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Norovirus Cross-Contamination during Food Handling and Interruption of Virus Transfer by Hand Antisepsis: Experiments with Feline Calicivirus as a Surrogate†

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ABSTRACT

While there is good epidemiological evidence for foods as vehicles for norovirus transmission, the precise means of spread and its control remain unknown. The feline calicivirus was used as a surrogate for noroviruses to study infectious virus transfer between hands and selected types of foods and environmental surfaces. Assessment of the potential of selected topicals in interrupting such virus transfer was also made. Ten microliters of inoculum of feline calicivirus deposited onto each fingerpad of adult subjects was allowed to air dry and the contaminated area on individual fingerpads was pressed (10 s at a pressure of 0.2 to 0.4 kg/cm^2) onto 1-cm-diameter disks of ham, lettuce, or brushed stainless steel. The virus remaining on the donor and that transferred to the recipient surfaces was eluted and plaque assayed. Virus transfer to clean hands from experimentally contaminated disks of ham, lettuce, and stainless steel was also tested. Nearly 46 ± 20.3, 18 ± 5.7, and 13 ± 3.6% of infectious virus was transferred from contaminated fingerpads to ham, lettuce, and metal disks, respectively. In contrast, approximately 6 ± 1.8, 14 ± 3.5, and 7 ± 1.9% virus transfer occurred, respectively, from ham, lettuce, and metal disks to hands. One-way analysis of variance test showed that pretreatment (washing) of the fingerpads either with water or with both topical agent and water significantly ($P < 0.05$) reduced virus transfer to ≤0.9%, as compared with ≤2.3 and ≤3.4% transfer following treatments with either 75% (vol/vol) ethanol or a commercial hand gel containing 62% ethanol, respectively. Despite wide variations in virus transfer among the targeted items used, intervention agents tested reduced virus transfer significantly ($P < 0.05$) when compared with that without such treatments (71 ± 8.9%). These findings should help in a better assessment of the potential for cross-contamination of foods during handling and also assist in developing more effective approaches to foodborne spread of norovirus infections.

The Norwalk agent or closely related viruses, now placed in the genus *Norovirus* in the family *Caliciviridae (19),* cause >65% of the cases of nonbacterial gastroenteritis in the United States *(23, 24)* and continue to be a major threat to public health as evidenced by the recent outbreaks of gastroenteritis on cruise ships *(1),* in hospitals, and various community settings *(7, 17, 18).* These highly contagious viruses are spread by a variety of means, including the consumption of contaminated foods *(6, 7).* Ingestion of only 10 to 100 infectious virus particles may be sufficient to initiate infection *(14)* leading to severe diarrhea and dehydration. Although the disease is usually self-limiting, outbreaks due to them cause considerable economic losses *(12).* The higher numbers of foodborne outbreaks of noroviral gastroenteritis being recorded may be due to a general increase in the consumption of fresh fruit and vegetables and also as a result of the changing eating habits in industrialized countries *(23).*

While consumption of raw or improperly cooked shellfish remains the major risk factor for norovirus gastroenteritis *(6, 20, 22),* many types of fruits and vegetables are increasingly being implicated as vehicles *(8, 9, 15, 26).* The higher numbers of foodborne outbreaks of noroviral gastroenteritis being recorded may be due to a general increase in the consumption of fresh fruit and vegetables and also as a result of the changing eating habits in industrialized countries *(23).* Such foods become contaminated either at their origin, during processing, or by infected food handlers *(1, 11, 12, 16, 21, 28).* Previously, Bidawid et al. *(4)* reported that nearly 9% of hepatitis A virus was transferred from artificially contaminated fingerpads to lettuce during the types of contact that normally occur in food handling. The objectives of this study were to investigate the potential role of food handlers in the cross-contamination of foods and environmental surfaces with caliciviruses and to determine the extent of transfer of virus transfer between foods and food handlers. Furthermore, the efficacies of select intervention agents to interrupt virus transfer from contaminated fingerpads to foods and surfaces were also assessed in this project. Because noroviruses cannot be grown in the laboratory, the feline calicivirus (FCV), which can readily be cultured and plaque assayed *(5),* was used as a surrogate *(13, 27).*

