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Enteric Virus Survival during Household Laundering and Impact of Disinfection with Sodium Hypochlorite▽

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This study was conducted to determine whether enteric viruses (adenovirus, rotavirus, and hepatitis A virus) added to cotton cloth swatches survive the wash cycle, the rinse cycle, and a 28-min permanent press drying cycle as commonly practiced in households in the United States. Detergent with and without bleach (sodium hypochlorite) was added to washing machines containing sterile and virus-inoculated 58-cm^2 swatches, 3.2 kg of cotton T-shirts and underwear, and a soiled pillowcase designed to simulate the conditions (pH, organic load, etc.) encountered in soiled laundry. The most important factors for the reduction of virus in laundry were passage through the drying cycle and the addition of sodium hypochlorite. Washing with detergent alone was not found to be effective for the removal or inactivation of enteric viruses, as significant concentrations of virus were found on the swatches (reductions of 92 to 99%). It was also demonstrated that viruses are readily transferred from contaminated cloths to uncontaminated clothes. The use of sodium hypochlorite reduced the number of infectious viruses on the swatches after washing and drying by at least 99.99%. Laundering practices in common use in the United States do not eliminate enteric and respiratory viruses from clothes. The use of bleach can further reduce the numbers of enteric viruses in laundry.

Soiled laundry has been implicated as a potential route of disease transmission. Laundry has been associated with outbreaks of salmonellosis, bacterial and viral meningitis, Q fever, and ringworm (1, 2, 22, 27). Many of these instances were due to "contact with linens or aerosols associated with bed making, linen sorting, or related activities" (5, 28). Furthermore, it has been shown that damp laundry may promote the growth of bacteria (2).

Previous research on the survival of virus during laundering has been limited to vaccinia virus (22) and poliovirus (9). Little or no research has been undertaken on other enteric viruses likely to be present in laundry. To this end, the survival of several enteric viruses during laundering with and without chlorine bleach was studied. The viruses used in this study were rotavirus, hepatitis A virus (HAV), and adenovirus, which are all transmitted by the fecal-oral route. The purpose of this study was to determine their survival on clothes after washing and drying in a home washing machine using procedures commonly practiced in today's households.

Enteric viral pathogens are the most important causes of diarrhea and enteritis in children under 2 years of age. Among reported cases of diarrhea in children younger than 2 years, rotavirus was the etiological agent in 46 to 52% of the cases (4, 8). Nosocomial rotavirus infections are common. In one study, 17% of the children hospitalized for nondiarrheal illness developed diarrheal illness associated with rotavirus (20). HAV is an excellent candidate for study because of its known resistance to inactivation by high temperatures. HAV infection is probably severely underreported because of the large number of subclinical cases (17) and poor reporting of diagnosed cases by physicians. HAV is the most prevalent cause of viral hepatitis, accounting for 45 to 65% of cases (16). HAV or infectious hepatitis is spread by the fecal-oral route, and transmission is "markedly influenced by the level of sanitation" (14). HAV is most communicable during the incubation period (14) before clinical symptoms develop. A free-chlorine residual level of 10 mg/liter will inactivate HAV after 15 min at 20°C (14).

Slightly less than half of the adenovirus serotypes cause some type of respiratory or enteric disease (15). Five percent of the acute respiratory disease in children under 5 years of age is caused by adenovirus (6). Furthermore, adenovirus is the second leading cause of viral diarrhea in children. Adenoviruses appear more sensitive to chlorine than some of the other enteric viruses (12, 26). All of the enteric viruses selected for study are excreted in the feces for 3 days to several months, with peak concentrations of 10^{10} to 10^{11}/g of feces (14, 29).

MATERIALS AND METHODS

Washing and drying procedures. Cotton cloths were laundered and cut into 58-cm^2 swatches, which were wrapped in paper and autoclaved. Sets of swatches were inoculated with 10 ml of viral suspensions in Tris-buffered saline (Sigma Chemical Co., St. Louis, MO) and left at room temperature for 30 min before washing. Approximately 10^6 to 10^9 50% tissue culture infective doses of virus was added to four swatches. Adenovirus could not be grown to as high a titer as poliovirus and rotavirus; thus, lower concentrations were inoculated onto the swatches.

