3
2011/10/24	KR	>40							
2011/11/14	RV	32.34	8.42×10^2	100[c]	8.42×10^2	35.07	9.78×10^2	100[c]	9.78×10^2
2011/12/05	RV	32.87	5.94×10^2	31	1.91×10^3				
2012/01/30	KR	35.2	1.46×10^{2a}	283	1.46×10^2	34.96	1.02×10^3	283	1.02×10^3
2012/02/20	RV	34.13	2.72×10^2	79	3.45×10^2				
2012/02/27	KR	>40							
2012/03/19	RV	35.17	1.48×10^{2a}	75	1.96×10^2				
2012/05/21	RV					35.59	7.90×10^2	191	7.90×10^2
2012/06/25	KR					35.41	8.48×10^2	155	8.48×10^2
2012/07/02	KR					36.98	5.08×10^{2a}	81	6.24×10^2
2012/07/30	KR					37.05	5.00×10^{2a}	349	5.00×10^2
2012/08/20	RV					33.48	2.22×10^3	83	2.67×10^3
2013/02/18	RV	35.41	1.33×10^2	60	2.23×10^2	36.12	6.54×10^2	60	1.10×10^3
2013/02/25	KR					37.94	4.20×10^{2a}	334	4.20×10^2
2013/03/18	RV					>40			
2013/04/15	RV					>40			
2013/05/13	RV					34.38	1.35×10^3	270	1.35×10^3
2013/06/10	RV					34.25	1.44×10^3	121	1.44×10^3
2013/09/16	RV					35.89	1.36×10^3	14	9.76×10^3
2013/10/21	KR	34.89	1.78×10^2	235	1.78×10^2				
2013/11/11	RV	36.02	9.60×10^1	63	1.52×10^2	34.83	2.10×10^3	63	3.33×10^3
2014/02/17	RV	33.34	4.52×10^2	147	4.52×10^2	35.15	1.81×10^3	147	1.81×10^3
2014/03/17	RV	>40				36.18	1.23×10^3	21	5.85×10^3
2014/04/14	RV	34.73	1.95×10^2	176	1.95×10^2	34.29	2.72×10^3	176	2.72×10^3

KR, Klip River; RV, Rietspruit River; SR, Suikerbosrant River.

[a]These values were outside the range of the standard curve and were extrapolated.

[b]Extraction efficiencies exceeding 100% are ascribed to clumping of mengovirus stocks.

[c]The extraction efficiency was not estimated for this sample and was assumed to be 100%.

the recorded temperature of the water ranged from 8 to 18 °C and the pH ranged from ~7 to 8.

Risk estimates from the QMRA

A difference in the daily probabilities of infection was observed depending on the type of exposure, NoV genogroup, and dose-response model used (Figure 1). Daily risk estimates from all four dose-response models were lower for recreational water (RW) exposure (min–max: 5.29×10^{-7} to 6.42×10^{-1}) in contrast to drinking water (DW) exposure (min–max: 2.33×10^{-5} to 7.24×10^{-1}). Also, daily risk estimates using all four dose-response models were lower for GI (min–max: 2.91×10^{-8} to 7.02×10^{-1}) versus GII (min–max: 1.19×10^{-7} to 7.24×10^{-1}). Daily risk estimates were highest for the ${}_1F_1$ hypergeometric dose-response model when the mean dose was less than approximately 2,000 gc.

A difference in the estimated daily probabilities of infection was observed for the drinking water exposure for GII versus GI (Figure 2) as well as for the different dose-response models. In general, the estimated probability of infection was higher for GII (min–max: 2.33×10^{-5} to 7.24×10^{-1}) as opposed to GI (min–max: 1.04×10^{-5} to 7.02×10^{-1}) and was highest for the ${}_1F_1$ hypergeometric dose-response model. All exposures, no matter which genogroup or dose-response model, exceeded the allowable daily risk of infection of <1 in 10^6 infections.

