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Confirming the need for virus disinfection in municipal subsurface drinking water supplies

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Abstract

Enteric viruses pose the greatest acute human health risks associated with subsurface drinking water supplies, yet quantitative risk assessment tools have rarely been used to develop health-based targets for virus treatment in drinking water sourced from these supplies. Such efforts have previously been hampered by a lack of consensus concerning a suitable viral reference pathogen and dose-response model and difficulties in quantifying pathogenic viruses in water. A reverse quantitative microbial risk assessment (QMRA) framework and quantitative polymerase chain reaction data for norovirus genogroup I in subsurface water supplies were used herein to evaluate treatment needs for subsurface drinking water supplies. Norovirus was not detected in over 90% of samples, which emphasizes the need to consider the spatially and/or temporally intermittent patterns of enteric pathogen contamination in subsurface water supplies. Collectively, this analysis reinforces existing recommendations that a minimum 4-log treatment goal is needed for enteric viruses in groundwater in absence of well-specific monitoring information. This result is sensitive to the virus dose-response model used as there is approximately a 3-log discrepancy among virus dose-response models in the existing literature. This emphasizes the need to address the uncertainties and lack of consensus related to various QMRA modelling approaches and the analytical limitations that preclude more accurate description of virus risks.

Keywords

Groundwater, quantitative microbial risk assessment (QMRA), norovirus

1.0 Introduction

Human enteric viruses commonly occur in subsurface water supplies (Borchardt et al., 2003; Fout et al., 2017; Moreira and Bondelind, 2017) and are generally understood to contribute to a significant number of waterborne outbreaks of gastroenteritis related to groundwater consumption (Moreira and Bondelind, 2017; WHO 2017). Consistent with this, they are generally more prevalent in the subsurface than other pathogens such as protozoan cysts (Moulton-Hancock et al., 2000). The scientific community generally agrees that the multi-barrier approach is effective in reducing risks from enteric viruses and other waterborne pathogens in drinking water by characterizing those risks in source water and ensuring that effective treatment is in place to deliver safe drinking water to consumers. Despite this general consensus, various approaches (some of which are more risk-based than others) are utilized to identify "groundwater"-based drinking water supply systems vulnerable to fecal contamination and decide upon appropriate types and levels of treatment required to achieve public health protection goals. In jurisdictions such as Canada and the United States, specific (albeit widely variable) approaches are utilized to differentiate pathogen risks in these systems, which are categorized as "groundwater" or "groundwater under the direct influence of surface water" with the latter term reserved for systems with purportedly higher risk of pathogen contamination (Chaudhary et al., 2009; U.S. EPA, 2016; British Columbia Ministry of Health, 2017). Quantitative microbial risk assessment (QMRA) is commonly relied upon to establish required levels of treatment that must be applied to adequately reduce disease burden associated with exposure to pathogenic microorganisms in a drinking water source. The general approach to determining the level of treatment required to make a drinking water supply acceptably safe for a particular type of hazard involves defining an acceptable degree of risk, determining anticipated

levels of exposure, and selecting an appropriate dose-response function that links exposure to risk (Regli et al., 1991; WRRF, 2013; WHO, 2017). This is especially challenging for waterborne viral pathogens (as well as protozoa) relative to similarly important pathogenic bacteria because less is known about their occurrence in subsurface water supplies and, in the case of viruses, their dose-response relationships. Notably, numerous investigations conducted over the past 20 years have demonstrated the presence of enteric viruses in subsurface supplies that were considered to be at low risk to fecal contamination (i.e., subsurface supplies considered "not under the direct influence of surface water") (Borchardt et al., 2003, 2004; Locas et al., 2007; Bradbury et al., 2013). Thus, it is critical to consider these observations within a QMRA framework to quantitatively assess and confirm the need for virus disinfection (or other treatment) in municipal subsurface drinking water supplies, as presented within this study.

2.0 Methodology

A reverse QMRA analysis was conducted using well water virus occurrence data to evaluate the level of virus treatment (typically chemical disinfection) needed to achieve a specified acceptable level of risk. Alternative dose-response models were used to characterize the effects of uncertainty in model form (e.g. mechanistic assumptions) and demonstrate the importance of judicious model selection in QMRA. The rationale for the chosen reference pathogen, the various modelling inputs selected, details of how the modelling work was carried out, and discussion of assumptions and limitations are presented below.

2.1 Norovirus as a Reference Waterborne Viral Pathogen

Ideally, treatment targets for a drinking water supply might be determined considering the risk posed by each type of waterborne pathogen due to varying abundance among source waters and considerable variation of dose-response relationships among different pathogens. This ideal

approach to understanding, prioritizing, and managing public health risks would require impractically detailed information about occurrence, treatment, and dose-response for every conceivable waterborne pathogen (WHO, 2017). Moreover, there are analytical limitations such as the current lack of a widely available and standardized methodology for efficient cell culturing and enumeration of human-infective norovirus. For these reasons, it is common practice to consider 'reference' or 'index' pathogens that are relatively abundant and also believed to be representative of a broader group of pathogens. "It is believed that if a drinking water treatment is effective in removing these index pathogens, adequate safety is warranted against other waterborne pathogens" (Schijven et al., 2011).

