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Virus transfer proportions between gloved fingertips, soft berries, and lettuce, and associated health risks

Katharina Verhaelen [a,b], Martijn Bouwknegt [a,*], Anna Carratalà [c,d], Froukje Lodder-Verschoor [a], Marta Diez-Valcarce [e,f], David Rodríguez-Lázaro [e,f], Ana Maria de Roda Husman [a,b], Saskia A. Rutjes [a]

[a] Centre for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands
[b] Faculty of Veterinary Medicine, Institute for Risk Assessment Sciences (IRAS), Utrecht University, P.O. Box 80.178, NL-3508 TD Utrecht, The Netherlands
[c] Department of Microbiology, Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[d] Environmental Chemistry Laboratory, École Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland
[e] Instituto Tecnológico Agrario de Castilla y León (ITACyL), Valladolid, Spain
[f] Faculty of Science, University of Burgos, Pza. Misael Bañuelos s/n, 09001 Burgos, Spain

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ABSTRACT

Multiple outbreaks of human norovirus (hNoV) have been associated with fresh produce, such as soft berries and lettuce. Even though food handlers are considered an important source for the introduction of hNoV into food chains, their contribution to public health risks associated with hNoV remains unknown. To assess to which extent food handlers contribute to the introduction and spread of hNoV in fresh produce chains quantitative virus transfer data are needed. We estimated transfer proportions of hNoV GI.4, GII.4, murine norovirus (MNV-1), a culturable surrogate of hNoV, and human adenovirus (hAdV-2), a human pathogen proposed as an indicator for human faecal pollution, between gloved fingertips and raspberries, strawberries, and lettuce, by quantitative RT-PCR and cell culture if applicable. Virus transfer proportions were corrected for virus–matrix specific recoveries, and variability and uncertainty of the parameters were estimated. Virus transfer from gloves to soft berries was generally lower as compared to lettuce, with mean transfer proportions ranging between 0.1 to 2.3% and 9 to 10% for infectious MNV-1 and hAdV-2, respectively. Transfer from produce to glove was mostly greater than transfer from glove to produce, adding to the likelihood of virus transfer due to cross contamination from contaminated produce via food handlers. HNoV GI.4 and hNoV GII.4 showed no significant difference between their mean transfer proportions. Using the estimated transfer proportions, we studied the impact of low and high transfer proportions on the public health risk, based on a scenario in which a food handler picked raspberries with contaminated fingertips. Given the made assumptions, we could show that for a pathogen as infectious as hNoV, low transfer proportions may pose a greater public health risk than high transfer proportions, due to a greater viral spread. We demonstrated the potential of food handlers in spreading hNoV in food chains, showing that prevention of virus contamination on food handlers' hands is crucial for food safety. Nevertheless, complete prevention of virus contamination on fresh produce cannot be achieved in reality, and reliable and effective intervention measures are consequently required. We estimated that, especially for low transfer proportions, a robust one $\log_{10}$-unit reduction of infectious hNoV on contaminated produce, and on food handlers' hands, could lower the public health risk substantially. Using the obtained data in quantitative risk assessment will aid in elucidating the contribution of food handlers in hNoV transmission.

1. Introduction

Human norovirus (hNoV) is the most important foodborne pathogen in terms of disease outbreaks, with an estimated 58% of foodborne outbreaks being associated with this virus in the US (Scallan et al., 2011). Fresh produce, such as lettuce and soft berries are common vehicles for hNoV transmission (EFSA, 2011; Hall et al., 2012). Food handlers are assumed to be the most likely source of hNoV for ready to eat products (Baert et al., 2009; Hall et al., 2012) and in the US, about 50% of hNoV outbreaks have been linked to ill food handlers (Widdowson et al., 2005).