The estimated probabilities of infection were lower for domestic water exposure (Figure 3) than for drinking water. Similar to the drinking water exposure, the largest estimates were for GII (min–max: 1.19×10^{-7} to 5.19×10^{-1}) and for the ${}_1F_1$ hypergeometric dose-response model. The exceedances for GI were slightly lower for this exposure scenario at 94% for the fractional Poisson, 96% for the ${}_2F_1$ with immunity, 98% for the ${}_2F_1$ hypergeometric, and 100% for the ${}_1F_1$ hypergeometric. The exceedances for GII were ~100% for all dose-response models.

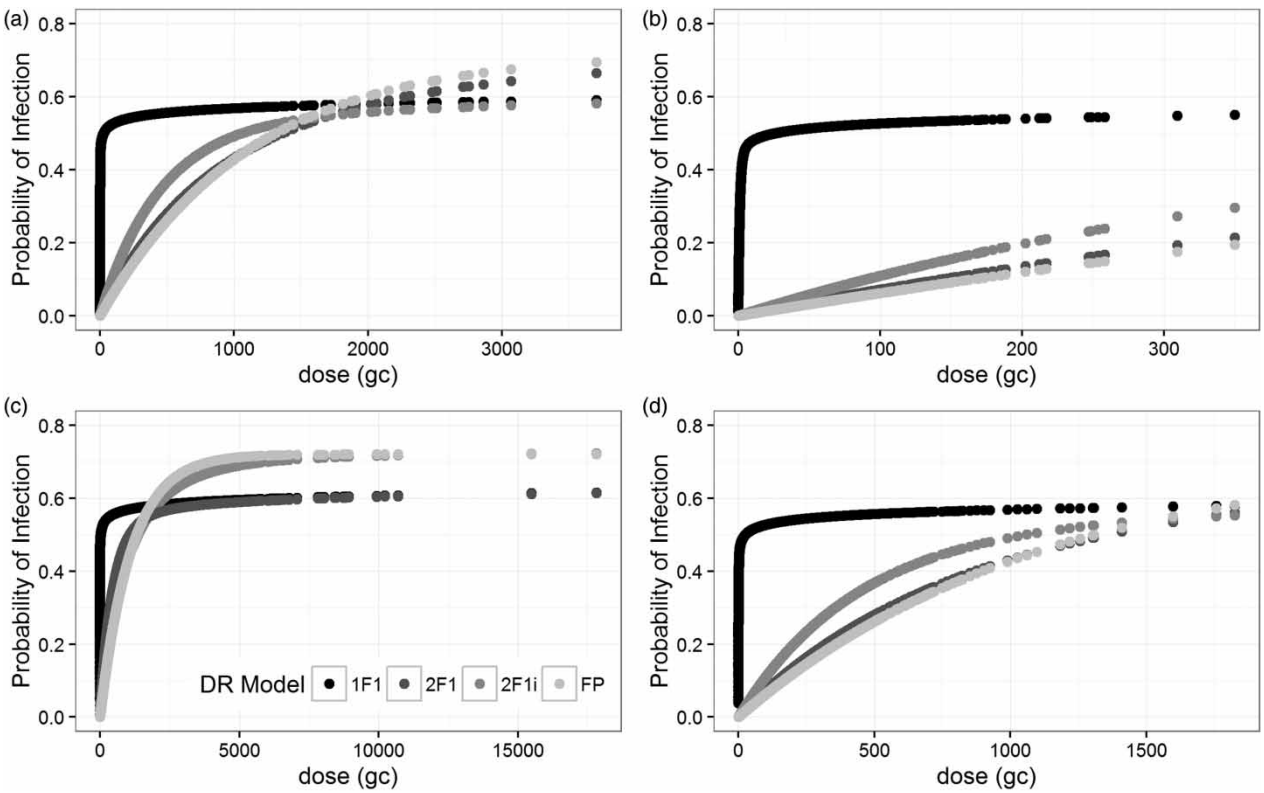

Figure 1 | Comparison of probability of infection (P_{inf}) values for exposure to drinking water (DW) and recreational (swimming) water (RW): (a) P_{inf} for DW exposure to norovirus GI; (b) P_{inf} for RW exposure to norovirus GI; (c) P_{inf} for DW exposure to norovirus GII; (d) P_{inf} for RW exposure to norovirus GII. 1F1 = ${}_1F_1$ hypergeometric, 2F1i = ${}_2F_1$ hypergeometric with immunity, 2F1 = ${}_2F_1$ hypergeometric, FP = fractional Poisson.