In this study, enteric viruses that are transmitted via the fecal-oral route through drinking water are addressed. While there are a great number of types of viruses that may possibly be transmitted through drinking water, relatively few are well understood. Norovirus is the most prominent candidate based on waterborne outbreaks in the United States; it has been identified as the etiologic agent responsible for twenty outbreaks between 2001 and 2014 (CDC, 2004; 2006; 2008; 2011; 2013; 2015; 2017). Only *Campylobacter* spp. has comparable case numbers attributed to waterborne outbreaks. Both *Campylobacter* spp. and *Giardia* share similar numbers of waterborne outbreaks with norovirus while larger numbers of relatively small outbreaks are attributable to *Legionella* spp. In Canada, fourteen outbreaks of waterborne disease between 1974 and 2001 were attributed to Norwalk-like viruses or rotavirus (Schuster et al., 2005). Only *Giardia*, *Campylobacter* spp., and *Salmonella* spp. were implicated in greater numbers of waterborne outbreaks, with slightly fewer *Cryptosporidium* spp. and Hepatitis A virus outbreaks. Norovirus has also been estimated to be the most common waterborne cause of endemic acute gastrointestinal illness from private and small water systems in Canada (Murphy et al., 2016).

Moreira and Bondelind (2017) summarize six additional outbreaks in Europe between 2002 and 2011 that were attributable to norovirus contamination of groundwater. In a study of 31 foodborne pathogens in the United States, it was estimated that norovirus accounts for 20,796,079 (57%) domestically acquired illnesses and 571 (22%) resulting deaths per year, yet it is believed that only about 26% of norovirus illnesses are foodborne (Scallan et al., 2011). An unknown portion of the remainder of cases would be waterborne, as direct (person-to-person) and indirect (surface) contact transmission are also significant norovirus exposure pathways. The other two viruses less commonly identified in waterborne outbreaks in the United States and Canada are hepatitis A virus and rotavirus. Hepatitis A virus was responsible for ten waterborne outbreaks in Canada between 1974 and 2001 (Schuster et al., 2005) and just three outbreaks in the United States between 2001 and 2014 (CDC, 2008; 2011; 2013) with a hundred-fold fewer outbreak-related cases than norovirus. Although it is more specifically attributable to waterborne transmission than norovirus, it is a less commonly detected pathogen with little information on dose-response (Pintó et al., 2009). Rotavirus is more readily quantifiable and a dose-response model is in common use, but it was not identified as the etiologic agent in any waterborne outbreaks in the United States between 2001 and 2014. It is unclear how many of the Canadian outbreaks attributed to Norwalk-like viruses or rotavirus between 1974 and 2001 were indeed rotavirus. Rotavirus is used as an example reference pathogen in the World Health Organization's Guidelines for Drinking Water Quality (WHO, 2017) but is not a suitable reference pathogen for this study because it is relatively specific to young children who are now frequently vaccinated against it in Canada and the United States (Tate et al., 2011; Le Saux, 2016) and evidence of a substantial waterborne disease burden for the general population is lacking.

The Drinking Water Contaminant Candidate List (U.S. EPA, 2009) includes adenovirus, calicivirus (which includes norovirus), enterovirus, and hepatitis A virus, but excludes rotavirus. Adenoviruses are mainly respiratory pathogens that primarily affect infants and children, and the two serovars that are implicated as waterborne pathogens do not grow well in culture (WHO, 2017). Enterovirus occurrence and treatment information together with rotavirus dose-response is used as the reference waterborne viral pathogen in the Netherlands (Schijven et al., 2011), but it is not known if waterborne transmission is a significant exposure route despite fecal excretion and abundance of enteroviruses in many waters (WHO, 2017).

There is no consensus on which waterborne virus constitutes the best reference viral pathogen for drinking water risk assessments. As "no single virus has all of the characteristics of an ideal reference virus", the Guidelines for Canadian Drinking Water Quality concerning enteric viruses (Health Canada, 2011) combine the relatively extreme rotavirus dose-response model with more conservative treatment efficiencies for hepatitis A virus. Specific recommendations for source water monitoring of viruses are lacking, however, and published groundwater occurrence data are sparse with variable reliability due to analytical challenges. Norovirus is selected as the reference pathogen herein because it is a prolific pathogen for which both relevant occurrence data from environmental waters (specifically including subsurface water supplies) and dose-response information are available.

2.2 Defining an Acceptable Degree of Risk

There are two values of acceptable risk that are in common use in the drinking water industry. The first is 10^{-4} infections/person/year (Regli et al., 1991; Schijven et al., 2011; WRRF, 2013). A major limitation of this threshold is that it does not yield particularly meaningful comparisons among pathogens because the consequences of infection for one type of pathogen may be much

more severe than for another. Moreover, it cannot be directly compared with other types of risks such as chronic illnesses caused by disinfection by-products. The Disability-Adjusted Life Year (DALY) is a more standardized metric of health consequences from various types of hazards, with a common acceptable risk threshold of 10^{-6} DALYs/person/year (Health Canada, 2011; WHO, 2017). Major limitations of this approach are that 1) dose-response with illness as the endpoint is often more poorly understood than infection dose-response, and 2) asymptomatic infections are asserted to pose no risk. Norovirus shedding is prolific and prolonged in both symptomatic and asymptomatic infections (Teunis et al., 2015) and it is known that extensive secondary transmission can occur within households and other settings in which people live in close proximity. Zelner et al. (2010) analyzed norovirus transmission patterns in an outbreak and asserted that secondary transmission is increased by asymptomatic infections. Accordingly, the present scenario of norovirus in untreated groundwater necessitates a risk characterization approach recognizing potential public health consequences of asymptomatic infections and the infection endpoint of 10^{-4} infections/person/year was therefore used herein.