* Corresponding author at: National Institute for Public Health and the Environment, P.O. Box 1, NL-3720 BA Bilthoven, The Netherlands. Tel.: +31 30 274 7002; fax: +31 30 274 4434.
E-mail addresses: Verhaelen@gmail.com (K. Verhaelen), martijn.bouwknegt@rivm.nl (M. Bouwknegt), anna.carratala@epfl.ch (A. Carratalà), Froukje.Lodder@rivm.nl (F. Lodder-Verschoor), mdvalcarce@ubu.es (M. Diez-Valcarce), ita-rodlazda@itacyl.es (D. Rodríguez-Lázaro), Ana.Maria.de.Roda.Husman@rivm.nl (A.M. de Roda Husman), Saskia.Rutjes@rivm.nl (S.A. Rutjes).

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Not only the frequency, but also the reported size of outbreaks related to food handlers can be substantial, with up to several thousand infected individuals (Friedman et al., 2005).

The characteristics of hNoV facilitate the spread of the virus via food handlers. HNoV is the most infectious virus described with an estimated average probability of infection for a single specific Norwalk virus particle to be close to 50% (Teunis et al., 2008). At the same time hNoV is shed in extremely high numbers at a median of 9.5×10^{10} genomic copies/g faeces as measured by quantitative RT-PCR (Atmar et al., 2008). This means that the transfer of minuscule amounts of faeces can result in hNoV infection upon exposure. In addition, viral shedding preceding the onset of illness in up to 30% of infected persons (Rockx et al., 2002) and a high incidence of asymptomatic infections (Okabayashi et al., 2008; Phillips et al., 2010) make it virtually impossible to exclude food handlers shedding viruses from food production processes. These hNoV characteristics may explain why infected food handlers were more commonly identified in food-related hNoV outbreaks than in those caused by other pathogens (Lopman et al., 2003). To date, hNoV cannot be cultured efficiently and infectivity has to be studied using surrogate viruses. Previously feline and canine caliciviruses were used, but currently a cultivable norovirus, murine norovirus MNV-1 is used, which resembles more closely the properties of hNoV (Wobus et al., 2006).

Food handlers can contaminate produce in the (i) primary production phase e.g. at harvest, in the (ii) processing phase e.g. while sorting and packaging produce or in the (iii) food preparation phase e.g. preparing desserts or salads. They can contribute to the spread of hNoV either by introducing the virus onto food via poor hand hygiene, or by cross-contamination while handling contaminated food. The contribution of food handlers in the introduction and spread of hNoV in fresh produce chains and the associated health risk are difficult to assess. To determine a possible link between hNoV illness and a specific food item is intricate; attributing additionally the contamination source of this food item to the outbreak is a major challenge.

Quantitative microbial risk assessment (QMRA) can be used to prioritize the contribution of pathogen contamination sources to the overall food contamination and (Pires et al., 2009) can be applied to elucidate the role of food handlers in hNoV transmission. For this purpose, quantitative data on transfer coefficients of hNoV from finger pads to produce are needed and additionally from produce to finger pads to study the effects of possible cross-contamination. Only two recent studies have described the transfer of noroviruses from gloved fingertips to fresh produce (Sharps et al., 2012; Stals et al., 2013). However, besides studying different virus–matrix combinations, we also estimated the uncertainty and variability of the determined transfer proportions, crucial aspects for an accurate description of a public health risk by QMRA (Nauta, 2010). We quantified the transfer proportions of hNoV GI.4, hNoV GII.4, murine norovirus (MNV-1) and human adenovirus (hAdV-2) between gloved fingertips and raspberries, strawberries and lettuce and between raspberries and lettuce and gloved fingertips. Human adenovirus was included, because the virus is suggested as an indicator for viral contamination (Hundesa et al., 2006) and is furthermore a potential foodborne pathogen. Virus numbers were determined by molecular techniques and cell culture if possible. The obtained data on transfer proportions and their variations were used to estimate the number of produce a single food handler can contaminate and the corresponding contamination levels of viruses per food item, comparing a low and a high transfer proportion. Relating these data to dose–response models further allowed us to determine associated health risks.