MATERIALS AND METHODS

Cells and virus. Seed cultures of Crandell's feline kidney cell line (ATCC #CCL-94) and strain F9 of FCV (ATCC #VR-

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782) were obtained from the American Type Culture Collection (ATCC), Rockville, Md. The cells and the virus were propagated as described previously *(4)*. The virus pool, which consisted of unconcentrated supernatant from infected monolayers, was harvested and stored as 1-ml aliquots at −80°C until needed.

Plaque assays. Plaque assays were performed as described previously *(5)*.) In brief, Crandell's feline kidney cell monolayers were grown in 12-well cell culture plates (Costar, Fisher Scientific, Ottawa, Ontario, Canada) overnight at 37°C in a humidified 5% CO_2 atmosphere. A 100 μl portion of each virus dilution was inoculated into each of three wells. The virus was allowed to adsorb to the cells for 90 min at 37°C, and then 2 ml of an agarose-medium mixture overlay to each well. The plates were reincubated at 37°C for 36 h, and the monolayers were fixed and stained for counting plaques as described previously *(5)*.

Preparation of food items and metal disks. Metal disks (1 cm in diameter) were prepared from sheets of brushed stainless steel by the Engineering Department of the Rehabilitation Centre, Ottawa Hospital (Ottawa, Ontario, Canada). Locally purchased items of food tested in this study included Romaine lettuce and ham cold cuts. The lettuce and the metal disks were washed with a nongermicidal liquid soap (Ivory, Procter & Gamble, Toronto, Ontario, Canada), thoroughly rinsed in tap water for 2 min, and allowed to dry for approximately 20 min in a laminar flow hood. To further reduce microbial load that might interfere with the plaque assay, both sides of the lettuce leaves were exposed to UV light for 1 min, whereas the metal disks were autoclaved at 121°C for 15 min. The meat products were used without pretreatment. Several disks of each food item to be tested were cut by pressing the mouth of a sterile plastic scintillation vial with an inside diameter of 1 cm (Fisher Scientific) against each of the food items. The disks were stored in separate sterile Petri dishes and were used within 2 h of preparation.

Adult subjects. Prior permission was obtained from our institutional ethical review board to place FCV on the hands of adult subjects. Each potential subject was given information on the procedure to be used and the relative risks involved before signing an informed consent form. Immediately before the experimental contamination, the hands of each subject were carefully inspected to ensure their freedom from any damage to the skin. In case any damage was evident, the use of that subject was postponed until the skin had healed completely. Six adults (4 females and 2 males ranging in age from 24 to 45 years) participated in this study. Prior to starting each experiment, the subject to be used was asked to wash his or her hands thoroughly with a nongermicidal liquid soap (Ivory), rinse them well in running tap water, and dry them with a paper towel. About 5 ml of 75% (vol/vol) ethanol was placed in the cupped hands and the subject was asked to rub the alcohol over the entire surface of both hands until they became dry and ready for the experimental contamination.

An inoculum of a known concentration of FCV was deposited on demarcated areas on the fingerpads during the experimental procedure. At the end of the experiments, the fingerpads were decontaminated by pressing, for 4 min, on a piece of paper towel soaked in a 10% solution of domestic bleach. The hands were then washed thoroughly with a nongermicidal liquid soap (Ivory) and running tap water and dried with a paper towel.

Protocol for transfer of virus from hands to food and metal disks. The protocol used in this study was modified from a previously described procedure *(4)* to accommodate the use of the different food items of plant and animal origin and the stainless steel disks. A virus suspension of ca. 3×10^7 PFU/ml was made in Earle balanced salt solution (EBSS) containing a soil load (final protein concentration: 0.5 mg/ml mucin, 2.0 mg/ml bovine serum albumin, and 1.5 mg/ml tryptone) to simulate fecal material. In all experiments, 10 μl of viral suspension (ca. 3×10^5 PFU) was used for experimental contamination of each fingerpad or disk of food and metal. For in-put virus titer control, 10 μl of virus suspension was mixed with 990 μl of EBSS and serially diluted in EBSS and plaque assayed.

