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Contamination of Foods by Food Handlers: Experiments on Hepatitis A Virus Transfer to Food and Its Interruption

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Hepatitis A virus (HAV) is an important pathogen which has been responsible for many food-borne outbreaks. HAV-excreting food handlers, especially those with poor hygienic practices, can contaminate the foods which they handle. Consumption of such foods without further processing has been known to result in cases of infectious hepatitis. Since quantitative data on virus transfer during contact of hands with foods is not available, we investigated the transfer of HAV from artificially contaminated fingerpads of adult volunteers to pieces of fresh lettuce. Touching the lettuce with artificially contaminated fingerpads for 10 s at a pressure of 0.2 to 0.4 kg/cm^2 resulted in transfer of 9.2% ± 0.9% of the infectious virus. The pretreatments tested to interrupt virus transfer from contaminated fingerpads included (i) hard-water rinsing and towel drying, (ii) application of a domestic or commercial topical agent followed by water rinsing and towel drying, and (iii) exposure to a hand gel containing 62% ethanol or 75% liquid ethanol without water rinsing or towel drying. When the fingerpads were treated with the topical agents or alcohol before the lettuce was touched, the amount of infectious virus transferred to lettuce was reduced from 9.2% to between 0.3 and 0.6% (depending on the topical agent used), which was a reduction in virus transfer of up to 30-fold. Surprisingly, no virus transfer to lettuce was detected when the fingerpads were rinsed with water alone before the lettuce was touched. However, additional experiments with water rinsing in which smaller volumes of water were used (1 ml instead of 15 ml) showed that the rate of virus transfer to lettuce was 0.3% ± 0.1%. The variability in virus transfer rates following water rinsing may indicate that the volume of water at least in part influences virus removal from the fingerpads differently, a possibility which should be investigated further. This study provided novel information concerning the rate of virus transfer to foods and a model for investigating the transfer of viral and other food-borne pathogens from contaminated hands to foods, as well as techniques for interrupting such transfer to improve food safety.

Hepatitis A is a common form of acute viral hepatitis in many parts of the world. It is responsible for significant worldwide morbidity and occasional mortality (21, 27). Outbreaks of hepatitis A occur periodically throughout the world, and fecally contaminated food and water are the main vehicles (4). Although less than 10% of the cases of hepatitis A in the United States are associated with food-borne outbreaks (7), substantial costs are incurred by both society and the food industry as a result of these outbreaks (11). The foods implicated in these outbreaks include shellfish (13, 14, 18, 22, 28, 31), sandwiches, dairy products, baked products, salads, fruits, and vegetables (9, 15). Examples of such outbreaks include the outbreak in Denver, Color., in 1992, in which more than 5,000 individuals were exposed to hepatitis A virus (HAV) due to consumption of a variety of gourmet foods prepared by an infected food handler (11). A recent outbreak in Michigan, which resulted in more than 200 cases of infectious hepatitis in school children, occurred due to the consumption of imported contaminated strawberries (6, 20). In nearly 50% of hepatitis A cases, the mode and vehicle(s) of virus spread remain unidentified (17). A number of reports have suggested that infected food handlers may play an important role in food contamination in many cases (8, 11, 30). However, our understanding of the ability of hands to transfer viruses such as HAV to foods is limited, and this in turn hampers the institution of proper hand hygiene measures to reduce the risk of food contamination.

This study was designed to develop an experimental procedure to investigate the amount of HAV that is transferred from artificially contaminated hands of adult volunteers to lettuce, both with and without prior treatment of hands with water or with a topical agent followed by rinsing with water and drying, as well as alcohol treatment with air drying.

MATERIALS AND METHODS

Cells and viruses. Seed cultures of FRhK-4 cells and HAV strain HM-175, which were kindly provided by M. D. Sobsey of the University of North Carolina, Chapel Hill, were cultivated and maintained and virus pools were prepared as described previously (24, 25). The virus pools used consisted of unconcentrated harvested cells and were stored as 1-ml aliquots at −80°C until they were needed.

