AMERICAN JOURNAL OF EPIDEMIOLOGY
Vol. 113, No. 4
Copyright © 1981 by The Johns Hopkins University School of Hygiene and Public Health
All rights reserved
Printed in U.S.A.

HANDWASHING TO PREVENT DIARRHEA IN DAY-CARE CENTERS[1]

ROBERT E. BLACK, AUBERT C. DYKES, KERN E. ANDERSON, JOY G. WELLS, SUSANNE P. SINCLAIR, G. WILLIAM GARY, JR., MILFORD H. HATCH AND EUGENE J. GANGAROSA

Black, R. E. (CDC, Atlanta, GA 30333), A. C. Dykes, K. E. Anderson, J. G. Wells, S. P. Sinclair, G. W. Gary, Jr., M. H. Hatch and E. J. Gangarosa. Handwashing to prevent diarrhea in day-care centers. *Am J Epidemiol* 1981;113:445-51.

Diarrhea has been recognized as a frequent health problem among children enrolled in day-care centers. Thus, we evaluated the effect of a handwashing program in two day-care centers (HWC) on the incidence of diarrhea among children when compared to children in two control centers (CC). After the program was begun, the incidence of diarrhea at the HWC began to fall and after the second month of the study was consistently lower than that at the CC. The incidence of diarrhea in the HWC was approximately half that of the CC for the entire 35-week study period. Adenoviruses, rotavirus, *Giardia lamblia*, and enteropathogenic *Escherichia coli* were found in the stools of a small number of ill children, but no pathogen was identified in the stools of most children with diarrhea. These results suggest that a handwashing program will probably prevent at least some of the diarrhea in day-care centers.

adenoviruses; day care; diarrhea; *Escherichia coli; Giardia lamblia;* rotavirus

Day-care centers have been implicated as settings for the spread of communicable diseases, especially diarrhea, among young, susceptible children (1–5). Specific agents such as *Shigella, Giardia,* and rotavirus have been associated with diarrheal illness in day-care centers; however, the cause of most diarrhea cases in these institutions is unknown (2–7).

Shigella, 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 personal hygiene (1–5). Transmission is facilitated by the close contact these children have with one another, with the staff of the day-care center, and possibly with contaminated fomites such as toys.

Because organisms can be spread on the hands of children and personnel, handwashing after toilet activities and diaper changings has been recommended to prevent spread of enteric infections in day-care centers (2, 3, 5, 8). We report an attempt to evaluate the effect of careful handwashing by children and staff after toilet activities and before eating on the incidence of diarrhea among children in day-care centers.

Received for publication December 5, 1979, and in final form July 28, 1980.

Abbreviations: CC, control centers; HWC, handwashing centers.

[1] From the Bureau of Epidemiology and Bureau of Laboratories, Centers for Disease Control, Atlanta, GA 30333.

Reprint requests to Dr. R. E. Black, Center for Vaccine Development, University of Maryland School of Medicine, 29 South Greene Street, Baltimore, MD 21201.

The authors thank Nancy Puhr and Robert Pollard, Centers for Disease Control, for their assistance and the day-care personnel for their cooperation and active participation in the study.

Materials and methods

Epidemiologic investigation

We studied four day-care centers in suburban Atlanta, Georgia. They were part of a national chain of centers and were comparable in building design; number of employees; sex, age, and race composition of children enrolled; total number of children; and number of children less than three years of age enrolled. They also had similar diapering and toilet facilities and sinks, bar soap, and paper towels for handwashing by children and employees. At each center, we studied two groups of non-toilet-trained children in separate rooms—infants six months to one and one-half years old and toddlers one and one-half to two and one-half years old. Two centers were randomly used as experimental centers (handwashing) and two as control centers.

On June 7, 1976, we began monitoring diarrheal illness at the four centers. Day-care personnel recorded the daily attendance and occurrence of diarrhea for each child in the study groups. We defined diarrhea as a bowel movement that was judged by day-care personnel to be watery or more loose than usual for that child. However, we usually confirmed the presence of diarrhea by examining feces in the diapers when specimens were processed. The presence or absence of diarrhea was recorded only for days of attendance; no attempt was made to identify diarrhea at home. We collected these records each week from the centers until completing the study on April 1, 1977.

