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Probabilistic quantitative microbial risk assessment model of norovirus from wastewater irrigated vegetables in Ghana using genome copies and fecal indicator ratio conversion for estimating exposure dose

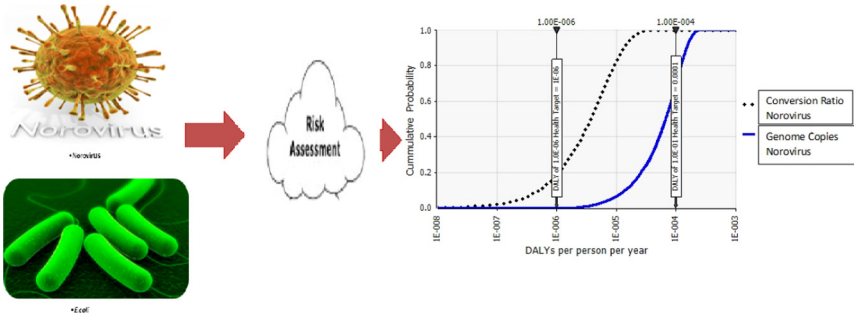
Emmanuel de-Graft Johnson Owusu-Ansah [a,*], Angelina Sampson [b], Samuel K. Amponsah [a], Robert C. Abaidoo [c], Anders Dalsgaard [d], Tine Hald [e]

[a] Department of Mathematics, Faculty of Physical and Computational Sciences, SCB-SF 24/B6, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[b] Department of Chemistry and Biochemistry, South Dakota State University, USA
[c] Department of Theoretical and Applied Biology, Faculty of Bioscience, Biology Block, A 32, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[d] Veterinary Diseases Biology, Faculty of Science, University of Copenhagen, Copenhagen, Denmark
[e] Genomic Epidemiology and Global Decision Support Initiative, National Food Institute, Soltofts Plads, Building 221 Room 250, Technical University of Denmark, 2800 Kgs Lyngby, Denmark

HIGHLIGHTS

- Study sought to improve estimates of QMRA model by using both field data and literature reported values
- Comparison of fecal indicator ratio conversion and gene copies of virus for dose estimation
- Fecal indicator conversion ratio underestimates the risk possess to vegetable consumers
- Modeling methodology used for estimating dose is a form of uncertainty in risk assessment modelling
- QMRA estimates based on virus of interest should be always encourage instead of fecal indicator measurements

GRAPHICAL ABSTRACT



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ABSTRACT

The need to replace the commonly applied fecal indicator conversions ratio (an assumption of $1{:}10^{-5}$ virus to fecal indicator organism) in Quantitative Microbial Risk Assessment (QMRA) with models based on quantitative data on the virus of interest has gained prominence due to the different physical and environmental factors that might influence the reliability of using indicator organisms in microbial risk assessment. The challenges facing analytical studies on virus enumeration (genome copies or particles) have contributed to the already existing lack of data in QMRA modelling. This study attempts to fit a QMRA model to genome copies of norovirus data. The model estimates the risk of norovirus infection from the intake of vegetables irrigated with wastewater from different sources. The results were compared to the results of a corresponding model using the fecal indicator conversion ratio to estimate the norovirus count. In all scenarios of using different water sources, the application of the fecal indicator conversion ratio underestimated the norovirus disease burden, measured by the Disability Adjusted Life Years (DALYs), when compared to results using the genome copies norovirus data. In some cases the difference was >2 orders of magnitude. All scenarios using genome copies met the 10^{-4} DALY per person per year for consumption of vegetables irrigated with wastewater, although these results are considered to be highly conservative risk estimates. The fecal indicator conversion ratio model of stream-water and

* Corresponding author.
E-mail address: edjowusu-ansah.cos@knust.edu.gh (E.-G.J. Owusu-Ansah).

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drain-water sources of wastewater achieved the 10^{-6} DALY per person per year threshold, which tends to indicate an underestimation of health risk when compared to using genome copies for estimating the dose.

1. Introduction

To secure and increase crop production, irrigation has become a principal water use in many countries, where it has traditionally been dependent on rain water (Anonymous, 2014; Jiménez, 2006). In developing countries, the increase in wastewater usage has ensured that farmers produce adequate food throughout the year, and millions of small-scale farmers in urban and peri-urban areas are dependent on irrigation to grow high-value edible crops for urban markets (Qadir et al., 2010); irrigation has also contributed to improved nutrition and employment opportunities for farmers. The use of wastewater provides nutrients needed for plant growth and reduces the costs of using fertilizer on plants (Jiménez, 2006). It permits higher crop yields, year-round production, and enlarges the range of crops that can be grown, particularly in (but not limited to) arid and semi-arid areas.

