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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