Protocol for transfer of virus from hands to food and metal disks: (a) virus elution from experimentally contaminated surfaces. To establish a baseline for virus-recovery efficiencies from the fingerpads, lettuce, ham, and the metal disks before and after virus drying, areas on these surfaces were demarcated by pressing the mouth (8 mm inside diameter) of an empty, sterile 1.8-ml plastic vial (Sarstedt Inc., St. Laurent, Quebec, Canada) onto these surfaces (except the metal disks). This was followed by depositing 10 μl of the virus suspension at the center of each demarcated area, and the virus inocula were recovered from at least three of each type of the inoculated surfaces either immediately (time zero, T0) or after air drying for 20 min (T20). The virus was recovered from the fingerpads by pressing the mouth of a 1.8-ml plastic vial containing 990 μl EBSS onto the virus-containing demarcated area on the fingerpad, and the vial was inverted and held in place for 10 s, allowing full contact between the EBSS and the inoculated area, followed by 20 full inversions with the vial still in place. The same process was repeated for a second time, and the surface of the fingerpad was scraped on the inside rim of the vial to recover as much of the fluid as possible. Serial, 10-fold, dilutions of the eluates were made in EBSS for the plaque assay. To determine virus recovery efficiencies at T0 from the food and metal disks, 10 μl of the virus suspension were deposited onto lettuce, ham, and the metal disks, following which the inoculated items were immediately picked up with a pair of sterile forceps and placed inside scintillation vials containing 990 μl EBSS. The same process was repeated to determine T20 except that the virus deposited onto the foods and disks was allowed to air dry (air temp 23 ± 2°C; relative humidity 45%) for 20 min before placing the inoculated items into separate scintillation vials. Each of the vials was vortexed for 30 s, following which serial dilutions were made in EBSS and plaque assayed. Virus recovery efficiencies from the fingerpads, food items, and the disk were determined by calculating the percentage of virus titer obtained before and after drying as follows: baseline = (titer of recovered virus after drying)/(titer of virus at T0) × 100, where T0 represents the titer of FCV when 10 μl of the virus was inoculated on lettuce and immediately recovered in 1 ml and plaque-assayed.

Protocol for transfer of virus from hands to food and metal disks: (b) virus transfer protocol. To test virus transfer from fingerpads to disks of foods and metal (Fig. 1A), 10 μl of the virus suspension was deposited onto each fingerpad and the inoculum allowed to dry for 20 min before allowing the contaminated area of each fingerpad to come in contact with a disk of lettuce, ham, and metal, placed in separate weighing plastic boats resting on the pan of a balance (Sartorius, Mississauga, Ontario, Canada), for 10 s at a pressure of 0.2 to 0.4 kg/cm^2. The virus remaining on the fingerpads and that transferred to each of the contacted items was recovered and plaque assayed. The same basic procedure was also used to study virus transfer from experimentally contaminated disks of foods or metal to clean fingerpads (Fig. 1B).

