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Occurrence of norovirus in raw sewage – A systematic literature review and meta-analysis

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ABSTRACT

Human noroviruses (NoV) are a leading cause of recreational waterborne illnesses and responsible for the majority of viral-associated gastrointestinal illnesses nationwide. We conducted a systematic literature review of published peer-reviewed publications to identify NoV density data in wastewater influent, and provided an approach for developing pathogen density distributions, using the NoV data. Literature review inclusion criteria included scope, study quality, and data availability. A non-parametric bootstrap statistical model was used to estimate the NoV distribution in wastewater influent. The approach used accounts for heterogeneity in study-specific distribution curves, sampling locations, and sampling season and provides a comprehensive representation of the data. Study results illustrate that pooling all of the available NoV data together in a meta-analysis provides a more comprehensive understanding of the technical literature than what could be appreciated from individual studies. The studies included in this analysis indicate a high density of NoV in wastewater influent (overall mean = 4.6 $\log_{10}$ genome copies (GC)/liter (L)), with a higher density of NoV genogroup (G) II (overall mean = 4.9 $\log_{10}$ GC/L) than for GI (overall mean = 4.4 $\log_{10}$ GC/L for GI). The bootstrapping approach was also used to account for differences in seasonal and geographical occurrences of NoV GI and GII. The methods presented are reproducible and can be used to develop QMRA-ready density distributions for other viral pathogens in wastewater influent, effluent, and ambient waters. To our knowledge, our results are the first to quantitatively characterize seasonal and geographic differences, which could be particularly useful for future risk assessments.

1. Introduction

Human noroviruses (NoV) are the leading cause of gastroenteritis in the United States (US) and worldwide among persons of all ages (Mead et al., 1999; Scallan et al., 2011; Patel et al., 2009; CDC, 2008; WHO, 2003). Scallan et al. (2011) estimated 31 known pathogens cause over 37 million cases of illness annually, and that NoV are responsible for ~21 million of those illnesses – more than any other known pathogen. Similarly, Hall et al. (2013) concluded NoV cause on average 570–800 deaths and 19–21 million total illnesses each year in the US. NoV infection is primarily spread via the fecal-oral route with transmission occurring through person-to-person transmission, ingestion of contaminated food or water, or through contact with contaminated media (such as surfaces) (Pouillot et al., 2015).

NoV comprise at least five genogroups (Patel et al., 2009), which can be further subdivided into >30 genotypes (Kroneman et al., 2013). Three of the genogroups (GI, GII, GIV) appear capable of causing illness in humans (Atmar 2010). Human NoV have only recently been cultured (Jones et al., 2014), but methods for culture-based quantification of environmental water samples have not yet been developed (Thorne and Goodfellow, 2014; Papafragkou et al., 2014). NoV enumeration in environmental media has been performed through molecular methods including quantitative reverse-transcriptase polymerase chain reaction or (RT-qPCR), with densities of nucleic acids in units of copies per volume (Patel et al.,

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2009). NoV can be highly infectious – exposure to few virions can yield a high probability of human infection, although the reported range of infectivity currently remains uncertain or is widely variable (Teunis et al., 2008; Van Abel et al., 2016; Atmar et al., 2014; Messner et al., 2014; Schmidt, 2015). Viral shedding of NoV occurs in large numbers for a prolonged duration with NoV infection, even after symptoms resolve (Atmar et al., 2008). Repeated infections can occur throughout life with re-exposure, likely because immunity is short term and there is lack of complete cross-protection against the diverse NoV genogroups (Patel et al., 2009).

The risk of NoV infection and subsequent illness from waterborne exposure is an emerging research topic (Messner et al., 2014; Soller et al. 2010a, 2010b, 2014, 2015; Arnold et al., 2013; Viau et al., 2011; Griffith et al., 2016). Although viruses have long been suspected as likely etiologic agents responsible for swimming-associated illness in fecally contaminated recreational waters (WHO, 2003; Cabelli et al., 1982), little information has been available until recently to provide specific evidence in this regard. Recent research, however, suggests human enteric viruses, and NoV in particular, are responsible for a large portion of recreational water illnesses in fresh and marine waters impacted by treated wastewater effluent and by urban stormwater runoff (Soller et al., 2010a, 2015; Arnold et al., 2013, 2016; Viau et al., 2011; Arnold et al., 2016). Human enteric viruses, and particularly NoV, may also be important etiologic agents with respect to health risks associated with exposure to recycled water (Soller et al., 2016).

The U.S. Environmental Protection Agency (EPA) is currently in the process of developing Recreational Water Quality Criteria recommendations for coliphage, a viral indicator, to protect public health from viral illnesses in ambient waters designated as primary contact recreation (U.S. EPA, 2015). A quantitative microbial risk assessment (QMRA) based approach can be used to derive such criteria. For this purpose, a QMRA would rely on availability of densities of key viral pathogens and coliphages in wastewater influent. However, to date, NoV densities in the literature have not been summarized in a manner that is conducive for use in such QMRA evaluations. An increasing amount of literature has been published over the last decade that reports NoV densities in WWTP influent, effluent, and in surface waters (Aw and Gin, 2010; Rose et al., 2004; Katayama et al. 2006, 2008; Lodder and de Roda Husman, 2005, van den Berg et al., 2005). The objectives of this work are to summarize the results of a systematic literature review and to characterize the density of NoV in raw sewage, accounting for season and geographic region. The results from this work will also offer utility to other QMRA applications, such as risk characterizations from consumption of recycled wastewater and shellfish, and exposure to recreational waters and biosolids.

