

Title: The likelihood and rate of human norovirus transfer to small fruits during handling

Final Progress Report

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Objectives: This study investigates the rate of norovirus transfer from contaminated hands to small fruits by replicating two negligent worker scenarios surrounding restroom use in a harvest setting. In the absence of hand sanitation, contamination of fruit by both an ill worker and a healthy worker subsequently using the same restroom facilities as the ill worker is demonstrated.

Specifically, we examined:

- The rate of transfer between norovirus soiled or contaminated:
 - toilet paper to hands
 - hands to restroom door handles
 - soiled hands to fruits
 - door handles to previously clean hands
 - previously clean hands to fruits
- We then determine the likelihood of transfer when
 - An ill person uses the restroom, leaves the restroom without washing their hands and subsequently handles a food item
 - a healthy person touches a door handle that has been previously contaminated by an ill person, leaves the restroom and subsequently handles a food item without hand washing.

By imitating real-world scenarios, the goal of this research project is to explore the likelihood of NoV transfer to fruits surrounding restroom use, in the absence of hand sanitation. Knowing amount of NoV contamination likely to occur in realistic scenarios will be the basis of future research projects that will examine the efficacy of common and novel interventions for virus removal from hands, restroom facility surfaces, and fruits. Other possible studies will include the development of educational modules for employee training that have a direct parallel to the job they are performing, which may in turn, improve worker compliance to hand hygiene regulations.

Justification: The importance of proper hand sanitation for foodhandlers is well recognized. Human noroviruses (NoV) have emerged as the leading cause of outbreaks of foodborne gastroenteritis, causing 30-50% of all foodborne illnesses in the U.S. (11). Of outbreaks involving fruits, 67% were attributed to NoV in the US in 1990-2005, by far exceeding the contribution of all bacterial foodborne pathogens, including *E. coli* O157H7 and *Salmonella* (5). While a large proportion NoV outbreaks are linked to food handler contamination during food service, national and international outbreaks involving fresh and

frozen raspberries or raspberry products have called in to question the role of foodhandlers in NoV contamination during harvesting and food processing (7, 9).

A previous study involving norovirus transfer using feline calicivirus as a surrogate showed 13- 46% of virus contaminated on hands can transfer to lettuce, ham and food contact surfaces upon handling (2). Because NoV can be shed in feces at high rates (up to 10^{12} virus copies/g) for symptomatic as well as asymptomatic individuals (1), it can persist in the environment for >1 week (3, 4) and as few as 1-100 NoV particles is enough to cause illness (1) stringent hand hygiene procedures must be followed in the harvest environment to minimize NoV contamination of small fruits. Here, the impact of hand hygiene negligence is demonstrated.

Methodologies

Virus stock preparation and quantification. A stool specimen obtained from an outbreak of human NoV genogroup II cluster 4 (GII.4) (a kind gift of Andrea Maloney, South Carolina Department of Health & Environmental Control) was prepared in a 20% suspension in PBS and stored at -80°C until use. Viral RNA was extracted using a Viral RNA mini-kit (Qiagen, Valencia, CA) and amplified by real time-RT-PCR (Step One, Applied Biosystems, Foster City, CA) using NoV specific primers and probes (8, 10) and a Quantitect one-step RT-PCR kit (Qiagen). Viral RNA was quantified by comparison to a standard curve of NoV RNA transcript of a known concentration and the stock was determined to be 1.25×10^{11} virus particles/ml.

Preparation of food items and food contact surfaces. All fruits (strawberries, raspberries, red grapes, and blueberries) were soaked in a 10% bleach solution for 10 minutes, rinsed twice in Milli-Q sterile DI water, dried for 30 minutes and exposed to germicidal UV for 10 minutes in a sterile weigh boat before use. Stainless steel coupons (5 cm x 2 cm finish #4) were soaked in 70% ethanol for 1 hour, rinsed with water, then autoclaved at 121°C for 30 minutes at 17 psi and stored in a sterile beaker until use. One-ply toilet paper and latex gloves were exposed to UV for 10 minutes on each side prior to use. Six sheets of 1-ply toilet paper were folded over to make a single stack of 6 sheets and a 1" x 1" square was cut out using sterilized scissors.