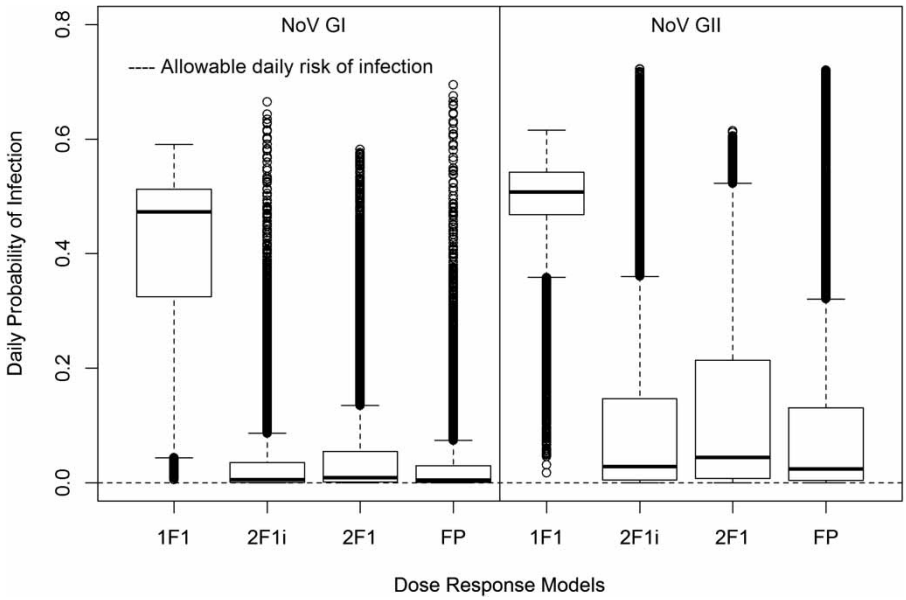

Figure 2 | Daily probability of infection values for drinking water (DW) exposure to norovirus GI and GII. 1F1 = ${}_1F_1$ hypergeometric, 2F1i = ${}_2F_1$ hypergeometric with immunity, 2F1 = ${}_2F_1$ hypergeometric, FP = fractional Poisson; Box = Interquartile range (IQR) (upper = 75%, middle = median, lower = 25%), Whiskers = 1.5*IQR, Points = outliers (i.e. distant from rest of data).

To compare to an annual risk of illness benchmark for recreational water, the probability of illness was estimated. The annual probabilities of illness for recreational exposures varied depending on the type of exposure with the highest risks being from boating (min–max: 3.91×10^{-6} to 5.43×10^{-1}) and swimming (min–max: 6.20×10^{-6} to 6.42×10^{-1}) while lower risks were observed from playing by the riverside (min–max: 5.30×10^{-7} to 5.48×10^{-1}). Again, a difference was observed between the different dose-response models (Figure 4). Also, the