2. Materials and methods

2.1. Data sources

Two information sources were used to obtain the data: (i) the peer-reviewed literature and (ii) surveillance data collected by the US and Canadian governments (Pouillot et al., 2015).

We performed a systematic literature search of the peer-reviewed literature for articles reporting NoV densities in raw wastewater in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Web of Science. The search included the keywords “norovirus or Norwalk” AND “sewage OR influent OR effluent OR ambient OR lake* OR river* OR stream* OR marine OR ocean OR estuary* OR “surface water” OR “ground water” OR groundwater”. The literature search was limited to peer-reviewed publications written in English between 2003 and September 2015. NoV methodologies, particularly the most popular NoV primers, were not available prior to 2003.

Study inclusion criteria including scope, study quality, and data availability were applied to each publication. For the first step, to be considered within scope, the article needed to have occurrence data for NoV in water, including wastewater influent, effluent, or ambient water. All abstracts were reviewed and classified into “not likely to have occurrence data” and “likely to have occurrence data.”

Publications that likely had occurrence data were retrieved and the full text was evaluated for scope. Specifically, the article needed quantitative data for NoV in wastewater influent. Wastewater influent was further limited to wastewater collected by municipal sewage systems (not septic systems) collected either before or after primary screening and settling. Publications reporting only presence/absence data were excluded. The following information for publications within the scope was recorded: information on water type, genogroup(s) quantified, collection season and months, study geographic region, and whether the summary data points or individual data points were provided.

Study quality was assessed independently by two senior scientists. Studies had to pass all of the following criteria:

- Assay Type – Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) and most probable number RT-PCR (MPN RT-PCR) were considered acceptable for quantifying NoV. Methods estimating NoV densities using amplicon band intensity in gels were not included. When other publications were cited for methods, the original method papers were evaluated.
- Clarity – Publications were not included if the study and associated methodologies were not documented clearly enough to assess study quality.
- Controls – The study needed to identify controls used, or cite a peer-reviewed method paper that described the use of controls. Both positive and negative controls needed to have been performed. We trusted authors handled the results appropriately based on the controls.
- Reported Detection Limit – Detection or quantification limits (unless there were no samples below the detection limit) needed to have been reported.
- Inhibition – If inhibition was discussed and addressed in an appropriate manner, data from the study was included. If a study described data that seemed affected by PCR inhibition, but lacked either a discussion of the inhibition or clarification via author correspondence, the study was excluded. Evaluation of inhibition was particularly important if results seemed counter-intuitive (e.g., viral densities greater in effluent than in influent).

Publications with missing information were not immediately disqualified; rather the corresponding authors were emailed for clarifications. In some cases, the authors provided additional information that addressed study deficiencies identified during the review. In other cases, the authors were not reachable or their response did not provide needed information.

After a publication passed both the scope and quality criteria, it was evaluated for data availability. Individual data points, not means and medians, were required for this analysis. In some cases, individual data points were available in a table or could be digitized from figures using GetData Graph Digitizer, Version 2.25 (http://www.getdata-graph-digitizer.com/). When individual data points were not available, individual data points were requested from the study authors. If the author sent data, and those data aligned with the published data, those datasets were included. For example, Pouillot et al. (2015) used data collected by the Food and Drug Administration (FDA), and FDA provided the individual data points collected for that study. If a research group published multiple

papers using the same dataset, only one publication was used to represent the dataset.

Some studies used primers that separated genogroups I (GI) and II (GII), other studies used primers that amplified all genogroups. Only studies differentiating between GI and GII were included. Subsequent statistical analyses evaluated the two NoV genogroups (GI and GII) both together (hereafter referred to as "pooled genogroups") and separately (hereafter referred to as "genogroup-specific").

2.2. Modelling approaches

For this analysis, a data point was characterized by the month of collection, the virus type (NoV GI or NoV GII), and the measured virus density in raw wastewater. Viral density units are reported in $\log_{10}$ genome copies per liter (GC/L) for NoV. Measurements below the limit of detection (LOD) were substituted with the LOD obtained as reported in the peer-reviewed publication.

In order to estimate variability in the reported NoV densities, as well as to better inform future modelling decisions, a two-stage bootstrap approach was designed. Conceptually, bootstrapping is a general tool for assessing statistical accuracy, which usually estimates the population distribution by using a number of resamples from the observed measurements (Efron and Tibshirani, 1994; Hastie et al., 2001). In this approach, if the NoV densities obtained from the literature (sample distribution) are representative of "all" NoV densities in raw sewage (population distribution) and the data sample size is large, then the sample distribution approaches the true population distribution of NoV densities. Additionally, as the number of bootstrap resamples taken with replacement from the observed sample increases, the bootstrap distribution approaches the population distribution. The bootstrap approach also accounts for the amount of data reported by each study and the fact that samples were taken at various geographic regions and over different seasons (Mooney et al., 1993).

