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Efficacy of handwashing as an aid in the control of rotavirus and *Giardia* transmission

Manthriratna, Gothami Anoma, M.S.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

EFFICACY OF HANDWASHING AS AN AID IN THE CONTROL OF ROTAVIRUS AND *GIARDIA* TRANSMISSION

by

Gothami Anoma Manthriratna

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STATEMENT BY AUTHOR

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SIGNED: Ipanthirratna

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Charles P. Gerba
C. P. Gerba
Professor of Microbiology
and Immunology

Sept. 10, 1989
Date

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ABSTRACT

Diarrhea caused by rotavirus and *Giardia* is a major health problem among children attending day-care centers because of inadequate personnel hygiene. Epidemiological evidence suggesting person-to-person transmission of enteric pathogens has long been recognized. This study was initiated to investigate the effectiveness of handwashing for the removal of rotavirus and *Giardia* from contaminated hands. The palms of participant hands were inoculated with approximately 10^3 *Giardia* cysts or 10^5 plaque forming units of rotavirus and the effect of washing using tap water alone, a liquid soap or a bar soap on their removal was assessed. Handwashing with liquid soap was found to be very effective in the removal of rotavirus and *Giardia* cysts as compared to washing with bar soap or tap water alone. The overall recovery of viruses in both bar soap and liqid soap was low (0.03 - 22.5%), probably due to virus inactivation by the detergent.

INTRODUCTION

The provision of day-care for pre-school aged children has expanded rapidly in the United States as well as in other countries because of the increasing number of women employed outside the home and the increasing number of single parent families. Recently, it has been recognized that these facilities may serve as significant reservoirs of infectious disease for an estimated 11.4 million children who spend a minimum of ten hours a week in these day care centers (Child Day-care Infectious Study Group, 1984). Facilities that bring young children in close contact have increased the risk of spread of communicable diseases within this highly succeptible population. Viral infections such as hepatitis and bacterial infections such as shigellosis have long been recognized to spread readily within day-care nurseries because of inadequate personnel hygiene and lack of toilet traning (Weissman et al,1975 and Williams et al,1975).

Day-care aquired infectious diseases have been well documented in medical literature. During the first five weeks of enrollment in a day-care center, children have an average diarrhea attack rate of 25% (Shuman, 1983). The lack of prior exposure makes the children particularly suceptible to infection and their lack of hygenic practices enhances the spread of disease. Family members and the community are also at high risk because children are often asymptomatic carriers of infections that can be clinically manifested in adults.

Acute infections of diarrhea in the children in day-care centers can be caused by many enteropathogens. Infections caused by Giardia, Shigella, Salmonella, rotavirus, enteropathogenic Escherichia coli, Campylobacter, Yersinia, Entamoeba histolitica and adenovirus have affected children, employees and household contacts (Shuman,1983). All of these organisms are transmited by the fecal-oral route.

Giardia and enteric virus infections are most likely to be spread by the fecal-oral route among young non-toilet trained children who have not yet learned good personnel hygiene. Person to person transmission of viruses and parasites is more important than that of bacteria because of their low infectious dose.The organisms are transmitted directly by person to person spread or indirectly by contaminated fomites or through ingestion of contaminated food or drink. The children too young to have learned personnel hygiene practices have close contacts with one another, with the staff of the day-care center and possibly with contaminated fomites such as toys. This will further facilitate the transmission of Giardia and enteric viruses in day-care centers.

Direct contact is an important route in the transmission of diseases. Hand contamination with enteric pathogens and the subsequent hand to hand contamination is responsible for the spread of diseases in day-care centers and in pediatric hospital wards. Day-care centers provide a situation in which the children are gathered into an environment in which the person to person contact is accentuated and the potential spread of many infectious diseases is increased. Furthermore, the environment in day-care centers can be heavily contaminated with enteric bacteria and viruses as was demonstrated by Keswick et al (1983) in a study of environmental surfaces in a day-care center during three non-outbreak periods. Of the 25 samples collected from apparently clean surfaces and from teacher's hands, 16% were positive for rotavirus. Laboratory investigations were made on the survival of two animal viruses, rotavirus SA 11 and poliovirus type 1, and bacteriophage f2 on similar environmental surfaces. Rotavirus SA 11 could be recovered after a 30 minute exposure on a dry surface.

Sattar et al (1985) assessed the potential of fomites and environmental surfaces as vehicles in the transmission of rotavirus diarrhea, under varing temperature and relative humidity conditions. Under low relative humidity conditions (25%) approximately 10% of the virus remained infectious even after ten days. These studies suggested that rotavirus can remain viable on contaminated surfaces long enough to be transmitted to suceptible children.

There is ample evidence that a variety of bacteria can be transmitted by hands (Albert and Condie, 1981; Salzman et al, 1967; Rammelkamp et al, 1964; Casewell and Phillips, 1977 and Knittle et al, 1975). Transmission by hand contact occurs not only with bacteria but also with viruses and parasites. Hand to hand transmission of rhinoviruses have been shown and experimental transfer from the hands to others by an environmental surface has been described (Gwaltney and Hendley, 1982; Gwaltney et al,1978). Rhinoviruses were transmitted from experimentally infected volunteers to succeptible recipients: Virus on volunteers hands were transfered to recipients fingers during 71% of ten-second hand contact exposures. Respiratory syncytial virus is also shown to be transfered from contaminated surfaces to hands (Hall et al, 1980 as cited by Dashner, 1985).

ROTAVIRUS AND DAY-CARE DIARRHEA

In devoloped and temperate countries, about half of all diarrhea in children that requires hospitalization is caused by rotavirus infection. In hospital based studies in Washington DC up to 78% of pediatric diarrhea admissions in winter months were associated with rotavirus infection (Kapikian et al, 1976). In developing and tropical countries, rotavirus accounts for 25% - 50% of hospitalized childhood diarrhea (Feachem et al,1983). In community based studies in Brazil and Bangladesh rotavirus were identified in 4-19% of diarrhea episodes among children under age two (Black et al, 1981; Guerrant et al, 1983). However, in rural Bangladesh 46% of dehydrating diarrhea episodes were rotavirus associated (Black et al, 1981).

Rotavirus particles are spherical, 70nm in diameter and made up of double stranded RNA in two distinct capsid layers that on electron microscopy gives the appearance of a wheel, hence the name rotavirus. A description of the virus, classification, epidemiology and diagnostic methods are given by Bartlett et al (1987). Rotavirus enteritis is generally a disease of young children and infants particularly those in the six month to three year age group with a peak incidence at 9 - 12 months. It is estimated that up to 10^{10} particles per gram of feces is shed by children with diarrhea. Shedding may also occur with children without diarrhea (Melnick and Rennick, 1980; Steinhoff, 1980).

Barron-Romero et al (1975) reported a study carried out in four day-care centers in Mexico city to understand the frequency of asymptomatic rotavirus infections. Rotaviruses were detected in 169 (29.9%) of 564 children without diarrhea. These viruses were present in 62 (20.5%) of 302 adults without diarrhea. These observations indicated that asymptomatic rotavirus infections are not a rare event in children.

Pickering et al (1988) studied the asymptomatic excreation of rotavirus before and after rotavirus diarrhea in children in day-care centers. Asymptomatic rotavirus excreation occured in 50% of the children tested on the day before diarrhea occured, 31% two days before diarrhea and 9% in day 3 through 5 before diarrhea. During the week after cessation of diarrhea, 32% had positive specimens. Twelve percent had positive stool specimens during the second week after diarrhea episodes. Asymptomatic rotavirus shedding before and after a diarrhea episode, as identified in this study, represented a source of transmission that could best be avoided by appropiate handwashing procedures and minimizing fecal contamination of the environment.

Keswick et al (1983) estimated the prevalence of rotavirus shedding in the absence of illness in children in Houston day-care centers. Of the children tested 12.4% were positive for rotavirus, indicating that rotavirus is prevalent in day-care centers and may be transmitted to previosly exposed children or to family contacts because of the close association and the poor personnel hygiene of infants and young children.

In contrast to this study in the U.S., a higher prevalence rate (17.2%) of asymptomatic rotavirus infection was identified in Nigerian day-care centers (Abiodun et al, 1985). This high incidence of asymptomatic rotavirus infection also suggested the need for better hygenic conditions including adequate handwashing practices.

The high incidence of rotavirus infections in day-care centers and their potential for person to person transmission are also revealed by Bartlet et al (1985 a,b) in day-care centers in Pheonix, AZ and by Pickering (1981). Rotavirus was found in 6% of the children and was the second most commonly identified pathogen.

The incidence of rotavirus infection and diarrhea in Houston infant-toddler day-care centers was studied by Bartlett et al (1988b). They also studied the pattern of rotavirus in these day-care centers compared with rotavirus illness in the community, patterns of entry and transmission in the day-care child groups and the role of asymptomatic rotavirus infection in these groups. The rate of diarrhea for the first 12 months of study was 2.62 episodes per child-year. Rotavirus was identified in 49 (8.4%) of these episodes. These rotavirus diarrhea episodes represented only 40% of all rotavirus infections identified; an additional 74 episodes were asyptomatic.

GIARDIA LAMBLIA AND DAY-CARE DIARRHEA

Giardia lamblia, a protozoan parasite, is transmitted in a cyst form that may survive for months in a moist environment. Giardiasis is an infection of the small intestine of man by the flagellated protozoan *G. lamblia*. Symptoms may be absent, but when present may include frequent diarrhea with greasy, foul-smelling stools. There may be fatigue, abdominal cramps, flatulence, anorxia, fever and vomitting. During the infection damage to the intestinal epithelium may take place. The damage leads to malabsorption of carbohydrates, fats, fat-soluble vitamins and vitamin B12. Malabsorption and bile duct inflamation are the most serious complications of giardiasis. As many as 400 million cysts may be passed in the stools during one day.(Feachem et al, 1983).

In one study (Bonner and Dale, 1986) of children entering the first grade, a significantly higher prevalence of giardiasis was found in children from day-care centers compared to children who had not attended a day-care. The prevalence of giadiasis in day-care centers is highest among children under three years old. At this age, children crawl on the floor, are still in diapers and need help with toiletting and handwashing. If the staff is over worked or undertrained in enteric disease control, their own poor hygiene may abet infection spread.