2.3 Determining Virus Occurrence in Subsurface Water Supplies

To evaluate treatment needs, infectious norovirus data from subsurface water supplies capturing both spatial variability between wells and temporal variability at each well would be desirable. Such data have not been available due to a lack of standardized culture methods for detection of infectious noroviruses, though new methods continue to be developed (e.g. Ettayebi et al., 2016). Semi-quantitative methods using polymerase chain reactions (PCR) have commonly been used for risk assessment, yet these are criticized because they may quantify genomes from inactive viruses. For this study, norovirus GI qPCR data from raw well water collected in association with a community-level intervention study in 14 small Wisconsin communities (pop. 1,363-

164 8,300) relying on untreated groundwater were used (Borchardt et al., 2012). The study consisted
165 of four 12-week monitoring periods in the Spring and Autumn of both 2006 and 2007 with a
166 crossover of ultraviolet irradiation intervention between the two study years. Of 1596
167 measurements, only 392 were for raw well water. Tap water samples collected from various
168 homes throughout each community's distribution system were not used in this study to exclude
169 the effect of the UV disinfection (where applicable) and any post-treatment variation associated
170 with the distribution systems. In general, the norovirus concentrations were lower in tap water
171 (not shown) with periods of UV disinfection prior to distribution corresponding to lower
172 frequency of detection and concentrations at the tap.
173 Of 392 well water measurements in units of genomic equivalent copies per liter (gec/L), 360
174 (91.8%) are non-detects. The few positive samples range from 0.00621-264 gec/L. The
175 arithmetic mean, counting non-detects as zeros, is 3.84 gec/L, and the 95^{th} and 99^{th} percentiles
176 are 9.66 and 119 gec/L respectively. These data are summarized in Figure 1 as the fraction of
177 samples exceeding various concentration values. With the exception of two communities for
178 which all data were non-detects, individual communities had average concentrations ranging
179 from 0.24 gec/L to 20.88 gec/L. There were nine communities with averages over 1 gec/L, of
180 which five were over 10 gec/L.

181 **2.4 Norovirus Dose-Response Models**

182 Due to the lack of scientific consensus on norovirus dose-response (Schmidt, 2015), seven
183 different dose-response models available in the published literature were used herein (Table 1,
184 Figure 2). The comparison of results obtained using several dose-response models is included in
185 this research to demonstrate the range of possible outcomes using available models and to
186 illustrate the implications of differences among these models. Because the analysis is intended to

emphasize differences between alternative models rather than uncertainty in the fit of any one chosen model, consideration of parametric uncertainty for each fitted model is beyond the scope of this research.

The first model (Rota) is the rotavirus dose-response model that was obtained for healthy adults prior to the availability of vaccinations. The rotavirus dose-response model is commonly used as a worst-case model for enteric viruses (e.g. Health Canada, 2011; Schijven et al., 2011). An approximate beta-Poisson dose-response model has often been used for rotavirus (with parameter values such as α=0.253, and β=0.422 or N_{50}=6.17). In order to use the approximate model while invoking single-hit theory, the approximation must be validated against the conditions $\beta >> \alpha$ and $\beta >> 1$ (Teunis and Havelaar, 2000; Schmidt et al., 2013). The published approximate beta-Poisson models for rotavirus were excluded from consideration herein due to clearly violated approximation criteria (β<1), and an exact beta-Poisson model is used instead.

Six of the models have been fit to data from various norovirus dose-response experiments (including only healthy adult 'secretors' who are not believed to be inherently immune to norovirus genogroup I) and implicitly assume equivalence between quantified genomes and infectious viruses in the administered doses. Teunis et al. (2008) developed a model with a fit (Noro 1A) that suggested a high degree of virus aggregation in the first of two utilized stock suspensions. It has become common practice (e.g. U.S. EPA, 2014; WHO, 2016) to assume disaggregation for environmental noroviruses and to change the aggregation parameter accordingly so that the dose-response model form is simplified from one including a ${}_2F_1$ hypergeometric function to one including just a ${}_1F_1$ confluent hypergeometric function (Noro1B). Messner et al. (2014) developed the simple fractional Poisson model that partitions the study population into fully susceptible (r=1) or fully immune (r=0) subjects, with fraction P

210 being fully susceptible. The model was fit to a collection of data from several experiments
211 including the original data from Teunis et al. (2008). Good fit to the data was established
212 (Noro2A) by presuming virus aggregation in several of the experiments, and this model has
213 subsequently been modified by setting the mean aggregate size to μ=1 to represent
214 disaggregation (Noro2B) as may be appropriate for environmental noroviruses.
215 To explore the validity of specific major assumptions in the preceding norovirus dose-response
216 models—specifically, the assumptions of no uncontrolled sources of immunity among subjects
217 and complete susceptibility among the non-immune in the Noro1A and Noro2A models,
218 respectively—the generalized aggregated exact beta-Poisson with immunity model was
219 developed by Schmidt (2015). The fit of this model to the data compiled by Messner et al.
220 (2014), with best fit denoted as Noro3A in this study, challenged these assumptions by revealing
221 evidence of both uncontrolled sources of immunity among subjects and incomplete susceptibility
222 among non-immune subjects, and also suggested a much lower degree of virus aggregation than
223 previously asserted. The data were deemed insufficiently informative to distinguish best fit
224 among the three model forms (Noro1A, Noro2A, and Noro3A) to determine which is most
225 mechanistically appropriate. Nonetheless, it was noted that manipulating the aggregation
226 parameter to represent disaggregation would cause risk values to differ by several orders of
227 magnitude among the three disaggregated models (Noro1B, Noro2B, and Noro3A) at the low
228 doses encountered in acceptably safe treated drinking waters.
229 A final alternative (Noro3B) is obtained by zeroing the immunity parameter in Noro3A. This is
230 not only conservative for populations with differing levels of immunity but also ensures that the
231 risk borne by a person who has no reason to know that they are non-immune is not under-stated
232 due to inclusion of others who are immune in the risk characterization.

