Transfer of noroviruses between fingers and fomites and food products

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Human norovirus (NoV) contaminated hands are important routes for transmission. Quantitative data on transfer during contact with surfaces and food are scare but necessary for a quantitative risk assessment. Therefore, transfer of MNV1 and human NoVs GI.4 and GII.4 was studied by artificially contaminating human finger pads, followed by pressing on stainless steel and Trespa® surfaces and also on whole tomatoes and cucumber slices. In addition, clean finger pads were pressed on artificially contaminated stainless steel and Trespa® surfaces. The transfers were performed at a pressure of 0.8–1.9 kg/cm² for approximately 2 s up to 7 sequential transfers either to carriers or to food products. MNV1 infectivity transfer from finger pads to stainless steel ranged from 13 ± 16% on the first to 0.003 ± 0.009% on the sixth transfer on immediate transfer. After 10 min of drying, transfer was reduced to 0.1 ± 0.2% on the first transfer to 0.013 ± 0.023% on the fifth transfer. MNV1 infectivity transfer from stainless steel and Trespa® to finger pads after 40 min of drying was 2.0 ± 2.0% and 4.0 ± 5.0% respectively. MNV1 infectivity was transferred 7 ± 8% to cucumber slices and 0.3 ± 0.5% to tomatoes after 10 min of drying, where the higher transfer to cucumber was probably due to the higher moisture content of the cucumber slices. Similar results were found for NoVs GI.4 and GII.4 transfers measured in PCR units. The results indicate that transfer of the virus is possible even after the virus is dried on the surface of hands or carriers. Furthermore, the role of fingers in transmission of NoVs was quantified and these data can be useful in risk assessment models and to establish target levels for efficacy of transmission intervention methods.

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1. Introduction

Human noroviruses (NoVs) are the leading cause of gastroenteritis affecting people of all age groups (Koopmans, 2009). The virus is a major threat to public health due to numbers of outbreaks in closed settings like hospitals, nursing homes, cruise ships, and long term care facilities (Hansen et al., 2007; Harris et al., 2010; Koopmans, 2009; Koopmans et al., 2006). Though the NoV infection is usually self-limiting in otherwise healthy persons, due to the high incidence of disease and closing of wards in hospitals and nursing homes, and due to absence from work the burden of disease is high (Friesema et al., 2012; Johnston et al., 2007). Transmission of the human NoV occurs directly through person to person contact or indirectly via consumption of contaminated food and water and contaminated surfaces (Koopmans and Duizer, 2004; Rodriguez-Lazaro et al., 2012). The person to person transfer has been reported as a major means of transmission in NoV outbreaks in closed settings (Kroneman et al., 2008) and hands are thought to be the main vehicle for the transmission (Tuan Zainazor et al., 2010).

Virus transfer between hands and surfaces or food can be quantified by determination of the fraction of the virus on artificially contaminated hands that is transferred to the receiving fomites or food surfaces. Previous studies have shown that many bacteria (Harrison et al., 2003; Rusin et al., 2002; Zapka et al., 2011) and bacteriophages MS2 and φX174 are readily transferred via hand contact (Julian et al., 2010). Similar results have been found for pathogenic viruses like rotavirus (Ansari et al., 1988), hepatitis A virus (HAV) (Mbithi et al., 1992), human parainfluenza virus-3 and rhinovirus (Ansari et al., 1991). Transfer of NoV has been estimated by studying feline calicivirus (FeCV), which belongs to the same family Caliciviridae, and the infectivity transfer was estimated as 13% from a finger pad to stainless steel when pressed for 10 s after air drying (Bidawid et al., 2004).