Epidemiologic evidence suggesting person to person transmission of *Giardia lamblia* has long been documented (Black et al 1977). These findings have been confirmed by investigations of *G. lamblia* outbreaks at two day-care centers by Keystone et al (1978). An epidemiologic surveillance for endemic G. lamblia infections in Vermont also indicated that person to person transmission play a role in non - outbreak related giardiasis (Birkhead and Vogt, 1989).

Keystone et al (1984) surveyed for the intestinal parasites in 900 children and 146 staff attending 22 day-care centers in metropolitan Toronto. The prevalence of giadiasis in the children ranged from 2% to 16%. The results of this study showed that giardiasis is endemic in day-care centers in metropolitan Toronto. Previous studies by Black et al (1978) have shown that person to person transmission of intestinal parasites in day-care centers occurs in the young mobile children who are not toilet trained. In contrast, the highest prevalence rates in this study were in older children, 7 - 9 years of age. Infection may have spread to them from younger sibblings.

Polis et al (1986) investigated an outbreak of giardiasis in one urban day-care center in which 35% of the children were infected. Infection was transmitted to at least one household contact of 47% of the infected children, indicating person to person transmission of Giardia lamblia. Pickering et al (1984) evaluated the excreation of G.lamblia in children in day-care centers in Houston. In the two prevalence surveys, they identified Giardia cysts in 21% and 26% of the children respectively.

In a two year prospective study of diarrheal illness in day-care centers in Phoenix, AZ Bartlett et al (1985 a,b) identified Giardia lamblia, rotavirus, and Campylobacter jejuni as the most common pathogens. Giardia lamblia was identified at sometime in 22 (79%) of the 28 participated day-care centers. Giardia was found to be significantly more common in toddlers than in infants. In an Australian day-care center study (Boreham and Shepherd, 1984) 19.7% of the children were infected with G. lamblia and the greatest prevalence was observed in the toddler age group.

The children attending day-care centers in Hampton County, South Carolina showed a prevalence rate of 26% (Sealy and Shuman, 1983). The first graders were identified to have at least six times as much infection as those who do not attend day-care.

SIGNIFICANCE OF HANDWASHING

Giardia lamblia and rotavirus thus appear to be the most important enteropathogens that cause nosocomial infections in day-care centers. Hand washing is considered as the single most important procedure in preventing the spread of nosocomial infections (Steere and Mallison, 1975). Because organisms can be spread from the hands of children and personnel, hand washing after toilet use and diaper changing has been recomended to prevent the spread of enteric infections in day-care centers (Black et al, 1981). The center for disease control recomends a rigorous washing with soap under a stream of water for at least ten seconds (Nahata, 1985).

Although epidemiologists agree that the contaminated hands apparently play a major role in the transmission of pathogenic viruses and protozoa, its experimental evaluation has only begun in recent years. Faix (1987) investigated whether handwashing techniques effectively remove Cytomegalovirus from the hands and if some hand washing agents are more effective than others against Cytomegalovirus. This study suggested that hand washing is an effective means of irradicating Cytomegalovirus from the contaminated hands of caretakers. Ordinary hand soap appeared to be as effective as other more expensive handwashing agents such as chlorohexidine gluconate and povidone- iodine. The results also suggested that in the absence of soap or other agents, rinsing with water alone will limit persistence of virus on hands.

Schurman and Eggers (1985) evaluated the effectiveness of hand washing for the removal of poliovirus. They found that 5 minute washing with soap and water decreases the virus concentration on hands by 2 - 4 logs. They suggested that polio virus binding to the skin was reversible.

Black et al (1981) evaluated the effect of handwashing by children and staff after toilet activities and before eating, on the incidence of diarrhea among children in day-care centers. The incidence of diarrhea was found to decline after the hand washing program was initiated.

Bartlett et al (1985), in a two year study of day-care centers in Maricopa County (Phoenix, Arizona), have shown that day-care centers adhering to specific hygenic procedures, specially child and staff handwashing, had lowered the risk of diarrheal illness compared to those that did not. In the third year of the study, they conducted a trial of day-care center staff training in handwashing and other hygenic practices without monitoring staff practices after training (Bartlett et al, 1988 a). The approach and results of this study differed greatly from those of the previous studies (Black et al, 1981; Bartlett et al, 1985). Although no difference in diarrhea rate in the pre and post - training years was encountered, a significant decrease in the number of infections by *Giardia lamblia* was experienced post-training.

OBJECTIVES

The importance of *Giardia lamblia* and rotavirus in day-care environments has long been noted by epidemiologists. Many studies have been conducted on the prevalence of these two organisms and their potential for person to person transmission in infant-toddler day-care centers. Although handwashing has shown to reduce the incidence of diarrhea in day-care centers, at present no attempt has been made to evaluate how effective handwashing could be in the actual removal of these two organisms. No one has previously studied quantitatively how much virus and *Giardia* remain on the hands and/or are removed after handwashing. Even a few organisms left on the hands after washing could have the potential for transmission to another person, causing infection, because of the low infectious dose for viruses and *Giardia* cysts.

The purpose of this study was to evaluate the removal and retention of rotaviruses and *Giardia* cysts on the hands after washing with liquid and bar soaps (Dial corporation, Scottsdale, Arizona). Simian rotavirus, SA 11 was used as a model for human rotaviruses. Because of the morphological, biochemical and antigenic similarities with the human agent (Schaub et al, 1977; McNulty et al, 1978), SA 11 served as a useful rotavirus model system. *G. muris* cysts were used as a model for *Giardia lamblia*.

MATERIALS AND METHODS

The most common techniques used for evaluating qualitative and quantitative bacteriology of hands include the use of finger streak method in which the subject places the fingers on the surface of a petri plate filled with a selective sampling medium and the cotton swab method in which the hands are sampled with sterile cotton swabs which are then immersed in a sampling medium (Larson, 1984). The cotton swab method of sampling has also been used for the recovery of cytomegalovirus from contaminated hands (Faix, 1987).

During the present study, an attempt was made to simulate the actual handwashing. No standard procedures are available for evaluating virus and protozoa removal by handwashing. A protocol for virus adsorption and elution from hands has been developed using coliphage MS 2 (Joan B. Rose, Unpublished data). MS 2, a RNA tailess phage was applicable as a model for enteric viruses since it has simillar morphological and survival characteristics to the enteroviruses (Bitton,1980). This same methodology was applied to the study of rotavirus removal during handwashing. The palms of the hands were innoculated with suspension of the microorganism and spread over the palms by rubbing the hands together. The cysts or the viruses were then recovered from the hands using a variety of techniques.

The Center for Disease Control recommends a vigorous washing with soap under a stream of tap water for at least 12 seconds (Center for Disease Control, 1975; as cited by Nahata, 1985). In order to meet these conditions, hands were washed under warm running tap water for 12 - 15 seconds in all of the experiments.

ROTAVIRUS SA 11 STUDIES

PREPARATION OF THE VIRUS STOCK

Simian rotavirus SA 11 was propagated in MA 104 (Microbiological Associates, Bethesda, MD) rhesus monkey kidney cells. The cells were grown in 32 oz bottles for 5 days, until a complete monolayer was formed. The cells were innoculated with 0.1 ml of virus suspension, and allowed to adsorb to the cells in a 37^{O} C incubator for 1 hour. After the adsorption period, 50 ml of Eagle's Minimum Essential Media (Gibco laboratarıes, Grand Island, NY) without serum was added to each 32 oz bottle after which they were returned to the incubater.

When nearly 90% of the cell monolayer exhibited virus cytopathogenic effects (5 - 6 days), the bottles were frozen (-70^{O} C) and thawed three times, then a volume equal to the volume of virus suspension of freon (1,1,2 Trichlorotrifluoro ethane, Aldrich Chemical Company, Milwaukee, WI) was added and the resulting mixture was stirred for 30 minutes. The virus - freon mixture was then centrifuged at 9000 x g for 10 minutes to pellet the cell debris. The supernatant containing the monodispersed virus was stored in 3 ml aliquots at -70^{O} C until needed. The concentration of the virus stock was determined by plaque assay (Smith et al,1979). The purified virus stock contained 2.32 x 10^{6} pfu/ml (plaque forming units per milliliter).

CELL CULTURE

MA 104 rhesus monkey kidney cells were grown in Eagle's Minimum Essential Media (MEM) containing sodium bicarbonate (7.5%, Mallinckrodt Inc.,Paris, KY), Hepes buffer (1M, Research Organics, Cleveland, OH), glutamine (200 mM, Fisher Scientific Company, Fair Lawn, NJ), antibiotics (Penicillin/Streptomycin, Kanamycin and Mycostatin, United States Biochemicals, Cleveland, OH), non - essential amino acids (Irvine Scientific, Santa Ana,CA) and 8% fetal bovine serum (Gibco Laboratories, Grand Island, NY). After the cells were grown in 8% media for 3 days, the media was changed to MEM containing 4% fetal bovine serum. When a confluent monolayer had formed cells were used for assays.

SUBJECTS

Three volunteers in our laboratory participated in the hand washing experiments.

VIRUS ADSORPTION AND ELUTION TO AND FROM THE HANDS

Suspensions of virus (0.6 ml) were innoculated onto one palm of the subject and distributed over the hands by rubbing the hands together for approximately 30 seconds. Wrists and top sides of hands were purposely not exposed. After drying for another 30 seconds, virus was recovered off the hands using the following procedures.

(a) Hands were rinsed sequentially with three 300 ml volumes of 1% Tryptic Soy Broth (Difco Laboratories, Detroit,MI) at pH 8.50.

Measurements of pH were done with a Beckman I70 pH meter (Beckman Instruments Inc., Irvine, CA). Each eluate was collected and pH adjusted to 7.20. Subsamples of 20 ml were placed into sterile vials, while stirring the eluents. Each sample was then filter sterilized through 0.45um and 0.22um pore size filters (Deltaware membrane systems, Kimble Division of Owens - Illinois, Toledo, OH). The filters were pre - treated with beef extract (pH = 7.2, BBL Microbiology Systems, Cockeysville, MD). The samples were assayed by the plaque forming unit method.