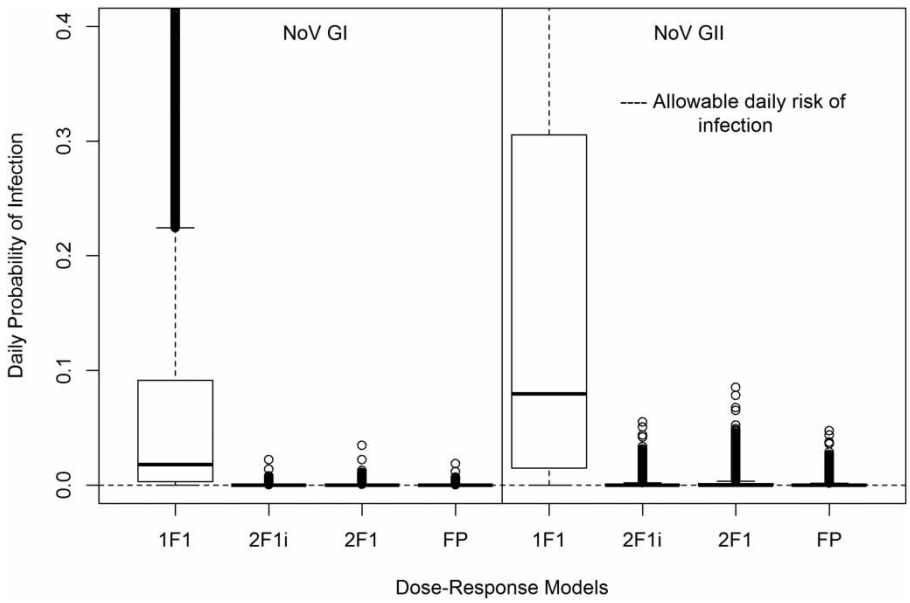

Figure 3 | Daily probability of infection values for domestic water exposure to norovirus GI and GII. 1F1 = ${}_1F_1$ hypergeometric, 2F1i = ${}_2F_1$ hypergeometric with immunity, 2F1 = ${}_2F_1$ hypergeometric, FP = fractional Poisson; Box = Interquartile range (IQR) (upper = 75%, middle = median, lower = 25%), Whiskers = 1.5*IQR, Points = outliers (i.e. distant from rest of data).