The two-stage bootstrap approach was implemented as follows. In Stage 1, the objective was to understand variation in the distribution of NoV densities caused by specific factors, such as geographic region and season (Table 2). The dataset was first separated into subsets based on geographic region and season, respectively. For 10,000 iterations, a sample with replacement and with equal weight was drawn from each subset, resulting in a distribution of estimated means, standard deviations and 95% confidence intervals. To account for region or season, Stage 2 focused on incorporating variation contributed by each subset (geographic region and season), using a stratified bootstrap approach. Unlike in Stage 1, the number of samples randomly selected from each subset were allowed to vary. In this study, we assumed that number of samples were taken from each subset is proportional to the subset sample size. Thus, the approach accounts for the fact that the number of NoV samples taken under different seasons and locations vary considerably. For the Stage 2 analysis, the sampling step is repeated for 100, 500, 1,000, and 3000 iterations; the samples are pooled; and the means, standard deviations and their 95% confidence intervals are estimated.

2.3. Significance testing

Two sample T-tests with unequal variances were used to compare the means of the observed NoV densities for pooled and genogroup-specific NoV samples by geographic region and by season. To compare the bootstrapped distributions we used the Kolmogorov-Smirnov test (Lehmann and D'Abrera, 2006). Data extraction and management was performed using Excel (Microsoft Corporation, 2013). Bootstrap analyses were performed using R version 3.0.2 (R Core Team, 2013) The statistical T-tests were performed in Stata version 13 (StataCorp, 2013).

Code availability: Computer code and bootstrapped distributions are available upon request from the corresponding author.

3. Results

3.1. NoV density data in raw sewage

Fig. 1 illustrates the overall literature search strategy and number of titles retrieved. A total of 483 titles remained after duplicates from the two databases were removed. Literature Search 1 was conducted in January 2015, and it was updated with Literature Search 2, which was conducted in September of 2015. After full-text review, 48 references passed study scope, of which 29 passed our study quality criteria.

Fifteen studies satisfied the inclusion criteria for scope, study quality, data availability, and they differentiated between NoV genogroups (Pouillot et al., 2015; Aw and Gin, 2010; da Silva et al., 2007; Flannery et al., 2012; Francy et al., 2012; Grondahl-Rosado et al., 2014; Hellmer et al., 2014; Hewitt et al., 2011; Kauppinen et al., 2014; Masclaux et al., 2013; Miura et al., 2015; Montazeri et al., 2015; Nordgren et al., 2009; Perez-Sautu et al., 2012; Sima et al., 2011). Together these studies provided a total of 850 individual NoV density data points (Table 1, GI: $n = 389$; GII: $n = 461$). These samples were collected under various conditions and quantified by different analytical techniques. All included studies used RT-qPCR methods. Approximately 15% of the data reported were below the LOD. The literature search yielded references with relevant quantitative data representing four geographic regions, including 12 countries; Europe (Norway, Sweden, Switzerland, Spain, France, Finland, Ireland), North America (US and Canada), Asia (Japan and Singapore) and New Zealand (Table 1).

3.2. Descriptive statistics

Table 2 presents the summary statistics of the observed NoV densities for both the pooled and genogroup-specific data. The observed densities for the pooled NoV ranged from 0.02 to 9.17 $\log_{10}$ GC/L. Approximately 70% of all NoV samples were collected in North America and Europe, with the remaining 30% from Japan, Singapore and New Zealand (Table 2). Both pooled and genogroup-specific NoV densities were significantly lower in North America and New Zealand (medians < 4 $\log_{10}$ GC/L) than those in Europe and Asia (medians > 5 $\log_{10}$ GC/L, p-value <0.001) (Fig. 2A and C). In terms of seasonality, NoV densities were significantly higher in winter and spring compared to summer and fall (Fig. 2B and D) (p-value <0.001).

Additionally, the observed median densities of NoV GII were consistently significantly higher compared to median densities of NoV GI, a phenomenon independent of geographic region (Fig. 2C) and season (Fig. 2D). The exception was in Asia, where the median of NoV GI was significantly higher than GII, although the means are identical.

3.3. Bootstrap analysis

3.3.1. Pooled genogroups results

The bootstrap analysis results for the pooled NoV genogroups are presented in Table 3 and Fig. 3. Geographic region and season-specific bootstrapped distributions obtained in Stage 1, all are significantly different from each other (Kolmogorov-Smirnov test, p-value < 0.001, comparisons among regions and seasons). However, the means of the bootstrapped distributions obtained from Stage 2, which account for geographic region and season, are not