This study was conducted with a NoV GI.4 strain and an epidemic NoV GII.4 strain (Siebenga et al., 2010). As human NoV cannot be cultured (Duizer et al., 2004), their transfer was determined by quantitative PCR. Additionally, cultivable murine NoV (MNV1) was used as a model virus (Wobus et al., 2004) to study transfer of infectious viruses. MNV1 has been described as a more suitable model virus than FeCV, as MNV belongs to the same genus as human NoV, is an enteric virus and...
resistant to low pH (Cannon et al., 2006). We studied transfer of the virus from fingers to stainless steel or Trespa® and vice versa and to whole tomato and cucumber slices to better understand hands as a vehicle for transmission of human NoV. Quantitative data on transfer of the virus from fingers to different fomites and food products were collected, since this information will be helpful for quantitative risk assessment and to develop effective transmission intervention methods.

2. Materials and methods

2.1. Viruses, cell line and propagation of virus

Viruses used for the tests were MNV1 (Mu/NoV/GV/MNV1/2002/USA), human NoV GI.4 (Hu/NoV/GI.4/946/2009/Netherlands) and human NoV GII.4 (Hu/NoV/GII.4/1803/2008/Netherlands). MNV1 stock was prepared by infecting monolayers of murine macrophage cell line Raw-264.7 as described before (Tuladhar et al., 2012b). Briefly, monolayers of the cells were infected at a multiplicity of 0.1 infective particles per cell and incubated at 37 °C at 5% CO₂ atmosphere. After 2 to 3 days, cytopathic effect was visible and the virus was harvested by freeze-thawing twice, followed by removal of cell debris by centrifugation at 1512 × g for 15 min at 4 °C.

Human NoV suspensions were prepared as 10% w/v stool homogenates in Dulbecco’s Modified Eagle Medium (DMEM, Gibco, Germany) as described before (Svraka et al., 2009) and filtered through 0.2 μm pore size filter. The suspensions were free of all other enteric viruses tested (rotavirus, enteric adenoviruses, astroviruses and sapoviruses) as determined before by PCR assays (Svraka et al., 2009). The virus stocks were then stored at −80 °C. Reverse transcription PCR units (RT-PCR) were determined using slopes of standard curves of quantitative assays. The standard curve was made by plotting cyclic threshold (Ct) value versus log RT-PCRU of 10 fold dilutions of the virus stock.

2.2. Preparation of sterile stool suspension

Stool suspension from a healthy volunteer was tested for presence of viral RNA or DNA as described before (Svraka et al., 2009) and found free of all the enteric pathogens tested (human NoVs GI and GII, rotaviruses, enteric adenoviruses, astroviruses and sapoviruses) by PCR. A 20% (w/v, wet weight) stool suspension was prepared in phosphate buffer (0.01 M, pH 7.2) and sterilized by autoclaving at 121 °C for 15 min. The suspension was vortexed, centrifuged at 1512 × g for 20 min, and then the supernatant was aliquoted and stored at −20 °C.

2.3. Cleaning and sterilization of carriers

Stainless steel (AISI type 304 standard, Netherlands) and Trespa® (Framing panel, a solid fiber board which consists of wood fibers with a phenolic resin-based binder) carriers used were 2.2 cm × 2.2 cm in size and 1.5 mm and 2.5 mm thick for stainless steel and framing panel respectively. Trespa is used for bench surfacing in kitchens, offices and laboratories. The carriers were cleaned with soap and water, then soaked in 70% (v/v) ethanol for 30 min and dried, then sterilized by autoclaving at 121 °C for 15 min and transferred to individual wells of a six well plate.

2.4. Washing food materials (tomato and cucumber)

Tomatoes (Roma, Holland) and cucumbers were purchased from a local market (The Netherlands). Both products were washed twice under running tap water (15 °C) and dried for 30 min at room temperature. The cucumbers were cut into slices with a diameter of 4.8 ± 0.2 cm and thickness of 0.9 ± 0.2 cm. The small size tomatoes were about 4 cm in width and about 2.5 cm in height and were used without cutting. Each product was then marked with a circle of about 2 cm diameter to mark the contamination site.