(b) Hands were rinsed under running tap water rubbing the palms together for 12 seconds (approximately 600 ml of tap water) followed by a single wash with 300 ml of 1% Tryptic Soy Broth. Tap water was dechlorinated by adding sodium thiosulfate (Fisher Scientific, Fair Lawn, NJ) at a concentration of 2 mg / L. The pH of all eluents were adjusted to 7.20, filter sterilized and assayed.

(c) Hands were wetted with 100 ml of tap water and then washed with one pump (2 - 3 ml) of Dial liquid soap (Dial Corporation, Scottsdale, Aizona) for 30 seconds and finally rinsed under running tap water for 12 seconds. Tap water was dechlorinated by addition of sodium thiosulfate. A second elution was conducted immediately using 300 ml of 1% Tryptic Soy Broth. All the washings and eluents were collected, pH adjusted, soap samples were freon extracted, filter sterilized and assayed.

(d) Hands were wetted with 100 ml of tap water and then washed with Dial bar soap for 30 seconds and finally rinsed under running tap water for 12 seconds. Tap water was dechlorinated by addition of sodium thiosulfate. A second elution was conducted immediately using 300 ml of 1% Tryptic Soy Broth. All the washings and eluents were collected, freon extracted, filter sterilized and assayed.

The flow chart below illustrate the handwashing procedure adapted.

wash hands with Tryptic Soy Broth,
tap water, liquid soap or bar soap

↓

washings ⟶ assay

↓

elution with Tryptic Soy Broth
to recover the viruses remaining
on the hands

↓

eluents ⟶ assay

A positive control was run in each of the above steps to determine the number of viruses placed on the hands and to determine if tap water, liquid soap or bar soap affected the plating efficiency of the virus. This was accomplished by placing the same amount of virus that was added on to the hands (0.6 ml) directly into 600 ml of tap water, Tryptic Soy

Broth, liquid soap or bar soap as was desired.

PLAQUE ASSAY FOR ENUMERATION

To determine the titer of the virus stock and the number of viruses recovered off the hands, an agar overlay technique was used (Smith et al, 1979; Ramia and Sattar, 1979).

After formation of a complete monolayer in 25 cm sq. plastic tissue culture flasks (Corning Glass Works , Corning, NY) the maintanance medium was poured off the MA 1Ø4 cells and washed three times with serum free MEM. The cells were then innoculated with Ø.3 ml of the sample (or virus dilution) and incubated for 1 - 1 1/2 hours at 37^{O} C. To enhance virus adsorption to cells the flasks were rotated every 15 minutes, during the period of adsorption. The 2 x MEM containing sodium bicarbonate, Hepes, glutamine, antibiotics, trypsin (1ØØ ug/ml) and DEAE dextran (2ØØØØ ug/ml, Pharmacia Fine Chemicals, Uppsala, Sweden) was prepared. The pH of the 2 x MEM was adjusted to 7.2Ø using 1N HCl. At the end of the adsorption period 2 x MEM was mixed with an equal volume of 1.5% Bacto agar, maintained at 45^{Ø} in a liquid state (Difco Laboratories, Detroit, MI). Eight ml of the agar overlay medium was added to the opposite side of the cells and then rotated to cover the monolayer. The agar media was allowed to solidify and then placed in the incubator. Each sample was assayed in duplicate. After 5 days of incubation at 37^{O} C all the agar in the flasks was shaken out and the cells were stained with Ø.5% crystal violet (Matheson Coleman and Bell,Los Angles,CA). The number of plaques in each flask was counted.

GIARDIA MURIS STUDIES

GIARDIA MURIS STOCKS

G. muris cysts isolated from Gerbil feces were obtained from Diane Swabby (Swabby Gerbco Inc., Pheonix, AZ) and were stored in water at 4^{o} C until needed.

SUBJECTS

Three volunteers in our laboratory participated in the handwashing experiments.

INNOCULATION AND ELUTION

Approximately $1Ø^4$ G. muris cysts (in Ø.6 ml) were pipetted on to one palm of the subject and distributed over hands by rubbing the hands together for 3Ø seconds. After drying for another 3Ø seconds the cysts were recovered off hands using the following procedures.

(a) Hands were rinsed sequentially with three 3ØØ ml volumes of Tris buffered saline (pH = 7.2Ø, Sigma Chemical, St. Louis, MO). Each elution was collected and tagged using flourescent antibodies (Goat anti rabbit, Judy Sach, U.S. Environmental Protection Agency, Cincinnati, OH).

(b) Hands were rinsed under running tap water for 12 seconds (approximately 6ØØ ml of tap water) followed by a single wash with

Tris bufferd saline. Eluents were collected and tagged.

(c) Hands were wetted with 100 ml of tap water and then washed with one pump (2 - 3 ml) of Dial liquid soap for 30 seconds and then finally rinsed with tap water for 12 seconds. A second elution was run immediately with 300 ml of Tris.

(d) Hands were wetted with 100 ml of tap water and then washed with Dial bar soap for 30 seconds and finally rinsed with tap water for 12 seconds. A second elution was run immediately with 300 ml of Tris.

The flow chart below illustrates the handwashing procedure adapted.

wash hands with Tris, tap water,
liquid soap or bar soap

↓

washings ⟶ assay

↓

elution with Tris to recover
the cysts remained on the hands

↓

eluents ⟶ assay

<u>Giardia</u> cysts were added directly to 600 ml of Tris, tap water and/or soapy tap water (produced by washing hands) as positive controls to evaluate the number of cysts placed on the hands and to determine

adequate antibody staining of cysts in Tris, tap water or soapy waters.

FLOURESCENT ANTIBODY TECHNIQUE FOR ENUMERATION

The indirect immunofluorescence assay for the detection of Giardia cysts, as described by Riggs et al (1983) and Sauch (1985) was used to detect the cysts in eluents from the hands.

A membrane filter (Nuclepore Corporation, Pleasanton, CA) with a pore size of 5 um was cut using a #7 cork pore and placed in the 15 mm diameter filter housings (Nuclepore Corporation, Pleasanton, CA). Volumes of 1 - 4 ml of samples were passed through filters using a syringe and washed twice with 10 ml of PBS (Phosphate Buffered Saline). After the sample had been passed through the filter, parafilm (American Can Company, Greenwich,CT) was used to plug the bottom of the filter housing and three drops of the primary antibody (Judith Sach, U.S. Environmental Protection Agency, Cincinnati, OH) was added onto the filter and incubated at room temperature for 30 minutes. Antibodies have previously been titered to optimal concentrations. The filters were washed twice with PBS using 10 ml syringes and three drops of the secondary antibody (Goat anti - Rabbit immunoglobulin G conjugated to fluorescein isothiocyanate, obtained from Judith Sach) was added and incubated for another 30 minutes at room temperature. The filters were washed twice with PBS and mounted on a glass slide with a drop of 95% glycerin/PBS solution, a cover slip was added. The slides were read using a Olympus BH-2 epiflourescent microscope (Scientific Instruments Co.,Tempe,AZ) and the number of Giardia cysts were counted on the entire filter and calculated per volume initially filtered.

CALCULATIONS AND STATISTICAL ANALYSIS

The limit of detection (the minimum value that can be detected in the assay) for rotavirus assay was determined in a 600 ml sampling solution per hand with 0.3 ml aliquots assayed in duplicate for each subject. Thus, if one plaque forming unit (PFU) was observed among the two replicates, the limit of detection would be:

$$\frac{1 \text{ PFU} \times 600 \text{ ml/ hand}}{2 \text{ flasks} \times 0.3 \text{ ml/flask}} = 10^3 \text{ viruses / hand}$$

The limit of detection for G. muris assay was determined in a 600 ml sampling solution per hand with 4 ml aliquots assayed in duplicate for each subject. If one cyst was observed among the two replicates, the limit of detection would be:

$$\frac{1 \text{ cyst} \times 600 \text{ ml/ hand}}{2 \text{ replicates} \times 4 \text{ ml/ replicete}} = 75 \text{ cysts / hand}$$

For statistical purposes, one half of the limit of detection was used to estimate the actual values when no viruses or Giardia were recovered. This estimate is better than using zero, which causes difficulties in statistical analysis (John E. Heinze, The Dial Corporation, Scottsdale AZ; personnel communications).

A statistical computer program (CoStat Statistical Software; CoHort Software, Berkley, CA) was used for one - way Analysis of Variance

(ANOVA) to determine significant differences between the eluents. The arcsin (or angular) transformation of percent recoveries were used in Analysis of Variance in order to satisfy the necessary assumptions for valid ANOVA (Sokal and Rohlf, 1981). The angular transformation of data was done using the following equation.

Angular transformation $\emptyset = \arcsin \sqrt{p}$ where p = proportion.

RESULTS

SA - 11 STUDIES

Table 1 summarizes the number of SA 11 rotaviruses recovered from the hands after washing sequentially with 300 ml of 1% Tryptic Soy Broth. The percent recovery of viruses from the hands is given in Table 2 and Figure 1. The average number of viruses recovered in the first elution were 6.03×10^3 (25.1%), 6.32×10^3 (23.9%), and 6.45×10^3 (28.7%) for the three subjects. However, a substantial amount of virus still remained on hands after the first wash. In the second elution of the series, 1.64×10^3 (6.6%) virus particles were recovered. In the third elution of the series 2.5% of the viruses were recovered. The sequential washing of hands resulted in a total recovery of 35.1%. Previous studies by Joan B. Rose (unpublished data) using MS - 2 bacteriophage resulted in similar percent recoveries after washing hands with Tis Buffered Saline.

The results of tap water washing of rotavirus from the hands are summarized in Tables 3 - 4 and Figure 2. The average percent recovery for washing with tap water alone was 35.1%. The immediate elution with 300 ml of 1% Tryptic Soy Broth resulted in an average of 3.3% recovery. The average concentration of virus placed on hands before washing with tap water was 2.65×10^5.