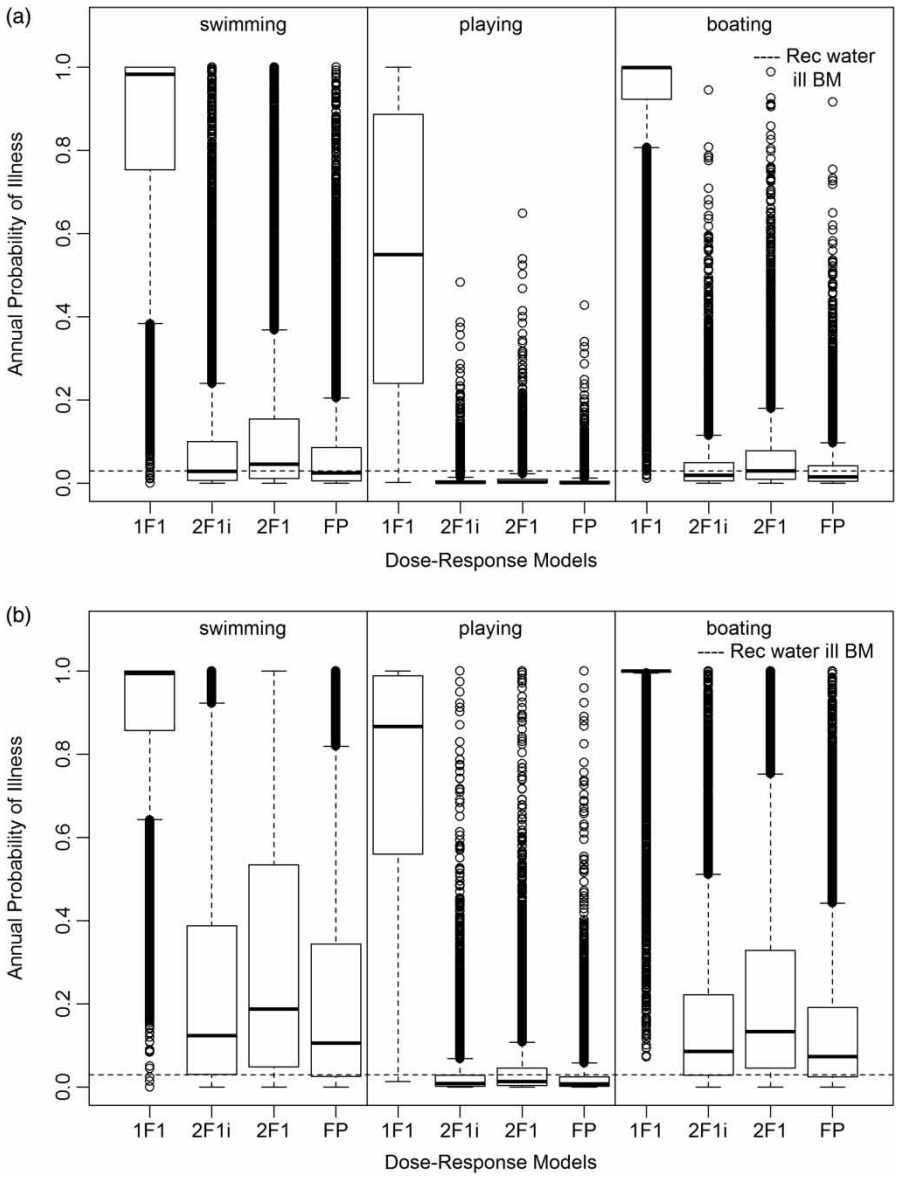

Figure 4 | Annual probability of illness values for all recreational water (RW) exposures to norovirus: (a) GI and (b) GII. 1F1 = ${}_1F_1$ hypergeometric, 2F1i = ${}_2F_1$ hypergeometric with immunity, 2F1 = ${}_2F_1$ hypergeometric, FP = fractional Poisson, BM = benchmark, which is the recreational water illness benchmark. Box = Interquartile range (IQR) (upper = 75%, middle = median, lower = 25%), Whiskers = 1.5*IQR, Points = outliers (i.e. distant from rest of data).

risks were higher for GII as opposed to GI. The exceedances summarised in Table 4 varied greatly depending on the genogroup and dose-response model selected with GII having more exceedances as compared to GI and ${}_1F_1$ hypergeometric consistently predicting the most exceedances.

In general, the percentage of exceedances for the corresponding benchmark was greatest for drinking water exposures followed by domestic water exposures followed by the recreational water exposures.

DISCUSSION

The use of surface water for recreation, drinking, and domestic purposes is associated with potential public health

Table 4 | Exceedances (%) for recreational water exposures for norovirus GI and norovirus GII from all dose-response models

	Swimming				Playing				Boating			
Norovirus genogroup	**1F1**	**2F1i**	**2F1**	**FP**	**1F1**	**2F1i**	**2F1**	**FP**	**1F1**	**2F1i**	**2F1**	**FP**
GI	96	49	58	46	97	5	9	4	100	38	50	34
GII	96	76	82	74	100	24	33	21	100	74	82	71

1F1, ${}_1F_1$ hypergeometric; 2F1i, ${}_2F_1$ hypergeometric with immunity; 2F1, ${}_2F_1$ hypergeometric; FP, fractional Poisson.

risks. This study provided a unique opportunity to investigate the health risks posed by exposure to selected water sources in SA because the concentration of NoV was quantified for the QMRA. Moreover, the concentrations of NoV GI and GII were estimated separately. While the samples had been frozen for a number of years, GI and GII genogroups from 50 and 74% positive samples, respectively, were quantified. A review of the data indicated that the time frozen did not significantly impact the NoV concentration results presented in this paper (data not shown). This was advantageous as many QMRAs do not have quantification data for viruses but extrapolate the concentration from indicator and surrogate data (Howard *et al.* 2006; Seidu *et al.* 2008; Labite *et al.* 2010; Machdar *et al.* 2013; Petterson *et al.* 2016). This is especially true in developing countries where few data exist on the concentration of pathogens in water sources (Enger *et al.* 2012).