2.5. Volunteers

2.5.1. Permission

Test persons participating in the transfer experiments were previously informed about the procedure and the risks before signing the informed consent form. The study protocol was reviewed and approved by the medical ethics review committee of Wageningen University (METC number: 12/01; NL number: 39407.081).

2.5.2. Inspection and preparation of hands for the test

One male and two females participated in this experiment. Prior to every test, both hands of each panelist were inspected carefully to make sure that they were free from any apparent cuts, scratches, or damages. Each panelist then washed their hands thoroughly using non-medicated soap (Hevron Cosmetics, Netherlands) and running tap water (15 °C) for 40 s and dried the hands with sheets of paper towel. Approximately 2 ml 70% (v/v) ethanol was dispensed onto the palm of panelist hands, which was rubbed over the entire surface of hands and fingers until dry to disinfect the hands. The test procedure was then initiated by dropping 10 μl of virus suspension on a finger pad.

2.5.3. Survival of virus on fingers

To determine the number of infective virus particles on finger pads at 0 min, 10 μl of the test virus was placed on each finger pad and eluted immediately by placing the inoculated area over the open mouth of a croyval (3.6 ml capacity: Nunc, Denmark) containing 1 ml DMEM. The vial was inverted 20 times with the finger pad still pressed to it, so that the skin was rinsed with the medium. The vial was then turned upright and the finger pad was scraped in downward position against the inside rim of the vial to recover as much of the remaining fluid as possible into the vial as described before (Mbithi et al., 1992). For the dried controls, finger pads were contaminated with 10 μl test virus and dried for 10 min (visibly dry) inside a biosafety cabinet at room temperature (25–26 °C, 40–45% RH). The dried inoculum was then eluted as described above. Three finger pads from each individual (n = 9) were tested for both baseline controls (0 and 10 min).

2.5.4. Decontamination of finger pads

After the tests, the finger pads were immediately decontaminated by pressing for 1 min onto tissue paper soaked in 5000 ppm free chlorine solution in a petridish. Thereafter the hands were washed thoroughly with water and soap as described before.

2.6. Transfer experiment

The experiment with MNV1 was performed in presence of 1% stool, as enteric viruses always contaminate along with fecal material or vomit. Human NoVs GI and GII were mixed in equal volumes and used in the same experiments as 10% feces suspension. As human NoVs were prepared as stool homogenate, no extra stool was added. All the experiments were performed inside a biosafety cabinet at room temperature (25–26 °C, 40–45% RH). The experiments were repeated three times with three individuals at different times (n = 9). Three fingers of each individual were tested per experiment. The titer of the virus stock used was MNV1: 5.3 × 10⁶ TCID₅₀/ml and 7.1 × 10⁸ RT-PCR/ml. The titers of NoVs GI.4 and GII.4 in the suspension used were 5.6 × 10⁶ and 7.5 × 10⁶ RT-PCR/ml, respectively. Transfer experiments were performed by applying a pressure varying between 0.8–1.9 kg/cm² for 2 s per finger-carrier contact.

2.6.1. Transfer of viruses from fingers to carrier

Transfer of virus from finger pads to surfaces (stainless steel and Trespa®) was performed directly (0 min) and after drying for 10 min
after virus application. Sterile stainless steel and Trespa® carriers were kept in wells of a 6 well plate inside a biosafety cabinet. The plate was placed on a top loading balance (Sartorius, Germany) to measure the weight equal to the pressure applied during the transfer. Finger pads were contaminated with 10 μl virus suspension and pressed for 2 s on the sterile carrier sequentially up to 7 carriers (approximate time 14 s in total). These experiments were repeated with other finger pads up to 3 fingers from each individual. For the transfer experiments with dried virus suspension, the virus was applied on the finger pads and then dried for 10 min inside the biosafety cabinet (25–26 °C, 40–45% RH) and pressed onto the sterile carriers sequentially as explained above. Then 1 ml DMEM was added onto the carrier immediately and the virus was recovered by flushing the carrier with the medium 20 times by pipetting the medium up and down. The virus suspension was collected and stored at −20 °C until analysis.