Plaque assay. HAV titers were measured by performing plaque assays (24). Briefly, cell monolayers were grown overnight in a 12-well culture plates (Costar/Fisher Scientific, Ottawa, Ontario, Canada) at 37°C in the presence of 5% CO_2. A 100-μl portion of a virus dilution was inoculated into each of three wells. The virus was allowed to adsorb to the cells for 90 min at 37°C in the presence of 5% CO_2, and then 2 ml of a semisolid agarose-containing overlay was added to each well. The plates were incubated in a humid atmosphere at 37°C in the presence of 5% CO_2 for 8 days. The process used to fix and stain the monolayers for plaque counting has been described previously (29).

Topical agents, water, and drying. Standard hard water (3) and two topical agents were tested to determine their ability to interrupt transfer of HAV. One of the topical agents (designated P1) was nonmedicated Ivory soap (Procter & Gamble Co., Toronto, Ontario, Canada), and the other (designated P2) was the commercially used medicated (antimicrobial) topical agent Septiline (Wood Wyant Inc., Mississauga, Ontario, Canada). Alcohol-based Purell hand rub gel (Gojo Industry Inc., Akron, Ohio) containing 62% alcohol and aqueous 75%

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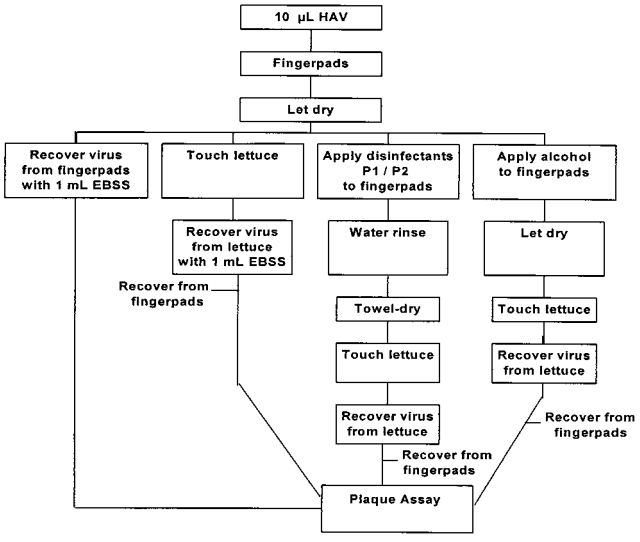

FIG. 1. Schematic illustration of the procedure used to determine the rates of HAV transfer from contaminated fingerpads of human volunteers to lettuce. Ten-microliter portions of HAV were inoculated onto demarcated areas on fingerpads of volunteers. After air drying, the contaminated fingerpads, before and after treatment with topical agents, were pressed gently on lettuce. The virus was then recovered from the fingerpads and the lettuce and plaque assayed in order to determine rates of virus transfer. P1 and P2 are topical disinfectants. The amount of virus remaining on the fingerpads was determined after the lettuce was touched.

alcohol were also included in this study. Autoclaved square pieces (ca. 7 by 7 cm) of paper towel were used for fingerpad drying.

Inoculation of lettuce with HAV. Romaine lettuce, purchased locally, was used as a representative vegetable in this study. Individual lettuce leaves were cut into rectangular pieces (ca. 6 by 7 cm), washed with nongermicidal liquid Ivory soap (99.4% pure), thoroughly rinsed in water for 2 min, and allowed to dry for approximately 20 min in a laminar flow hood. Each side (front and back) of each lettuce piece was then exposed to UV light for 1 min to reduce and/or inactivate contaminating microorganisms that might interfere with the plaque assay. Each piece of lettuce was then placed in a clean, UV-disinfected weighing boat.

To establish a baseline for recovery of virus from lettuce, 10 μl of an HAV preparation (10^5 PFU) was spread evenly over a demarcated area (approximately 2 by 1 cm) on a lettuce midrib and was allowed to dry in a laminar flow hood for 20 to 30 min. The virus was then recovered (see below) from the lettuce, its titer was determined, and the percentage of virus recovered (baseline for lettuce) was determined as follows: baseline = (titer of recovered virus after drying)/(titer of virus at time zero) × 100. The titer of virus at time zero was the titer of HAV obtained when 10 μl of a virus preparation was inoculated onto lettuce and then immediately recovered with 1 ml and plaque assayed.