In a two-month baseline period (June–July), we observed that children and staff in all four centers infrequently washed hands and that the toilet-trained children were rarely supervised when using the toilet. Then the handwashing program was begun in two centers. Employees washed their hands before handling food and after arriving at the center, helping a child use the toilet, or using the toilet themselves. When children entered the center, used the toilet, were diapered, or prepared to eat, employees washed their hands using bar soap and paper towels. Children using the toilet were supervised by the staff to ensure that they did not place their hands in their mouths. These practices were rigorously monitored by the investigators and by the senior staff of the centers. The two control day-care centers were observed for diarrhea in the same manner as the experimental centers, except that handwashing practices in the control centers were not changed.

Informed consent was obtained from the parents of each child in the study. Positive laboratory results were reported to parents and to the children's physicians. Treatment with quinacrine or metronidazole was recommended for infections with *Giardia lamblia*.

The incidence of diarrhea was calculated for each week from June 7, 1976, to April 1, 1977, by dividing the number of new (at least four days without recorded diarrhea after a previous episode) episodes of diarrhea by the number of children present for at least two of five days in that week (child-week). By the conclusion of the study, 116 children had been observed for 2242 child-weeks.

Laboratory procedures

In July 1976, we obtained baseline stool specimens from all children participating in the study and later from additional children as they were enrolled in the centers. These specimens were examined for parasites and bacteria including enterotoxigenic and invasive *Escherichia coli*. From July 1 until the end of the study, we collected a stool specimen from each child with diarrhea and, when possible, from a well child present in the same room that day. Diarrhea and control specimens were examined for parasites, bacteria and certain viruses.

Fecal specimens were obtained directly from soiled diapers and were either processed immediately or kept refrigerated until being processed on the same day. Two stool swabs were taken and placed in cold Cary-Blair transport media for bacterial analysis. The Cary-Blair specimens were held refrigerated until laboratory processing within 24 hours. A small quantity of feces was placed into vials, one containing 5 per cent formalin and the other polyvinyl alcohol, for parasitic examination. A vial of stool was also frozen at −20 C for viral studies.

The Cary-Blair specimens were examined for *Salmonella* (9), *Shigella* (10), pathogenic *Vibrios* (11), *Yersinia enterocolitica* (12) and enterotoxigenic, enteropathogenic and invasive *E. coli*. Five lactose positive and two lactose negative colonies if present were selected from each specimen. The isolates were tested for heat-labile enterotoxin production by the Y-1 adrenal cell assay (13), for heat-stable enterotoxin production by the infant mouse assay (14) and for invasiveness by the Sereny test (15). Stools were examined by the fluorescent antibody technique of Thomason et al. (16) for the presence of enteropathogenic serogroups O26:K60, O55:K59, O86:K61, O111:K69, O125:K70, O126:K71, O127:K63 and O128:K67. Isolates from positive stools were serologically confirmed by the tube agglutination technique (9).

Formalin-preserved stool specimens were examined for parasites with direct microscopic study and with formalin-ether concentrations after being treated with Dobell's iodine and normal saline. Polyvinyl alcohol-preserved stools were stained with Wheatley's trichrome and examined.

For electron microscopic studies for viruses, stool specimens were suspended in four volumes of phosphate-buffered saline pH 7.4, centrifuged at 1400 × g for 20 minutes to remove large debris and examined with the pseudoreplica technique (17). Efforts to cultivate and characterize viral particles seen with electron microscopy have been reported (18).

Results

Epidemiologic evaluation of the handwashing program

During the eight-week baseline observation period, the incidence of diarrhea at the handwashing centers (HWC) was higher than that at the control centers (CC) (table 1). After the handwashing program was begun in the ninth week of the study, the incidence of diarrhea at the HWC began to fall (figure 1). The incidence at the CC fluctuated more widely and was consistently higher than that of the HWC after the second month of the study. The two HWC were similar to each other in their incidence of diarrhea as were the two CC. The incidence of diarrhea in the CC was nearly twice that in the HWC for the entire 35-week study period (table 1).

During the study period the incidence of diarrhea (table 1) among children six months to one and one-half years old at the HWC was significantly lower than that at the CC ($\chi^2 = 9.2, p < 0.001$). The incidence among children one and one-half to two and one-half years old at the HWC was similarly lower, but not statistically different, than the incidence for comparable children at the CC. Although the incidences of diarrhea at the HWC and CC were apparently higher for the older of the two groups of study children, neither difference was statistically significant.