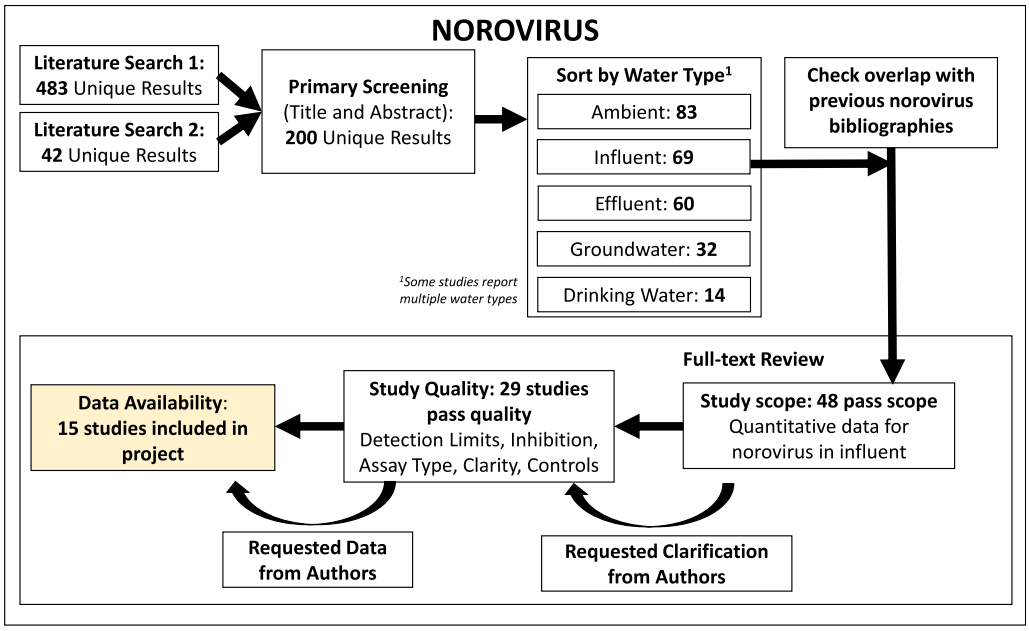

Fig. 1. Overview of the NoV literature search process.

Table 1
Description of NoV GI and GII in raw sewage datasets.

First author, year	Location	Number of data points				LOD	Source
		NoV GI		NoV GII			
		Measured	Nondetects	Measured	Nondetects		
Aw, 2010	Singapore	18		18			Author
DaSilva, 2007	France	43	37	75	6	3.7, 2.3 [a]	Digitized
Flannery, 2012	Ireland	48		48			Author
Francy, 2012	USA	19					Author
Grondahl-Rosado, 2014	Norway	8		9			Digitized
Hellmer, 2014	Sweden	5	2	6		1	Table
Hewitt, 2011	New Zealand	15		13			Author
Kauppinen, 2014	Finland	19		19			Digitized
Masclaux, 2013	Switzerland	0		44	16	4.3	Table
Miura, 2015	Japan	0		18			Digitized
Montazeri, 2015	USA	12		12			Digitized
Nordgren, 2009	Sweden	12		11	1	4	Table
Perez-Sautu, 2012	Spain	49	5	53	1	3.6	Digitized
Sima, 2011	France	15	1	14	2	2.4	Digitized
Pouillot, 2015 (USFDA/CFSAN Data)	USA & Canada	28	53	89	6	3.0, 3.1[b]	Author
Total		291	98	429	32		

Studies that do not include LOD did not have any non-detects.
[a] The first value is for GI and the second is for GII.
[b] The first value is for analysis done before 2008, and the second is for analysis done after 2008.

statistically different, with NoV densities of 4.6 (95% CI: 4.2, 5.1) and 4.7 (95% CI: 4.3, 5.6) $\log_{10}$ GC/L, respectively (Table 3). Fig. 3 illustrates the distribution of NoV mean densities from Stage 1 bootstrap, by geographic region and by season. The figure shows that geographic regions have a strong impact on NoV density, with the lowest mean density in New Zealand and the highest in Asia (Table 3 and Fig. 3a). Spring and winter were associated with significantly higher NoV densities than summer and fall (Fig. 3b).

3.3.2. Genogroup-specific results

The Stage 1 bootstrap analysis shows a significant difference between mean NoV GI and GII densities for North America (p-value<0.001), but not for Europe, New Zealand or Asia (Table 4). A difference in mean NoV density by season was also observed, with the greatest differences between spring and fall for both GI and GII, with a winter peak for GII (Table 4 and Fig. 5). For GI, there is no significant difference in mean density distributions between North America and New Zealand (Fig. 4a), and for GII, there is no significant difference in the mean density distributions between North America and Europe (Fig. 4b). In North America, the NoV GII density is significantly higher than the density of NoV GI (4.7 $\log_{10}$ GC/L versus 3.3 $\log_{10}$ GC/L, p-value <0.001) (Table 4).

For the bootstrap Stage 2, the sensitivity analysis to evaluate the impact of the number of sampling iterations on the bootstrapped NoV density distributions (for 100, 500, 1,000, and 3000 iterations) indicated little effect (Figs. S1 and S2 in Supplemental File). The results for 3000 iterations in this analysis are presented (Fig. 6). Geographic region and season specific bootstrapped distributions,

Table 2
Summary statistics of NoV samples in raw sewage.
- All units are $\log_{10}$ GC/L
- Specific countries within selected geographic regions are: North America: USA and Canada; Europe: Norway, Sweden, Switzerland, Spain, France, Finland, and Ireland; Asia: Japan and Singapore.