2.6.2. Transfer of viruses from fingers to food product

Marked whole tomatoes and cucumber slices were kept in an individual sterile boat (50 ml reagent reservoir, Corning USA) inside a biosafety cabinet. As described above, the contaminated finger pads with 10 μl virus at 0 min and 10 min were successively pressed on the marked area of 7 food products: on skin of whole tomatoes and on cut face of cucumber slices. The pressure applied was measured as described above. The transferred virus was recovered immediately by flushing with 2 ml DMEM on the marked area for 20 times by pipetting. MNV1 suspensions were then filtered through a 0.2 μm pore size filter (Whatman, Germany) to prevent contamination in the cell culture assay and the sterile filtrate was collected for further analysis. The samples were then stored at −20 °C until analysis. There was no difference in PCR signals observed from filtered and non-filtered stocks.

2.6.3. Transfer of viruses from carriers to finger

Stainless steel and Trespa® carriers were kept in wells of 6 well plates separately. All carriers were individually contaminated with 10 μl of virus suspension and spread by a pipette tip over a round area of approximately 1 cm diameter. Thereafter, the carriers were dried for 40 min inside a biosafety cabinet (25–26 °C, 40–45% RH) until visibly dry. Test persons pressed their clean and dry finger pads (cleaned and disinfected with ethanol 70% as explained in Section 2.5.2) onto the contaminated spot sequentially. Sequential pressing was performed with five finger pads of each person. The virus on the finger pads was eluted by inverting the open mouth of a cryovial with 1 ml DMEM on the contaminated area on the finger pad as described above. The virus remaining on the carrier was eluted by flushing the carrier with 1 ml DMEM as described above. The collected samples were then stored at −20 °C until analysis. After the experiment, the volunteers decontaminated and then washed their finger pads as explained above.

2.7. Infectivity determination

The number of infective MNV1 was enumerated by titration on RAW-264.7 cells as described before (Tuladhar et al., 2012b). Briefly, 1 × 10⁵ cells/ml cell suspension was freshly prepared and 100 μl of the suspension was loaded on each well of a 96 well plate separately. 10 fold serial dilutions of the virus were titrated on the cells and the cytopathic effect was observed after 5 or 7 days of incubation at 37 °C in 5% CO₂. Virus titers were then calculated by the Spearman–Karber method (Karber, 1931).

2.8. Reverse transcription PCR (RT-PCR)

To allow comparison between the cultivable MNV1 and the non-cultivable human NoVs, quantitative RT-PCR assays were performed for all three viruses. The virus RNA extraction was performed using a Magna Pure Light cycle total nucleic acid extraction kit as described before (Svraka et al., 2009). Amplification of MNV1 (Bae and Schwab, 2008), NoV GI4 (Svraka et al., 2009) and GII4 (Tuladhar et al., 2012b) was performed as previously described. RT-PCR amplifiable units (RT-PCRU) were determined from slopes of standard curves made for each virus. The standard curve was made by plotting cyclic threshold (Ct) value versus log RT-PCRU of 10 fold dilutions of the virus stock. The highest dilution giving a positive result was assigned a value of 1 amplifiable unit. Samples of transfer 1, 3 and 5 of all the experiments were analyzed by RT-PCR assay.

2.9. Calculations and statistical analysis

The MNV1 infectivity and RT-PCRU recovery from the finger pads (0 and 10 min), carriers and food products were calculated as (virus recovered [infectivity or RT-PCRU] after time (t) from source/virus applied) × 100%. Student’s t-Test was performed to analyze and compare results.