Virus recovery from inoculated lettuce. The inoculated virus was recovered from lettuce by repeated (>25 times) pipetting of the demarcated area with 1 ml of phosphate-buffered saline (pH 7.6) by using the fine end of a sterile 1-ml tip that was attached to a model 1000P Gilson pipettor (Mandel, Toronto, Ontario, Canada). The eluent and any visible droplets remaining on the lettuce were collected from the boat by using the same tip, serially diluted, and plaque assayed. The same process was used to recover the virus from lettuce following transfer from experimentally contaminated fingers.

Volunteers. Eleven males and females (ages, 24 to 45 years) participated in this study. A known concentration of HAV was deposited on demarcated areas on the fingerpads of these volunteers during the experiment. Previous studies have demonstrated that a 1:10 dilution of domestic bleach containing ca. 5,000 ppm of available chlorine effectively reduces the titer of HAV by more than 4 $\log_{10}$ after 1 min of exposure, whereas exposure for 3 min reduces the virus titer (10^5 PFU/10 μl) to levels that are below the limit of detection (23, 24, 26). Therefore, at the end of each experiment, the fingerpads were decontaminated by pressing them for 4 min on a piece of paper towel soaked in a 10% solution of domestic bleach (Javex Bleach, Toronto, Ontario, Canada). The hands were then washed thoroughly with liquid soap and running tap water and dried with a paper towel.

Protocol for transfer of virus from hands to lettuce. The procedure used to assess virus survival on hands described by Ansari et al. (1, 2) was modified to incorporate virus transfer to lettuce (Fig. 1). In all experiments, 10-μl portions of a viral suspension (ca. 1.3×10^5 PFU, containing 5% fetal bovine serum to simulate an organic soil load) were used. For the input virus titer control, 10 μl of the virus suspension was serially diluted in Earle's balanced salt solution (EBSS) and plaque assayed.

Each fingerpad was demarcated by pressing it tightly against the mouth (inside diameter, 8 mm) of an empty sterile 1.8-ml plastic vial (Sarstedt Inc., St. Laurent, Quebec, Canada), and a 10-μl portion of a virus suspension was placed at the center of each demarcated area. The actual amount of virus in the inocula on the fingerpads (zero-time control) was determined by immediately recovering the deposited virus separately from four fingerpads (two on the left hand and two on the right hand) as follows. The contaminated area of a fingerpad was placed over and tightly pressed against the mouth of a plastic vial that was identical to the vial used for fingerpad demarcation but contained 990 μl of EBSS. The vial was inverted (upside down) and held in place for 10 s, which allowed full contact between the EBSS and the inoculated demarcated area; this was followed by 20 complete inversions. The process was repeated, and the surface of the fingerpad was scrapped on the inside rim of the vial to recover as much of the fluid as possible. Serial 10-fold dilutions of the eluates were prepared and used for the plaque assay.

To establish a baseline for virus recovery from the fingerpads after drying, 10-μl portions of a virus suspension were deposited on four fingerpads (two on each hand) and allowed to air dry, and then the virus was recovered from the fingerpads and plaque assayed as described above.

The effect of water on removal of the dried virus inoculum from fingerpads was determined by washing an inoculated area with hard water (i.e., a water rinse) by pressing the demarcated area of the fingerpad tightly onto the mouth of a 25-cm^2 tissue culture flask (catalog no. 152094; Canadian Life Technologies, Burlington, Ontario, Canada) containing 15 ml of hard water (in separate trials, 1 ml of water in a 1.8-ml plastic vial was used instead of 15 ml) and inverting the flask (or the vial) upside down five times. The fingerpad was towel dried by pressing it lightly on a presterilized piece of paper towel resting on a weighing balance (Sartorius, Mississauga, Ontario, Canada) until the indicator on the balance showed a pressure of 0.2 to 0.4 kg/cm^2, which was maintained for 10 s (2, 14). The virus was then recovered as described above. The rate of virus transfer to lettuce following water treatment was determined by the same procedure, except that after towel drying the fingerpad was pressed for 10 s (pressure, 0.2 to 0.4 kg/cm^2) onto a demarcated area on a piece of lettuce placed in a weighing boat resting on the balance. The virus was then recovered from the lettuce and the fingerpads, and each preparation was plaque assayed.