		Number of observations total (GI | GII)	Mean total (GI | GII)	Median total (GI | GII)	Range (min-max) or standard deviation total (GI | GII)
Total observations		850 (389 | 461)	4.80 (4.59 | 4.98)	4.74 (4.07 | 5.00)	(0.02–9.17) (1.00–9.17)|(1.00–9.17)
Observations by geographic region	North America	219 (112 | 107)	3.95 (3.27 | 4.65)	3.88 (3.10 | 4.47)	1.10 (0.67 | 1.03)
	Europe	549 (244 | 305)	5.06 (5.16 | 4.98)	5.04 (4.99 | 5.06)	1.59 (1.58 | 1.60)
	New Zealand	28 (15 | 13)	3.42 (3.06 | 3.83)	3.25 (2.93 | 4.19)	1.04 (0.79 | 1.16)
	Asia	54 (18 | 36)	6.39 (6.39 | 6.39)	6.35 (6.43 | 6.29)	0.67 (0.35 | 0.79)
Observations by season	Spring	208 (96 | 112)	5.33 (5.23 | 5.42)	5.48 (5.24 |5.62)	1.60 (1.63 | 1.57)
	Summer	180 (81 | 99)	4.28 (3.94 | 4.55)	4.21 (3.70 | 4.64)	1.33 (1.18 | 1.38)
	Fall	158 (80 | 78)	4.12 (4.02 | 4.23)	3.94 (3.70 | 4.33)	1.29 (1.27 | 1.31)
	Winter	304 (132 | 172)	5.11 (4.88 | 5.28)	5.04 (4.63 | 5.22)	1.61 (1.82 | 1.40)

For New Zealand, the following season definitions are used: Spring (September to November), Summer (December to February), Fall (March to May), Winter (June to August) For countries in the Northern Hemisphere, the following season definitions are used: Spring (March to May), Summer (June to August), Fall (September to November), Winter (December to February) (source: http://www.timeanddate.com/calendar/aboutseasons.html).

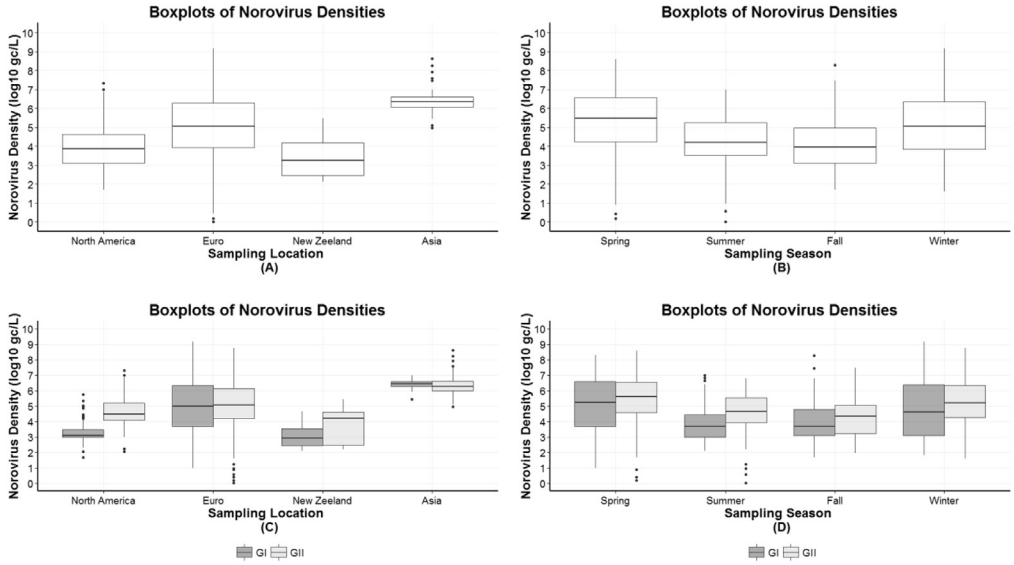

Fig. 2. NoV densities in raw sewage, boxplots by geographic region and season for pooled and genogroup-specific NoV. (A) NoV densities by geographic region for pooled genogroups; (B) NoV densities by season for pooled genogroups; (C) NoV densities by region and genogroups; (D) NoV densities by season and genogroups. For boxplots, the bottom and top of the box represent the first and third quartiles (Q1 and Q3, respectively); the line in the middle of the box is the median. The length of the box is the interquartile range (IQR). The upper whisker extends to the greatest observed value $\leq$ Q3+1.5*IQR; the lower whisker extends to the smaller observed value $\leq$ Q1-1.5*IQR. The symbols represent outliers (those measurements greater than the upper whisker, or lower than the lower whisker).

obtained in Stage 1, are all significantly different from each other for both NoV GI and GII (Kolmogorov-Smirnov test p-values<0.001 for comparisons among regions and among seasons). The results from the Stage 2 bootstrapping (4.4 and 4.5 log10 GC/L for GI and 4.9 log10 GC/L for GII), which account for geographic region and season, are a good estimate of the overall NoV density distributions provided by Stage 1 results. In addition, the confidence interval is tighter when geographic region and season are taken into account (Table 4).

4. Discussion

This work summarizes the currently available NoV density data in raw wastewater. The systematic literature review process conducted for identifying and retrieving relevant literature benefits from a rigorous study selection process, which captures important research attributes such as study relevance and quality (Moher et al., 2009). The review process is also efficient, reproducible, and easily modified to incorporate additional data, as they become available.

The subsequent two-stage bootstrap approach employed offers several advantages. For example, the analysis is non-parametric and does not assume a shape for the underlying statistical distribution. Our stratified approach also allows for the evaluation of and the accounting for subsets of data of various sizes, sampled at different geographic regions and over different seasons.

Study results illustrate that pooling all of the available NoV data together in a meta-analysis provides a more comprehensive and nuanced understanding of the technical literature than what could be appreciated from individual studies. The studies included in this analysis indicate a high density of NoV in wastewater influent (overall mean = 4.6 $\log_{10}$ GC/L), with a higher density of NoV GII (overall mean = 4.9 $\log_{10}$ GC/L) than for GI (overall mean = 4.4 $\log_{10}$ GC/L for GI). The densities are higher than those reported by

Table 3
Summary statistics for the bootstrapped NoV densities in raw sewage (genogroups pooled).