In the initial assays for the recovery of SA 11 from the hands using liquid soap and tap water as the eluents, plaques were not detected. However, 1.67×10^3 virus particles were recovered from the hands in the

TABLE 1 NUMBER OF ROTAVIRUSES RECOVERED FROM THE HANDS USING SEQUENTIAL ELUTION WITH TRYPTIC SOY BROTH

			Number recovered pfu(x1Ø^3)		
Titer on hands pfu(x1Ø^4)	Subject	Trial	Elution 1	Elution 2	Elution 3
2.25	MY	1	6.15	Ø.75	Ø.9Ø
2.49		2	6.9Ø	1.Ø5	Ø.45
2.5Ø		3	5.Ø3	1.5Ø	1.3Ø
2.49	GM	1	6.38	2.ØØ	1.ØØ
3.ØØ		2	6.23	2.5Ø	Ø.4Ø
2.49		3	6.38	2.5Ø	Ø.5Ø
1.99	MK	1	6.75	2.1Ø	Ø.2Ø
3.ØØ		2	6.45	1.35	Ø.4Ø
1.99		3	6.15	1.ØØ	Ø.45

TABLE 2 PERCENT RECOVERY OF ROTAVIRUS FROM THE HANDS USING SEQUENTIAL ELUTION WITH TRYPTIC SOY BROTH*

		PERCENT RECOVERY		
Subject	Trial	Elution 1	Elution 2	Elution 3
MY	1	27.3	3.3	4.0
	2	27.7	4.2	1.8
	3	20.1	6.0	5.2
	AVERAGE	25.0	4.5	3.7
GM	1	25.5	8.0	4.0
	2	20.8	8.3	1.3
	3	25.5	10.0	2.0
	AVERAGE	23.9	8.8	2.4
MK	1	33.8	10.5	1.0
	2	21.5	4.5	1.3
	3	30.8	5.0	2.2
	AVERAGE	28.7	6.7	1.5

* = Average concentration of virus placed on hands = 2.47×10^4

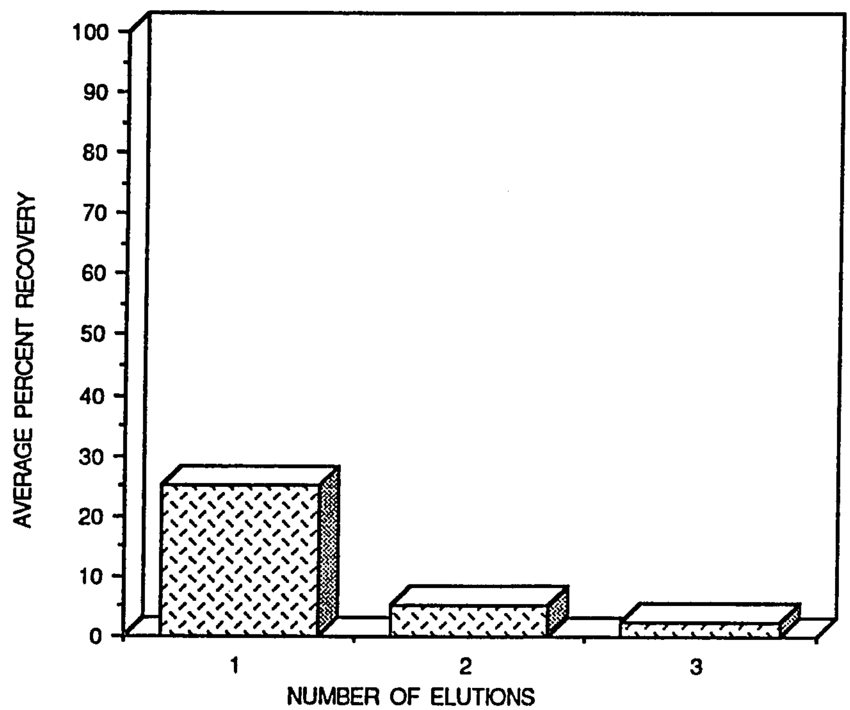

Fig. 1 Percent recovery of rotavirus from the hands using sequential elution with Tryptic Soy Broth

TABLE 3 NUMBER OF ROTAVIRUSES RECOVERED FROM THE HANDS AFTER WASHING WITH TAP WATER

			NUMBER RECOVERED pfu(x10^4)	
Titer on hands pfu(x10^5)	Subject	Trial	Tap water washing	TSB Elution[a]
1.99	GM	1	6.35	0.47
1.90		2	6.95	0.20
2.20		3	8.33	1.11
1.20	MK	1	3.36	1.00
3.80		2	3.30	1.35
2.70		3	9.50	0.85
2.70	MY	1	8.88	0.15
3.60		2	14.20	1.10
3.80		3	14.10	1.70

a = Elution with 300 ml of Tryptic Soy Broth.

TABLE 4 PERCENT RECOVERY OF ROTAVIRUS FROM THE HANDS AFTER WASHING WITH TAP WATER*

Subject	Trial	PERCENT RECOVERY Tap water Washing	TSB Elution[a]
GM	1	31.9	2.4
	2	36.6	1.1
	3	37.9	5.0
	AVERAGE	35.5	2.8
MK	1	28.0	0.8
	2	35.1	3.6
	3	35.2	3.2
	AVERAGE	32.8	2.5
MY	1	32.9	0.6
	2	39.4	3.1
	3	37.1	4.5
	AVERAGE	36.5	2.7

* = Average concentration of virus placed on hands = 2.65×10^5

a = Elution with 300 ml of Tryptic Soy Broth.

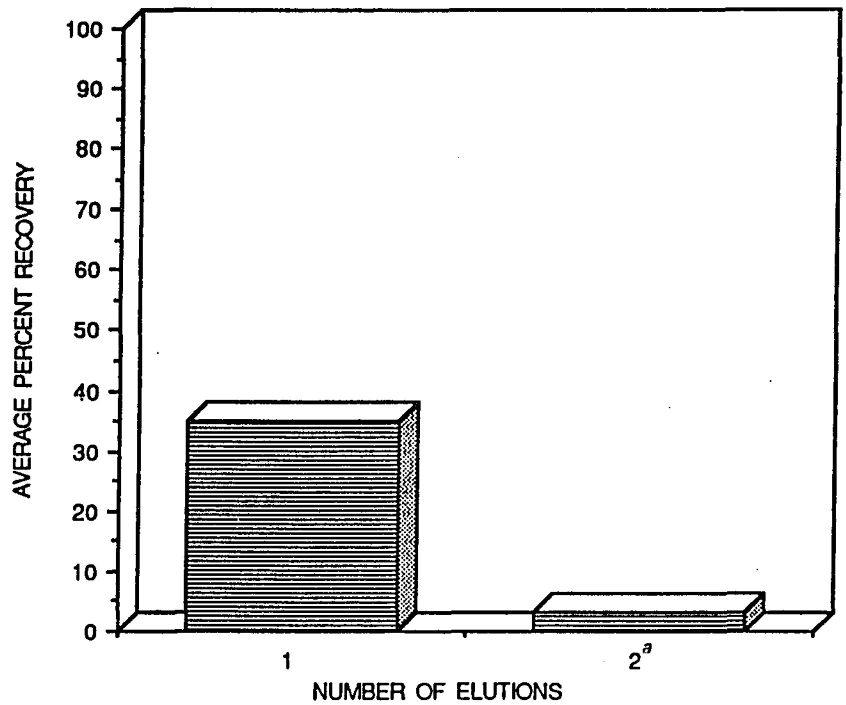

Fig. 2 Percent recovery of rotavirus from the hands after washing with tap water
a = Elution with 300 ml of Tryptic Soy Broth

immediate elution with 300 ml of 1% Tryptic Soy Broth. It was thought that the soap was inhibiting the adsorption of virus to the cell monolayer. Plaques were detected when DEAE dextran was added to the overlay media. When innoculated directly on to the cell monolayers, soap was found to inhibit plaque formation of viruses even at the high dextran concentration (60000 ug/ml). However, 10 - fold dilutions of the eluents in Tris gave detectable plaques. A further increase in dextran concentration caused deterioration of the cell monolayer.

The results of liquid soap washing of SA 11 from the hands are given in Tables 5 - 6 and Figure 3. The average concentration of virus placed on the hands before washing was 9.33×10^5. The number of viruses recovered from the hands after washing was 2.09×10^5 (22.5%). Although the percent recovery of SA 11 from the hands after washing with liquid soap was lower than that of the tap water or the first elution of the sequential elution, the immediate elution with 300 ml of 1% Tryptic Soy Broth yielded a very low percent recovery (0.2%). There was statistically less ($P \leq 0.5$) virus recovered from the hands in the 300 ml of Tryptic Soy Broth post elution with the soaps and more virus remained on the hands when washed with tap water or buffer alone.

When the cell monolayer was innoculated with bar soap eluents, no plaques were encountered even after the use of dextran. Plaques were not formed when the eluents were assayed in larger volumes (1.5 ml) using 75 cm sq. tissue culture flasks. However, the immediate elution with 300 ml of Tryptic Soy Broth resulted in an average recovery of 8.42×10^3 (0.6%).

TABLE 5 NUMBER OF ROTAVIRUS RECOVERED FROM THE HANDS AFTER WASHING WITH LIQUID SOAP

Titer on Hands pfu(x10^5)	Subject	Trial	Number Recovered: Liquid Soap Washing pfu(x10^5)	Number Recovered: TSB Elution[a] pfu(x10^3)
7.10	MK	1	1.57	2.50
10.20		2	2.24	2.50
11.30		3	1.89	0.50
5.60	GM	1	1.18	1.00
9.70		2	1.47	1.50
10.20		3	2.57	2.00
9.00	MY	1	2.15	2.50
10.30		2	3.14	1.50
10.60		3	2.64	1.00

a = Elution with 300 ml of Tryptic Soy Broth

TABLE 6 PERCENT RECOVERY OF ROTAVIRUS FROM THE HANDS AFTER WASHING WITH LIQUID SOAP*

		PERCENT RECOVERY	
Subject	Trial	Liquid soap Washing	TSB Elution[a]
MK	1	22.1	Ø.4
	2	21.9	Ø.3
	3	16.7	Ø.1
GM	1	21.1	Ø.2
	2	15.2	Ø.2
	3	25.2	Ø.2
MY	1	23.9	Ø.3
	2	3Ø.5	Ø.2
	3	24.9	Ø.1

