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Review

A review on recent progress in the detection methods and prevalence of human enteric viruses in water

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ABSTRACT

Waterborne human enteric viruses, such as noroviruses and adenoviruses, are excreted in the feces of infected individuals and transmitted via the fecal-oral route including contaminated food and water. Since viruses are normally present at low concentrations in aquatic environments, they should be concentrated into smaller volumes prior to downstream molecular biological applications, such as quantitative polymerase chain reaction (qPCR). This review describes recent progress made in the development of concentration and detection methods of human enteric viruses in water, and discusses their applications for providing a better understanding of the prevalence of the viruses in various types of water worldwide. Maximum concentrations of human enteric viruses in water that have been reported in previous studies are summarized to assess viral abundances in aquatic environments. Some descriptions are also available on recent applications of sequencing analyses used to determine the genetic diversity of viral genomes in water samples, including those of novel viruses. Furthermore, the importance and significance of utilizing appropriate process controls during viral analyses are discussed, and three types of process controls are considered: whole process controls, molecular process controls, and (reverse transcription (RT)-)qPCR controls. Although no standards have been established for acceptable values of virus recovery and/or extraction-(RT-)qPCR efficiency, use of at least one of these appropriate control types is highly recommended for more accurate interpretation of observed data.

Abbreviations: AdV, adenovirus; AiV-1, Aichi virus 1; AstV, astrovirus; BEV, bovine enterovirus; BKPyV, BK polyomavirus; BoNoV, bovine norovirus; CoSV, cosavirus; Ct, threshold cycle; dPCR, digital PCR; EV, enterovirus; FCV, feline calicivirus; FRNAPH, F-specific RNA coliphage; HAV, hepatitis A virus; HEV, hepatitis E virus; HGV, hepatitis G virus; PSC, primer sharing control; HuNoV, human norovirus; ICC-PCR, integrated cell culture-polymerase chain reaction; JCPyV, JC polyomavirus; MgV, mengovirus; MNV, murine norovirus; MPC, molecular process control; NaPP, sodium polyphosphate; NGS, next-generation sequencing; NoV, norovirus; NoV-GI, norovirus of genogroup I; NoV-GII, norovirus of genogroup II; NoV-GIII, norovirus of genogroup III; NoV-GIV, norovirus of genogroup IV; PBV, picobirnavirus; PMMoV, pepper mild mottle virus; PV, poliovirus; PyV, polyomavirus; QMRA, quantitative microbial risk assessment; qPCR, quantitative polymerase chain reaction; RT, reverse transcription; RV, rotavirus; RVA, group A rotavirus; SaliV, salivirus; SaV, sapovirus; TTV, torque teno virus; VIRADEL, virus adsorption and elution; WHO, World Health Organization; WPC, whole process control; WWTP, wastewater treatment plant.

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Contents

1. Introduction

Human enteric viruses, such as noroviruses (NoVs), adenoviruses (AdVs), and enteroviruses (EVs), are excreted at high concentrations in the feces of infected individuals (up to 10^{11} viruses/g-feces), with or without symptoms, and are transmitted via the fecal-oral route including contaminated food and water (Bosch, 1998; Wyn-Jones and Sellwood, 2001; Prüss et al., 2002). Currently, treated or untreated sewage and combined sewer overflows from urban areas are the major sources of environmental pollution from human enteric viruses in surface waters (Fong and Lipp, 2005; Rodríguez et al., 2012). Since human enteric viruses are not able to grow outside their host cells, efficient removal and/or inactivation of these viruses at wastewater treatment plants (WWTPs) can contribute greatly to reducing the amount of viruses discharged into an environment. Installation of appropriate wastewater treatment systems can result in controlling the risk of viral infection via various routes, such as contact with recreational waters, ingestion of potable water, or consumption of virus-contaminated shellfish. However, it is very difficult to achieve the complete removal of viruses with conventional wastewater treatment processes (Sano et al., 2016).

Many studies have focused so far on the detection of human enteric viruses in various types of aquatic environments, such as raw and treated wastewater, surface water, groundwater, seawater, and even treated drinking water (Fong and Lipp, 2005; Gerba et al., 2013). Since human enteric viruses are present generally at relatively low concentrations in environmental water samples, it is essential to begin these types of studies by concentrating the viruses into smaller sample volumes to enhance the usefulness of detection assays. The development and application of methods for concentrating viruses have contributed significantly to the detection of diverse viruses using culture- or molecular-based assays (Ikner et al., 2012; Cashdollar and Wymer, 2013).

The rapid advancement of molecular biological techniques, such as quantitative polymerase chain reaction (qPCR), has enabled us to obtain quantitative information about the viral genomes present in water. Although qPCR itself provides quantitative data with high accuracy, concentrations obtained via this method should be interpreted very carefully because of potential losses in efficiency during the detection process, including virus concentration steps, DNA/RNA extractions, reverse transcription (RT), and qPCR.

Use of process controls or internal/external controls is now becoming common practice in evaluating the efficiency of virus recovery and level of inhibition during the detection process. During the virus detection process, there are a few points at which a process control can be added to a sample. Since different types of process controls are recommended depending on the point of inoculation, use of process controls should be considered prior to starting a field survey to determine the prevalence of viruses in that particular water sample.

Despite great efforts, viruses sometimes are not detected in tested water samples. These data, called “non-detects” should be considered for more appropriate data treatments; however, previous studies rarely have considered this issue important.

This review paper describes the latest knowledge regarding detection methods of human enteric viruses in aquatic environments, including molecular biological techniques, such as (RT-)qPCR, cloning-sequencing, and viral metagenomic analysis, as well as their applications for detecting and evaluating various types of indigenous viruses in water worldwide. In addition, we describe the importance of using appropriate process controls in these types of studies. To the best of our knowledge, this is the first paper summarizing, in detail, the current knowledge about the application of process controls to virus detection in water.

2. Methods for concentrating and detecting human enteric viruses in water

2.1. Methods for concentrating viruses

Quantities of human enteric viruses can vary greatly depending on the types of water in which they are found. High concentrations of target viruses may be detected easily from relatively small volumes of wastewater or sludge samples (<100 mL), whereas for surface, recreational, and drinking waters, larger volumes