Analysis	N	Mean (95% CI)	SD (95% CI)
Stage 1			
Geographic Region			
North America	219	4.0 (3.8, 4.1)*	1.1 (1.0, 1.2)
Europe	549	5.1 (4.9, 5.2)***	1.6 (1.5, 1.7)
New Zealand	28	3.4 (3.0, 3.8)**	1.0 (0.9, 1.2)
Asia	54	6.4 (6.2, 6.6)***	0.7 (0.5, 0.9)
Season			
Spring	208	5.3 (5.1, 5.5)***	1.6 (1.4, 1.8)
Summer	180	4.3 (4.1, 4.5)***	1.3 (1.2, 1.5)
Fall	158	4.1 (3.9, 4.3) ***	1.3 (1.2, 1.4)
Winter	304	5.1 (4.9, 5.3) ***	1.6 (1.5, 1.7)
Stage 2			
Accounting for geographic region		4.6 (4.2, 5.1)	1.6 (1.3, 1.9)
Accounting for season		4.7 (4.3, 5.6)	1.5 (1.3, 1.9)

Significant comparisons between regions and between seasons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. All units are $\log_{10}$ GC/L.

numerical evaluation approach, and due to the timing of our analysis, inclusion of data from several newly published studies (Grondahl-Rosado et al., 2014; Hellmer et al., 2014; Kauppinen et al., 2014; Miura et al., 2015; Montazeri et al., 2015). Our meta-analysis relied on a higher number of individual NoV densities (850 pooled; GI: n = 389; GII: n = 461), compared with 819 (GI: n = 253; GII: n = 566) from Pouillot et al. (2015). The different study inclusion criteria resulted in only seven overlapping data sources between the two meta-analyses (Pouillot et al., 2015; da Silva et al., 2007; Flannery et al., 2012; Francy et al., 2012; Hewitt et al., 2011; Nordgren et al., 2009; Sima et al., 2011). Another important difference we identified was the treatment of LODs. Pouillot et al. (2015) used a systematic approach for calculating the theoretical LOD for each of the included studies. In our approach, we used the LOD reported in the papers or contacted the authors to obtain the experimental LOD for the study (inability to identify an LOD from a study resulted in the study being excluded). In general, the theoretical LODs used by Pouillot et al. (2015) were lower than our LODs,

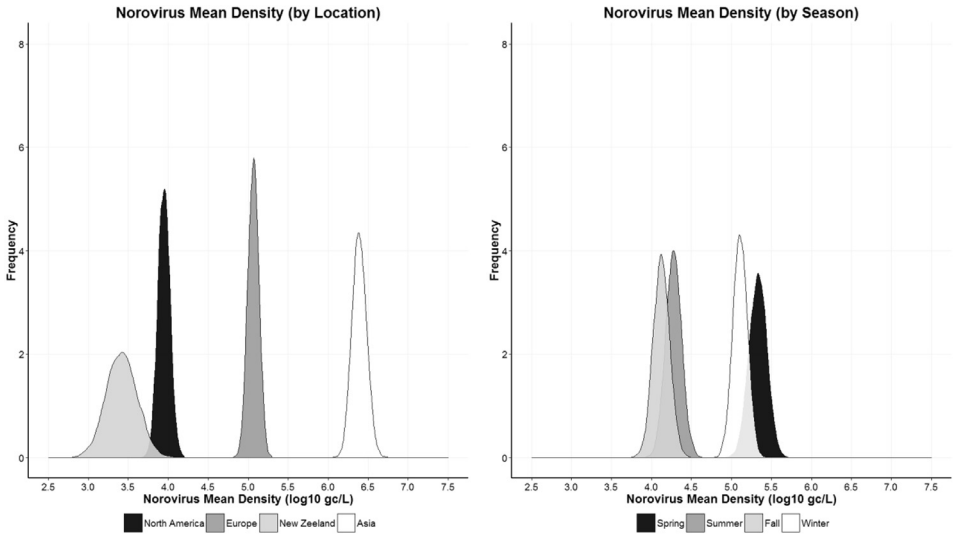

Fig. 3. Distribution of NoV mean density (pooled genogroups) by geographic region (left) and season (right).

Table 4
Summary statistics for the bootstrapped genogroup-specific NoV densities in raw sewage.