The estimated concentration of NoV GI and GII in these SA surface water samples (8×10^1 to 9.76×10^3 gc/L: Table 3) was similar to reported viral concentrations in water sources in sub-Saharan Africa. For open storm drains in Uganda, the estimated concentration of human adenovirus (HAdV) F & G ranged from 1.35×10^2 ($\pm1.9\times10^2$) to 2.65×10^4 ($\pm1.9\times10^4$) gc/L and for rotavirus (RV) from 3.44×10^2 ($\pm4.86\times10^2$) to 2.98×10^4 ($\pm3.66\times10^4$) gc/L, while unprotected spring water had an estimated concentration of HAdV of 7.64×10^0 ($\pm1\times10^1$) gc/L (Katukiza *et al.* 2013a, 2013b). In SA, water samples from the Buffalo River had estimated corrected viral concentrations in gc/L ranging from 3.11×10^1 to 2.48×10^3 for HAdV, 2.25×10^3 to 9.68×10^4 for hepatitis A virus (HAV), 7.93×10^2 to 2.79×10^3 for RV, and 2.79×10^1 to 1.25×10^2 for enterovirus (EV) (Chigor *et al.* 2014), while in Accra, Ghana, the concentration of NoV in stream water, drains, and waste stabilisation pond influent and effluent ranged from $<3.13\times10^3$ to $1.58\pm0.28\times10^5$ gc/L (Silverman *et al.* 2013). The concentration of NoV was, however, lower than the concentration of sapoviruses found in the water samples from the same SA source where average monthly samples ranging from 4.24×10^6 to 6.04×10^8 gc/L (Murray *et al.* 2013b). The concentration of NoV GI and GII in these surface water samples was considered to be clinically relevant because the median infectious dose for NoV has been estimated at approximately 26–2,800 gc (Teunis *et al.* 2008; Atmar *et al.* 2011). The observed higher prevalence of NoV GII compared to NoV GI in the water sources was similar to previous studies in SA where GII was found in 43% and GI in 14% surface water samples from the same sites, respectively (Mans *et al.* 2013). Similarly, the prevalence of NoV GII was 63% while NoV GI was 29% in wastewater samples from SA (Murray *et al.* 2013a). The difference in NoV GI and NoV GII prevalence in surface water sources was not unusual and has been reported in recreational water from nine countries in Europe (Wyn-Jones *et al.* 2011), in surface water in Singapore (Aw & Gin 2011), in urban surface water in Kenya (Kiulia *et al.* 2014) and river water samples in Japan (Kishida *et al.* 2012).

The daily risk of infection from the consumption of untreated surface water as DW ranges from 2.3×10^{-5} to 6.4×10^{-1} for NoV GI and for GII, from 7.3×10^{-5} to 7.2×10^{-1}. At these high probability of infection values, the daily health benchmark of <1 infection in 10^6 individuals is exceeded 100% of the time (Figure 2). The daily risk of infection from domestic water use is less (range GI: 1.3×10^{-7} to 4.8×10^{-1}, GII: 3.6×10^{-7} to 5.1×10^{-1}). The daily health benchmark for domestic water was however still exceeded 97% of the time for GI and 100% of the time for GII when all dose-response models were used to quantify the risk. The daily risk of infection, which was higher for NoV GII when compared to NoV GI, was not

unexpected because the concentration and prevalence of GII was higher than for GI in the surface water samples. In addition, the daily risk of infection was higher when using the water for drinking water rather than for domestic use because the volume of water consumed was orders of magnitude higher for drinking (1.3 L) than domestic water (1–10 mL) use. However, even with the small volume of water consumed during domestic exposures, the daily risk of infection still consistently exceeds the benchmark. It should be noted that protective measures are not often taken during domestic exposures, which could contribute to the higher risks from this pathway (Yapo *et al.* 2014). The risk of infection after drinking and domestic water use exposures are similar to other QMRA results from sub-Saharan Africa. In SA, the consumption of untreated drinking water stored in containers led to an unacceptable risk of infection from *Escherichia coli* and *Salmonella* spp. (Steyn *et al.* 2004), while an individual consuming just 100 mL of untreated contaminated surface water had up to a 26% chance of falling ill from seven enteric pathogens (Le Roux *et al.* 2012). Similar risk results, albeit slightly higher for the maximum risk, were estimated by a study from Ghana that found for RV the risk from drinking water ranged from 1.5×10^{-5} to 3.3×10^{-1} (Machdar *et al.* 2013). In Cote d'Ivoire collecting and washing plastic bags in wastewater canals for domestic water use, a common practice in this country, was associated with the highest risk of infection (Yapo *et al.* 2014).