3. Results

3.1. Recoveries of viruses from human finger pads

The MNV1 titer loaded on finger pads was 5.0 ± 0.2 × 10⁴ TCID₅₀ per 10 μl. The MNV1 infectivity recoveries at 0 and after 10 min drying were 57 ± 28 (n = 9) and 20 ± 18% (n = 9), respectively. Although approximately 80% of loaded infectious viruses were lost; still over 10,000 infectious MNV1 was retrieved from the finger pad after 10 min drying. The MNV1 RT-PCRU recoveries at 0 and after 10 min drying were 63 ± 9 (n = 9) and 28 ± 3% (n = 9), respectively. For NoVs GI4 and GII4, 2.8 × 10⁴ and 3.8 × 10⁴ RT-PCRU were loaded on the finger pads (n = 9), respectively. The RT-PCRU recoveries of NoV GI4 from finger pads at 0 and 10 min were 80 ± 25% and 31 ± 13%, respectively and for NoV GII4 recoveries were 28 ± 12% and 9 ± 6%, respectively. The RT-PCRU recovery of NoV GI4 was significantly higher (P < 0.05) than NoV GII4.

3.2. Viruses transfer from finger pads to stainless steel and Trespa® carriers

The sequential MNV1 infectivity transfers from finger pads to stainless steel at 0 and 10 min are shown in Fig. 1A. The infectivity transfer of MNV1 at immediate transfer (t = 0 min) on stainless steel and Trespa® were 13 ± 16% and 13 ± 3% respectively. The infectivity transfer from the finger pad to stainless steel and Trespa® after 10 min drying were reduced to 0.1 ± 0.2 and 0.1 ± 0.1%, respectively and were significantly lower (P < 0.05) at 0 min, however the transfers were similar for stainless steel and Trespa® (data for Trespa® not shown). Infectious virus was detectable up to 5–6 out of 7 consecutive transfers to both types of carriers at 0 and 10 min of drying.

The RT-PCRU transfers of MNV1 and NoVs GI4 and GII4 from finger pads at time 0 and 10 min to stainless steel are shown in Fig. 1B. The RT-PCRU transfers of MNV1 and the NoVs GI4 and GII4 in the first transfer to stainless steel (t = 0) was 50 ± 18%, 69 ± 31% and 19 ± 10% respectively, and after 10 min when the finger pads were dry, the transfer reduced to 1.1 ± 0.9%, 2.2 ± 3.1% and 0.6 ± 1.6%, respectively. The RT-PCRU transfers of MNV1 and the NoVs GI4 and GII4 in the first transfer to Trespa® (t = 0) were 54 ± 23%, 54 ± 32% and 13 ± 14% respectively. After 10 min when the finger pads were dry, the transfers were reduced to 3.7 ± 2.7%, 1.7 ± 2.2% and 1.1 ± 3.2% respectively. There was a significantly different (P < 0.05) in RT-PCRU transfers at 0 and 10 min for both stainless steel and Trespa® but the transfers were not significantly different (P > 0.05) between the two carriers (data not shown). RT-PCRU transfers of MNV1 and NoV GI4 is similar (P > 0.05), but the transfer of NoV GI4 to stainless steel and Trespa® at immediate transfer (t = 0 min) was significantly lower (P < 0.05) than MNV1 and NoV GI4. However, there was no significant difference (P > 0.05) in transfer among the NoVs after drying for 10 min. Comparing the MNV1 infectivity and RT-PCRU transfers, a significant difference (P < 0.05)
was observed at both immediate transfer and after drying at 1, 3 and 5 transfers. MNV1 RT-PCR transfers were higher than infectivity transfers.

### 3.3. Virus transfer from contaminated stainless steel and Trespa® to human finger pads

The sequential transfer of MNV1 infectivity from contaminated stainless steel and Trespa® carriers to finger pads after 40 min drying are shown in Fig. 2A. The MNV1 infectivity at first transfer to the finger pad from stainless steel and Trespa® carrier was 1.8 ± 1.8% and 3.8 ± 4.8% respectively. There was no significant difference (P > 0.05) in infectivity transfer from the two carriers to the finger pads.