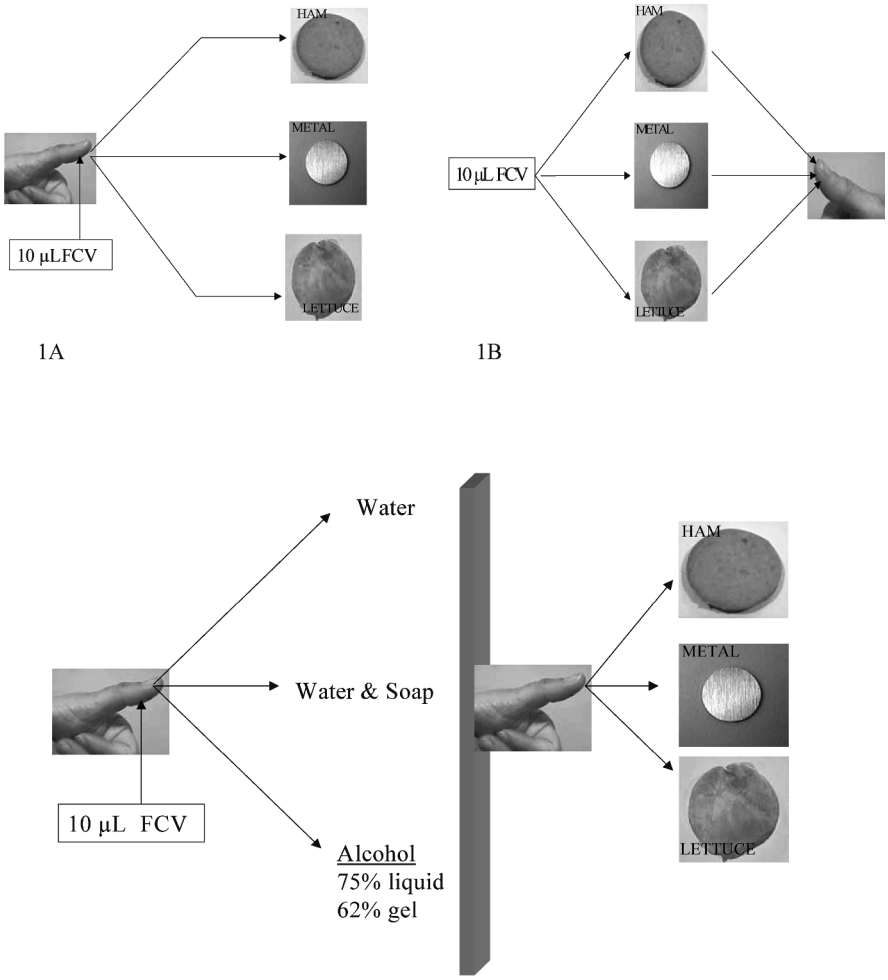

FIGURE 1. *Protocol to investigate the extent of feline calicivirus (FCV) transfer and its interruption: (A) virus transfer from inoculated fingerpads to ham, lettuce, and stainless steel metal disks, (B) virus transfer from inoculated ham, lettuce, and stainless steel metal disks to uncontaminated fingerpads, (C) pretreatment of contaminated fingerpads with interruption agents (water, water and topical agent, and alcohol) prior to touching the food and stainless steel metal disks.*

Protocol for transfer of virus from hands to food and metal disks: (c) interruption of virus transfer (Fig. 1C). The ability of a number of hand decontamination agents, i.e., water with a standard hardness of 200 ppm as $CaCO_3$, liquid soap and water, 62 and 75% alcohol-based rubs, to interrupt virus transfer from the fingerpads to foods and metal disks was investigated as follows: 10 µl of virus suspension was deposited onto each fingerpad, and allowed to air dry for 20 min (air temp 24 ± 2°C; relative humidity 45%). The inoculated fingerpads were then treated with individual intervention agents as follows.

(i) The effect of water on the removal of the dried virus inoculum from the fingerpads was assessed by washing the inoculated area with hard water by pressing the demarcated area of the fingerpad tightly onto the mouth of a 1.8-ml-capacity plastic vial containing 990 µl of hard water, and the vial was inverted upside down maintaining contact between the water and the virus inoculum for 10 s, following which the vial was rocked back and forth five times. The fingerpad was towel dried by pressing slightly on a presterilized piece of paper towel (autoclaved pieces ca. 7 cm by 7 cm), resting on a weighing balance, for 10 s at a pressure of 0.2 to 0.4 kg/cm^2 *(2, 4).* The virus was then recovered as described above. The extent of virus transfer to the foods and metal disks following water treatment was determined by the same procedure except that, after towel drying, the fingerpad was repressed for 10 s (0.2 to 0.4 kg/cm^2 pressure) onto pieces of lettuce, ham, and the metal disks and placed in a weighing boat resting on the balance. The titer of the virus recovered from the foods, the metal disks, and the fingerpads was determined by plaque assay.