Analysis	N GI | GII	Mean (95% CI) GI | GII	SD (95% CI) GI | GII
Stage 1			
Geographic Region			
North America	112 | 107	3.3 (3.2, 3.4) | 4.7 (4.5, 4.9)**	0.7 (0.6, 0.8) | 1.0 (0.9, 1.2)
Europe	244 | 305	5.2 (5.0, 5.4) | 5.0 (4.8, 5.2)	1.6 (1.5, 1.7) | 1.6 (1.5, 1.7)
New Zealand	15 | 13	3.1 (2.7, 3.4) | 3.8 (3.2, 4.4)	0.8 (0.6, 1.1) | 1.2 (0.9, 1.5)
Asia	18 | 36	6.4 (6.2, 6.6) | 6.4 (6.1, 6.7)	0.4 (0.2, 0.5) | 0.8 (0.6, 1.0)
Season			
Spring	96 | 112	5.2 (4.9, 5.6) | 5.4 (5.1, 5.7)	1.6 (1.5, 1.8) | 1.6 (1.3, 1.9)
Summer	81 | 99	3.9 (3.7, 4.2) | 4.6 (4.3, 4.8)*	1.2 (1.0, 1.4) | 1.4 (1.2, 1.6)
Fall	80 | 78	4.0 (3.8, 4.3) | 4.2 (3.9, 4.5)	1.3 (1.1, 1.5) | 1.3 (1.1, 1.5)
Winter	132 | 172	4.9 (4.6, 5.2) | 5.3 (5.1, 5.5)	1.8 (1.7, 2.0) | 1.4 (1.3, 1.6)
Stage 2			
Accounting for geographic region		4.4 (4.0, 4.8) | 4.9 (4.5, 5.4)	1.7 (1.4, 2.0) | 1.4 (1.1, 1.8)
Accounting for season		4.5 (4.1, 5.0) | 4.9 (4.4, 5.3)	1.6 (1.3, 1.9) | 1.5 (1.2, 1.9)

Significant comparisons between GI and GII means by regions and seasons: * $p < 0.05$, ** $p < 0.001$. All units are $\log_{10}$ GC/L.

Pouillot et al. (mean 3.9 $\log_{10}$ GC/L for NoV GII and mean 1.5 $\log_{10}$ GC/L for NoV GI). Several reasons for the mean NoV GI density differences include: different study inclusion criteria, a different and roughly 41% of the Pouillot et al. (2015) data points were below the LOD.

The bootstrapping approach used in this work accounts for the

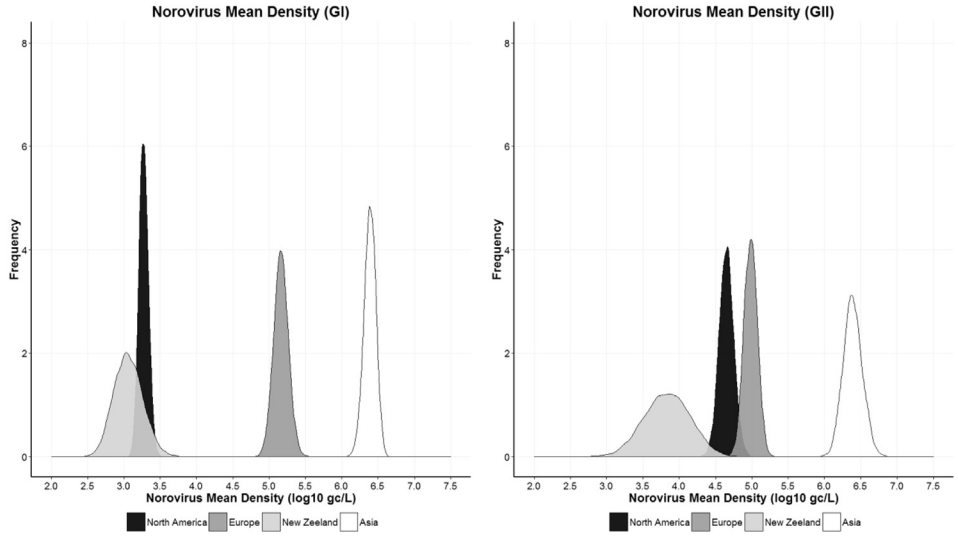

Fig. 4. Distribution of NoV mean density by geographic region for GI (left) and GII (right).

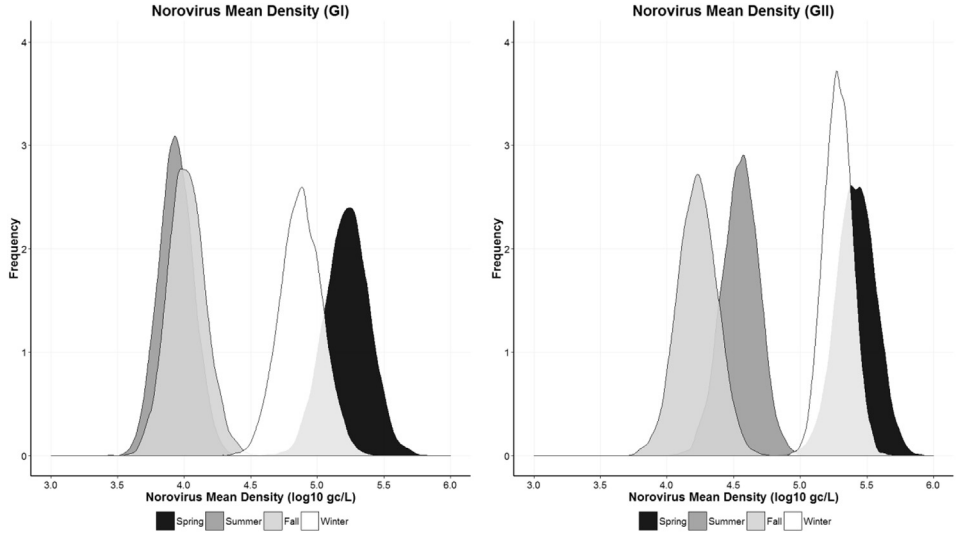

Fig. 5. Distribution of NoV mean density by season for GI (left) and GII (right).