The RT-PCRU transfers from contaminated stainless steel and Trespa® to finger pads are shown in Fig. 2B. For NoV GI.4, transfer from stainless steel and Trespa® to the finger pads was 4.2 ± 6.6% and 3.2 ± 3.2% respectively. The transfer of NoV GI.4 was 3.5 ± 3.4 and 2.4 ± 3.8% respectively and for MNV1 was 1.8 ± 1.2 and 2.4 ± 1.3% respectively. The RT-PCRU transfer of NoVs GI.4 and GII.4 was not significantly (P > 0.05) different from RT-PCRU transfer of MNV1 for both carriers. There was no significant difference (P > 0.05) in MNV1 infectivity and RT-PCRU transfer from both the carriers to finger pads. An average of 3.5 log_{10} infective MNV1 still remained on the carriers after 5 sequential transfers.

### 3.4. Transfer from fingers to food products (whole tomatoes and cucumber slices)

Infectivity transfer of MNV1 from finger pads to whole tomatoes and cucumber slices at 0 and 10 min are shown in Figs. 3A and 4A respectively. The virus was transferred to all the seven food products touched sequentially. The MNV1 infectivity transferred to whole tomatoes and cucumber pieces at immediate transfer (t = 0) were similar: 14 ± 6% and 12 ± 11% respectively. However, after 10 min drying, transfer to cucumber slices was 7 ± 8% which was significantly higher (P < 0.05) than the transfer of 0.3 ± 0.5% to tomatoes at 10 min. For tomatoes, there was a significant difference (P < 0.05) for infectivity transfer at 0 and 10 min but not for cucumber slices. On both the food products, infectivity was detected even on the 7th transfer as shown in Figs. 3A and 4A. Dried finger pads became wet on pressing the cucumber slices resulting in higher percentage of transfer than on tomatoes.

The RT-PCRU transfers of MNV1 and NoVs GI.4 and GII.4 to tomatoes at immediate transfer (t = 0) were comparable (P > 0.05), whereas the
transfer of NoV GII.4 to tomatoes at immediate transfer (t = 0 min) is significantly lower (P < 0.05) than MNV1 and NoV GI.4. The RT-PCRU transfers from finger pads to cucumber slices are shown in Fig. 4B. On cucumber slices, RT-PCRU transfers of MNV1, NoV GI.4 and GII.4 at 0 and 10 min were significantly different (P < 0.05).

4. Discussion

In the majority of human NoV outbreaks, person to person transmission has been described as one of the most important routes. Cross contamination from contaminated fingers to food and fomites has been described as significant means of transmission (Tuan Zainazor et al., 2010; Weber et al., 2010). The main aim of this study was to quantify the transfer of NoVs from fingers to fomites and foods to gain a better understanding of the spread of the viruses. We have chosen stainless steel and Trespa® as carriers representative for nonporous inanimate surfaces which can be found in kitchens, hospitals, and health care settings as for example food contact surfaces or other surfaces often touched by humans and found to be contaminated by NoV (Boxman et al., 2009a,b, 2011). Norovirus has been shown to be persistent on environmental surfaces like soil, stainless steel (Cannon et al., 2006; Fallahi and Mattison, 2011) formica, ceramic (Liu et al., 2009), and also capable of attaching and surviving on food materials like lettuce (Tian et al., 2011) and strawberry (Mattison et al., 2007).