(ii) The combined effect of a nongermicidal liquid soap (Ivory) and rinsing with hard water on FCV dried onto the fingerpads was assessed by inverting a vial containing 1 ml of the soap upside down onto the fingerpad while maintaining contact for 20 s. The vial was then removed without scraping the fingerpad. The fingerpad was exposed to hard water and was towel dried and the remaining virus on the fingerpad was recovered and plaque assayed as described above. The same procedure was repeated to determine the extent of virus transfer to the foods and disks after treatment with the topical agent and water except that, after towel drying, the fingerpad was repressed (0.2 to 0.4 kg/cm^2 pressure) for 10 s onto pieces of lettuce, ham, and metal disks and placed in a weighing boat resting on the balance. The pressure applied in these experiments equals that exerted while handling of objects and in contacting environmental surfaces during food preparation. The titer of the virus recovered from the foods, the metal disks, and the fingerpads was determined by plaque assay.

(iii) The same procedure was repeated to investigate the effect of a commercial 62% (wt/vol) ethanol-based gel and a 75% (vol/vol) ethanol solution on the inactivation and/or removal of FCV from fingerpads, except that, following the 20 s contact with the gel-based alcohol (10 s for the liquid 75% alcohol), the fingerpads were allowed to air dry rather than being towel dried. The remaining virus was recovered and assayed in the same fashion. Similarly, the extent of virus transfer to the lettuce, ham, and the

TABLE 1. *Percent (%) transfer of infectious feline calicivirus from donor to recipient surface before and after interruption of such transfer through various treatments*

		% infectious virus recovered (mean ± SEM) from recipient after contact									
				After treatment with:							
Donor surface[a]	Recipient surface	No treatment[b]	*P*[c]	Water alone[b]	*P*	Soap and water[b]	*P*	Ethanol (62%)[b]	*P*	Ethanol (75%)[b]	*P*
Finger	Ham	46.0 ± 20.3		0.9 ± 0.3		0.6 ± 0.2		3.4 ± 0.9		2.3 ± 0.7	
	Lettuce	18.0 ± 5.7	<0.006	0.6 ± 0.1	<0.003	0.4 ± 0.1	<0.004	2.1 ± 0.5	<0.001	1.2 ± 0.3	<0.001
	Metal	13.0 ± 3.6		0.5 ± 0.1		0.5 ± 0.1		1.2 ± 0.2		0.7 ± 0.1	
Ham	Finger	6.0 ± 1.8		ND[d]		ND		ND		ND	
Lettuce	Finger	14.0 ± 3.5	<0.002	ND		ND		ND		ND	
Metal	Finger	7.0 ± 1.9		ND		ND		ND		ND	

[a] Ten microliters of inoculum deposited and allowed to dry.

[b] Percent virus recovered. Virus recovery efficiencies for the ham, lettuce, and metal were determined in separate trials and were used as the 100% baseline.

[c] *P*, one-way ANOVA was used for the statistical analysis of data.

[d] ND, not done.

metal disks following alcohol treatments was determined as described above.

Calculations and statistical analysis. To determine the percent (%) virus transfer, virus recovery efficiency (baseline) from each of the test items (i.e., fingerpads, ham, lettuce, and metal) was first calculated using the following formula:

$$\text{baseline} = \frac{\text{titer of recovered virus after drying}}{\text{titer of virus at time 0}} \times 100$$

Using the baseline as the actual (100%) virus inoculum, the percent transfer was calculated as follows:

$$\%\ \text{transfer} = \frac{\text{titer of recovered virus}}{\text{baseline}} \times 100$$

For each experiment, four randomly selected fingers (two on each hand) were used, and the experiment was repeated with at least two different subjects. The one-way analysis of variance statistical test was used to analyze and compare the results of eight sets of data ($n = 8$) among the four independent different test items (fingerpads, ham, lettuce, and metal disks) as well as among the four different treatments (water, soap and water, 62% alcohol, and 75% alcohol) *(3)*.