a = Elution with 3ØØ ml of Tryptic Soy Broth

* = Average concentration of virus placed on hands = $9.33x1Ø^5$

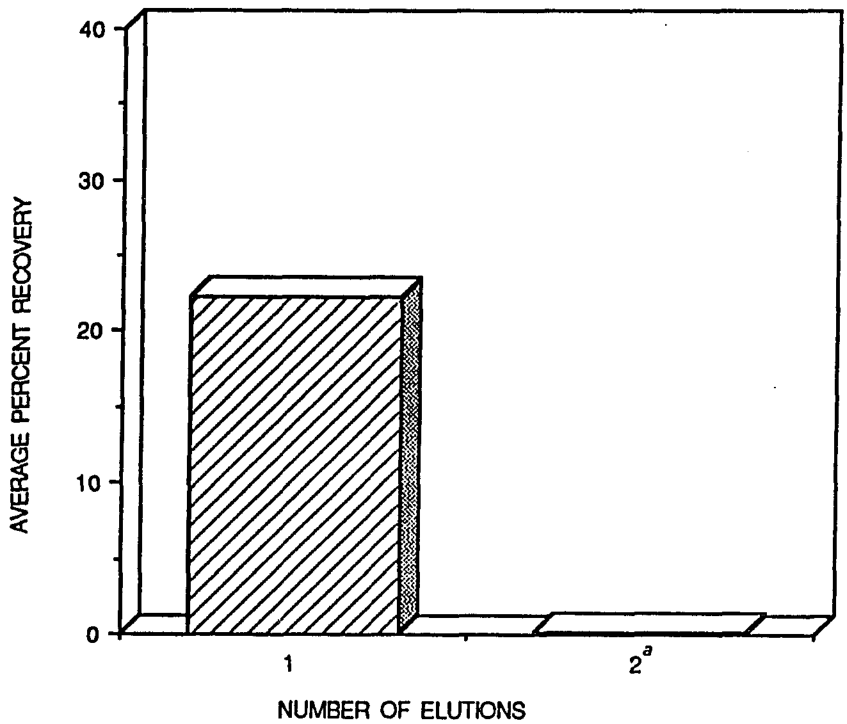

Fig. 3 Percent recovery of rotavirus from the hands after washing with liquid soap
a= Elution with 300 ml of Tryptic Soy Broth

The results for SA - 11 rotavirus recovery from the hands after washing with bar soap are summarized in Tables 7 - 8. The percent recoveries of SA - 11 encountered in the immediate elution with 300 ml of Tryptic Soy Broth were not significantly ($P \leq 0.5$) different whether hands were rinsed with liquid soap or bar soap (Appendix B).

The results for three sequential elutions with 300 ml of Tryptic Soy Broth, tap water, liquid soap and bar soap washings of rotavirus from contaminated hands are compared in Figure 4. This showed that less viruses were recovered from the hands after the first wash with the soaps.The results of completely randomized one - way Analysis of Variance to determine the effects of different handwashing procedures on the recovery of rotavirus from the contaminated hands are given in Appendix A. This showed that washing with Trptic Soy Broth or liquid soap is statistically different ($p \leq 0.5$) than washing with tap water or bar soap.

The plating efficiency of the virus was shown to be affected by the tap water, liquid soap and bar soap. The virus titers obtained for each of the above controls were significantly different ($p \leq 0.5$) from that for Tryptic Soy Broth control. The inhibition of plaque formation was less with the liquid soap than with the bar soap.

GIARDIA *MURIS* STUDIES

The results of sequential elution of *G. muris* with Tris are summarized in Tables 9 - 10 and Figure 5. Of an average of 2.29×10^4 cysts placed on hands, 1.37×10^4 (59.7%), 3.67×10^3 (15.8%) and 2.27×10^3 (9.9%)

TABLE 7 NUMBER OF ROTAVIRUS RECOVERED FROM THE HANDS AFTER WASHING WITH BAR SOAP

Titer on hands pfu(x10^6)	Subject	Trial	NUMBER RECOVERED Bar soap washing	TSB elution[a] pfu(x10^3)
1.98	MK	1	0.0	5.30
1.00		2	0.0	3.00
1.30		3	0.0	6.00
1.70	GM	1	0.0	2.00
1.30		2	0.0	11.00
1.50		3	0.0	8.00
1.60	MY	1	0.0	2.40
1.70		2	0.0	8.50
1.70		3	0.0	8.00

a = Elution with 300 ml of Tryptic Soy Broth

TABLE 8 PERCENT RECOVERY OF ROTAVIRUS FROM THE HANDS AFER WASHING WITH BAR SOAP*

Subject	Trial	PERCENT RECOVERY Bar soap Washing	TSB Elution[a]
MK	1	0.0	0.3
	2	0.0	0.3
	3	0.0	0.5
GM	1	0.0	0.1
	2	0.0	0.8
	3	0.0	0.5
MY	1	0.0	0.2
	2	0.0	0.5
	3	0.0	0.5

* = Average concentration of virus placed on hands = 1.53×10^6

a = Elution with 300 ml of Tryptic Soy Broth.

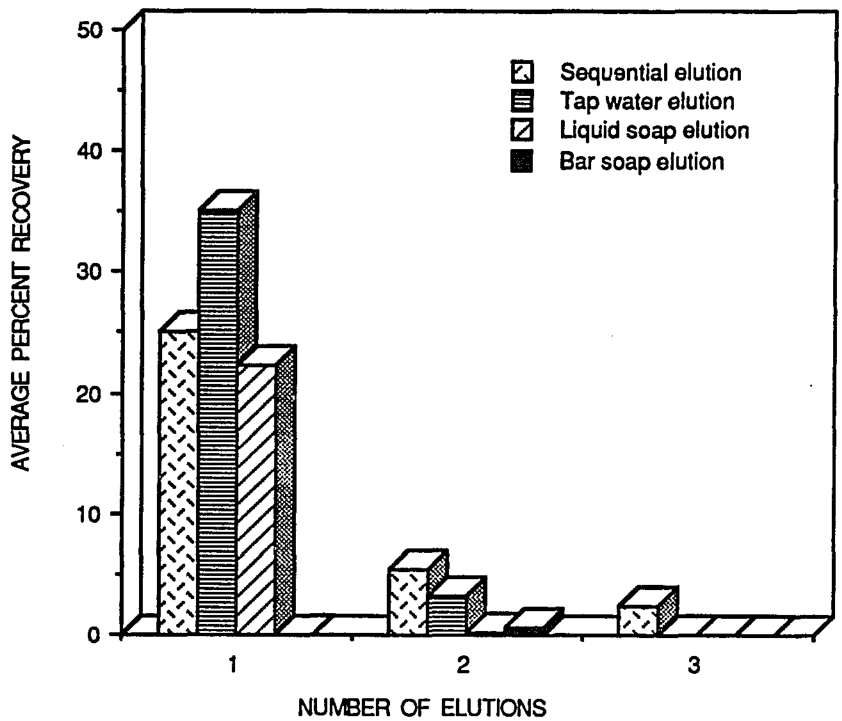

Fig. 4 Percent recovery of rotavirus from the hands using different hand washing procedures

TABLE 9 NUMBER OF *GIARDIA* CYSTS RECOVERED FROM THE HANDS USING SEQUENTIAL ELUTION WITH TRIS

Nunber of cysts placed on hands cysts ($x10^4$)	Subject	Trial	Number of cysts recovered cysts ($x10^3$)				
			1	2	3	4	5[b]
2.67	JR	1	14.1	4.5	3.0	2.7	1.2
2.54		2	13.9	3.9	2.3	1.7	7.5
2.21		3	15.0	2.7	1.8	1.1	4.5
2.06	MK	1	13.5	3.2	2.0	1.1	0.3
2.25		2	13.8	3.9	2.1	1.1	0.5
2.27		3	13.9	3.6	2.6	1.2	0.5
2.18	GM	1	13.2	3.9	2.0	1.2	0.6
2.22		2	13.2	3.6	2.4	0.9	0.5
2.25		3	12.5	3.3	2.4	1.5	0.3

b = Numbers 1 - 5 refer to the sequential elutions

TABLE 10 PERCENT RECOVERY OF G. MURIS FROM THE HANDS USING SEQUENTIAL ELUTION WITH TRIS*

		PERCENT RECOVERY				
Subject	Trial	1	2	3	4	5[b]
JR	1	52.8	16.9	11.2	10.1	4.5
	2	55.0	15.4	8.9	6.5	3.0
	3	68.0	12.2	8.2	4.8	2.0
	AVERAGE	58.6	14.8	9.4	7.1	3.2
MK	1	65.7	15.3	9.5	5.1	1.5
	2	61.3	17.3	9.3	4.7	2.0
	3	61.6	15.9	11.3	5.3	2.0
	AVERAGE	62.9	16.2	10.0	5.0	1.8
GM	1	60.7	17.9	9.0	5.1	2.8
	2	59.5	16.2	10.8	4.1	2.7
	3	55.6	14.7	10.7	6.7	1.3
	AVERAGE	57.9	16.3	10.1	5.4	2.3

* = Average concentration of cysts added onto hands = 2.29×10^4

b = Numbers 1 - 5 refers to the sequential elutions.

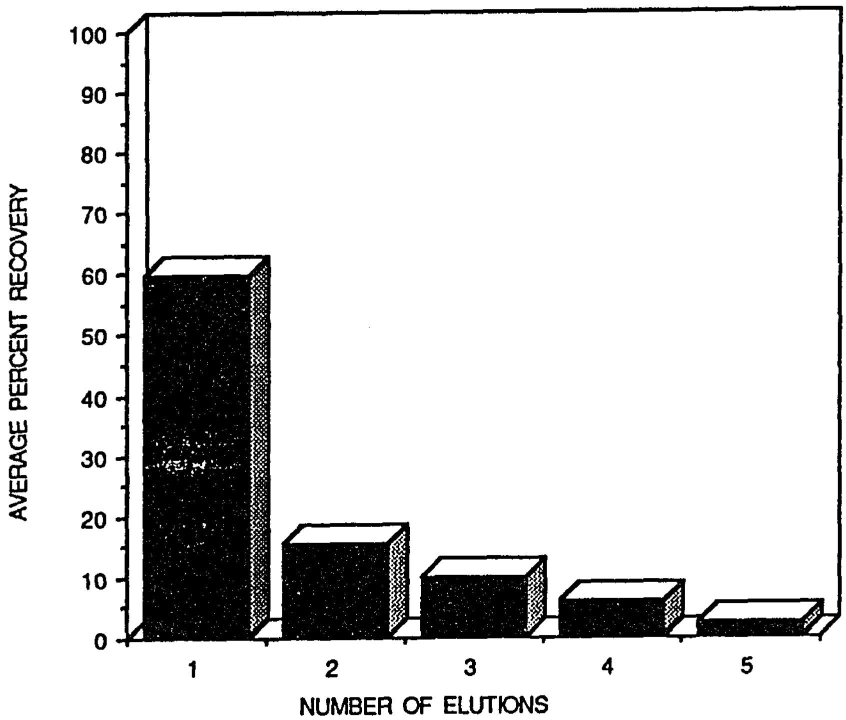

Fig. 5 Percent recovery of G.muris from the hands using sequential elution with Tris

were recovered in the first, second and third elutions respectively. An additional 13.6% was recovered in the fourth and fifth elutions combined. These results suggested that after the first wash with 300 ml of Tris, 40% of the cysts still remained on the hands.