In Ghana, the use of wastewater for vegetable farming is widespread, particularly in and around the more populated cities, where safe water is scarce (Seidu et al., 2008; Keraita et al., 2013). This places Ghana among the countries with the highest volume of raw wastewater usage worldwide, and represents an appropriate study area in relation to wastewater usage in agriculture within developing countries (Amoah et al., 2005; Jiménez, 2006; Amoah et al., 2006; WHO, 2006; Amoah et al., 2007; Raschid-sally and Jayakody, 2008; Hall et al., 2009; Mara and Sleigh, 2009; Drechsel et al., 2009; Mara and Sleigh, 2010a, 2010b; Labite et al., 2010; Fung, 2011; Ackerson and Awuah, 2012; Tiimub et al., 2012; Lundqvist and Raschid-Sally, 2013; Mok and Hamilton, 2014).

The use of raw wastewater for irrigation is, however, not without public health risks. Quantitative Microbial Risk Assessment (QMRA) has over the years become a powerful tool for estimating health risk through the use of probabilistic modelling. Recent studies estimating the risks of gastroenteritis applied the QMRA methodology to determine the disease burden associated with the consumption of vegetables irrigated with wastewater (Barker et al., 2014; Barker et al., 2013a, 2013b; Mok et al., 2014). Barker (2014) has indicated that the common challenge to conduct such QMRA has been the limited availability of input data on an actual pathogen, in particular virus concentrations as well as virus dose response models. To establish virus concentration, a fecal indicator conversion ratio has therefore been used to express the relationship between the occurrence of fecal indicators (typically *E. coli*) and the virus. This approach is also used in the current WHO guidelines (WHO, 2006), and has been adopted by subsequent QMRA studies (Barker et al., 2014; Barker et al., 2013a, 2013b; Mara and Hamilton, 2010; Mara and Sleigh, 2010a, 2010b; Travis et al., 2010; Mok and Hamilton, 2014; Ackerson and Awuah, 2012).

In this study, QMRA model is developed that uses the conversion ratio based on *E. coli* and norovirus genome copies data that was measured in wastewater to estimate final dose. The model employs a parametric approach to characterize the distribution of norovirus genomic copies to estimate the dose. The study further assesses the impact and likelihood of underestimating or overestimating the health risk (DALY) as a result of using the fecal indicator conversion ratio to estimate the dose of norovirus for risk assessment, when compared to using the genome copies data.

2. Methods

2.1. Exposure assessment model

The objective of the exposure assessment model was to estimate the dose of norovirus that consumers are exposed to through the consumption of wastewater-irrigated lettuce and cabbage. Lettuce and cabbage were selected as the main vegetables to represent crops commonly consumed raw in Ghana and other African countries as main ingredients in street foods (Fung, 2011). Water types used for irrigation in developing countries typically include wastewater from streams, drain-water and partially treated wastewater from Waste Stabilization Ponds (WSP). Data from all three types of wastewater were included in the model.

The norovirus dose that the consumer is exposed to on any day (d: number of virus particles ingested per person per exposure) resulting from consumption of either salad (cabbage or lettuce) irrigated with stream, drain or WSP water was estimated as

$$\mathrm{d} = \frac{C_q \times C_z \times V_p \times \mathrm{W}}{kt} \quad (1)$$

where d is the daily dose of norovirus ingestion (genome copies/meal/person/day) by consumers, *Cz* is the daily consumption of vegetable per person (g/meal/person/day), C_q is the concentration of norovirus in irrigation water (gc/mL), *Vp* is the volume of irrigation retained by the salad (mL/g), W is the reduction of viruses by washing during preparation (day^{-1}), k is the pathogen kinetic decay constant (per day) and t is time between last wastewater irrigation event and harvest consumption/storage (days). In this study, it is considered that half of all norovirus genome copies are infectious and each infectious virus is capable of causing an infection (Teunis et al., 2008; Teunis and Havelaar, 2000).