The effect of a viscous topical agent (P1 or P2) on HAV dried on fingerpads was assessed by inverting a vial containing 1 ml of P1 or P2 upside down on a fingerpad while maintaining contact for 20 s instead of the 10 s used for liquid agents. The vial was then removed without scraping the fingerpad. The fingerpad was washed with hard water and towel dried, and the virus remaining on the fingerpad was recovered and plaque assayed as described above. The same procedure was used to determine the rate of virus transfer to lettuce after treatment with disinfectants (P1 and P2) and water, except that after towel drying, each fingerpad was pressed (pressure, 0.2 to 0.4 kg/cm^2) for 10 s on a demarcated area on a piece of lettuce. The virus was then recovered from both the lettuce and the fingerpad and plaque assayed.

The same procedure was used to investigate the effects of a 62% alcohol-based gel and 75% aqueous (liquid) alcohol on inactivation of HAV and/or removal of HAV from fingerpads, except that following contact for 20 s (10 s for the 75% aqueous alcohol), the fingerpads were air dried rather than towel dried. The remaining virus was recovered and assayed in the same fashion. Similarly, the rate of virus transfer to lettuce following alcohol treatment was determined as described above for the P1 and P2 treatments.

Statistical analysis. For each experiment, four fingers (two on each hand) were used, and the experiment was repeated with at least two different volunteers. Since the plaque assay for each finger was done in triplicate, at least 24 sets of data were obtained, and the data were averaged. The baseline level of virus recovery was determined by calculating the percentage of the virus titer recovered from the fingerpads or the lettuce compared to the titer of virus deposited onto the surfaces. The rate of virus recovery from the fingerpads following treatment or following touching of the lettuce was determined by calculating the percentage of the virus titer obtained from the fingerpads or the lettuce compared to the baseline value. The extent of virus transfer to the lettuce was determined by calculating the percentage of the virus titer recovered from the lettuce compared to the baseline recovery value for lettuce. The eight sets of data ($n = 8$) were analyzed by Student's t test (5).

RESULTS

Table 1 shows the extent of virus recovery from fingers and lettuce, as well as the rates of transfer of HAV from artificially contaminated fingerpads of human volunteers to lettuce. The rates of recovery of the dried virus inoculum from fingerpads and lettuce were 70.5% ± 3.5% and 75.8% ± 3%, respectively. These values were considered the baseline values (100%) for all other virus recovery and transfer data obtained in this study.

Following treatment of the inoculated fingerpads with water alone, with P1 or P2 and water, or with 75% alcohol, a maxi-

TABLE 1. Recovery of HAV from contaminated fingerpads of human volunteers and rates of transfer to lettuce after contact with fingerpads

Surface[a]	Drying	Disinfection procedure[b]	Lettuce touched	% Virus recovery (mean ± SE) from:	
				Fingers	Lettuce
Finger	−	None	−	77.5 ± 6.9	
Lettuce	−	None	−		88.5 ± 3.7
Finger	+	None	−	70.5 ± 3.5	
Lettuce	+	None	−		75.8 ± 3.1
Finger	+	Water, towel	−	3.7 ± 0.6	
	+	None	+	53.4 ± 4.9	7.0 ± 0.6 (9.2 ± 0.9)[c]
	+	Water (15 ml), towel	+	6.2 ± 0.7	0
	+	Water (1 ml), towel	+	5.9 ± 0.8	0.23 ± 0.05 (0.31 ± 0.07)
	+	P1, water, towel	−	6.5 ± 1.2	
	+	P1, water, towel	+	2.0 ± 0.4	0.30 ± 0.06 (0.39 ± 0.08)
	+	P2, water, towel	−	4.1 ± 0.8	
	+	P2, water, towel	+	5.2 ± 0.8	0.26 ± 0.05 (0.34 ± 0.7)
	+	62% ethanol (gel), dry	−	64.3 ± 4.0	
	+	62% ethanol (gel), dry	+	45.7 ± 5.0	0.49 ± 0.07 (0.64 ± 0.09)
	+	75% ethanol, dry	−	24.1 ± 2.8	
	+	75% ethanol, dry	+	18.8 ± 3.5	0.35 ± 0.06 (0.46 ± 0.08)

[a] A 10-μl inoculum contained 1.29×10^5 PFU of HAV.
[b] P1 and P2 are topical disinfection agents (see text).
[c] The numbers in parentheses are mean percentages of HAV transferred from fingers to lettuce ± standard errors.

mum of 24% of the virus was recovered from the fingerpads, compared to approximately 70% before treatment.