When the hands were rinsed under a stream of tap water for 12 - 15 seconds an average of 3.25×10^3 *G. muris* cysts were recovered (Table 11). Table 12 and Figure 6 summarize the percent recoveries of *G. muris* cysts using tap water washing. Fifty seven percent of cysts were recovered with the wash and 13.8% with the two sequential elutions. The results for the tap water washing were significantly ($P \leq 0.5$) different from those for sequential elution of *G. muris* and fewer cysts were recovered from the hands.

Tables 13 - 14 summarize the data for liquid soap washing for *G. muris*. A very high average percent recovery was observed (78.2%) in the soap washings. *G. muris* cysts were not detected in the immediate elution with 300 ml of Tris. Fifty ml of the immediate Tris elution was centrifuged, resuspended in 8 ml of Phosphate Buffered Saline, and all 8 ml were assayed. *Giardia* cysts were not detected. The percent recovery of cysts in the liquid soap wash was significantly ($P \leq 0.5$) greater than the first washing with Tris or tap water or bar soap.

Tables 15 - 16 and Figure 7 summarize the results for bar soap washing of *G. muris* contaminated hands. An average of 3.21×10^3 cysts (54.5%) were recovered from the hands after the bar soap wash. Between 0.6% - 5% of the cysts were transfered to the bar soap and could be

TABLE 11 NUMBER OF *GIARDIA MURIS* CYSTS RECOVERED FROM THE HANDS AFTER WASHING WITH TAP WATER

Number of cysts placed on hands cysts (x10^3)	Subject	Trial	Number recovered cysts (x10^2)		
			Tap water Washing	Tris Elution 1	Tris Elution 2
44.40	JR	1	104.0	40.5	25.5
3.80		2	23.7	3.5	1.3
3.60		3	19.8	4.0	1.8
3.70	MK	1	24.9	3.8	1.3
3.60		2	22.2	4.8	1.3
3.85		3	22.1	4.8	1.0
3.50	GM	1	23.5	3.3	1.0
3.65		2	22.3	3.3	1.0
3.30		3	21.1	2.8	1.5

TABLE 12 PERCENT RECOVERY OF _G. MURIS_ CYSTS FROM THE HANDS AFTER WASHING WITH TAP WATER*

Subject	Trial	PERCENT RECOVERY Tap water Washing	TRIS Elution 1	TRIS Elution 2
JR	1	23.5	8.8	5.5
	2	62.4	9.2	3.3
	3	53.1	11.1	4.9
	AVERAGE	47.0	9.7	4.5
MK	1	67.3	10.1	3.4
	2	61.7	13.2	3.5
	3	57.3	12.3	2.6
	AVERAGE	62.1	11.9	3.2
GM	1	67.0	9.3	2.9
	2	61.1	8.9	2.7
	3	64.1	8.3	4.5
	AVERAGE	64.1	8.8	3.4

* = Average concentration of cysts added to hands = 8.16×10^{3}

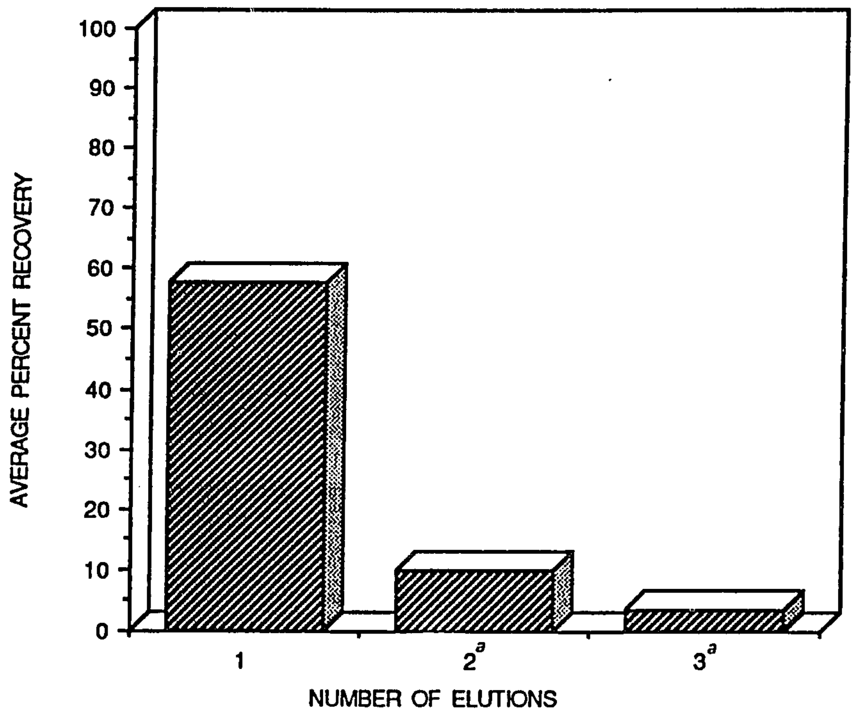

Fig.6 Percent recovery of G.muris from the hands after washing with tap water
a = Elution with 300 ml of Tris

TABLE 13 NUMBER OF *GIARDIA* CYSTS RECOVERED FROM THE HANDS AFTER WASHING WITH LIQUID SOAP

Number of cysts placed on hands cysts(x10^4)	Subject	Trial	Number of cysts recovered: Liquid soap washing cysts(x10^3)	Number of cysts recovered: Tris elution[a]
1.13	JR	1	8.69	0.0
1.25		2	9.99	0.0
1.11		3	8.83	0.0
1.29	MK	1	10.30	0.0
1.85		2	13.30	0.0
1.07		3	8.60	0.0
1.13	GM	1	9.07	0.0
1.25		2	9.57	0.0
1.26		3	9.83	0.0

a = Elution with 300 ml of Tris

TABLE 14 PERCENT RECOVERY OF G. MURIS FROM THE HANDS AFTER WASHING WITH LIQUID SOAP*

		PERCENT RECOVERY	
Subject	Trial	Liquid soap Washing	TRIS Elution[a]
JR	1	76.9	0.0
	2	79.9	0.0
	3	79.6	0.0
	AVERAGE	78.8	0.0
MK	1	79.8	0.0
	2	71.9	0.0
	3	80.4	0.0
	AVERAGE	77.4	0.0
GM	1	80.7	0.0
	2	76.6	0.0
	3	78.0	0.0
	AVERAGE	78.4	0.0

* = Average concentration of cysts added to hands = $1.26x10^4$

a = Elution with 300 ml of Tris

TABLE 15 NUMBER OF <u>GIARDIA</u> CYSTS RECOVERED FROM THE HANDS AFTER WASHING WITH BAR SOAP

			Number of cysts recovered cysts ($x10^2$)		
Number of cysts placed on hands cysts ($x10^3$)	Subject	Trial	Bar soap wash	Tris elution[a]	Bar wash[b]
13.10	JR	1	75.8	1.13	1.50
2.55		2	14.8	0.00	0.00
8.18	MK	1	36.9	0.75	37.50
6.90		2	37.6	0.00	0.00
5.85		3	32.3	0.00	1.88
3.08	GM	1	16.2	1.88	1.50
3.45		2	19.2	0.00	0.00
4.43		3	23.6	0.00	1.50

a = Elution with 300 ml of Tris

b = Washed the soap bar with 300 ml of Tris

TABLE 16 PERCENT RECOVERY OF GIARDIA CYSTS FROM THE HANDS AFTER WASHING WITH BAR SOAP*

Subject	Trial	PERCENTAGE RECOVERY		
		Bar soap washing	Tris elution[a]	Bar wash[b]
JR	1	57.9	Ø.9	1.2
	2	58.Ø	Ø.Ø	Ø.Ø
	Average	57.9	Ø.4	Ø.6
MK	1	45.1	1.Ø	Ø.5
	2	54.5	Ø.Ø	Ø.Ø
	3	55.2	Ø.Ø	3.2
	Average	51.6	Ø.3	1.2
GM	1	52.6	6.1	4.9
	2	55.8	Ø.Ø	Ø.Ø
	3	53.4	Ø.Ø	3.4
	Average	53.9	2.Ø	2.8

a = Elution with 3ØØ ml of Tris

b = Washed the soap bar with 3ØØ ml of Tris

* = Average concentration of cysts added on to hands = $5.94x1Ø^3$

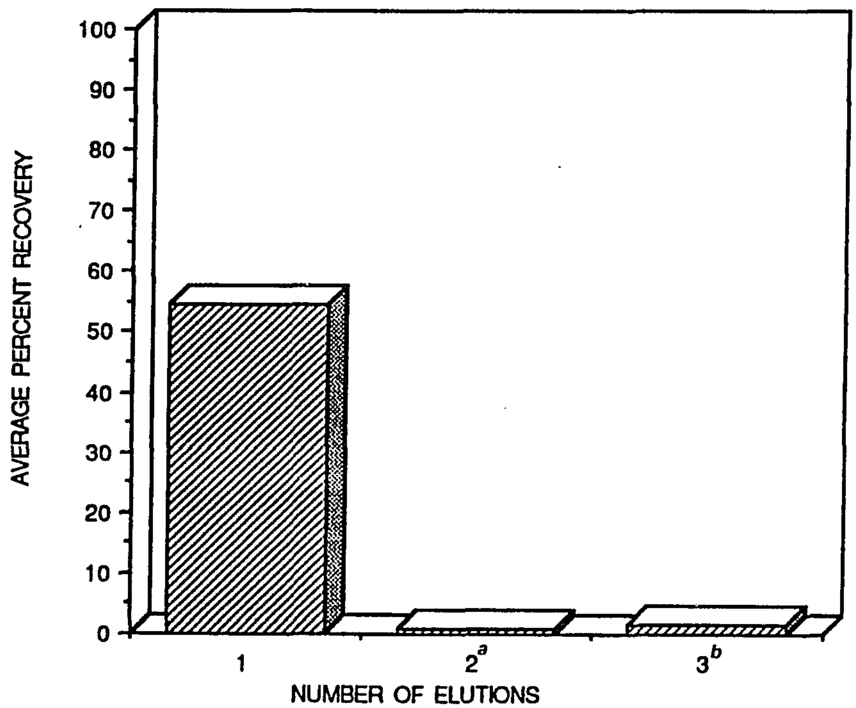

Fig. 7 Percent recovery of G.muris from the hands after washing with bar soap
a = Elution with 300 ml of Tris
b = Washed the soap bar with 300 ml of Tris

recovered. The average percent recovery of G. muris after washing with liquid soap and bar soap was significantly (P ≤ 0.5) different. Bar soap was found to be less effective in removing cysts from the hands. The immediate wash with 300 ml of Tris resulted in an average percent recovery of 0.9%.