The incidences of diarrhea at the HWC and the CC were calculated by the length of attendance for children who enrolled after the study began. In the study period, newly enrolled children totaled 38 in HWC and 36 in CC and their first four weeks of attendance contributed 148 and 143 child-weeks to the HWC and CC, re-

TABLE 1

Incidence of diarrhea in handwashing and control centers during baseline and study periods, 1976–1977

Type of center and age of children	Baseline period		Study period	
	Ep/ch-wk*	Inc/100 ch-wk†	Ep/ch-wk	Inc/100 ch-wk
Handwashing centers				
6–17 months	17/88	19.3	22/629	3.5
18–29 months	15/108	13.9	17/298	5.7
All	32/196	16.3	39/927	4.2
Control centers				
6–17 months	11/112	9.8	40/517	7.7
18–29 months	3/117	2.6	32/373	8.6
All	14/229	6.1	72/890	8.1

* New episodes of diarrhea/child-weeks at risk. (Child-week = the number of children present for at least two of five days in a week.)

† Diarrhea incidence/100 child-weeks.

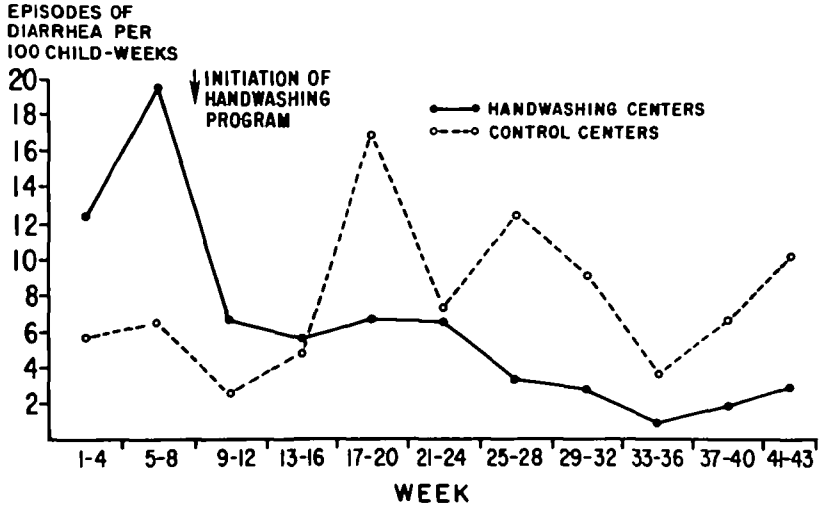

FIGURE 1. Incidence of diarrhea at handwashing and control day-care centers. (Child-week = the number of children present for at least two of five days in a week.)

spectively. As seen in figure 2, the incidence peaked within two to four weeks after the children started at the centers; however, during this period, and with minor exceptions thereafter, the diarrhea rates were lower at the HWC than those at the CC.

Laboratory evaluation of diarrhea

Baseline stool specimens were obtained from 47 (76 per cent) of 62 children enrolled at the HWC and 54 (92 per cent) of 59 children at the CC. *G. lamblia* cysts (in five stools) and *Y. enterocolitica* (in two stools) were the only pathogens identified. The *Y. enterocolitica* strains were both Nilehn 1, Wauter 2 biotypes and were isolated from children at two different day-care centers.

The laboratory findings on stools from children with diarrhea and from matched controls are shown in table 2. Since few pathogens were identified and the identification rate for the HWC was comparable to that for the CC, results are listed together.

In addition to the five children who had *G. lamblia* cysts in initial fecal specimens,