Touching the lettuce with contaminated fingerpads prior to treatment or interruption with water and/or topical agents resulted in transfer of approximately 9.2% ± 0.9% of the virus to the lettuce. In contrast, touching the lettuce after treatment of the fingerpads with water, with P1 (or P2) and water, or with alcohol (62 or 75%) resulted in transfer of at most 0.64% of the virus to the lettuce. The percentages of virus recovered from the fingerpads after the lettuce was touched were 2 to 6% when topical agents and water were used, 19% when 75% alcohol was used, and 46% when the gel compound containing 62% alcohol was used.

DISCUSSION

Various outbreaks of hepatitis A have been associated with foods contaminated by infected food handlers. The objective of this study was to determine the amount of HAV transferred from artificially inoculated fingerpads of human volunteers (simulating infected food handlers) to lettuce through contact before and after treatment of the fingerpads with different topical agents and water (simulating different kinds of hygienic practices).

Although the same steps, reagents, and techniques were used for all volunteers, variations among the different test subjects in some aspects of the testing procedure were commonly observed. This was shown by the wide range of virus drying times (6 to 14 min) (data not shown) for the fingerpads of different volunteers, the variable rates of virus recovery, and the differences in the amount of virus remaining on the fingerpads or transferred to lettuce following the various steps. Most likely, the skin texture, moistness, dryness, and thickness, as well as other factors, contributed to the variations observed.

The rates of recovery of HAV from fingerpads and lettuce (70.5 and 75.8%, respectively) were not significantly different ($P > 0.05$). The slightly lower rate of recovery from the fingerpads may have been due to the more complex texture and surface variation of the fingerpads than of lettuce.

An important outcome of this study is the finding that the highest level of virus transfer from contaminated fingerpads to lettuce (approximately 9%) occurred after the lettuce was touched with soiled fingerpads without any prior treatment of the fingerpads with interruption agents. In a previous study, Cliver and Kostenbader (10) found that nearly 66% of porcine enterovirus type 3 was recovered from the surface of a tomato touched by a human finger that was artificially contaminated with porcine enterovirus type 3-containing fecal material. Hollinger and Ticehurst (19) found that the amount of HAV excreted in feces of infected individuals ranged from 10^6 to 10^9 virus particles per g. However, the actual minimal infectious dose of HAV required to cause human infection is unknown, although one infectious unit might be sufficient to cause infection. Similarly, the amount of fecal material that might be present on human hands which become soiled due to unhygienic practices is not known and could vary widely for different individuals. Nevertheless, considering the amount of virus present in feces, even a small amount of fecal material (e.g., 1 mg) could easily contain 10^3 to 10^6 viruses. Assuming that the ratio of infectious virus to virus particles is 1:79 (12), at least 13 to 13,000 infectious units could be present in 0.001 g. If a worst-case scenario of a 9% rate of transfer of HAV from fingerpads to lettuce as demonstrated in our study is used, at least 1 to 1,300 infectious HAV units could be transferred to lettuce by touching, which most likely would be sufficient to initiate infections in susceptible individuals.

Treatment of the fingerpads with either water or a topical agent (P1 or P2) followed by water rinsing significantly ($P < 0.05$) reduced the amount of virus remaining on the fingerpads and resulted in a significant ($P < 0.05$) reduction in the amount of virus transferred to the lettuce (≤0.64%). If this finding is applied to the fecally soiled hand scenario described above, the three treatments would reduce the probability of lettuce contamination by about 25- to 100-fold and thus reduce the possibility of virus spread and subsequent infection, particularly if only a few virus infectious units were initially present on the fingerpads prior to treatment. An unexpected finding was that rinsing with 15 ml of water alone removed HAV more efficiently (to levels below detectable levels) from the fingerpads than any of the other topical agents used removed HAV. We used a large volume of water (15 ml, as compared to 1 ml of other topical agents) and the procedure described by Ansari et al. (1) in an attempt to more closely simulate a real-life hand-