Comparison of the average percent recoveries of G. muris from the hands for the four different handwashing procedures (Tris, tap water, liquid soap and bar soap) are shown in Figure 8. Liquid soap removed approximately 20% more cysts than other handwashing procedures. There was no significant difference (p ≤ 0.5) in Giardia cyst counts between Tris and tap water or Tris and bar soap in the first washing, however in the second elution fewer cysts recovered from the hands. The results of the completely randomized one - way Analysis of Variance to determine the effects of different eluents on the recovery of G. muris cysts from the hands are given in Appendix C.

Comparison of G. muris and rotavirus recoveries for the different hand washing procedures are given in Figures 9 - 11. Higher percent recoveries of G. muris cysts were obtained compared to rotavirus in all the procedures.

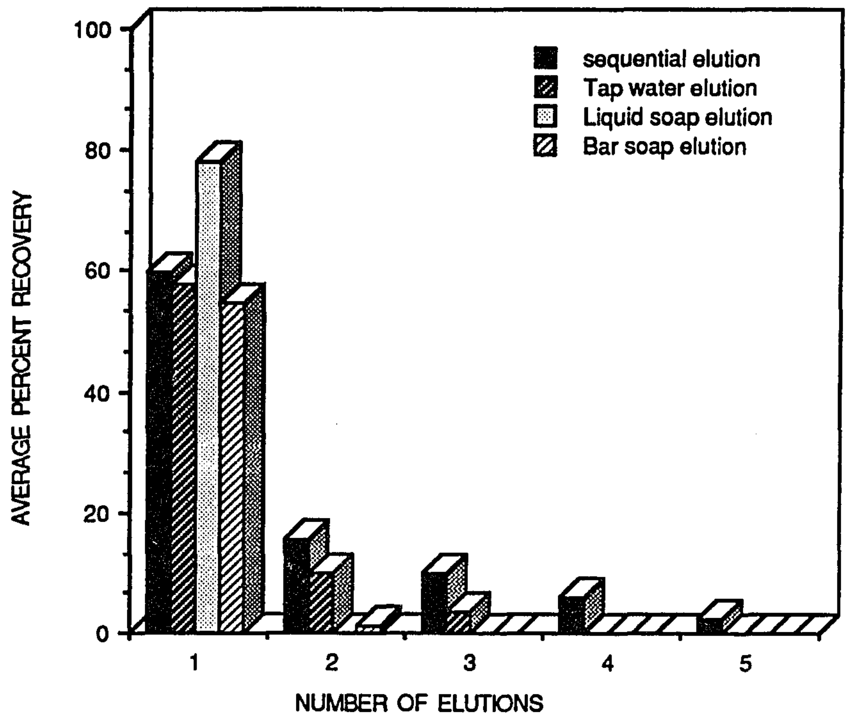

Fig. 8 Percent recovery of G.muris from the hands using different handwashing procedures

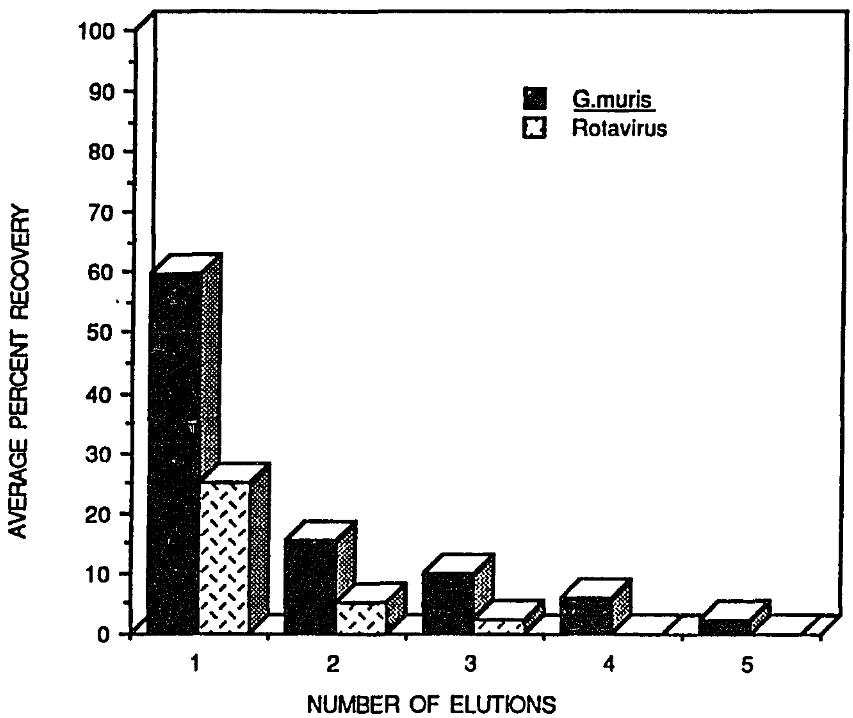

Fig. 9 Percent recovery of rotavirus and G.muris from the hands using sequential elution with Tryptic Soy Broth for rotavirus and Tris for G.muris

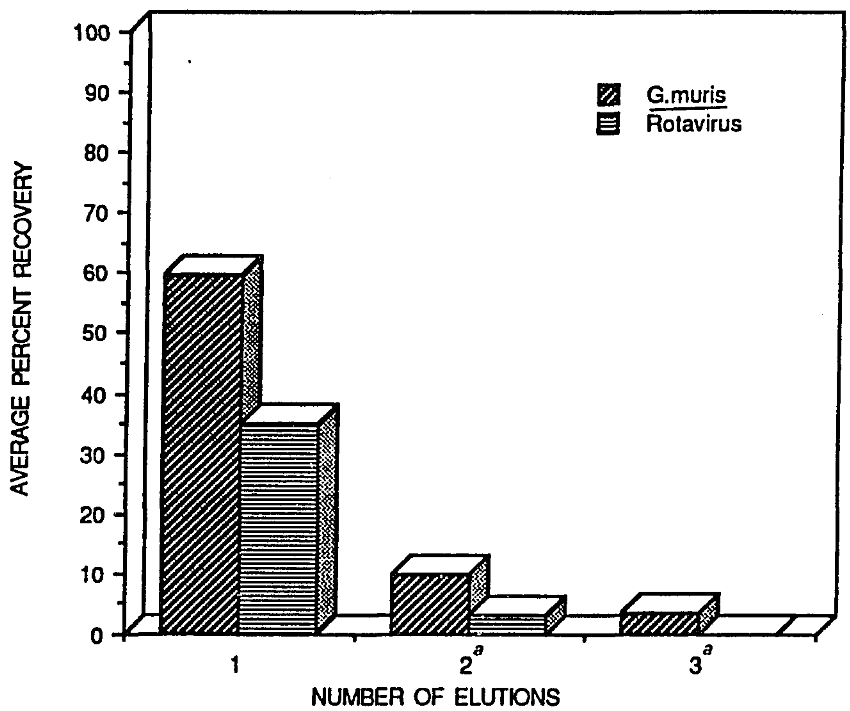

Fig. 10 Percent recovery of G.muris and rotavirus from the hands after washing with tap water

a= Elution with 300 ml of Tris for G.muris and with 300 ml of Tryptic Soy Broth for rotavirus

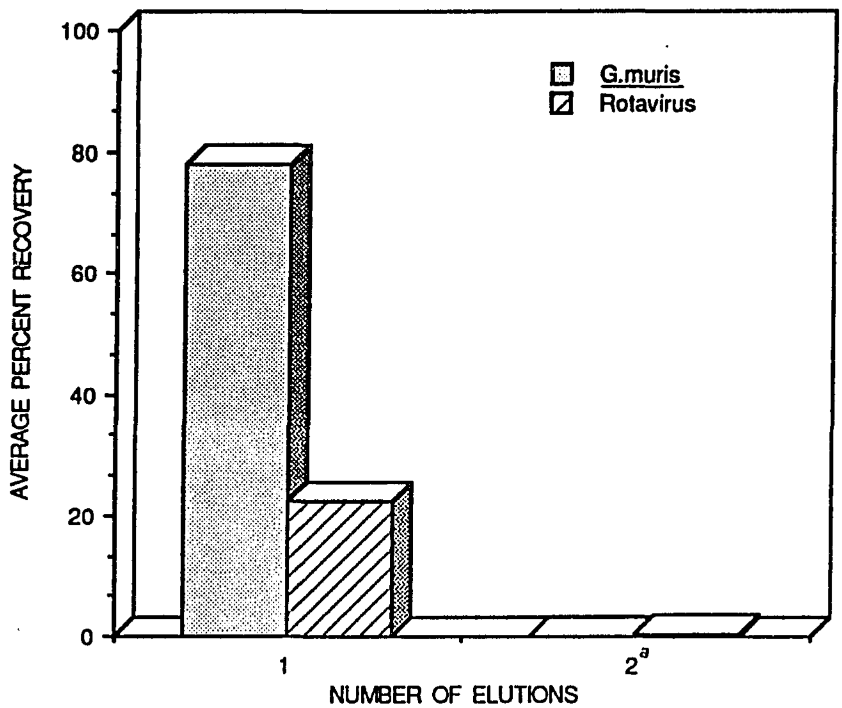

Fig. 11 Percent recovery of G.muris and rotavirus from the hands after washing with liquid soap

a = Elution with 300 ml of Tris for G.muris and with 300 ml of Tryptic Soy Broth for rotavirus

DISCUSSION

The results of this study suggest that both liquid and bar soaps are effective in limiting the persistence of rotavirus on the hands. Washing the hands with tap water alone was also found to be effective in the removal of rotavirus from the hands. However, washing with tap water alone was not as effective as washing with either liquid soap or bar soap as it was demonstrated that 2.7%, 0.2% and 0.6% of the virus remained on the hands after tap, liquid soap and bar soap washings respectively.