2.5 Reverse QMRA Methodology

Drinking water QMRA generally involves supplying information about raw water pathogen concentrations, treatment efficacy, consumption volumes, and dose-response to evaluate risk. In contrast, reverse QMRA can be used to evaluate the treatment efficacy or performance that is needed to yield a specified value of risk. A mean consumption volume of 1.45 L/person/day, based on a study of water consumption habits in south-western Ontario (Pintar et al., 2009), is used herein. The following acceptable treated water virus concentrations ($C_{treated}$, in units of gec/L) were determined assuming year-round exposure and using the seven dose-response models described above together with the risk value of 10^{-4} infections/person/year: 4.1×10^{-7} (Rota), 1.5×10^{-4} (Noro1A), 4.5×10^{-7} (Noro1B), 2.8×10^{-4} (Noro2A), 2.6×10^{-7} (Noro2B), 2.5×10^{-4} (Noro3A), and 1.8×10^{-4} (Noro3B). The target log-reduction for each positive raw groundwater norovirus concentration (C_{raw}) was evaluated as $log_{10}(C_{raw}/C_{treated})$ with all others being given a target log-reduction of zero. This incorporates an assumption that the variability among the considered data is more representative of well-to-well variability than temporal variability within wells so that temporal variability need not be considered (because each value of concentration is assumed to apply year-round for its source rather than a temporal distribution of concentrations). Detailed risk characterization from a time series of data to support decision-making, rather than a proof-of-concept analysis to illustrate the need for basic treatment of many subsurface water supplies, would require methodology accounting for temporal variability. The assessment of treatment needs in this research emphasizes the mean, 95th percentile, 99th percentile, and maximum concentration values, which are relatively unaffected by non-detects. In particular, use of the arithmetic mean concentration with non-detects included as zeros is unbiased (Parkhurst and Stern, 1998), and handling of microbial non-detects as censored concentration values below

a detection limit is inappropriate (Chik et al., 2018). Consideration of random measurement errors—the disparity between the actual concentration in the source and the value estimated from a sample by a laboratory (Schmidt and Emelko, 2011)—in all of these norovirus concentration estimates (not only the non-detects) is beyond the scope of this study. Such error is presumed to be trivially small compared to the >4 orders of magnitude of variation among concentration values.

2.6 Limitations of Study Methodology

This study includes numerous simplifying assumptions and presumed representativeness of model inputs and data. Table 2 provides a list of assumptions (categorized relative to various aspects of the methodology) and the direction of bias that each introduces to the analysis is indicated wherever possible. The direction of overall bias remains unclear. This information could form the basis for collection of more or better empirical data or for more detailed modelling to further refine the analyses in this study in the future.

3.0 Results and Discussion

For each of the seven considered dose-response models, the target log-reduction values calculated from the norovirus occurrence data are summarized in Figure 3 as the fraction of samples needing at least the specified virus log-reduction in order to meet the 10^{-4} infections/person/year risk target. With a 4-log treatment level, 7-8% of the sample concentrations would pose a risk exceeding the annual infection risk target depending upon which dose-response model is used. Notably, the seven dose-response models cluster into two distinct groups. The first group consists of the three models that were fit to the available norovirus dose-response data without subsequent manipulation of the aggregation parameter (Noro1A, Noro 2A, Noro 3A), as well as the exact beta-Poisson with immunity model with the

279 immunity parameter zeroed (Noro 3B), and these yield target log-reductions as high as 4-6 log.
280 The second group, which yields target log-reductions as high as 7-9 log, consists of the rotavirus
281 dose-response model (Rota) and the two norovirus dose-response models in which the fitted
282 aggregation parameter was changed to represent presumed disaggregation in environmental
283 exposures (Noro 1B, Noro 2B). While aggregation reduces risk in environmental exposures
284 relative to equivalent mean doses of disaggregated viruses (Nilsen and Wyller, 2016), changing
285 the aggregation parameter that was fit to dose-response data so that it represents disaggregation
286 increases the calculated risk. Notably, the desired level of treatment for norovirus differs by
287 approximately 3-log depending upon which dose-response model is used. This evaluation
288 emphasizes the need for further consideration of these parameters in modelling approaches and
289 scientific consensus-building concerning norovirus dose-response in particular.
290 Figure 4 illustrates the calculated log-reduction values corresponding to the following
291 concentration statistics: the arithmetic mean (3.84 gec/L), the 95th percentile (9.66 gec/L), the
292 99th percentile (119 gec/L), and the maximum (264 gec/L). Based on these results, a minimum
293 target of 4-log virus reduction is justified as a general guideline for treatment of municipal
294 subsurface water supplies. This analysis reinforces the Guidelines for Canadian Drinking Water
295 Quality (Health Canada, 2011), which state that "a minimum 4-log reduction and/or inactivation
296 of viruses has been established as a health-based treatment goal."
297 An examination of Figure 4 underscores the significant uncertainty that currently exists around
298 various QMRA modelling approaches and the need for clear articulation of model limitations
299 and assumptions as well as consensus approaches, if not standardization. Considering the
300 alternative dose-response models utilized herein, the rotavirus model may be too conservative
301 with respect to norovirus risk and could lead to overly stringent treatment requirements. This is