The risk from recreational exposure was dependent on the type of exposure (Figure 4), with playing by the riverside demonstrating the least percentage of exceedances of the annual recreational water illness benchmark of 0.03 (GI: 4–97%, GII: 21–100%) and swimming yielding the highest risk and exceedances (GI: 46–96%, GII: 74–96%) (Figure 4). Variable results were reported from other QMRAs depending on exposure and water source. In SA, recreational exposure to HAdV-contaminated water was within the acceptable USEPA limits (van Heerden *et al.* 2005), but exposure to HAV was not (Venter *et al.* 2007). In a later QMRA study from SA, that did not specify the recreational exposures, benchmark exceedances for exposure to dam water contaminated with viruses were reported, while the non-dam water exposures were within acceptable limits (Chigor *et al.* 2014). A QMRA in Ghana that found the major transmission route for waterborne diseases was the ingestion of contaminated water by children playing by roadside drains (Labite *et al.* 2010), while in Uganda incidental ingestion exposure to contaminated surface water from open drainage channels contributed the highest disease burden in an urban slum (Katukiza *et al.* 2013a). The differences in QMRA results found to be within acceptable benchmark values could be due to different assumptions made including the volume of water ingested, the number of events per year, and the choice of dose-response model.

An important result from this study was that the recreational exposure risk was dependent on the dose-response model used. This was previously observed in a deterministic QMRA of drinking and water exposures that compared all available NoV dose-response models published in the literature (Van Abel *et al.* 2017). For recreational water exposures, where the intake doses are lower than for consumption of untreated drinking water, the risk of illness estimates from the four dose-response models was disparate (Figure 4). The ${}_1F_1$ hypergeometric model consistently predicted the largest probability of infection values leading to the highest percentages of exceedances at >95% (Table 4). The disparity was most extreme for playing by the river where the risk from GI ranges from ~3 to 100% depending on the dose-response model used. This a consideration because the ${}_1F_1$ hypergeometric function, which leads to the largest risk estimates, is the dose-response model most commonly used in QMRA (Van Abel *et al.* 2017). Use of only this dose-response model in QMRA would be extremely protective of human health, but may also be overestimating the risk burden and could lead to different risk management strategies than if another model was selected for use in the QMRA. The recommendation to use multiple dose-response models in NoV QMRA, even though the risk estimates predicted by the four dose-response models are disparate (~6 orders of magnitude), ensures the complete picture of risk is described including the conservative estimates that may be more protective of public health. Without more information on which norovirus dose-response model is 'best' then no one model can be put forward, thus the recommendation is to select multiple appropriate NoV dose-response models considering aggregation, genogroup, and secretor status for each NoV QMRA (Van Abel *et al.* 2017).

The calculated risk of infection for the drinking and domestic use of NoV-contaminated water places the greatest risk burden on individuals of LSES, as these individuals may have regular access to safe water in their community, but intermittent interruptions in the water supply may result in communities using surface water for drinking or domestic purposes. Even a few days of interrupted water supply has been shown to destroy the health benefits of treated water (Hunter *et al.* 2009). In contrast, recreational exposure to contaminated surface water can impact both LSES and USES individuals. Both LSES and USES individuals could swim in the surface water, which places both of these groups at increased risk of NoV infection or illness. The risk from playing next to the river, mainly associated with LSES, had the lowest risks. Boating was also associated with an increased risk of illness, which would probably only impact the USES individuals. Overall, the risk burden falls more heavily on LSES than USES which confirms QMRA results for HAV infection in individuals from the same geographical area (Venter *et al.* 2007). In addition to SES, vulnerable populations must be addressed. Susceptible individuals who are at a higher risk of (NoV) infection include the very young, the elderly, the malnourished, pregnant women and immunocompromised individuals, including human immunodeficiency virus/acquired immunodeficiency patients (HIV/AIDS) who may present with chronic NoV infection due to supressed immune systems (Gerba *et al.* 1996; Wingfield *et al.* 2010; Haas *et al.* 2014). The high prevalence of HIV/AIDS in SA predisposes a significant portion of the population to chronic NoV infection or illness, which could lead to a serious public health threat in this vulnerable population (Mans *et al.* 2013).