The low percent recoveries (0.03 - 34.84%) of rotavirus from the hands in all of the experiments indicated that the virus survival on the hands was less than anticipated. The inability to recover rotavirus after washing with bar soap could also reflect inactivation of the virus. Exposure on the hands and the drying which occured at the surface may be one mechanism of inactivation. The simian rotavirus SA - 11 was reported to be more sensitive to drying than either poliovirus or bacteriophage f2 (Keswick et al, 1983). The reason for this greater sensitivity is unclear.

Considering the low recoveries encountered in the immediate Tryptic Soy Broth elution, it is concluded that washing with liquid soap is more effective than that of bar soap in removing the viruses from the contaminated hands. The efficacy of liquid soap over the other handwashing procedures can be explained by the fact that more (2 - 3 ml) soap was on the hands when washed with liquid soap. Also, the active ingredient in Dial liquid soap is Triclosan (2,4,4'- trichloro-2'hydroxydiphenyl ether, at 0.2%) where as that of Dial bar soap is Triclocarban (3,4,4'-trichlorocarbanilide, at 0.8%, John E. Heinze, Dial Corporation, personnel communications). Some of the virus particles may actually be inactivated by the detergent or the detergent may inhibit the virus from forming plaques (even after using dextran in the overlay medium), thus rendering their detection difficult.

The MS - 2 bacteriophage studies by Joan B. Rose (unpublished data) resulted in percent recoveries ranging from 19.4% - 62.4% for bar soap and 8.7% - 68.5% for liquid soap and the soaps did not appear to inhibit plaquing. The percentage of phage remaining on the hands was 0.89% and 1.3% for liquid bar soaps respectively. These results for MS 2 were simillar to the rotavirus results in the present study.

According to Samadi et al.(1983) rotavirus has been detected in handwashings (with tap water) of the attendents of children with rotavirus diarrhea.

Faix (1987) also reported that liquid soap is effective against the removal of cytomegalovirus from contaminated hands. In contrast to these results were the studies by Schurmann and Eggers (1985). In their studies of handwashing with soap and water reduced the number of poliovirus on hands by 2 - 4 $\log_{10}$. However, they concluded that soap and water handwashing was less effective than rinsing in buffer (Phosphate Buffered Saline) alone in decontaminating poliovirus 1 contaminated hands.

A previous study (Schurmann and Eggers, 1983) using an alcohol based hand disinfectant, showed that after contamination of hands with a defined amount of virus, a large fraction of the virus could be recovered when non-enveloped viruses were tested. However, when enveloped viruses were used, more than 20 times less virus was recovered by the same procedure.

The high recovery of non-enveloped viruses was attributed to their being less subject to decay, due to chemical constituents of the skin such as hydrogen ions or fatty acids to which non-enveloped viruses are less succeptible than enveloped virions. The soaps apparently inhibit virus plaquing. This could be somewhat overcome with dextran, at least with liquid soap. Diethylaminoethyl dextran has been used in plaque assay as a facilitator to enhance plaque formation (Smith et al, 1979). The mode of action of DEAE dextran in enhancing virus infectivity is not clear. DEAE dextran, a cationic polymer, probably enhances the electrostatic attraction between the virus and the host cell. The cationic polymers are proposed to aid virus diffusion (Wallis and Melnick, 1968) or more likely enhance virus adsorption and penetration (Kaplan et al, 1967). Thus, it may be that the soaps are interfering with viral adsorption to the cells. This does not necessarily reflect lowered infectivity in humans as adsorption mechanics may be very different in-vivo.

No studies have been conducted to evaluate quantitatively how many Girdia cysts will remain on hands after handwashing. However, handwashing practices in day -care centers have resulted in a significant decrease in the number of Giardia lamblia infections (Bartlett et al, 1988a).

The results of this study showed that the liquid soap is more effective than washing with bar soap or rinsing with tap water or Tris in the removal of Giardia cysts from the contaminated hands. The cysts left on bar soap, if not inactivated, could be transfered to another person who use the soap. When liquid soap is used, it is unlikely that the soap dispensor can get contaminated with the microorganisms. The number of infectious virions of rotavirus or *Giardia* cysts which may end up on the hands changing a baby diaper is not known. However, it has been reported that large numbers of rotavirus particles and *Giardia* cysts are excreted by children with diarrhea. It has been estimated that up to 10^{10} virus particles /gm of feces is shed by children with diarrhea (Melnik and Rennik, 1980). In giardiasis, up to 9×10^8 cysts may be passed daily (Barnard and Jackson, 1984). These figures demonstrate that a high number of virus particles and *Giardia* cysts can be placed on the hands during a diaper changing in a day care center. Considering the high number of viruses or *Giardia* cysts excreated, it is possible that 10^5 - 10^6 plaque forming units of virus and 10^4 *Giardia* cysts (as was used in this study) could be placed on the hands during a single diaper changing in a day care center.

Data from this study showed that 10^3 viruses and 10^2 *Giardia* cysts can be left on the hands after the first wash. The residual number of viruses and cysts may be significant, as the minimal infectious dose of rotavirus is low (less than 10 PFU). Also, Rendtorff (1954) demonstrated that *Giardia* infection can be caused by ingestion of as few as 10 to 100 cysts.

Handwashing with 2 - 3 ml of liquid soap for more than 12 seconds may be recommended for decontaminating rotavirus and *Giardia* contaminated hands. Washing with two soap changes may also aid in the removal of viruses and cysts from the hands.

CONCLUSIONS

The retention of rotavirus and *Giardia* on hands after washing with a liquid soap and a bar soap was studied. Collectively, large numbers of virus and parasites were recovered from the hands after washing. Handwashing with liquid soap was found to be more effective than washing with tap water alone or washing with bar soap in the removal of *Giardia* cysts. Handwashing with liquid and bar soap was found to be effective than washing with tap water alone in the removal of rotavirus from contaminated hands.

Handwashing with soap is an effective means of enhancing removal of rotavirus and *Giardia* from contaminated hands and therefore may be a reliable technique for decreasing the risk of rotavirus and *Giardia* transmission in day-care centers and in pediatric hospital wards. During handwasing with soap and water, 10^5 rotavirus particles and 10^3 *Giardia* cysts were recovered from the hands after the first wash. An average of 10^3 virus particles still remained on hands after washing with either liquid soap or bar soap. Although no *Giardia* cysts were recovered from the hands in the immediate wash with 300 ml of Tris after washing with liquid soap, approximately 10^2 cysts were detected on the hands after washing with bar soap. These results suggested that without appropriate handwashing practices (washing the hands with 2-

3 ml of liquid soap for more than 12 seconds, if possible with two soap changes), the potential for the transmission of rotavirus and Giardia lamblia infections in day -care centers can be fairly high.

Since the indirect immunofluorescence assay used for the detection of Giardia cysts does not differenciate between viable and non viable cysts, it will be important to determine the cyst viability in the washings from the hands. Future work should be directed towards the viability studies of Giardia cysts recovered from the hands after washing. The effect of drying the hands with paper towels need to be evaluated. Studies are also needed on Cryptosporidium and other viruses (calicivirus, adenovirus and astrovirus) associated with childhood diarrhea.

Since various soap types were not tested, it is difficult to speculate whether Dial soap is better than any other soap in removal of rotavirus and Giardia from contaminated hands. However, Dial liquid soap appeared to be very effective and would be recommended over bar soap because more soap is utilized on the hands, better removal is achieved and microorganisms cannot be transfered to the liquid soap dispensor as it is to the bar.

APPENDIX A

COMPLETELY RANDOMIZED ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE EFFECTS OF DIFFERENT HANDWASHING PROCEDURES ON THE RECOVERY OF ROTAVIRUS FROM CONTAMINATED HANDS.

Treatment	Mean[a]	SD[b]	n[c]	Significance[d]
TSB	30.4889	3.0246	9	a
Tap water	36.1778	2.1341	9	b
Liquid soap	28.1111	3.1931	9	a
Bar soap	1.0000	0.1473	9	c

a = mean percent recovery

b = standard deviation

c = number of observations

d = treatments with different letters are significantly different

APPENDIX B

COMPLETELY RANDOMIZED ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE SIGNIFICANT DIFFERENCES ON THE RECOVERY OF ROTAVIRUS IN THE SECOND ELUTION WITH TRYPTIC SOY BROTH.

Treatment	Mean[a]	SD[b]	n[c]	Significance[d]
TSB	14.6667	3.0400	9	a
Tap water	8.9811	3.1045	9	b
Liquid soap	2.6000	0.9000	9	c
Bar soap	3.5666	0.9900	9	c

a = mean percent recovery

b = standard deviation

c = number of observations

d = treatments with different letters are significantly different

APPENDIX C

COMPLETELY RANDOMIZED ONE WAY ANALYSIS OF VARIANCE TO DETERMINE THE EFFECTS OF DIFFERENT HANDWASHING PROCEDURES ON THE RECOVERY OF GIARDIA MURIS CYSTS FROM THE HANDS.

Tretment	Mean[a]	SD[b]	n[c]	Significance[d]
Tris	50.8444	2.9339	9	a
Tap water	49.2889	8.0838	9	a
Liquid soap	62.1889	1.9088	9	b
Bar soap	47.3125	2.3418	9	a

a = mean percent recovery

b = standard deviation

c = number of observations

d = treatments with different letters are significantly different

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