Virus inoculation and transfer. The starting item for each transfer was inoculated with 10 one- μl iterations of stool suspension and either allowed to dry for 30 min (dry) or immediately used (wet). For transfer experiments, two scenarios were replicated for field harvester contamination of fruit; 1) the hand of a field worker is contaminated in the bathroom which touches the bathroom door handle, then proceeds to hand pick fruit 2) a non-shedding individual touches a contaminated bathroom door handle, then proceeds to hand pick fruit. Using the right index finger of a gloved hand, pressure was applied over the inoculated area with 50 g pressure \pm 5 grams for 5 seconds. Four experiments were run, each replicating a separate transfer. Triplicate experiments were performed with the right index finger of a single individual.

Elution of Items. After viral transfer, stainless steel coupons, glove tips, and toilet paper were placed in a 50 ml tube containing 10 ml of 0.1M PBS with 1M NaCl pH 7.5 and vortexed for 30 seconds. A 200 μl aliquot was transferred to a sterile Eppendorf tube for RNA extraction. Fruit was placed in a sterile

weigh boat and virus was eluted by repeated pipetting 25x over the contaminated area with 1 ml of 0.1M PBS with 1M NaCl and 0.05% v/v Tween 20. The entire eluate underwent RNA extraction. All samples either were immediately used for RT-PCR or stored at -80°C for later usage.

Transfer Scenarios. 1) The gloved finger tip is inoculated, following by applying pressure to a coupon. That same glove tip then touches various fruit at the specified pressure. Elution from coupon and fruit occurred immediately post-transfer. 2) A stainless steel coupon is inoculated with virus, then a clean (gloved) finger tip applies pressure. The same glove tip then touches various fruit at the specified pressure. Elution from coupon and fruit occurred immediately post-transfer.

Results

To first determine the effectiveness of the elution methods, percent recovery of virus from stainless steel coupons, gloves, toilet paper and fruits was determined. Percent recoveries were $93 \pm 11\%$ and $90 \pm 3\%$ for stainless steel coupons, $87 \pm 7\%$ and $51 \pm 19\%$ for glove tips, and $13 \pm 2\%$ and $8 \pm 4\%$ for toilet paper using wet and dry virus inoculation procedures, respectively (data not shown). For fruit, percent recoveries were $55 \pm 5\%$, and $8 \pm 7\%$ for strawberries, $25 \pm 8\%$ and $15 \pm 4\%$ for raspberries, $108 \pm 14\%$ and $65 \pm 15\%$ for red grapes and $72 \pm 14\%$ and $53 \pm 21\%$ for blueberries using wet and dry virus inoculation procedures, respectively (data not shown).

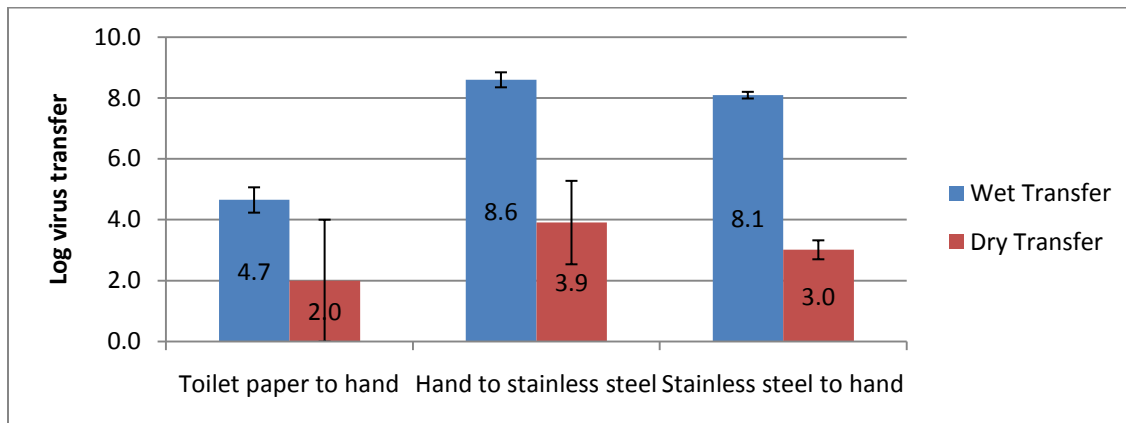


Figure 1: Norovirus transfer between contaminated surfaces and hands. Beginning with a $9.1 \log_{10}$ inoculum (wet or dry), \log_{10} transfer between surfaces (toilet paper, stainless steel) and hands was determined. Average transfer values are reported for 3 replicates and error bars indicate the standard deviations.