Fingers are the most active part of the hands that come in contact with different surfaces and foods. In this study, the infectivity transfer of MNV1 to stainless steel, Trespa® and to tomatoes was high (10 to 100 times the infectious dose up to 5 or 6 transfer) when transferred immediately and significantly decreased as the virus was dried on the fingers for 10 min. This is consistent with data on transfer of HAV which also showed a decrease on transfer when drying time increased (Mbithi et al., 1992). The MNV1 infectivity transfer was approximately 0.1% on both the carriers after 10 min drying on the finger pads. These transfers are lower than transfer of rotavirus (16%; (Ansari et al., 1988)), HAV (27%; (Mbithi et al., 1992)) and FeCV (13%; (Bidawid et al., 2004)) to stainless steel carriers after 20 min drying on the finger pads. The higher HAV transfer might be explained by a different transfer technique (rotating the finger in half circles for ten times over 10 s contact (Mbithi et al., 1992)), whereas the high rotavirus transfer might be due to its higher recovery on the finger pads anyway, namely >60% in 20 min for rotavirus compared to 20% of the MNV1 in 10 min. Similarly, higher FeCV transfer might also be due to the higher survival of the virus (71% in 20 min; (Bidawid et al., 2004)) than MNV1. Overall, the transfer is lower after drying than on immediate transfer. This might be explained by the fact that moisture facilitates the transfer (Boone and Gerba, 2007; Mbithi et al., 1992). As a lower percentage of the viruses are transferred after drying, and the number of infective viruses transferred was lower than the number required for the infection in some of the transfers, perhaps risk is associated with a higher level of shedding than the transfer after drying. Nevertheless, the observed transfer rates showed considerable contribution of hands in spreading the virus.

Fig. 3. MNV1 infectivity transfer (A) and RT-PCRU transfer of MNV1, human NoVs GI.4 and GI.4 (B) from finger pads to whole tomato at 0 min and 10 min on sequential transfer. Horizontal lines show detection limit. Error bars show standard deviation of series (n = 9). The transfer percentage is calculated as fraction of the number of viruses applied.

Fig. 4. MNV1 infectivity transfer (A) and RT-PCRU transfer of MNV1, human NoVs GI.4 and GII.4 (B) from finger pads to cucumber slices at 0 min and 10 min on sequential transfer. Horizontal lines show detection limit. Error bars show standard deviation of series (n = 9). The transfer percentage is calculated as fraction of the number of viruses applied.
Fresh products such as tomatoes, cucumber and raspberries contaminated with NoVs via food handlers are implicated in a number of outbreaks (Baert et al., 2009). These products are frequently eaten raw individually or combined with salads. Visually observed higher moisture level on the cucumber slices compared to tomatoes may account for observed higher transfer percentage of MNV1 infectivity from the dried finger pad. This shows that transfer from hand to moist and wet vegetables could be higher than to dried surfaces and thus moisture might increase the risk of infection. A similarly high transfer was also reported for FeCV (46%) to moist ham (Bidawid et al., 2004). A previous study indicated nearly 66% transfer of porcine enterovirus type 3 by a fecally contaminated human finger to whole tomato (Cliver and Kostenbader, 1984), which is higher than what we showed in this study for MNV1. This difference in transfer may be due to a number of different factors including differing influences in virus recovery method and binding affinity of the virus to the product which was also reported for FeCV and echovirus 11 on butter head lettuce (Vega et al., 2008). Thus different binding affinity of the viruses might have a role in different transfer capabilities. In addition, transfer capabilities might differ per individual due to variability in moisture, soil and skin composition of the hands.

The amount of the NoV excreted in feces from infected individuals ranged from $10^3$ to $10^6$ particles per g (Ozawa et al., 2007). The amount of fecal material that can be present on human hands due to unhygienic practices is unknown. Nevertheless assuming the amount of fecal material possibly present on hand as 1 mg, the amount of virus would be $10^3$ to $10^6$ virus particles. Since not all virus particles may be infectious, if we assume that half of the particles are infectious as estimated by Teunis et al. (2008) for NoV GI.1 then 1 mg of fecal material would contain between $5 \times 10^2$ and $5 \times 10^6$ infectious viruses. If the upper limit is used and in the case of approximately 14% transfer to tomatoes and 12% to cucumber slices, approximately $6.8 \times 10^2$ and $6.2 \times 10^4$ infectious virus particles would be transferred respectively, which would be largely sufficient to initiate infection in susceptible individuals on consumption.