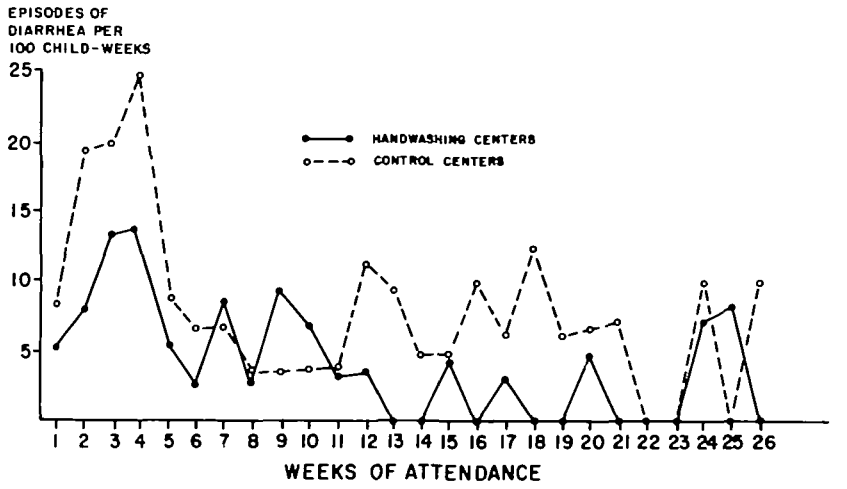

FIGURE. 2. Incidence of diarrhea by length of attendance at day-care centers among children newly enrolled during the study. (Child-week = the number of children present for at least two of five days in a week.)

TABLE 2

Identification of parasites, bacteria, and viruses from children with diarrhea and from controls at four day-care centers, 1976–1977

Organism	Diarrhea patients		Controls	
	Positive	No. tested	Positive	No. tested
Adenovirus	7*	79	1†	33
Giardia lamblia	4	67	1	33
Enteropathogenic *Escherichia coli*	4	67	1	19
Rotavirus	4	79	1	33
Toxigenic *E. coli*	0	85	1	37
Invasive *E. coli*	0	85	0	37
Salmonella	0	85	0	37
Shigella	0	85	0	37
Vibrio	0	85	0	37
Yersinia enterocolitica	0	85	0	37

* Noncultivable.

† Cultivable, type 1.

four children with diarrhea and one control child later passed *G. lamblia* cysts. No *Salmonella, Shigella, Vibrio, Y. enterocolitica* or toxigenic or invasive *E. coli* organisms were isolated. Four ill children from one of the HWC's were infected with enteropathogenic *E. coli* serogroup O:26, as was one well child at a CC.

Using direct electron microscopy, we found rotavirus particles in the stools of four (5 per cent) of 79 children with diarrhea and in the stool of one (3 per cent) of 33 control children. The five positive specimens were collected at a CC on two days in mid-January. Adenovirus particles were seen in seven (9 per cent) of 79 stools from children with diarrhea and in the stool of one (3 per cent) of 33 well children. An adenovirus type 1 was isolated from the control specimen but, as reported earlier, virus could not be cultivated from six of the specimens from children with diarrhea (18). The specimens from children with diarrhea were collected in two centers between September 28 and October 14.

Discussion

For more than 100 years, handwashing has been known to help prevent nosocomial infections transmitted by hospital personnel (19, 20). Since enteric infections in day-care centers are probably spread by the hands of children and employees, handwashing has been recommended as a preventive measure (2, 3, 5, 8). The potential usefulness of this technique is further suggested in the report of a study in school children from Cali, Colombia (21), which estimated that at least 44 per cent of the cases of diarrhea were related to school toilet hygiene.

Full compliance with a handwashing program by personnel of a hospital or day-care center is difficult to ensure. Whereas in hospitals the risk of cross-infection is generally recognized, in day-care centers there is the added problem of general skepticism concerning the usefulness of handwashing. Thus, we attempted to determine whether a rigorously supervised program could make a detectable reduction in diarrheal incidence. Admittedly, the frequent visits by the investigators to monitor the program make it difficult to generalize the findings to a less-supervised setting. Furthermore, because there was no objective measure of compliance with the handwashing program in the four centers, handwashing was not documented to be responsible for the lower incidence of diarrhea in the two experimental centers, even though over a 35-week period the incidence of diarrhea in these two centers was only 52 per cent of that in the control centers.

Community-based studies of pediatric illnesses in the United States indicate that young children may have one or two episodes of diarrhea per year (22, 23). These studies also indicate that children who attend school are more likely to transmit diarrheal illnesses to their families than are children of the same age who do not attend school (22). Studies of illnesses in day-care centers suggest a higher than average incidence of diarrhea (4), and our observations in the two control centers in this study indicate that the children in these groups had an average of four episodes of diarrhea per year.

Although rotavirus, *G. lamblia,* and enteropathogenic *E. coli* were all found in the stools of a small number of ill children, no pathogen was identified in the stools of most children with diarrhea. No enterotoxigenic or enteroinvasive *E. coli* were found in the stools of any children, which supports several other reports that these agents are rarely isolated from children with diarrhea in the United States (6, 7).