To simulate scenarios of virus transfer to fruits by hand harvesting, the percent transfer and level of transfer (\log_{10} virus transfer) between hands (gloved finger tips) and contact surfaces or fruits was first examined individually using a $9.1 \log_{10}$ virus inoculum. While percent transfer was less than 0.01% for virus transfer from toilet paper to hand, the actual amount of virus transferred was high (average \log_{10} values were 4.7 ± 0.4 and 2.0 ± 2.0 under wet and dry inoculation conditions, respectively) (Figure 1). Percent transfer was much higher for a contaminated hand to a stainless steel surface with a wet inoculum ($71\% \pm 27\%$), but similar ($<0.01\%$) for a dry inoculum. Likewise, the amount of virus detected on stainless steel after transfer was high (8.6 ± 0.3 and $3.9 \pm 1.4 \log_{10}$ virus) under wet and dry conditions, respectively). Contamination of previously clean gloved finger tips by touching a

contaminated stainless steel surface occurred at a rate of $17 \pm 5\%$ and $<0.01\%$ for wet and dry inoculations, translating to 8.1 ± 0.1 and $3.1 \pm 0.3 \log_{10}$ viruses under wet and dry respective conditions (Figure 1).

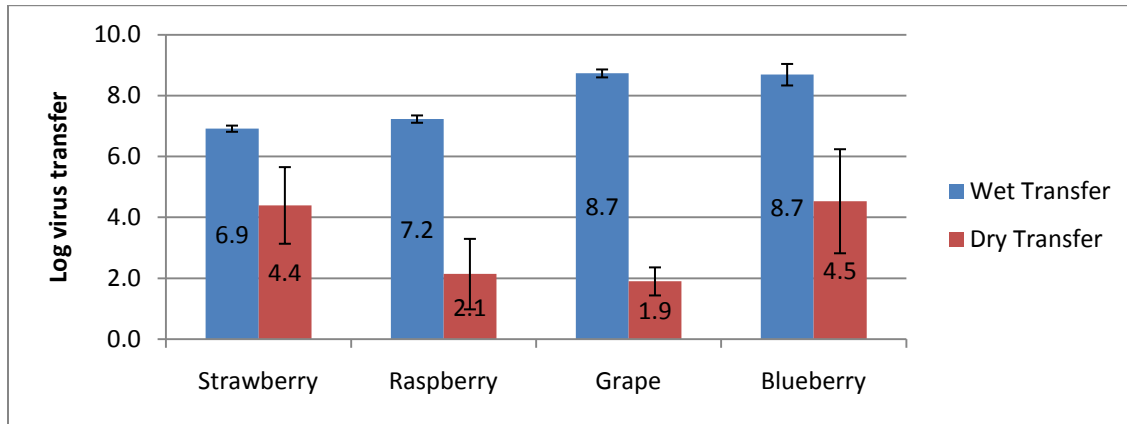


Figure 2: Norovirus transfer between from contaminated hands to fruits. Beginning with a $9.1 \log_{10}$ inoculum (wet or dry), \log_{10} transfer between hands and fruits (strawberry, raspberry, grape, blueberry) was determined. Average transfer values are reported for 3 replicates and error bars indicate the standard deviations.

Percent transfer from contaminated hands to strawberries was $11 \pm 2\%$ and $0.4\% \pm 0.5\%$ while percent transfer to raspberries was $22 \pm 6\%$ and $0.01 \pm 0.01\%$ (data not shown). Virus detected on strawberries after transfer from contaminated hands measured $\log_{10} 6.9 \pm 0.1$ and 4.4 ± 1.3 , while raspberries had $\log_{10} 7.23 \pm 0.12$ and 2.14 ± 1.16 for their respective wet and dry transfer conditions (Figure 2). Percent transfer of virus to grapes was $50 \pm 1\%$ and $< 0.01\%$, and blueberries had a measured transfer of $43 \pm 31\%$ and $0.4 \pm 0.7\%$ for wet and dry transfer, respectively (data not shown). The amount of virus transferred from contaminated hands to grapes and blueberries was high, 8.7 ± 0.13 and $1.9 \pm 0.5 \log_{10}$ for grapes and 8.7 ± 0.4 and $4.5 \pm 1.7 \log_{10}$ for wet and dry transfer to blueberries (Figure 2).