Surfaces contaminated with NoV can be a source of transmission (Baie and Schwab, 2008; Escudero et al., 2012; Liu et al., 2009). Indirect transmission of the virus from contaminated surfaces by touching with hands has been thought to be a potent source of transmission (Evans et al., 2002; Lopman et al., 2012). In the case of 13% infectivity transfer in wet conditions, transfer would be $6.5 \times 10^4$ infectious viruses from a contaminated finger pad to surfaces and from the dried finger pad (10 min) approximately $5.0 \times 10^5$ infectious virus (0.1%) transfer to the surfaces. Such contaminated surface might remain a potent source of transmission if not cleaned and disinfected. When approximately $6.5 \times 10^4$ infectious virus would be present on the surfaces transferred from a wet finger pad, after 40 min drying about 40% of the virus (data from this experiment) would still be infectious ($2.6 \times 10^5$). In the case of approximately 4% transfer from the contaminated surface to the finger pad, an approximate $1 \times 10^4$ infectious virus particles would be transferred to the finger pad, which would be sufficient to initiate infection on direct ingestion or indirectly by further transmission from the hand. Continuing this calculation, a level of contamination on toilet surfaces after leaving the toilet needs to be reduced to as low as 200 infectious particles. Then the risk of infection after touching the contaminated surfaces will be minimal, as the transfer percentage is approximately 4% (8 infectious particles) and the number of the infective virus required for infection has been described to be low as 8 particles (Teunis et al., 2008). With this scenario it seems that the risk of infection is associated with the high level of shedding ($>10^5$) (Ozawa et al., 2007). The high level of shedding of NoV has been found both in symptomatic and asymptomatic patients (Atmar et al., 2008; Sukhrie et al., 2012). However, symptomatic patients might be of higher risk as a recent study (Sukhrie et al., 2012) showed that symptomatic shedders are more often involved in transmission events of the virus than rare involvement of asymptomatic shedders in hospital setting.

We presented RT-PCR data on transfer potential of NoVs. The amount of viral RNA estimated by RT-PCR does not necessarily correlate with the number of infectious viruses quantified by cell culture assays (Tuladhar et al., 2012a). It is unlikely that all the genomic copies detected by RT-PCR were associated with infectious viruses as a larger fraction of noninfectious viruses is present in all virus stocks (Jakus, 1997). However, the data on RT-PCR transfer provided an estimate of transfer potential of the NoVs since an amount of infectious particles higher than the number of RT-PCRU is rather unlikely. Both NoVs GI and GII have been implicated in NoV outbreaks, however, GII strains are more often associated with outbreaks (Blanton et al., 2006). In our study, transfer percentage of MNV1 and NoV GII.4 was comparable but NoV GII.4 transfer percentage was significantly lower when transferred from hand to stainless steel and Trespa® at immediate transfer. The reason of proportionally higher number of outbreaks by GII strains was attributed to possible environmental stability or host susceptibility (Liu et al., 2009), and higher levels of shedding (Ozawa et al., 2007). In addition, it might also be due to a lower transfer percentage and different binding affinity of different genogroups of noroviruses as shown for shellfish (Maalouf et al., 2011) and lettuce (Tian et al., 2011). However, more in-depth studies are needed to confirm lower transfer percentage of NoV GII.4 than NoV GI.4 since the lower transfer cannot be shown after drying. The lower transfer might also be due to lower initial concentrations of NoV GII.4 than NoV GI.4 in the stock suspensions.

The transfer of the virus is possible even after the virus is dried on the surface of hands or carriers. Control and prevention of human NoV should focus on interruption of the virus transmission through hand hygiene practices (Malik et al., 2006) and disinfection of the contaminated surfaces (Tuladhar et al., 2012b) giving a specific focus on high risk areas in health care settings and food preparations facilities. The quantitative data on transfer of the viruses will help to determine efficacy of implementing prevention methods and can be useful in risk assessment models and to establish target levels for efficacy of transmission intervention methods.

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