Inoculated swatches were washed with 3.2 kg of sterile ballast material consisting of men's cotton T-shirts and underwear. An equal number of sterile swatches were included in some experiments to evaluate virus transfer among the fabrics. Ballast clothing was sterilized by autoclaving before each experiment. One pillowcase containing an organic load was then added. This synthetic organic load (31.2 g sebum base, 2.4 g triethandamine, 3.78 g gelatin; Sigma Chemical Co., St. Louis, MO) was added to simulate changes in pH, oxidant degradation, undissolved solids, turbidity, and staining and soil which occur in average laundry loads. Use of the synthetic organic load allows for a constant detergent or chlorine demand for each washer load. The organic load is designed to provide a demand for the chlorine in the range found in the average laundry

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TABLE 1. Enteric virus survival after laundering with detergent only

Sample	Log_{10} titer (SD)[b]		
	Rotavirus	HAV	Adenovirus
Inoculated swatch	6.52 (0.19)	6.42 (0.23)	5.19 (0.19)
Swatch after washing[a]	3.64 (0.10)	3.68 (0.42)	4.08 (0.09)
Swatch after drying	3.32 (0.20)	3.39 (0.12)	2.72 (0.69)
Transfer swatch after washing	3.54 (0.43)	3.18 (0.00)	3.40 (0.67)
Transfer swatch after drying	3.35 (0.430)	3.43 (0.07)	3.40 (0.67)

[a] 58-cm^2 surface area.
[b] Three to six replicates.

load found in a regular household (E. Shaheen, Clorox Company, personnel communication).

A heavy-duty vertical-axis washing machine was used. All experiments were performed with the high water level setting, which had a capacity of 69 liters of water. A 12-min wash cycle was followed by a 3-min rinse. The water temperature was adjusted to 20 to 23°C by adding ice to the water in the machine. This is the average temperature of a cold-water wash in the United States (E. Shaheen, Clorox Company, personnel communication). Ballast clothing was placed in the washing machine before it was filled with water. When the washer was full, powdered household detergent (linear alkyl benzene sulfonate, sodium carbonate, alkyl sulfate—an anionic surfactant) and, in some tests, 1 cup (236 ml) of household bleach (5.25% sodium hypochlorite) were added and the water was allowed to agitate for a few seconds to mix. The detergent was added at the concentration (61.1 g or 0.88 g/liter) recommended by the manufacturer. An organic load (urea, sebum base, triethanolamine; Sigma Chemical Co., St. Louis, MO) added to one of the pillowcases resulted in free-chlorine levels in the wash load of 114 to 125 mg/liter, which are typical of those found in household laundry (E. Shaheen, personal communication). The free-chlorine levels in the wash water were found to be between 114 and 125 mg/liter in experiments in which bleach and detergent were added together. Experiments without bleach had free-chlorine levels of less than 0.1 mg/liter. The test swatches were added last, and the machine then began the 12-min wash period. After the wash and rinse cycles were completed, the clothes were allowed to remain in the washer for 30 min before being transferred to a heavy-duty tumble dryer. This was done to simulate typical practices in the home. After the wash and rinse cycles, 5 ml of water was collected and added to an equal volume of 2× Tris-buffered saline (Sigma Chemical Co., St. Louis, MO) containing 2% sodium thiosulfate. The pH of the buffer was then adjusted to 7.0 by addition of 1 N HCl if necessary. The sample was then passed through a 0.45-μm-pore-size membrane filter (Gelman Sciences, Ann Arbor, MI) to remove bacteria. Two milliliters of Trypticase soy broth (Difco, Sparks, MD) was first passed through the filter to prevent adsorption of the virus to the filter. The clothing was dry to the touch upon removal from the dryer. The temperature of the clothing after drying was 55°C. All experiments were repeated three or four times. Each virus was tested separately. The washer was decontaminated between experiments by running two loads without ballast with 500 ml of household bleach and hot water (~40°C). The dryer was decontaminated by running at 55°C without ballast for 2 h.