Information on actual volumes of consumed vegetables in Ghana is scarce and various QMRA studies (Barker, 2014; Ackerson and Awuah, 2012; Seidu et al., 2008) have therefore used estimates of salad consumption. Fung (2011) reported that salad mainly consisted of lettuce and cabbage (>75%) with a salad serving size of 20 g/meal/person/day. This meal size is higher than the estimated value of 10 g/meal/person/day–12 g/meal/person/day of lettuce per meal per day reported by Seidu et al. (2008), in Ghana, there is lack of comprehensive study on salad servings contaminated from norovirus, hence all servings were assumed to be contaminated as a worst case scenario. The estimated value for C_z in this study is based on a uniform distribution to cater for the different portion sized used in the earlier studies (Table 1), again, a uniform distribution was used for all year round frequency of consumption of vegetable.

Volume of water V_p retained on the surface of vegetables consumed in Australia (Hamilton et al., 2006; Mok et al., 2014) was used as an approximate estimate for this study, as previous studies in Ghana have shown that such values are appropriate (Barker et al., 2014). Uniform distribution was used for cabbage and normal distribution truncated at zero was used for lettuce to characterize the volume of water retained by the two vegetables (Table 1).

The kinetic in-field decay constant k for norovirus was assumed to be normally distributed (Fiona Barker and Hamilton, 2013; Mok et al., 2014; Hamilton et al., 2006). However, cabbage and lettuce are perishable and consumption of these products is usually done soon after harvest. Hence, post-harvest virus decay beyond 48 h was considered insignificant and was not included in this study.

Time for withholding water '*t*' was assumed to be within 0 to 2 days after irrigation. A uniform distribution was chosen to cover zero to a maximum of two days as vegetables in hot climatic conditions as in Ghana must be irrigated frequently, typically daily, to keep fresh.

Table 1
Distributions of input parameters.

Parameter	Notation	Units	Distribution type (value) ~ [mean]	Reference
Salad consumption	C_z	g/meal/person/day	Uniform (10–20) ~ [14.1]	Seidu et al., 2008; Fung, 2011
Volume of irrigation water retained by product				
Cabbage	V_p	mL/g	Uniform (0.00775, 0.108) ~ [0.0580]	Mok et al., 2014; Barker et al., 2013a, 2013b; Hamilton et al., 2006; Shuval et al., 1997
Lettuce	V_p	mL/g	Normal (0.108, 0.019) ~ truncated at zero ~ [0.108]	Mok et al., 2014; Barker et al., 2013a, 2013b; Hamilton et al., 2006; Shuval et al., 1997
Pathogen kinetic decay constant	k	Day^{-1}	Normal (1.07, 0.07) ~ truncated at zero ~ [1.07]	Barker et al., 2013a, 2013b; Hamilton et al., 2006; Petterson, 2001; Petterson, 2001
Irrigation cessation period before harvest	t	Days	Uniform (0, 2) ~ [1.0]	Barker et al., 2013a, 2013b
Virus reduction after post-harvest washing during food preparation	w	Log_{10}units	Pert (0.1, 1.0, 2.0) ~ [1.0]	Mok et al., 2014; Baert et al., 2009; Baert and Uyttendaele, 2008; Ndiaye et al., 2011; Croci et al., 2002; Mitakakis et al., 2004

Washing of vegetables during preparation is common practice in Ghanaian food stalls and households (Amoah et al., 2007; Fung, 2011; Seidu et al., 2008). Although reports on varying degree of efficiency of bacterial removal by washing and disinfection are available (Amoah and Drechsel, 2007; WHO, 2006), similar information on reduction of viruses are scarce. Allwood and Malik (2004) pointed out that viruses may be more resistant to washing than bacteria, and viruses are generally more resistant to environmental stress (Mattison, 2011), moreover, there is also the possibility of recontamination of viruses as a result of washing in poor quality water. As used in other studies, pert distribution was used for describing pre-consumption washing practices (Mok and Hamilton, 2014; Ayuso-Gabella et al., 2011; Mara and Sleigh, 2010a, 2010b; Fiona Barker and Hamilton, 2013; Seidu et al., 2008). Distributions of input parameters are reported in Table 1.

2.2. Pathogen concentration in wastewater

Data of ratio concentration and genome copies of human norovirus in drain- water (domestic wastewater from adjacent house complexes joining the drainage system and mixing with storm water), and stream-water (including WSP effluent and rivers) were obtained from the literature and other laboratory studies (Silverman et al., 2013; Hassine-Zaafrane et al., 2014; La Rosa et al., 2010; Katayama et al., 2008; Haramoto et al., 2006) (Table 2). The recovery rate using paired count estimation as described by Petterson et al. (2007) was used to account for the sensitivity of the detection method used in cases where recovery method were not reported.