washing process in which more water (compared to soap) is used to rinse the soap off the hands. Reducing the water rinse volume to 1 ml resulted in levels of virus removal from the fingerpads comparable to those obtained with 1-ml portions of topical agents. These results suggest that the volume and the force produced by the 15 ml of water (as the flask was being inverted upside down) resulted in removal of more virus from the fingerpads. In contrast, the gentle contact of the 1-ml portions of topical agents for 10 to 20 s with the inoculum did not exert the same amount of force and thus was not as efficient in removing the virus from the fingerpads. It is also possible that the viscous nature of the topical agents caused some deaggregation of the virus on the fingerpads and thus increased the viral counts in the plaque assay or that contact (without lathering) of the topical agents resulted in thin residues which shielded the virus from the water rinse. The finding that water exhibited unexpectedly strong antiviral activity was similar to a previous finding of Cliver and Kostenbader (10), who reported that ordinary tap water exhibited unexplained strong antiviral activity compared to other disinfectants tested in their study. Additional studies to address these observations are warranted.

The reason for the higher rate of virus removal from the fingerpads with disinfectant P2 than with disinfectant P1 is also unclear, although the medicated nature of the P2 topical agent might have contributed to possible virus inactivation on the fingerpads.

Treatment of soiled fingerpads with either the 62% alcohol-based gel or 75% aqueous alcohol before the lettuce was touched significantly ($P < 0.05$) reduced the amount of virus transferred to the lettuce to levels comparable ($P > 0.05$) to those obtained after other treatments (i.e., water and topical agents). Although the amounts of residual virus on the fingerpads treated with 75% alcohol were greater (ca. 19%), these amounts were not significantly different ($P > 0.05$) than the amounts remaining on the fingerpads following treatment with water and/or topical agents. However, significantly ($P < 0.05$) more virus (ca. 46%) remained on the 62% alcohol-treated fingerpads after the lettuce was touched. It is possible that mere contact of the gel with the virus inoculum on the fingerpads for 20 s might not have been sufficient to inactivate and/or remove HAV from the fingerpads compared to the intended use of this preparation as a rubbing compound. The viscous nature of the gel may have shielded the virus, deaggregated it, or fixed it to the fingerpads. Our results indicated that although both 62 and 75% alcohol resulted in significant reductions in the amount of virus transferred to lettuce, the amounts of HAV remaining on the fingerpads treated with these agents, particularly 62% alcohol, may result in greater potential that virus would be transferred to other foods by repeated touching or handling. Therefore, treatment of hands of food handlers with alcohol alone may not be as effective as other treatments in reducing the amount of virus present on fingerpads.

In conclusion, our results demonstrated that hand washing with water, topical agents, and alcohol-based solutions can significantly reduce the probability that virus will be transferred from contaminated fingerpads to produce during food handling. In particular, water and topical agents seemed to be the most effective agents for reducing virus titers on fingerpads. Consequently, the risk of virus spread and infection through foods can be significantly ($P < 0.001$) reduced. Although none of the treatments completely removed or inactivated HAV on the fingerpads, using these hygienic practices properly (i.e., thorough lathering with topical disinfectants and rinsing with water) could reduce the numbers of viruses remaining on fingerpads. In a previous study, Cliver and Kostenbader (10) demonstrated that common disposable plastic gloves provided the best protection since no virus could penetrate the plastic either from within or from without. In view of our results, emphasis should be placed on proper hand washing by food handlers. In addition, the use of disposable gloves should be encouraged, particularly when preparers are handling foods such as fresh-cut produce that require no processing before they are served to consumers. The method described in this paper provides a model for future studies in which workers investigate the transfer of viruses (or other microorganisms) from food handlers to foods and vice versa. Although the many steps in this method were meant to simulate a real-life scenario of food contamination by infected food handlers, certain aspects of the method may need to be modified and/or refined. Future studies may include examinations of the role of greater pressure and friction on virus transfer to foods, the effectiveness of lathering and rubbing on virus removal from hands, and the manner in which different topical agents affect virus removal and/or inactivation.

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