Recovery of virus from swatches. Viruses were recovered from the swatches by cutting the swatches into 1-cm^2 pieces with sterile scissors and placing them into plastic Ziploc bags with 10 to 100 ml of Bacto peptone (Difco, Sparks, MD) containing 0.1% sodium thiosulfate to neutralize any free chlorine. The swatches were then placed in a Waring blender (Waring Products, New Hartford, CT) and blended at high speed for 20 s. The mixture was then poured through a 0.45-μm-pore-size filter (Gelman Sciences, Ann Arbor, MI) to remove the larger pieces of fabric and bacteria from the solution and then assayed on the appropriate medium. This procedure resulted in an average recovery of 31% of the viruses studied after the swatches were allowed to dry for 30 min at room temperature.

Virus propagation and assay. HAV strain HM 175 (ATTC VR-1402) was propagated in the FhRK-4 line, rotavirus SA-11 (ATTC VR-899) was propagated in the MA104 cell line, and adenovirus type 40 (ATTC VR-931) was propagated in PLC/PRF5 cells. They were then lysed by three successive freezing-and-thawing steps in a 37°C water bath. The viral suspension was centrifuged at 2,500 × *g* for 10 min to remove gross cellular debris, and the supernatant was added to a sterile 250-ml centrifuge bottle. Nine grams of polyethylene glycol and 4.8 g of NaCl were added for every 100 ml of suspension. The resulting mixture was stirred overnight at 4°C and then centrifuged at 10,000 × *g* for 30 min. The supernatant was carefully poured off the pellet, which was then resuspended in Tris-buffered saline (Sigma) at 10 to 25% of the original volume of medium in a cell culture flask. This remaining solution was divided into 50-ml centrifuge tubes, and a maximum of 20 ml of Freon (Sigma) was added to each centrifuge tube. The solution was vigorously mixed until the two solutions were homogeneous. The solution was centrifuged at 2,500 × *g* for 15 min, and the upper aqueous layer was stored at −80°C until needed.

TABLE 2. Enteric virus survival after laundering with bleach and detergent

Sample	Log_{10} titer (SD)[b]		
	Rotavirus	HAV	Adenovirus
Inoculated swatch[a]	7.98 (0.18)	8.30 (0.19)	6.70 (0.00)
Swatch after washing	2.16 (0.11)	1.82 (0.39)	2.61 (0.23)
Swatch after drying	1.10 (1.01)	1.72 (0.18)	2.32 (0.00)

[a] 58-cm^2 area.
[b] Two or three replicates.

The virus concentration was determined by 50% tissue culture infective dose assay, and the results were calculated according to the method of Reed and Muench (18). Samples were diluted serially (0.1 ml in 0.9 ml) in Eagle's minimum essential medium containing 5% fetal calf serum for poliovirus and 10% fetal calf serum for rotavirus. Fifty microliters of each dilution was placed on four replicate cell monolayers in a 96-well tray. The monolayers were examined every other day for 10 days for viral cytopathic effects.

Data presentation. To calculate the amount of virus added to each washer load, the number of viruses surviving on the inoculated swatch after 30 min of air drying at room temperature was multiplied by the number of swatches added to the machine (usually eight swatches). The number of viruses recovered on the swatches surviving after washing, rinsing, or drying was divided by the number detected on the air-dried swatches before placement in the machines and multiplied by 100. Data were log transformed for presentation.

RESULTS

Table 1 shows the survival of the different enteric viruses after the wash and rinse cycles and after 28 min of drying with and without additives in the wash water. Washing with detergent resulted in a reduction of the viruses by 92 to 99% after the rinse cycle. Adenovirus appeared to be more difficult to remove by washing than HAV and rotavirus. Transfer of the viruses to the sterile swatches in the laundry was very efficient for all of the viruses. This indicates that these viruses can be transferred from contaminated laundry to uncontaminated laundry during washing. Drying resulted in some additional reduction in virus, but not as much as washing achieved. Bleach in the presence of detergent resulted in further virus reduction by at least 99.99% after washing (Table 2).