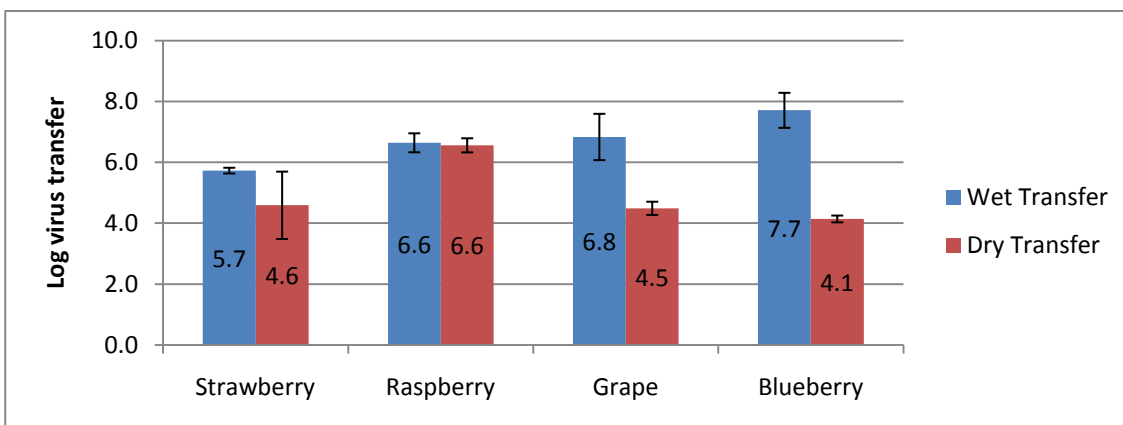


Figure 3: The likelihood of transfer of Norovirus from a contaminated hand to fruits after first touching stainless steel (representing a door knob). Beginning with a $9.1 \log_{10}$ inoculum (wet or dry) on gloved hands, \log_{10} transfer to fruits (strawberry, raspberry, grape, blueberry) was determined after touching a stainless steel coupon. Average transfer values are reported for 3 replicates and error bars indicate the standard deviations.

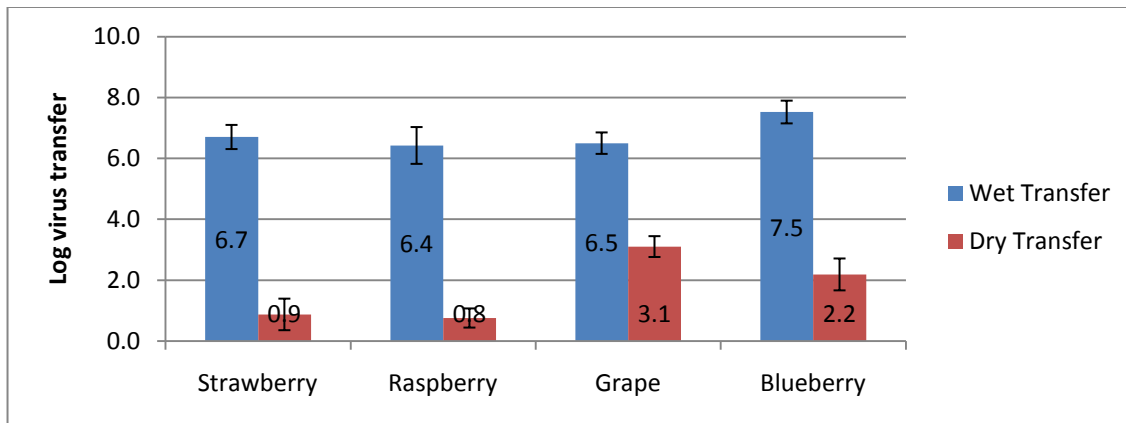


Figure 4: The likelihood of transfer of Norovirus to fruit from a previously clean hand that touches a contaminated door handle (stainless steel coupon) and then handles fruit. Beginning with a 9.1 log₁₀ inoculum (wet or dry) on a stainless steel coupon, log₁₀ virus transfer to fruits (strawberry, raspberry, grape, blueberry) was determined after handling by a hand coming into contact with a contaminated stainless steel coupon. Average transfer values are reported for 3 replicates and error bars indicate the standard deviations.