To characterize the virus concentration in wastewater used for irrigation, the study adopted two approaches; norovirus genomic copies and the ratio conversion of *Escherichia coli* (*E. coli*) to norovirus. The widely used $1{:}10^{-5}$ (indicator organism: viruses WHO, 2006) were used in this model, on part norovirus concentrations were estimated from fecal or thermotolorant coliform concentrations using pathogen specific ratios determined from published literature, the ratio values distribution were applied to thermotolorant or fecal coliform concentrations to estimate norovirus as

$$C_v = \text{ratio} C_{TC} \tag{2}$$

where C_v is the norovirus concentration, ratio is the distribution of literature values and C_{TC} is the concentration of *E. coli* coliforms. Norovirus gene copies concentrations for stream-water and drain-water were fitted with uniform distribution (minimum-maximum values in gc/L) to characterize the concentrations from each wastewater source. The one-way Analysis of Variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of norovirus concentration data, the data were assumed to be independent (unrelated) groups. This procedure compares the means between the groups of interest, in this case the norovirus concentrations and determines whether any of those means were statistically significantly different from each other. Specifically, it tests the null hypothesis: The norovirus concentration means were all statistically equal. Though the one-way ANOVA is an omnibus test statistic and cannot tell you which specific groups were statistically significantly different from each other, however, in this case, it does since no significant difference was found which intend warrants no further analysis to unmasked the groups with significant difference, based on the ANOVA method, the norovirus combined concentrations from the wastewater sources was then pooled after analysis of variance (ANOVA) showed no significant differences among the wastewater source for their log transformed data. The pooled data was best fitted with lognormal distribution (meanlog = 10.23, sdlog = 1.35(in gc/L)) and gamma distribution (shape $= 2.8 \times 10^{-1}$, rate 1.9×10^{-5}). The gamma distribution was selected because it has lower Akaike Information Criteria (AIC) value compared to the lognormal distribution to characterize the pathogen concentrations.

Table 2
Concentrations of norovirus and fecal indicator bacteria in raw wastewater, their distributions and fit parameters used in models.

Norovirus data	Indicator organism[a] CFU/mL)	Norovirus (gc/mL)[b]	Virus recovery	Ratio of means	Min ratio	Max ratio	References
Accra Drain-water	$10^2 - 10^6$	1.85×10^2	25%–50%	1.76×10^{-4}	1.85×10^{-5}	1.60×10^{-1}	Silverman et al. (2013), Hassine-Zaafrane et al. (2014)
Accra Stream-water	$10^2 - 10^5$	1.03×10^2	25%–50%	7.13×10^{-4}	1.03×10^{-4}	9.90×10^{-2}	Silverman et al. (2013), Hassine-Zaafrane et al. (2014)
Japan (sewerage/drain)	9.42×10^4 (EC)	4.1×10^3	11.1%	4.3×10^{-2}	2.2×10^{-4}	1.7×10^{-1}	Haramoto et al. (2006)
Japan (sewerage/drain)	9.42×10^4 (EC)	1.2×10^3	11.1%	1.2×10^{-2}	4.8×10^{-4}	4.8×10^{-2}	Katayama et al. (2008)
Italy (sewerage/drain)	2.11×10^5 (FC)	3.4×10^4	35.4%	1.6×10^{-1}	n/a	n/a	La Rosa et al. (2010)
Accra	$10^2 - 10^5$ (EC)	1.27×10^2	15%	2.15×10^{-3}	n/a	n/a	Antwi-Agyei (2015)
Accra	$10^2 - 10^5$ (EC)	1.21×10^2	15%	1.07×10^{-4}	n/a	n/a	Antwi-Agyei (2015)
Accra	$10^2 - 10^6$ (EC)	1.43×10^2	15%	1.03×10^{-4}	n/a	n/a	Antwi-Agyei (2015)
Combined data (pooled data)	$10^2 - 10^6$ (EC)	1.64×10^2		1.56×10^{-4}	1.64×10^{-5}	1.60×10^{-1}	Silverman et al. (2013)

[a] *E. coli* (EC); fecal coliform (FC).
[b] Genomic copies with recovery (gc).

Table 3
Distributions of input parameters for DALY.