DISCUSSION

Previous studies have been conducted to identify the common pathogens that can be isolated from household and hospital laundry and to elucidate those laundering procedures that are most effective at reducing pathogen numbers (7, 9, 23–25). These studies have been primarily concerned with bacterial survival. Bacterial survival, however, is not an entirely accurate measurement of viral survival. Viruses, in general, are far more resistant to disinfection by chlorination and detergents than are bacteria (3). Limited viral survival studies have ascertained to what extent vaccinia virus and poliovirus survival is affected by different fabrics, different wash temperatures, and different

disinfectants (9, 22). However, no previous studies have been conducted on the survival of enteric viruses of substantial epidemiological importance such as rotavirus, HAV, and adenovirus. If these viruses remain infectious throughout laundering, they may be transmitted to other individuals in a hospital or household setting through direct contact (laundry ⇒ hand ⇒ mouth) or through more indirect routes (laundry ⇒ hand ⇒ food ⇒ mouth). As observed in previous studies, the laundry steps most important in the reduction of viruses were the addition of bleach and drying in an automatic dryer (7). Dilution of pathogens within wash water is also considered to be an important factor in pathogen reduction (7).

Drying for 28 min was not very effective at reducing virus numbers (Table 1). Adenovirus appeared to be affected the most by this process. Interestingly, drying after washing with bleach appeared to have only a small effect on virus reduction. Bleach had the greatest impact in virus reduction (Table 2), with more than 99.99% reduction after the final rinse. Still, HAV and rotavirus were reduced by 99% after the rinse cycle.

Transfer of virus from contaminated to uncontaminated swatches was observed in this study (Table 1). Basically, one item of heavily contaminated clothing can serve to contaminate an entire laundry load.

Studies we have conducted on the occurrence of fecal coliforms on the undergarments of individuals 1 to 50 years of age suggest that 0.01 to 10 g of fecal material is present per garment (11). The reported concentration of enteric viruses in feces of infected individuals ranges as high as 10^{10} to 10^{11}/g (10). This suggests that significant concentrations are potentially present on undergarments before and after laundering.

As little as one cell culture infective virus has the potential to cause illness (13). Therefore, sufficient numbers of rotavirus and HAV survived to cause a risk of infection in laundry washed only with detergent and this is potentially a risk for infection, especially during the transfer of wet laundry from the washer to the dryer, when the hands may become contaminated. Infection may result when the hands are brought to the mouth or encounter food or fomites (13). This risk is especially important in young children (infants and toddlers), who acquire and transmit enteric viruses within their age group very readily. On the basis of this risk and the survival studies, using sodium hypochlorite seems to be an important and effective step in the avoidance of infection by agents transmitted by the fecal-oral route in the household. Bacterial and viral transfer from fomites to the hands and then to the mouth is very efficient (19, 21).

The only other studies on the fate of viruses in laundry were those by Sidwell and colleagues (23, 24), who studied poliovirus and vaccinia virus. Under cold-water washing conditions similar to those in our study, they found that poliovirus type 1 was reduced by about 99% with no detergent and by 99.98% with cationic or anionic detergents (25). Air drying of the fabrics for 20 h resulted in a total reduction of virus of 99.9992%. Machine drying was not studied. In another study, poliovirus was found to survive for 1 to 4 weeks in cotton fabrics and up to 20 weeks in washable wool fabrics (25).

The viruses we studied appeared to be somewhat more resistant to removal by washing with water and detergent. These differences could be due not only to the virus type but also to differences in the methods and laundering products used. Washing in warm or hot water does result in a greater reduction of virus (25). It is estimated that only 5% of all home laundering in the United States currently is done with hot water (E. Shaheen, Clorox Company, personal communication).

In conclusion, significant numbers of enteric viruses were found to survive washing and drying under conditions commonly practiced in households. Addition of sodium hypochlorite with detergent significantly reduced the numbers of viruses. Treatment with bleach alone reliably caused reductions of greater than 99.99%.

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