geographic and seasonal heterogeneity observed in the reported data. This approach yielded an opportunity to characterize the extent to which seasonal and geographic trends are important. Our analysis showed a large degree of seasonality, with a marked winter peak for both genogroups. We also observed significant geographic differences. Winter NoV peaks have been noted previously. As early as 1929, Zahorsky (1929) described a "winter vomiting disease" with a characteristic course of sudden-onset vomiting and diarrhea, and increasing prevalence during the colder months (Adler and Zickl, 1969; Zahorsky, 1929). Additionally, Mounts et al. (2000) analyzed 12 studies conducted during a 21-year period and noted the cold weather seasonality of NoV GI illness. Pouillot et al. (2015) also observed a winter peak in NoV densities. To our knowledge, our results are the first to quantitatively characterize both seasonal and geographic differences, which could be particularly useful for future risk assessments.

Our study includes several limitations. First, although over 400 publications were reviewed, the inclusion criteria yielded only 15 peer-reviewed publications, a relatively small number of studies, with less data available for New Zealand and Asia, than North America and Europe. However, the 15 studies provided a robust dataset ($n = 850$). Our search strategy could be expanded to include other sources of human waste (cesspools, open defecation, septic systems both traditional and advanced). Second, NoV strain and number of outbreaks vary from year to year and this study did not account for the year that the data were collected. Thus the potential effects of year-to-year variability were not evaluated. Third, although the bootstrapping approach accounts for left-censoring (values reported below the lower limit of detection) via substitution, bootstrapping may not be appropriate when studies have very limited number of quantifiable samples. Although we did have scarce data for certain geographic areas (Asia and New Zealand), available data are relatively robust in that none of the included studies had a large proportion of observations reported below detectable limits (Table 1). Fourth, the data used in this meta-analysis come from diverse laboratories that used various sample processing techniques, detection methods, and molecular assays. While heterogeneities are expected in a meta-analysis such as this,

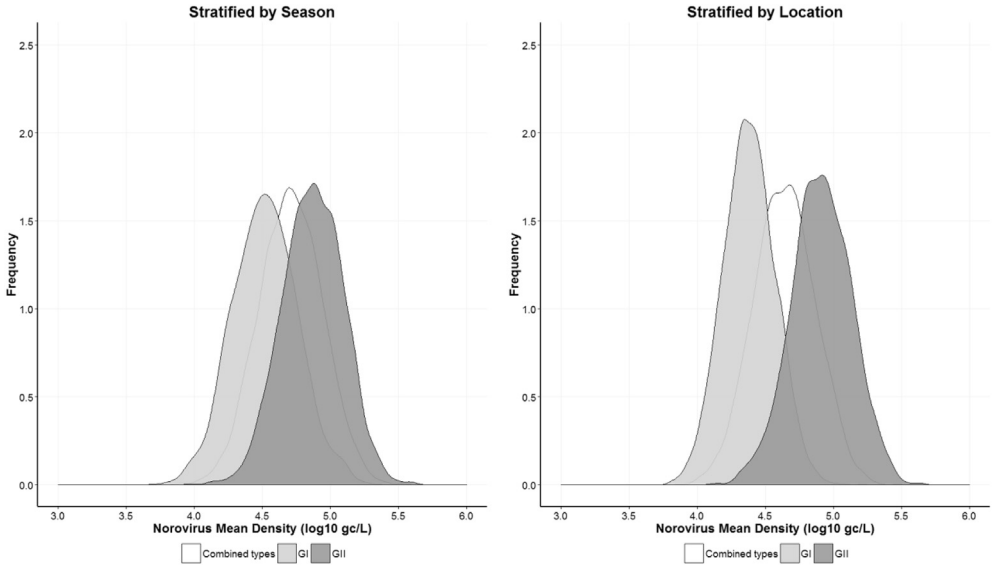

Fig. 6. Distribution of NoV mean density after stratified bootstrapping (Stage 2) by geographic region (left) and season (right).

some may raise questions about inter-study comparability. By employing strict study inclusion criteria, which only allowed the most scientifically rigorous methods to pass the study quality evaluation, the effect of heterogeneities between laboratories on overall study conclusions were minimized to the best extent possible. A major challenge in our study was the lack of reporting of detection limits, inhibition, and positive/negative controls. We encourage the scientific community to improve reporting for such study quality aspects to facilitate the inclusion of a larger number of papers in similar meta analyses. Additionally, future use of digital PCR, which does not require standard materials and can be less susceptible to inhibition, may also expand the amount of available data for use in similar meta analyses.

Until recently, recreational water quality criteria values were derived from epidemiological study results, establishing water quality-based relationships between gastrointestinal illness and the density of fecal indicator bacteria such as enterococci (Prüss, 1998; Wade et al., 2003; U.S. EPA, 1986; 2012). The results of this current study could provide information useful in characterizing human health risks associated with exposure to raw or treated wastewater.

5. Conclusions

The systematic literature review and a two stage bootstrap analysis provide robust methods for developing distributions for pathogen densities, which could be used in QMRA. The resulting distributions of NoV in raw wastewater will be useful in the future for a wide range of risk assessment applications, including water quality criteria derivation; water reuse; understanding recreational risks from epidemiological data; and potentially evaluating risks from viruses in biosolids and shellfish.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2017.01.017.

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