Two scenarios depicting the likelihood of food handler contamination of fruits after restroom use in the absence of hand sanitation are indicated in Figures 4 and 5. In the first scenario (Figure 3), an ill field worker contaminates his or her hand while using the restroom, then contaminates the door while upon exiting, and contaminates fruits after returning to work. The likelihood of fruit contamination by this individual is high, as up to 7.7 log₁₀ and 6.6 log₁₀ virus are detected on fruit post handling for the respective transfer of wet and dry virus suspension. For the second scenario (Figure 4), the likelihood of fruit contamination is examined for an individual who is not shedding virus. He or she instead, touches a contaminated door handle and subsequently harvest fruit. While dry contamination of fruit occurs to at a much lower degree (0.9 – 3.1 log₁₀ virus transferred), wet transfer is nearly as likely by the healthy worker as contamination by the ill worker (6.4 - 7.5 log₁₀ virus transferred).

Conclusions: This study examines the rate and level of norovirus transfer from hands to hand-harvested fruits in the absence of hand sanitation. Two scenarios are created to demonstrate the likelihood of virus contamination of fruit following restroom use by an individual shedding virus (ill) and by a healthy individual using the restroom after the ill worker. **Our results indicated that if hand washing is neglected, 10 - 100 million NoV particles (7-8 log₁₀) can be transferred (wet transfer) and detected on fruit post transfer by hands of ill individuals and healthy individuals sharing a restroom with an ill co-worker.** Norovirus transfer between hands and surfaces was interrupted when contaminated hands were given the opportunity to dry (time = 30 min) prior transfer. Similarly, transfer of virus from contaminated stainless steel that was allowed to dry to fruits by previously clean hands was greatly reduced.

These results indicate that the likelihood of a norovirus contamination is very high if proper hand sanitation procedures are not followed. It was evident that virus suspension that is still wet has a much higher propensity to transfer and cause contamination. Wet transfer is also more likely in real life scenarios as most of the items are handled soon after initial contamination. Even with greater variation

among dry transfer, a minimum of 6 viruses and a maximum of ~ 4 million viruses could be detected on fruits following transfer. Since as few as 1-100 virus particles can cause illness in humans, contamination even at this level creates a potential for the contaminated food to cause illness in humans.

One of the major limitations of the study is that quantitative RT-PCR was used to quantify virus transfer. Currently laboratory culture of NoV is not possible, despite 20+ years of trial (6). Therefore predicating NoV inactivation by experimental protocols and the infectivity of the virus stock we are using was not possible in the present study.

Hand washing is imperative for food safety on the field. Norovirus is a unique pathogen that can be shed in the feces at very high levels and is readily transferred between hands and surfaces. Requiring workers to stay home from work when ill and enforcing stringent hand washing procedures are necessary in any setting involving food. The use of gloves, no-wash hand sanitizers (to supplement hand-washing), thorough drying of hands and surfaces and regular sanitation of restroom facilities are all supplemental protective barriers against the spread of NoV surrounding restroom use.

Impact statement: Such results emphasize the importance of rigorous sanitation on the field. With proper measures taken at origin, through processing, and through food preparation, the likelihood of a norovirus outbreak can be greatly reduced. The results of this study will be presented in the poster section of the Southeast Region Fruit and Vegetable Growers Conference, Savannah, GA, Jan 7-9, 2010. Long-term objectives of this work will be to incorporate the transfer data into educational materials for food handlers that will clearly illustrate the ease of hand to surface and food transfer for NoV. Our group is also investigating novel hand and food sanitizers with efficacy against NoV that can be used to supplement current on-farm sanitation protocols.

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