especially the case for small groundwater systems where the need for an additional 3-log virus reduction (i.e., shifting from a requirement of 4-log to 7-log virus activation, as might be inferred from some of the dose response model outcomes in Figure 4) might necessitate costly treatment upgrades beyond basic chemical disinfection. The results obtained using the Noro1B and Noro2B models are also likely too conservative, as they may be skewed by problematic underlying assumptions (Schmidt, 2015). The same concerns about assumptions apply to the Noro1A and Noro2A models, but use of these models is more easily justified because they have good empirical fit to the available data (if all mechanistic assertions concerning virus aggregation and host immunity/susceptibility are ignored). These results highlight the need to reconsider the current recommended use of the Noro1B and Noro 2B dose-response models in waterborne microbial risk assessments with disaggregated norovirus (e.g. U.S. EPA, 2014; WHO, 2016). The Noro3A and Noro3B models are recommended as preferred alternatives without problematic mechanistic assumptions.

One challenge associated with imposing a generic, unilateral treatment guideline (e.g., minimum 4-log virus reduction for all water supplies) is that it may be overly stringent for some systems with exceptional source water quality while possibly not being protective enough for heavily contaminated sources. While it is widely believed that groundwater quality is generally less variable than surface water quality, the quality of groundwater-based water supplies can be quite variable both within and between wells. Substantial differences can exist between high-quality groundwater sources and groundwaters impacted by either surface water or subsurface wastewater discharges. Thus, there would be a desire by some water systems to demonstrate suitability of a lower treatment target through continued source water monitoring. For example, "a jurisdiction may allow a groundwater source considered less vulnerable to faecal

contamination to have less than the recommended minimum 4-log reduction if the assessment of the drinking water system has confirmed that the risk of enteric virus presence is minimal" (Health Canada, 2011). This may particularly be the case for subsurface water supplies with long underground retention times. California's regulations on groundwater replenishment using recycled water state that "for each month retained underground…the recycled municipal wastewater or recharge water will be credited with 1-log virus reduction" (California Code of Regulations, 2014).

Supporting guidance is needed to inform enteric virus monitoring in source waters using standardized methods that are readily accessible at commercial laboratories nationwide. Methods for the enumeration of oocysts of the protozoan pathogen *Cryptosporidium* spp. have been rigorously standardized and widely adopted among commercial laboratories (U.S. EPA, 2005), yet methods for enteric viral pathogen monitoring (e.g. U.S. EPA, 2012) are not in widespread use. While culture-based methods are commonly used for monitoring and dose-response of bacterial enteric pathogens and some types of viruses, continued challenges with culturing norovirus make qPCR methods most readily available. Consensus upon a norovirus enumeration method is needed so that water systems and regulators may have convenient access to a widely accepted method and may also directly compare data from multiple studies. A PCR method that quantifies all known pathogenic varieties of norovirus (e.g. GI.1 and GII.4) without also quantifying non-pathogenic varieties would be particularly desirable.

Development of conveniently simple and reliably informative pathogen monitoring programs for water systems is a complex task. In particular, they must properly capture temporal variability in pathogen occurrence, yet not be too logistically or financially onerous. This is particularly challenging given the abundance of non-detect samples that may occur in groundwater, as

observed in the data used in this study. Even a very large number of non-detect samples may not prove low risk in the case of norovirus and could provide a false sense of security. Unlike zoonotic pathogens that are relatively ubiquitous, norovirus may be truly absent when there is no virus shedding in the catchment. If such a well were highly impacted by septic discharges or other wastewaters, however, norovirus concentrations could be quite high if infections become prevalent within the catchment. Although the wells considered in this study were all classified as being free of surface water influence, positive samples were temporally clustered: 27 samples in Spring 2006, 4 samples in Fall 2006, 0 samples in Spring 2007, and 1 sample in Fall 2007. Thus, a prolonged monitoring program may be required to properly characterize impacts of annual variation and seasonality (Ahmed et al., 2013) in norovirus prevalence. Notably, analytical limitations such as those described above must be overcome to better enable monitoring and more accurate description of virus risks.

Another issue to consider in the development of treatment targets is temporal variability in treatment efficacy. Such variability can be assessed by challenge testing, but these analyses are cost-prohibitive for many water systems. Fortunately, adherence to treatment targets is easier for relatively predictable chemical disinfection processes than it is for inherently more dynamic treatment processes like chemically-assisted filtration. Treatment upsets, however, may lead to periods with treatment efficacy well below nominal values, which correspond to elevated risk. Nominal compliance with a treatment target may be sufficient for systems relying exclusively upon disinfection methods for virus inactivation if a water safety plan is in place to virtually eliminate treatment upsets and appropriately mitigate risks should treatment upsets occur.