One of the most important findings of this QMRA study was that the choice of dose-response model was critical to the outcome of the QMRA, a finding that has been described previously (Teunis & Havelaar 2000). Therefore, to account for the uncertainty in NoV dose-response, multiple models (Van Abel *et al.* 2017) were used in the present study to formulate a complete picture of the risk. Of concern was that the four dose-response models were determined from predominantly NoV GI.1 data. Therefore, the assumption must be made that GII is as infectious as GI, which has not yet been demonstrated. Overall, more information on NoV dose-response for both GI and GII is needed. The lack of adequate information on the recovery of NoV is another limitation. This limitation has also been identified by others and a request for more pathogen recovery data was made (Vivier *et al.* 2002; de Man *et al.* 2014). For NoV, the recovery efficiencies estimated for glass-wool adsorption elution were very disparate. Ultimately, the recovery data from two studies (Lambertini *et al.* 2008; Ruhanya 2013) were used to address this uncertainty.

Further uncertainty in the risk estimates results from the lack of NoV infectivity information and this work included the assumption that all viruses are infectious. For the quantification of NoV in water, PCR was used, which is a method that cannot distinguish between infectious and non-infectious particles, but does demonstrate, at the very least, viral contamination (Vergara *et al.* 2016). However, until recently no cell culture method for NoV has repeatedly demonstrated success (Jones *et al.* 2015; Ettayebi *et al.* 2016) so an assumption about the percentage of infectious NoVs was made (Girones *et al.* 2010; World Health Organization 2011). This QMRA also assumed that the LSES individuals consumed the raw surface water without boiling or treating it in any manner prior to consumption. Others have reported that in-home treatment interventions for drinking water may occur, but the follow-up periods on the review of these techniques is short and the results incomplete (Hunter *et al.* 2009). Ultimately, it is unclear what water treatment measures would be taken and whether they would be carried out completely to ensure inactivation of the virus. Thus, the assumption was made that the water was consumed without treatment. The daily risk of infection from drinking or domestic water exposures is exceptionally high, leading to exceedances of the daily infection health benchmark. For recreational water exposures, the results for the annual risk of illness, as well as the percentage of exceedances, demonstrated huge variability. It should be noted that both health benchmarks were developed for use in the United States and the European Union, which may not be representative of a developing country like SA. Considering the limitations and assumptions, an overestimation of the drinking water risk may be demonstrated. However, although the results of this analysis indicate a high risk of NoV infection and illness from exposure to river water in SA, this is congruent with other QMRA results. Unacceptably high risks to water users in the Olifants River catchment in SA from exposure to

seven pathogens was demonstrated and the greatest risk to water users was found to be exposure to NoV (Le Roux *et al.* 2012; Genthe *et al.* 2013).

CONCLUSIONS

The results of this assessment demonstrate that selected surface waters in SA are contaminated with NoV which can lead to unacceptable risks of infection from exposure via direct drinking of untreated surface water as well as domestic and recreational use. Furthermore, this work presented quantitative data on the concentration of NoV GI and GII in surface waters, which is useful for QMRA. The results of the QMRA found that: (1) a higher risk was observed for GII as compared to GI; (2) the daily probability of infection risk was higher for drinking water exposures as compared to domestic use of water; (3) the annual probability of illness from recreation exposures was highest for boating and swimming and lowest for playing by the river; and (4) the risk burden was greater for individuals of LSES who may use untreated surface water for multiple household and recreational uses. Finally, the choice of dose-response model was critical to the outcome of the QMRA, so multiple dose-response models must be used in NoV QMRA to account for uncertainty and to completely describe the risk.

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