RESULTS

Efficiencies of recovery of the dried virus inoculum from the ham, lettuce, metal disks, and the fingerpads were about 79 ± 17, 73 ± 14, 94 ± 12, and 71 ± 13%, respectively, with the highest recovery being from the metal disk.

The results of the reciprocal transfer of the virus from fingerpads to foods and metal disks and vice versa are illustrated in Table 1. Touching foods and the metal disks with virus-inoculated fingerpads resulted in virus transfer of 46 ± 20.3, 18 ± 5.7, and 13 ± 3.6% to ham, lettuce, and the metal disks, respectively. In the reverse-transfer process, whereby uncontaminated fingerpads were pressed onto pieces of virus-inoculated ham, lettuce, and metal disks, the amount of virus transferred to the fingerpads was found to be 6 ± 1.8, 14 ± 3.5, and 7 ± 1.9%, respectively. Throughout the experimental procedure, the greatest variability in virus recovery and/or transfer occurred in the ham as compared with the other test items. Pretreatment of the fingerpads with the intervention agents significantly reduced the extent of virus transfer as compared with that without pretreatment ($P < 0.004$). Pretreatment of the virus-inoculated fingerpads with water rinse alone or a nongermicidal liquid soap followed by a water rinse showed that the combined effect of the water and topical agent slightly outperformed the use of water alone. Following treatment of the virus-inoculated fingerpads with water, the amounts of virus transferred to the ham, lettuce, and metal disks were significantly ($P < 0.003$) reduced to 0.9 ± 0.3, 0.6 ± 0.1, and 0.5 ± 0.1%, respectively, as compared with 0.6 ± 0.2, 0.4 ± 0.1, and 0.5 ± 0.1% following treatment with the soap and water combination ($P < 0.004$). Virus transfer to ham, lettuce, and metal disks from contaminated fingerpads following treatments with 62 and 75% alcohols was significantly ($P < 0.001$) reduced to ≤3.4 and ≤2.3%, respectively (Table 1). Treatment of the contaminated fingerpad with any of the intervention agents significantly reduced the amount of detectable virus on the fingerpad from 71 to ≤13.8% ($P < 0.001$) as illustrated in Table 2. Following treatment with either alcohol, ≤13.8 ± 3.7% of the virus remained on the fingerpads as compared with ≤8.6 ± 1.4% following treatments with water or soap and water combined ($P < 0.05$). The amounts of virus recovered from the contaminated fingerpads, not treated with any of the intervention agents, after touching the ham, lettuce, and the metal disks were 36.3 ± 5.5, 59.6 ± 7.5, and 58.7 ± 6.3%, respectively (Table 2). In contrast, the amount of virus remaining on the fingerpads following pretreatment with the intervention agents and touching the food and metal disks ranged between 4.4 and 23% (Table 2).

DISCUSSION

Noroviruses are now believed to account for >65% of nonbacterial gastroenteritis outbreaks in the United States and Canada *(17, 18, 22)*. This lends even greater urgency to a better understanding of the mechanisms of spread of

TABLE 2. *Percentages (%) of feline calicivirus recovered from the fingerpads, with and without pretreatment with intervention agents and after contact with the recipient surface*

Treatment	% infectious virus (mean ± SEM) detectable on fingerpads[a] before and after treatment[b]	*P*[c]	% infectious virus (mean ± SEM) detectable on fingerpads[a] after treatment and contact with recipient surface[b]			
			Ham	Lettuce	Metal disk	*P*
No treatment	71 ± 8.9		36.3 ± 5.5[d]	59.6 ± 7.5	58.7 ± 6.3	<0.008
Water alone	8.6 ± 1.4		5.1 ± 1.0	6.2 ± 1.4	7.8 ± 1.9	<0.006
Soap and water	5.6 ± 1.3	<0.001	4.4 ± 1.2	7.0 ± 1.8	8.3 ± 2.3	<0.001
Ethanol (62%)	13.8 ± 3.7		16.6 ± 5.5	13.0 ± 4.3	23.0 ± 7.4	<0.009
Ethanol (75%)	11.2 ± 2.8		12.7 ± 4.3	17.0 ± 4.1	19.0 ± 5.0	<0.03