Parameter	Notation	Units	Distribution type (value) ~ [mean]	Reference
Diarrheal burden of norovirus	*B*	$Person^{-1}year^{-1}$	Uniform (1.06E−04–6.23E−03) ~ [3.16E−03]	Begg et al., 2007; Haagsma et al., 2008
Frequency of salad consumption	*n*	Day/year	Uniform (208–365)	Seidu et al., 2008; Mok et al., 2014

2.3. Dose response and risk characterization

The hyper – geometric beta - Poisson dose response model by Teunis et al. (2008) was used for estimating the probability of norovirus infection, along with the fit parameter values for the combined inoculate[1] dataset (8flla + 8fllb).[2] The estimated probability of norovirus infection (P_{inf}: $person^{-1}day^{-1}$) is given by

$$P_{\inf} = 1 - \left[\left({}_2F_1\left(\beta, \frac{d(1-a)}{a}, \alpha+\beta; a\right)\right)\left(\frac{1}{1-a}\right)^{\left(\frac{d(1-a)}{a}\right)} \right] \quad (3)$$

where ${}_2F_1$ is the Hypergeometric function, d is the dose of norovirus ingested per exposure, α and β are fit parameters of aggregate size distribution (logarithmic series). The Pfaff transformation fails at doses > 33,323 mL^{-1} hence the full Beta-Poisson model given (Eq. (4)) provides an adequate approximation as used by Fiona Barker and Hamilton (2013) and Teunis et al. (1999).

$$P_{\inf} = 1 - {}_1F_1(\alpha, \alpha+\beta, -d) \quad (4)$$

where ${}_1F_1$ is the confluent Hyper-geometric function (Teunis et al., 2008). Following the model, the conditional probability of illness given infection thus infected subjects Teunis et al. (2008) is estimated as:

$$P_{ill} = 1 - (1+\gamma d)^{-r} \quad (5)$$

where γ and r are explained by Teunis et al. (1999) and used by Barker et al. (2014), hence the probability of illness per dose per person is estimated as

$$P_{ill2} = P_{ill} * P_{\inf} \quad (6)$$

2.4. Annual risk of infection and illness

The annual risk of infection and illness were determined given the frequency of exposures (n) of an individual within a year and was modelled following the independent assumption of Karavarsamis and Hamilton (2010). The annual risk of infection or illness P is estimated as:

$$P = 1 - \prod_{\tau=1}^{n} [1 - P_\tau] \quad (7)$$

where P_τ is daily probability of illness or infection per exposure event in n exposures within a year (P_τ could either be $P_{\inf}$ or P_{ill} depending on the interest of the study). Each n was assumed to be a uniform distribution of 208 to 365 days, as consumption of lettuce or cabbage is all year round. The annual disease burden was estimated using the Disability Adjusted Life Year (DALY, per person per year (WHO, 2015) metric, which is used to measure all disease burden expressed as the number of years lost due to disability, illness or premature death. Hence estimated as

$$DALY = P_{ill} BS \quad (8)$$

where P_{ill} is the annual probability of illness or infection per given dose and B is the disease burden (DALY per case of diarrhea – the diarrhea burden of diseases in Ghana was estimated with uniform distribution. Table 3 and S is the proportion of population susceptible to the diseases or immune compromised.

2.5. Model implementation

Models used in this study were organized and implemented in different software (R, MatLab, Mathematica and @Risk) to serve as a check of model values to assess consistency. Different scenarios which combined the use of different wastewater sources and vegetables were assigned with probability distributions to serve as input parameters. All sets of distributions were simulated with 10,000 iterations to draw samples from the @ Risk Decision Tool software (@Risk 6.3 version, 2014). Hyper-geometric functions were implemented in R and rechecked using the advance code for solving hyper geometric functions (Pearson, 2009) in MatLab. Sensitivity analysis was performed to identify input parameters having significant impact on output parameters. The analyses, was done with the use of Spearman's correlation coefficient to measure the monotonic relationship of the input parameters and the probability of illness. This was carried out with values from 10,000 iterations of the exposure model. A non-parametric test statistic (The Wilcoxon–Mann–Whitney test) was used to test for the significance differences in the medians and the use of Fligner-Killean test was adopted to evaluate the variances. All input parameters were set on median values and run consecutively with 25th and 75th percentile fixed.