4.0 Conclusions and Considerations

- Treatment such as chemical disinfection of municipal subsurface drinking water supplies is well-justified for public health protection in absence of ongoing monitoring of appropriate reference viruses
- 4-log is a justifiable minimum virus reduction target for groundwater-based drinking water supplies in absence of better information resolving uncertainties in virus occurrence, enumeration, and dose-response. The requirement of higher levels of virus inactivation for subsurface water supplies is not justified at present due to 1) uncertainties and the lack of consensus related to various QMRA modelling approaches and 2) analytical limitations that preclude more accurate description of virus risks.
- Defining standardized treatment requirements for all subsurface water supplies without knowledge of well-to-well variability in pathogen occurrence can lead to inadequately mitigated risks in the most compromised water supplies and overly stringent treatment requirements for relatively uncontaminated, low risk water supplies.
- The development of a culture method for pathogenic norovirus strains is warranted, or at least standardization of a qPCR method that concurrently quantifies both pathogenic GI and GII noroviruses.
- Monitoring for well-specific virus occurrence can help to refine case-specific treatment targets (e.g. permitting lower treatment targets), but it must be frequent enough to capture temporal variability while not being too onerous.
- Numerous non-detect samples for a particular type of enteric pathogen may provide a false sense of security if they happen to coincide with a period when there are no infected individuals shedding pathogens in the catchment.

Declaration of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1 – Summary of Alternative Dose-Response Models

	Rota	Noro1	Noro2	Noro3
Model Reference	Schmidt (2017)[a]	Teunis et al. (2008)	Messner et al. (2014)	Schmidt (2015)
Data Source				
– Ward et al. (1986) – Rotavirus	X			
– Teunis et al. (2008) – NoV GI[b]		X	X	X
– Seitz et al. (2011) – NoV GI			X	X
– Frenck et al. (2012) – NoV GII[c]			X	X
– Atmar et al. (2014) – NoV GI			X	X
Model Type	Exact beta-Poisson (${}_1F_1$)	Exact beta-Poisson (${}_2F_1$ or ${}_1F_1$)	Fractional Poisson	Exact beta-Poisson (${}_1F_1$) with immunity
Immunity[d]	None assumed	None assumed	$\phi = 1 - P = 0.278$	$\phi = 0.2754$ (Noro3A) None (Noro3B)
Host Susceptibility	$r \sim \text{beta}(\alpha = 0.1673, \beta = 0.1920)$	$r \sim \text{beta}(\alpha = 0.040, \beta = 0.055)$	$r = 1$	$r \sim \text{beta}(\alpha = 2.910, \beta = 2734)$
Aggregation	N/A	$\mu = 396.4$ (Noro1A) None (Noro1B)	$\mu = 1{,}106$ (Noro2A) None (Noro2B)	None

[a] This model differs slightly from an equivalent model presented in Teunis and Havelaar (2000) because the number of subjects administered a mean dose of 0.009 viruses is incorrect therein.

[b] Data from two norovirus genogroup I (NoV GI) stock suspensions are included: 8fIIa (presumed aggregation), 8fIIb (disaggregated).

[c] Norovirus genogroup II (NoV GII) data are included by assuming that both genogroups share identical dose-response.

[d] For norovirus, the data are restricted to secretors to control for a known immunity mechanism; immunity parameter ϕ represents apparent immunity among secretors that is attributable to other mechanisms.

553 **Table 2 – Limitations of Study Methodology and Available Data**

Risk Assessment Category	Anticipated Effect upon Developed Treatment Goals		
	Possible Underestimation	**Possible Overestimation**	**Unclear Effect**
Hazard Identification	– Secondary infections arising from an initial waterborne transmission are not addressed		– Unclear representativeness of norovirus (used as a viral reference pathogen) for determining an appropriate treatment target for all enteric viral pathogens in subsurface water supplies
Raw Water Occurrence & Infectivity Assessment	– Utilized occurrence data represent only norovirus GI rather than all pathogenic norovirus strains (including norovirus GII.4) – Losses in sample preparation methodology (i.e. incomplete analytical recovery) are presumed to be negligible	– PCR-based detection methods can include quantification of genomes from inactivated/degraded viruses – Detected viruses in well waters are presumed to be disaggregated (and virus clustering reduces mean risk in single-hit dose-response models (Nilsen and Wyller, 2016))	– Unclear representativeness of utilized norovirus occurrence data set for subsurface waters in general – Possible imprecision of norovirus concentration data due to random sampling and analytical errors – Separation of temporal variability and well-to-well variability is not addressed
Treatment Efficacy & Water Distribution			– Implications of temporal variability in treatment efficacy not addressed in current approach to prescribe treatment targets (treatment upsets can lead to excessive instantaneous or mean risk) – Distribution system effects upon waterborne exposures (e.g. virus decay or intrusion) are not addressed
Tap Water Consumption	– Indirect waterborne transmission (washing food or surfaces, shower aerosols, etc.) are not addressed		– Person-to-person and/or temporal variation in tap water consumption patterns are not addressed
Dose-Response	– Norovirus/rotavirus dose-response experiments have used only healthy adults, while other subpopulations are hypothesized to be more susceptible	– Utilized norovirus dose-response models exclude data from subjects who are likely immune due to absence of essential epithelial cell secretions (non-secretors), thus overestimating risk for the non-secretor demographic	– Norovirus dose-response data rely principally upon GI.1 strain – Lack of consensus on most appropriate norovirus dose-response model form – Unclear implications of low-dose extrapolation error – Parametric uncertainty not built into risk characterization approach