[a] Ten microliters of virus inoculum deposited on fingerpad and allowed to dry.
[b] Percent virus recovered. The efficiency of recovery of virus remaining on the fingerpads after specific treatments was determined in separate trials and was used as the 100% baseline.
[c] *P*, one-way ANOVA was used for the statistical analysis of data.

these viruses in order to devise effective means for interrupting their spread. Many outbreaks of noroviruses are linked to ingestion of foods contaminated at origin or by infected food handlers. Cross-contamination of foods during handling and preparation is yet another way for norovirus transmission. This study was aimed at assessing the amount of virus transfer to and from the hands of adult subjects during the handling of selected food items and when contacting environmental surfaces commonly found in settings where foods are handled. Further, we examined the ability of hand wash and hand-rub agents to interrupt such virus transfer.

In general, ham exhibited the most variable extent of virus recovery when compared with the other items tested. The reasons for the difference are not known but may have been due to either the interference by lacerated meat tissue (due to vortexing) with virus adsorption and/or infectivity of cell culture in the plaque assay or as a result of variable adsorption affinities of the virus particles to these meat matrices.

The visually observed higher levels of moisture on the surface of ham as compared with lettuce and metal disks may account for the higher amounts of virus (46%) transfer to it from fingerpads. In contrast, there was only 6% virus transfer from the contaminated ham to clean fingerpads. This was most likely due to the penetration of the virus inoculum into deeper areas of the meat tissue and thus being unavailable for contact with the fingerpads. In previous studies on hepatitis A virus, Bidawid et al. *(4)* suggested that more than 9% of the virus was transferred from soiled fingerpads to lettuce, whereas Cliver and Kostenbader *(10)* indicated that nearly 66% of porcine enterovirus type 3 was recovered from the surface of a tomato touched by a human finger artificially contaminated with porcine enterovirus type 3–containing fecal material. Data from our current study show that FCV transfer (13 to 46%) from soiled fingerpads to touched foods and surfaces were overall higher than those observed in our previous study with hepatitis A virus. This may be due to a number of factors, including the modification in the virus-elution procedure and the difference in the types of food items tested. Nevertheless, the observed transfers further reaffirm the important role of food handlers in virus spread to foods and environmental surfaces. Previous reports have suggested that the amount of norovirus shed in stools of infected individuals could be in the range of 10^6 to 10^7 virus particles per gram and that the minimal dose of norovirus required to cause human infection could be between 10 and 100 infectious viral units *(14, 24)*. The amount of fecal material that might be present on human hands, which become soiled due to unhygienic practices, is unknown and could vary widely between different individuals. However, taking into consideration the number of virus particles present in feces, even a small amount of fecal material (e.g., 1 mg) could easily contain 10^3 to 10^4 virus particles. Assuming that only half (5×10^2) of the virus particles are infectious and using a worst-case scenario of a minimum of 18% virus transfer from fingerpads to lettuce, as seen in this study, indicates that at least 50 to 80 infectious viral units could be transferred to the lettuce by the process of touching, which most likely would be sufficient to initiate infection in susceptible individuals. While virus recovery from stainless steel disks was the highest, they demonstrated the least amount of virus being transferred to them from the fingerpads (13%), possibly because of the smooth, hard, and nonabsorbent nature of such material. They were also poor donors, where only 7% of the virus was transferred from them to clean fingerpads.