3. Results

3.1. Estimation of annual probability of infection and illness of norovirus gastroenteritis

The annual probability of norovirus infection ranged from 9.2×10^{-1} to 9.4×10^{-1} for all norovirus genome copies while the ratio conversion method provided a similar range from 8.8×10^{-1} to 9.1×10^{-1}. These findings are supported by the estimation of the annual probability of diseases at a given infection that ranged from 8.6×10^{-1} to 9.0×10^{-1} for the norovirus genome copies and 8.1×10^{-1} to 8.3×10^{-1} for *E. coli* ratio conversion (Table 4). Using the USEPA's threshold of 1×10^{-4} annual probability of infection and the recommended 1×10^{-6} risk of infection by Signor and Ashbolt (Signor and Ashbolt, 2009) daily risk target, all model scenario exceeded the thresholds, hence wastewater irrigated vegetables cannot be said to be safe for consumption.

3.2. Annual diseases burden

The median annual diseases burden ranged from 1.8×10^{-5} to 6.7×10^{-5} for all norovirus genome copies (stream-water, drain-water and pooled data) concentration with 95th percentile values ranged from 8.7×10^{-5} to 1.2×10^{-4}, while for *E. coli* ratio conversion (stream, drain and pooled data), the median annual diseases burden ranged from

[1] Number of surrogates administered to susceptible subjects.
[2] 8flla: dose response models without and with aggregation applied to susceptible subjects; 8fflb: dose response model (no aggregation) for susceptible subjects; (8ffla + 8fflb): combined 8flla and 8fflb experiments.

Table 4
Annual probability of gastroenteritis infection an illness and diseases burden (DALYs) per person per year.

Model scenarios	Annual probabilities		Diseases Burden (DB: DALY pppy)		
	Infection	Diseases/infection	5th percentile	Median	95th percentile
Stream genome copies norovirus	9.4×10^{-1}	8.6×10^{-1}	8.0×10^{-6}	4.1×10^{-5}	1.2×10^{-4}
Stream norovirus ratio	9.1×10^{-1}	8.1×10^{-1}	6.8×10^{-9}	1.2×10^{-7}*	5.8×10^{-7}
Drain water genome copies norovirus	9.3×10^{-1}	9.0×10^{-1}	1.3×10^{-6}	1.8×10^{-5}	8.7×10^{-5}
Drain water norovirus ratio	8.8×10^{-1}	8.2×10^{-1}	8.7×10^{-10}	1.4×10^{-8}*	7.1×10^{-8}
Pooled (combined dataset) genome copies norovirus	9.2×10^{-1}	8.9×10^{-1}	8.2×10^{-6}	6.7×10^{-5}	1.7×10^{-4}
Pooled (combined dataset) norovirus ratio	9.1×10^{-1}	8.3×10^{-1}	2.6×10^{-7}	3.7×10^{-6}	1.7×10^{-5}

* Meets the WHO threshold standard of 1/1000000 for median diseases burden.

1.4×10^{-8}* to 3.7×10^{-6} with 95th percentile ranges from 7.1×10^{-8} to 1.7×10^{-5}. All scenarios using norovirus genome copies diseases burden (DALY) were found to be approximately, an order of magnitude higher than the use of *E. coli* ratio conversion method of translating fecal indicator to norovirus. It should be noted that only scenarios involving *E. coli* ratio conversion achieved the health target of less than 1×10^{-6} DALY pppy, whereas scenarios involving the use of norovirus genome copies were an order of magnitude less than the DALY health target of 1×10^{-4} DALY pppy (Fig. 1) but higher than the health target of 1×10^{-6}. When pooled/combine dataset were used for both norovirus genome copies and *E. coli* ratio conversion, the median annual diseases burden achieved the DALY of 1×10^{-4} but not 1×10^{-6} (Fig. 2).

3.3. Sensitivity analysis

The sensitivity analysis was used to ascertain the model parameters that have strongest impact on the model output. As shown on Table 5, the probability of illness was very sensitive directly to water quality ($p < 0.05$), volume of irrigation water caught by the vegetable ($p < 0.05$). The probability of illness was inversely sensitive to the virus reduction due to food preparation ($p < 0.05$) and the time between the last irrigation and harvest ($p < 0.05$: cessation of irrigation).

4. Discussion

The disease burden of the different model scenarios was found to be acceptable under different thresholds of DALY. Nevertheless, it should be noted that the DALY figures are conservative estimates and might in reality be a few orders higher due to the challenges of recovering and enumerating norovirus from sample material as discussed in Papafragkou et al., 2013; Schrader et al., 2012; Laverick et al., 2004. This means, the true disease burden (DALY) from stream wastewater and drain wastewater might be some orders higher than found in this study.