2. Materials and methods

2.1. Viruses and cells

Two human norovirus strains, one belonging to genogroup I (hNoV GI.4) and one to genogroup II (hNoV GII.4), originating from clinical stool samples were kindly provided by Dr. Erwin Duizer (Laboratory for Infectious Diseases and Perinatal Screening RIVM, The Netherlands). Murine norovirus (MNV-1) (kindly provided by Dr. Herbert W. Virgin, Washington University, St. Louis, USA) and human adenovirus (hAdV-2) were made available, under agreement by the group of Dr. Franco M. Ruggeri (Istituto Superiore de Sanitá, Rome, Italy). Recombinant mengovirus (vMC_0) (Martin and Palmenberg, 1996), kindly provided by Dr. David Lees (CEFAS, Dorset, UK) was used as a process control virus to monitor the efficiency of virus recovery. MNV-1 was propagated in RAW-264.7 cells (ATCC-TIB-71) and hAdV-2 in A549 cells (ATTC-CCL-185) (for details see Verhaelen et al., 2012).

2.2. Virus spiking

Virus transfer from finger pads to fresh produce was studied using gloves (VWR, Nitrile), because gloves are commonly used by food handlers in food environments and because spiking unprotected fingertips with human pathogenic viruses entails a health risk. In total 50 μL of a virus suspension, consisting of hNoV GI.4, hNoV GII.4 (10% faecal suspension) and MNV-1, hAdV-2 (neat stock suspension), were spiked on the middle finger, index finger and thumb of a glove in a marked area of about 1 cm^2. The spike contained about 2×10^8, 4×10^8, 7×10^8 and 2×10^8 genomic copies of hAdV-2, MNV-1, hNoV GI and GII, respectively. The number of genomic copies of hAdV-2 and MNV-1 related to 5×10^6 infectious hAdV-2 particles and 5×10^4 infectious MNV particles, respectively. The inoculum was left to dry at room temperature for 2 h, until visibly dry. To study virus transfer from produce to gloves, raspberries were sliced into half and the outer surfaces were evenly spiked with 150 μL of the same virus suspension aiming for a homogenous distribution of virus particles on the raspberry surfaces. Halving the raspberry allowed a more consistent spiking of the berry. Transfer from lettuce to gloves was studied by spiking a 4 cm^2 piece of iceberg lettuce in the same manner. The inoculum was left to dry for 4 h, until visibly dry.

2.3. Virus transfer

Here, we describe the methodology of virus transfer from gloved finger tips to produce and vice versa. Raspberries and strawberries were picked up with gloved fingers using the middle finger, index finger and thumb and were transferred into 20 mL of tris-glycine beef extract buffer (TGBE) (1.21% Tris base, 0.38% glycine and 1% beef extract, (pH 9.5)) after holding it for 5 s. Lettuce was treated in the same manner for comparability of the transfer proportions. The gloves were carefully pulled off the hand and the spiked area on the three finger tips was cut out with a sterile scalpel and transferred into a 50 mL Greiner tube containing 5 mL of TGBE buffer. The experiment was successively repeated ten times for each transfer route on the same day.

Similarly, in experiments on virus transfer from produce to glove, the spiked produce was touched successively with the index finger, middle finger and thumb for 5 s. The three glove pieces and the three raspberry halves or iceberg lettuce pieces were pooled in 5 and 20 mL of TGBE, respectively. The experiment was successively repeated ten times for each transfer route on the same day. For strawberries, only transfer from glove to strawberry was studied to elaborate whether a difference in virus transfer from food handlers to strawberries and raspberries may explain why outbreaks of hNoV are more frequently connected to raspberries.

2.4. Measurement of the donor and recipient surfaces

For transfer of viruses from glove to produce, the complete spiked area of the glove was in touch with the produce. However, for transfer from produce to gloves, the area touched by the glove was smaller than the spiked area of the produce, leading to an underestimation of the transfer rate. Therefore, the surface fraction of the produce covered