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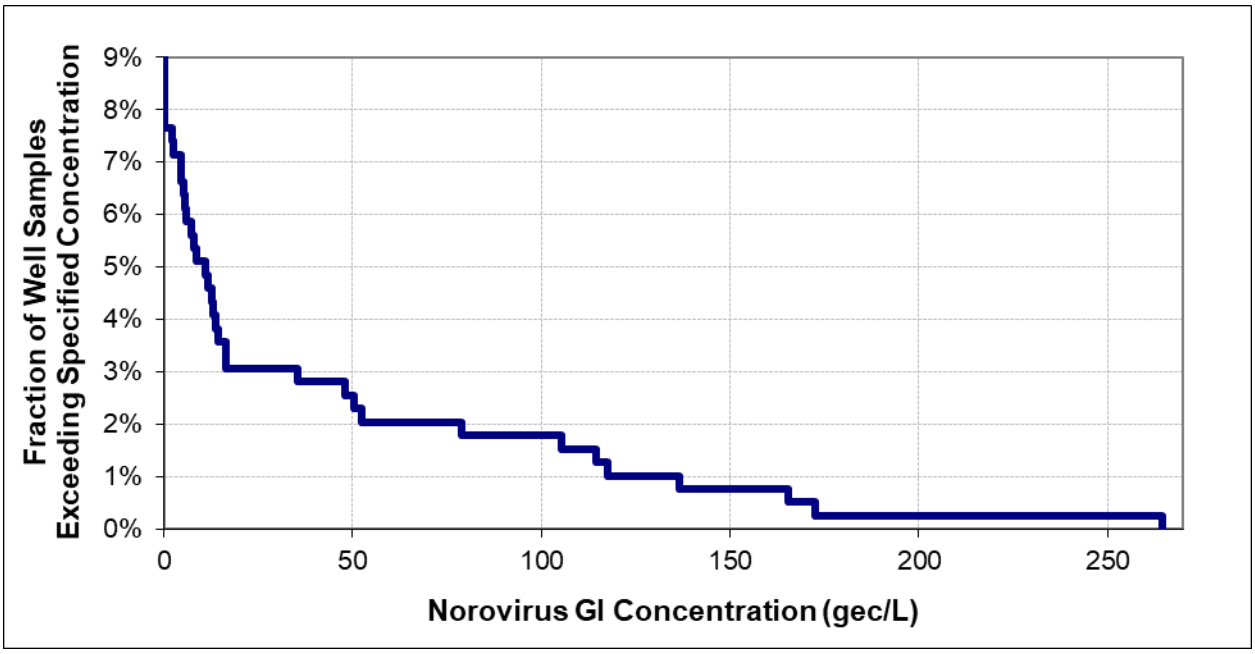