In the scenarios presented here, none of the models using genome copies of norovirus to predict the diseases burden found that it could establish the safety of consuming the produce i.e. the threshold of $\leq1\times10^{-6}$ pppy DALY was not met. In contrast, the use of ratio conversion met the threshold for the same model. The WHO guidelines states that, if the overall burden of diseases from other exposures is very high, setting a less stringent level of acceptable risk of 1×10^{-5} DALY pppy or 1×10^{-4} DALY pppy may be more realistic as was argued by Mara and Hamilton (2010). This assertion of WHO may guide the results of accepting the burden of disease levels for all model scenarios in this study. On the other hand, the ratio conversion method currently applied to estimate the diseases burden produces significantly lower estimate of DALY with 2 or more orders of magnitude lower than the use of genome copies norovirus concentration data. More importantly, the differences in diseases burden for stream wastewater and drain wastewater were insignificant; and both achieved the threshold of the health target of 1×10^{-4} DALY pppy, yet, the estimation of diseases burden in drain wastewater being less than that of stream wastewater hence making stream wastewater source having a greater diseases burden.

With emphasis placed on the differences of order of magnitude in DALYs as a result of the use of fecal indicator ratio conversion in estimating health risk in various QMRA models, Payment and Locas (2011) argued that, the use of *E. coli* as an indicator of fecal pollution does not represent well the presence of protozoa and other pathogen microorganisms. These Indicators are useful for monitoring hygiene such as in slaughter plants, but a high level fecal indicator does not necessarily mean a high level of pathogens (FAO), as this will depend on the prevalence and infectivity level of the pathogen in the source. On the part of Silverman et al. (2013), it is noted that "the current assumption of ratio conversion of 0.1 – 1 norovirus particles per 10^5 *E. coli* could underestimate virus dose with exposure to wastewater and surface water sample". Again, Mok and Hamilton (2014) indicated that, a standard pathogen concentration should be based on the pathogen of interest instead of indicator organism.

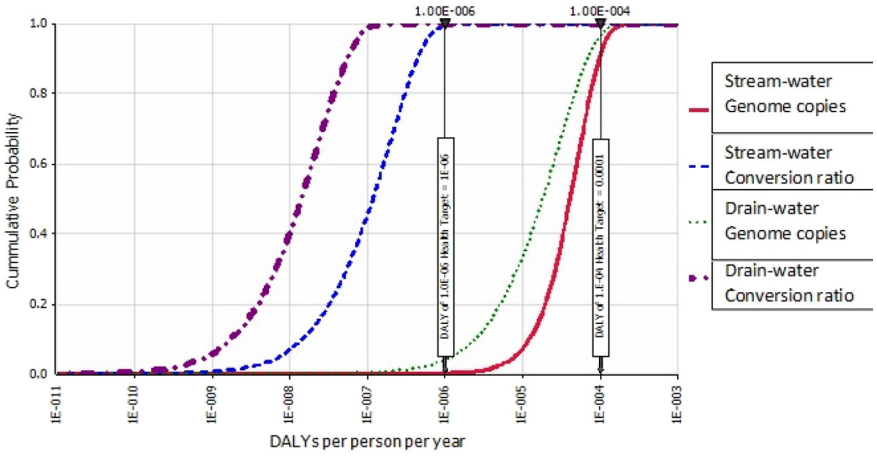

Fig. 1. Cumulative probability curve of diseases burden for stream and drain wastewater for actual norovirus and ratio conversion. Each cumulative probability represents the diseases burden for either the actual norovirus genome copies dose estimation or the conversion ratio dose estimation for stream water and drain water.