Our findings attest to the strong potential of norovirus cross-contamination of foods if they were touched by soiled hands. However, treatment of FCV-contaminated hands with water alone or a nongermicidal liquid soap and a water rinse prior to touching the food and metal disks reduced the amount of virus recoverable from the fingerpad to significantly lower levels (8.6%) as compared with 71% recoverable virus from untreated fingerpads ($P < 0.001$). Furthermore, the amount of virus transfer to foods and metal disks touched by pretreated fingerpads was significantly reduced to levels as low as 0.4% ($P < 0.004$). The standard error values associated with virus transfer data obtained in this study are reflective of a tightly controlled study in a laboratory setting and would most likely be of greater var-

iability in a natural environment. Although alcohol-based agents were not as efficient as water or water and soap in virus removal, nevertheless, they contributed to a significant ($P < 0.001$) reduction in virus titer from 71 to ≤13.8% on fingerpads treated with either 62 or 75% alcohol. Furthermore, treatment with alcohols contributed to lowering the amount of virus transfer significantly down to ≤3.4% from the virus remaining on the fingerpads as compared with ≤46% virus transfer from untreated fingerpads ($P < 0.006$).

A standard drying period of 20 min was selected to ensure that the virus inoculum was visibly dry on the hands of various subjects, thus confirming that at the time of transfer there was no visible moisture left on any of the fingerpads. Previously, our method of virus recovery from experimentally contaminated articles of food was by repeatedly pipetting the eluent over the contaminated area *(4)*. In this study, virus recovery from all test items was achieved by vortexing them in EBSS for 30 s. This modification may have resulted in higher and more uniform virus recoveries.

We used the protocol of Ansari *(2)* for the decontamination of the hands and this method is now a standard (#E-1838) of ASTM International (West Conshohocken, Pa.) to assess the virus-eliminating activity of hand wash and hand-rub agents. It represents a quantitative and closed system where the fingerpads of adult subjects are contaminated with a precisely measured volume of the test virus and the contaminated area exposed to a standardized amount of the test formulation. Friction to simulate hand washing is applied during the elution procedure and by scraping the skin on the inside lip of the vial with the eluent. The results obtained with this method have been found to be predictive of those using the whole-hand method *(2)*.

In conclusion, this study provides new information on calicivirus transfer from contaminated fingerpads to foods, which are most likely to be ingested without further processing, and to metal surfaces commonly found in settings where food is processed. It also addresses the effectiveness of selected hand washing and hand-rub agents in interrupting calicivirus transfer. These results emphasize the importance of proper hand washing as the easiest and most effective means of reducing the transfer of virus from the contaminated fingerpads, leading to a significant reduction in the risk of virus spread and infection. The combined effect of soap and water was most effective in removing virus contamination from the fingerpads, thereby significantly reducing the levels of virus transfer. The mechanical removal of FCV by hard water alone was nearly as effective as the combined effect of soap and water. In contrast, ethanol-based rubs were somewhat less effective as evidenced by a lower reduction in virus transfer as compared with that with the other treatments. Nevertheless, alcohol treatment significantly reduced the amount of residual virus present on treated fingerpads, and consequently contributed to a lesser virus transfer to foods and metal items.

Overall, all treatments significantly ($P < 0.03$) reduced the levels of virus transfer as compared with that without pretreatment, thereby reducing the risk of virus spread and infection through foods and surfaces. Although none of the treatments completely removed or inactivated FCV on the fingerpads, adhering to these hygienic practices with more emphasis on proper use, i.e., more thorough lathering with topical agents, rinsing with water, and proper drying of washed hands would be necessary to produce an incremental reduction in pathogen contamination on hands. Even though our data indicate that the application of soap and water only marginally outperformed the use of water alone in virus removal and inactivation, nevertheless, good lathering with soap (which was not done in this study) may assist in greater removal and inactivation of the virus than would water alone. Future studies using specifically designed protocols to address the efficacy of lathering with soap are needed to better clarify this issue.

Further work will be needed using the protocols reported here to assess the relative effectiveness of the wide variety of hand-wash and hand-rub agents in common use by food handlers. Ethanol-based hand rubs are generally very useful in the decontamination of hands between handwashings and the somewhat lower activity of such agents against nonenveloped viruses such as FCV should not detract from their continued use by food handlers as well as healthcare personnel.

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