Fig. 1 – Raw well water norovirus GI qPCR data

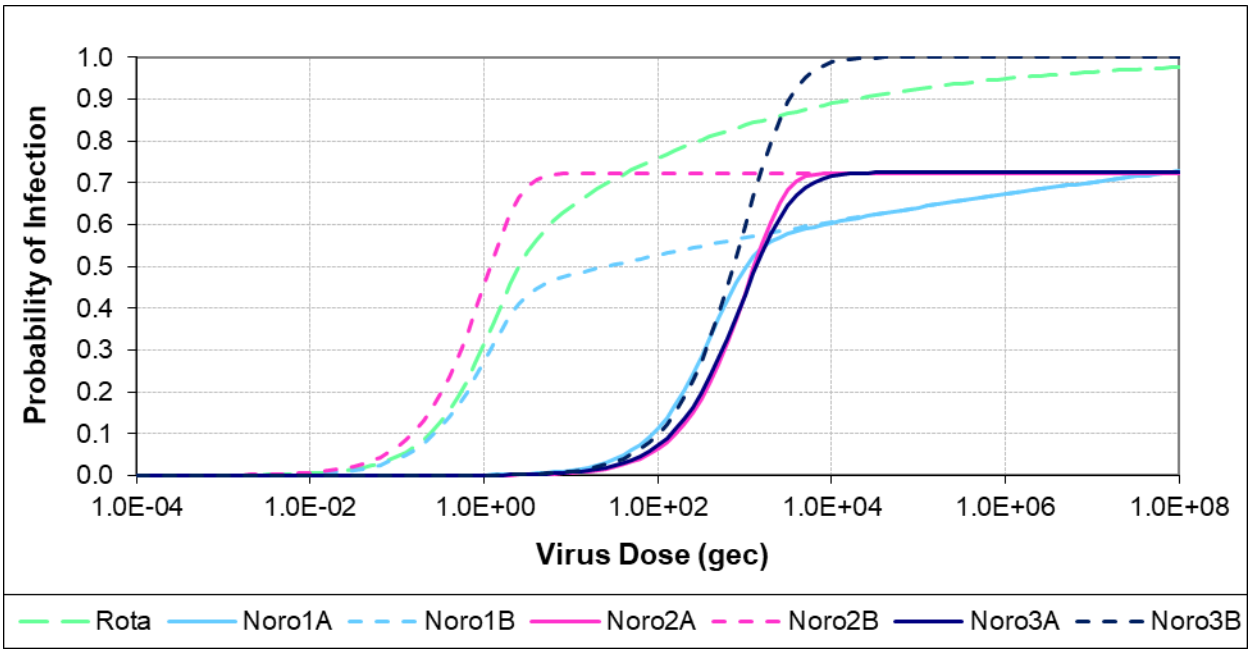

Fig. 2 – Alternative enteric virus dose-response models

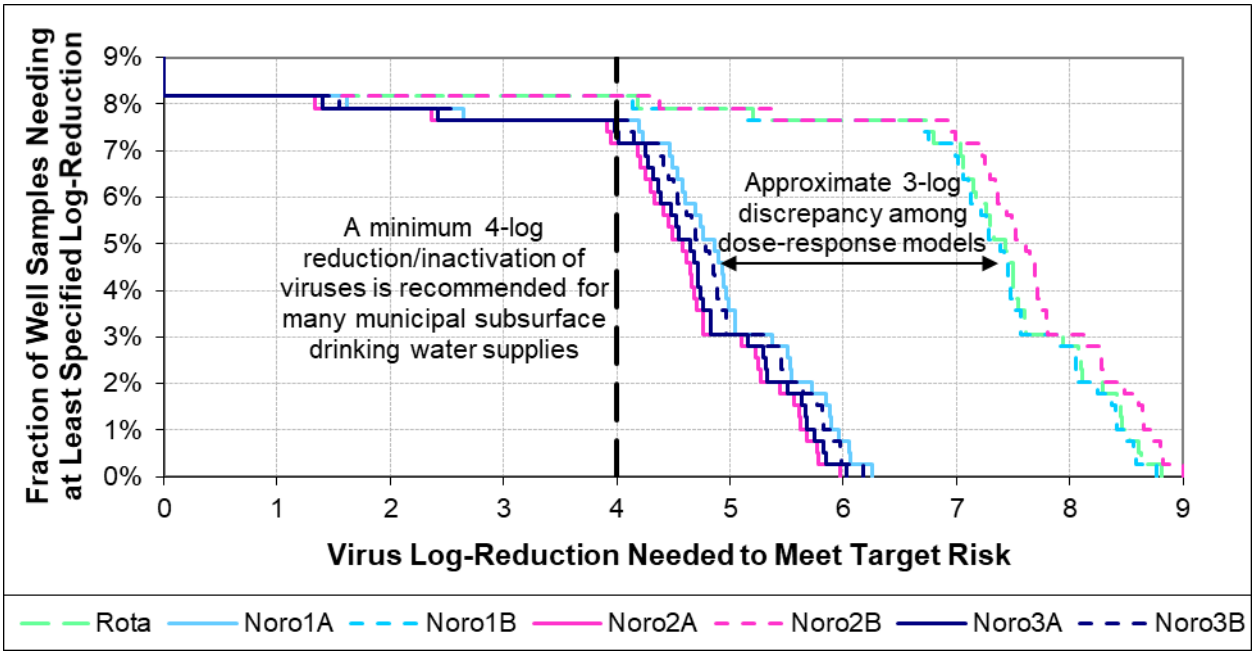

Fig. 3 – Virus log-reduction needed to reduce risk to acceptable level for well water samples with detected norovirus using seven alternative dose-response models

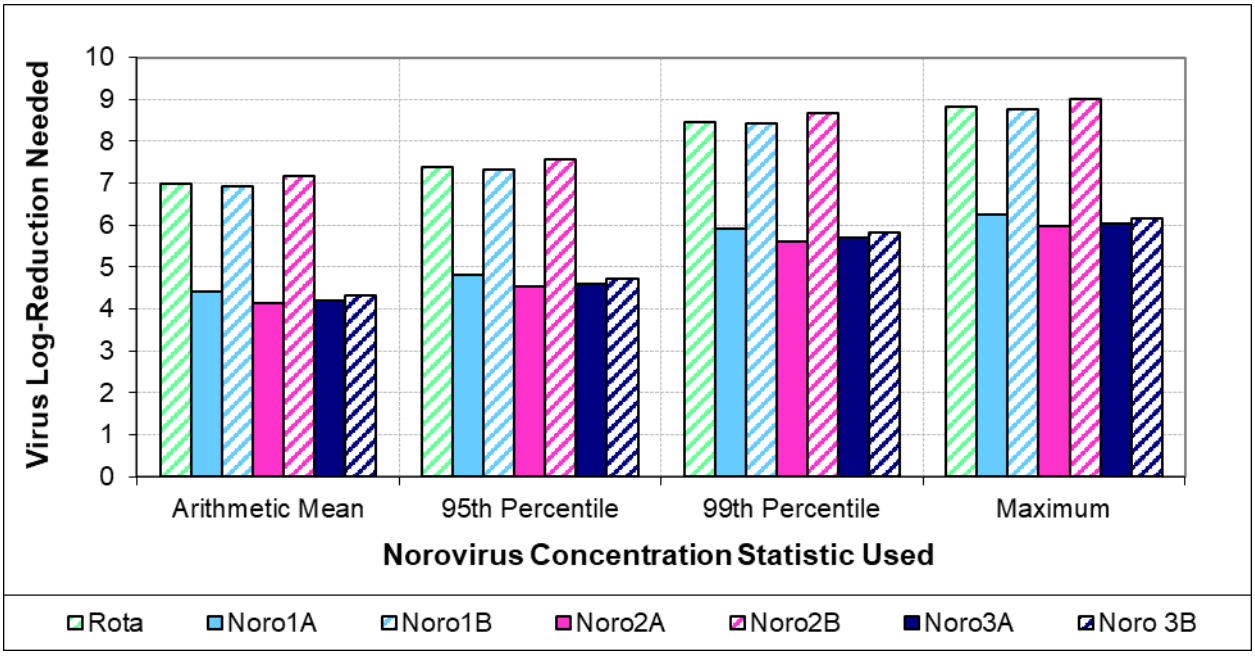

Fig. 4 – Virus log-reduction targets based on alternative norovirus concentration statistics and dose-response models