Adenoviruses have been associated with diarrhea in several studies, although their role as enteric pathogens is still debatable (7, 24–27). In recent investigations, using electron microscopy (EM), adenovirus-like particles have been observed but viruses could not be recovered in tissue cultures (7, 25–27). Laboratory investigation of EM-positive stools from this study, as well as from patients with sporadic cases of diarrhea, indicates that these noncultivable viruses represent a distinct adenovirus subgroup (18). Their presence in stools of seven of 79 ill children, but not in those of matched control children, suggests that they may be involved in diarrhea affecting children in day-care centers.

Most diarrhea among children in day-care centers is probably infectious, despite the fact that we identified a potential pathogen during only one quarter of diarrhea episodes in this study. This conclusion is supported by the lowered incidence of diarrhea associated with a handwashing program designed to reduce person-to-person transmission of enteric organisms. Furthermore, our observation that children enrolled in day-care centers are at higher risk of having diarrhea in the second to fourth weeks of attendance

suggests that illness follows initial contact with other infected children. Thus, a handwashing program will probably prevent at least some of these enteric infections.

References

1. Williams SV, Huff JC, Bryan JA. Hepatitis A and facilities for preschool children. J Infect Dis 1975;131:491-5.
2. Weissman JB, Gangarosa EJ, Schmerler A, et al. Shigellosis in day-care centers. Lancet 1975;1:8-14.
3. Thomas MEM, Tillett HE. Sonne dysentery in day schools and nurseries: an 18-year study in Edmonton. J Hyg Camb 1973;71:593-602.
4. Doyle AB. Incidence of illness in early group and family day care. Pediatrics 1976;58:607-13.
5. Black RE, Dykes AC, Sinclair SP, et al. Giardiasis in day-care centers: evidence of person-to-person transmission. Pediatrics 1977; 60:486-91.
6. Escheverria P, Blacklow NR, Smith DH. Role of heat-labile toxigenic *Escherichia coli* and reovirus-like agent in diarrhea in Boston children. Lancet 1975;2:1113-16.
7. Kapikian AZ, Kim HW, Wyatt RG, et al. Human reovirus-like agent as the major pathogen associated with winter gastroenteritis in hospitalized infants and young children. N Engl J Med 1976;294:965-72.
8. Hutchinson RI. Some observations on the method of spread of sonne dysentery. Monthly Bulletin of the Ministry of Health and Public Health Laboratory Service 1956;15:110-18.
9. Edwards PR, Ewing WH. Identification of Enterobacteriaceae. 3rd ed. Minneapolis: Burgess Publishing Company, 1972.
10. Morris GK, Koehler JA, Gangarosa EJ, et al. Comparison of media for direct isolation and transport of shigellae from fecal specimens. Appl Microbiol 1970;19:434-7.
11. Feeley JC, Balows A. Vibrio. In: Lennette EH, Spaulding EH, Truant JP, eds. Manual of clinical microbiology. 2nd ed. Washington, DC: American Society for Microbiology, 1974:238-48.
12. Morris GK, Feeley JC, Martin WT, et al. Isolation and identification of *Yersinia enterocolitica*. Public Health Laboratory 1977;35:217-32.
13. Sack DA, Sack RB. Test for enterotoxigenic *Escherichia coli* using Y-1 adrenal cells in miniculture. Infect Immun 1975;11:334-6.
14. Dean AG, Ching Y, Williams RG, et al. Test for *Escherichia coli* enterotoxin using infant mice: application in a study of diarrhea in children in Honolulu. J Infect Dis 1972;125:407-11.
15. Sereny B. Experimental *Shigella* keratoconjunctivitis. Acta Microbiol Acad Sci Hung 1955;2:293-6.
16. Thomason BM, Cherry WB, Davis BR, et al. Rapid presumptive identification of enteropathogenic *Escherichia coli* in faecal smears by means of fluorescent antibody. Bull WHO 1961;25:137-52.
17. Martin ML, Palmer EL, Middleton PJ. Ultrastructure of infantile gastroenteritis virus. Virology 1975;68:146-53.
18. Gary GW Jr, Hierholzer JC, Black RE. Characteristics of noncultivable adenoviruses associated with diarrhea in infants: a new subgroup of human adenoviruses. J Clin Microbiol 1979;10:96-103.
19. Steere AC, Mallison GF. Handwashing practices for the prevention of nosocomial infections. Ann Int Med 1975;83:683-90.
20. Taylor MRH, Keane CT, