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Sampling methods for recovery of human enteric viruses from environmental surfaces

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ABSTRACT

Acute gastroenteritis causes the second highest infectious disease burden worldwide. Human enteric viruses have been identified as leading causative agents of acute gastroenteritis as well as foodborne illnesses in the U.S. and are generally transmitted by fecal-oral contamination. There is growing evidence of transmission occurring via contaminated fomite including food contact surfaces. Additionally, human enteric viruses have been shown to remain infectious on fomites over prolonged periods of time. To better understand viral persistence, there is a need for more studies to investigate this phenomenon. Therefore, optimization of surface sampling methods is essential to aid in understanding environmental contamination to ensure proper preventative measures are being applied. In general, surface sampling studies are limited and highly variable among recovery efficiencies and research parameters used (e.g., virus type/density, surface type, elution buffers, tools). This review aims to discuss the various factors impacting surface sampling of viruses from fomites and to explore how researchers could move towards a more sensitive and standard sampling method.

1. Introduction

Acute gastroenteritis causes the second highest infectious disease burden worldwide with an estimated 1.45 million deaths per year (Ahmed et al., 2014). In the United States alone, acute gastroenteritis causes 178.8 million illnesses, 473,832 hospitalizations, and 5072 deaths (Scallan et al., 2011). There are approximately 31 major pathogenic agents known to cause acute gastroenteritis and/or foodborne illness including human enteric viruses such as astrovirus, rotavirus, hepatitis A virus (HAV), and human norovirus (hNoV) (Scallan et al., 2011). The most common enteric viruses that cause foodborne illnesses are hNoVs and HAV (Cliver, 1997; Koopmans and Duizer, 2004).

Generally, viral acute gastroenteritis is transmitted through food and water contamination, contaminated environmental surfaces, direct person-to-person contact, and other unknown sources (Wikswo et al., 2015). Furthermore, enteric viruses are spread by fecal-oral contamination, and there is growing evidence of viral transmission occurring through contaminated fomites in a variety of ways and settings including food preparation environments (Boone and Gerba, 2007; Rzezutka and Cook, 2004). Enteric viruses have been shown to maintain infectivity on fomites over prolonged periods of time (Escudero et al., 2012). For instance, seminal research by Kiseleva (1968) reported on the survival of echovirus, coxsackievirus, and poliovirus on representative surfaces (painted wood, glass, cotton fabric) in households and showed that these viruses maintained infectivity for two to more than 12 days. Human norovirus survival for up to 12 days has also been reported on carpets subject to vomiting episodes after an initial outbreak in a hospital ward (Cheesbrough et al., 1997). There are some studies focusing on the role of fomites and environmental contamination in the transmission of enteric viruses however this specific route of transmission is difficult to determine during outbreaks (Rzezutka and Cook, 2004).

To better understand the role of environmental surface transmission during outbreaks due to human enteric viruses, the persistence of viruses on various surface types must be investigated. To do this, a surface sampling method must be applied for recovery of viruses. For instance, understanding the persistence of human enteric viruses on inanimate fomite surfaces in relation to various environmental conditions could provide insight on ways to limit and prevent virus transmission and subsequent outbreaks. However, studies on surface sampling techniques are typically limited to swabs for application in environmental sampling during foodborne outbreaks or for investigation of baseline virus prevalence. As a result, information is lacking on evaluating tools used in laboratory sampling studies for the optimal recovery of viruses. Thus, this review aims to: (1) discuss and compare evaluations of surface sampling methods for optimal recovery of human enteric viruses from inanimate fomite surfaces and (2) explore how researchers could move towards one standard methodology for surface

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sampling of human enteric viruses and their surrogates.

2. Background

The most common foodborne viruses are categorized based on the type of disease they cause: (1) gastroenteritis (e.g. rotavirus, hNoV, Aichi virus A, coronavirus, and others), (2) enterically transmitted hepatitis viruses (e.g. hepatitis E and A), and (3) viruses that replicate in the human gut then migrate to other organs to cause disease (e.g. poliovirus) (Koopmans and Duizer, 2004). Enteric viruses are typically spread by vomiting or shedding into the stool and have a greater chance of transmission the longer the virus is able to survive outside the host. This survival is impacted by various environmental conditions such as pH, moisture, and temperature (Koopmans and Duizer, 2004; Rzezutka and Cook, 2004).

2.1. Enteric virus transmission due to environmental surface contamination

As indicated previously, enteric viruses have been shown to maintain infectivity on surfaces over prolonged periods. Human noroviruses have been detected on a variety of surfaces including cellular phones, public phones, televisions, chairs, keyboards, microwave ovens, bathroom light switches, various handles and knobs of kitchen and bathroom items, bed frames, and chairs (Boxman et al., 2011; Gallimore et al., 2006, 2008). Boxman et al. (2011) reported year round prevalence of hNoVs on environmental surfaces of catering facilities even without a recently reported outbreak of acute gastroenteritis. The authors reported that hNoV was recovered from 61.1% of catering settings with recent outbreaks in contrast to only 4.2% of catering settings without a recent outbreak. Elderly homes and pension/hotels catering company types had the highest prevalence of positive swab samples for hNoVs (Boxman et al., 2011). Moreover, multiple studies have shown institutional settings such as cafeterias and long-term facilities are more likely to have hNoVs on surfaces compared to food service settings (Boxman et al., 2011; Hall et al., 2014; Verhoef et al., 2013).

2.2. Current standard methods for surface sampling and analysis

For environmental surface sampling, the International Organization of Standardization (2017) recommends swabbing with a sterile cotton swab presoaked in PBS followed by RNA extraction and reverse transcription, real time PCR (RT-qPCR) analysis for HAV and hNoV sampling and detection on nonporous FCS. In the U.S., there is not a standardized method available. However, the Centers for Disease Control and Prevention (CDC, 2012) does recommend the use of swabs for obtaining norovirus from environmental surfaces; however, the CDC has also reported that swabbing is highly variable and that the interpretation of results should be conducted with caution.

Currently, hNoVs are most often detected by RT-qPCR due to its high sensitivity and low detection limits using measurements such as PCR amplifiable units (PCRU/ml). These PCRUs are determined by a standard curve produced from a 10-fold dilution series of the virus where one PCRU corresponds to the highest dilution with a quantifiable RT-qPCR value (or cycle threshold [C_T] value) (Knight et al., 2013; Tung et al., 2013). However, Knight et al. (2013) pointed out that the determination of PCRUs in correspondence to specific C_T values is dependent on the sample matrix and the standard used. Moreover, the cut-off C_T values (i.e. endpoint of detection) for hNoVs also vary across studies ranging from 32 to 40 (Knight et al., 2013). The presence of inhibitory components within some sample matrices could impact amplification efficiencies especially in contaminated food and environmental samples that typically have low viral loads (Knight et al., 2013; Sair et al., 2002). Regardless, RT-qPCR is primarily chosen for the analysis of viruses in environmental and food samples to allow for increased sensitivity to detect low viral concentrations that are typically present (Knight et al., 2013). However, as the authors of the review indicated, this method cannot determine infectivity since it may recognize intact or degraded viral nucleic acid, nonviable viruses, or defective viral particles (Knight et al., 2013). Consequently, the use of surrogates and other infectivity assays remain important in investigating enteric viral viability and infectivity in lab-based studies as further discussed in Section 2.3.2.

2.3. Factors impacting recovery of viruses from surfaces

Virus density, the rate of positive environmental samples of total samples collected, and exposure magnitude provide information about virus contamination on surfaces (Julian et al., 2011). However, these factors are impacted by the surface sampling method and detection assay selected. Subsection 2.3.1 to 2.3.5 will examine the variability among the many factors impacting recovery of viruses from surfaces, specifically surface type, virus type/density, drying time, elution buffers, and implement/recovery tool selection.

2.3.1. Surface type

Fomites are generally categorized as either nonporous or porous. Examples of nonporous surfaces are ceramic, glass, acrylic, and stainless steel, and examples of porous surfaces include carpets, lettuce, deli meats, wood, latex, and fruits. Surface type has been shown to have some effect on surface sampling recovery efficiencies (Table 1). Tung-Thompson et al. (2017) swabbed foods (cheese, apple, green pepper, tomato) and hard surfaces (stainless steel and ceramic) with wipes that were inoculated with 10 µl of varying PCR-units (PCRU)/ml of hNoV GII.4. The study obtained a mean range recovery efficiency of 74% to approximately 100% for all surfaces except for cheese, which was significantly different from the other surfaces with 29% to 69% recovery for high inoculum levels (10^4 to 10^6 PCRU) and no detection at low inoculum levels (10^2 to 10^3 PCRU) (Tung-Thompson et al., 2017). The authors were not able to determine if the lipid content of the cheese contributed to the possible absorption and recovery of the virus samples even though a previous study suggested this possibility for hNoVs (Fumian et al., 2009; Tung-Thompson et al., 2017).

Furthermore, surface properties can also impact recovery efficiencies in a variety of ways. For instance, stainless steel is a hydrophilic (contact angle of 58.2° in water, surface energy of 50.3 mJ/m^2) and negatively charged surface in which microorganisms have been shown to develop irreversible attachment within one minute potentially making surface recovery more difficult (Mafu et al., 1990; Mafu et al., 1991). The orientation of a surface could interfere with adequate surface sampling and collection as seen in a study involving vertical and horizontal stainless steel surfaces. Taku et al. (2002) determined that greater recovery efficiency could be obtained by allowing the elution buffer to sit on the surface for 15 min—something that cannot be performed on a vertical surface. The mean recovery for horizontal surfaces and sinks using the cell scraper-aspiration method ranged from 32% to 71% while vertical stainless steel surfaces only obtained a mean recovery of 11% since the buffer was not in contact with the surface long enough to facilitate virus recovery (Taku et al., 2002). Scherer et al. (2009) suggested physical properties of nonporous and porous could reduce virus recovery via trapping virus particles within the matrix/crevices or facilitate enhanced virus recovery by smooth/porous surfaces. Mattison et al. (2007) suggested the low mean recovery of feline calicivirus (FCV) from strawberries might be due to its surface texture and how the crevices may shield viruses against environmental conditions. Furthermore, the authors observed a pH change in the elution buffer from 7.2 to 5.5 when strawberries were immersed, which could impact virus recovery by either partial viral inactivation or interference with FCV recovery (Mattison et al., 2007). Overall, physical and chemical properties of nonporous and porous food and food contact surfaces could impact recovery efficiencies of enteric viruses. This review will focus on surface sampling techniques for enteric viruses from nonporous, inanimate surfaces.