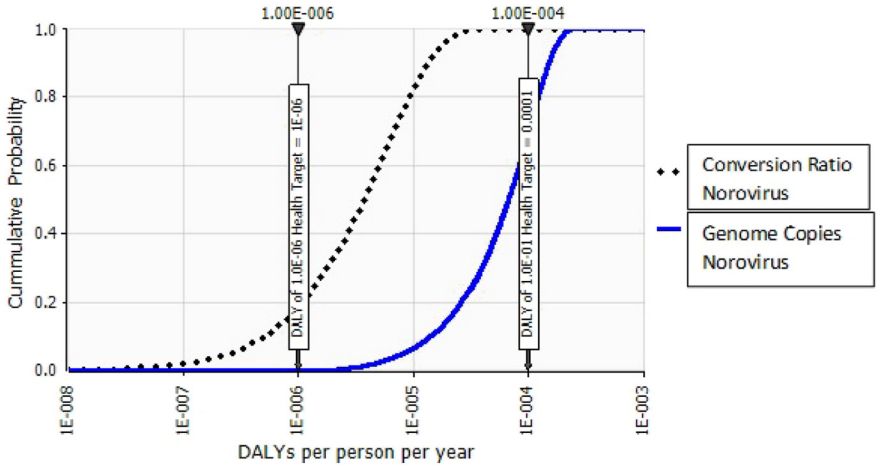

Fig. 2. Cumulative probability curve of diseases burden for pooled/combined dataset. This represents the combined dataset for stream and drain water, the cumulative probability graph shows the differences of using either the genomic copies or that of ratio conversion estimation for dose in modelling risk assessment.

This study shows that, a move away from using fecal indicator conversion rates can lead to more realistic risk estimation as shown clearly with ≥1 order of magnitude higher when genome copies norovirus particle concentration is used, though the values are considered conservative and by no means represent the actual norovirus concentration in streams, drain water and WSPs as reported by Silverman et al. (2013) due to factors such as the technique applied for the quantification and the insufficient number of samples used to characterize the concentration. Moreover, the unavailability of aggregation data for quantification of risk in dose response models might contributes as a model uncertainty, still, it gives a basis for a virus interest health risk assessment based on the concentration of genome copies of human norovirus and a corresponding fecal ratio conversion in order to establish specific health based targets. It should be noted that, the dose response model in this study is also a sources of uncertainty, hence representing a worst case scenario. Some recent dose response studies (Atmar et al., 2014; Messner et al., 2014) has shown a much slower response when used compared to the Teunis et al. (2008) dose response adopted. Nevertheless, the use of all published norovirus response models (1F1, 2F1 and fractional Poisson models) rely on questionable assumptions with respect to host susceptibility (Schmidt, 2015; Van Abel et al., 2016). Yet it is widely accepted that, there is no procedure or standard approach to the selection of a norovirus model (Van Abel et al., 2016). Atmar et al. (2014) and Messner et al. (2014) rely mainly on the immunity integration of susceptible population, however Payne et al. (2015) indicates that temporary immunity acquired by susceptible host from one genogroup or genotype does not confer protection from all, is not permanently sustained and hence reinfection of susceptible hosts inevitable throughout life. In this study, we settled on the use of Teunis et al. (2008), due to the aggregation of the data used as well as the non-available secretor status which forms the basis of the recent studies on dose response models. This led to view the dose response model from a mechanistic point of view which fully fits the assumptions of the Teunis et al. (2008) as used by most studies (Sokolova et al., 2015; Rygaard et al., 2014; Mok et al., 2014; Barker, 2014; Barker et al., 2013a, 2013b; Mara and Sleigh, 2010a, 2010b).

Table 5
Spearman rank order correlation coefficients for probability of illness.

Parameters	Correlation coefficient (spearman rank)
Water quality	0.62 (0.001)
Volume of irrigation water caught by vegetable	0.51 (0.005)
Kinetic decay constant	0.04 (0.051)
Virus reduction by food preparation	−0.24 (0.019)
Cessation of irrigation	−0.118 (0.013)

5. Conclusion

We estimated the risk of illness and the disease burden expressed in DALYs with the use of fecal indicator ratio conversion or genome copies of norovirus for the consumption of vegetables irrigated with wastewater in Ghana. A QMRA model was developed to estimate the differences in diseases burden, and the results showed that:

1. All model scenarios for consuming vegetables irrigated with wastewater (stream or drain) met the 1×10^{-4} DALY pppy threshold for norovirus. However, models using genome copies of norovirus are considered highly conservative estimates.
2. In all cases, stream water recorded a higher probability of illness and disease burden than the drain water sources and again represents conservative estimates.
3. In the model of the same scenarios, the use of fecal indicator ratio conversion tends to underestimate the risk of diseases burden DALY pppy as compared to the use of genome copies of norovirus data. This indicates that a shift from using fecal indicator to the actual pathogen (virus) of interest might give a more realistic output of the risk estimates.
4. In some cases, as high as 2 order of magnitude was recorded in terms of differences in DALY for fecal indicator ratio conversion and genome copies of norovirus for stream and drain wastewater. However, when pooled/combined dataset were used, >1 order magnitude difference was recorded.

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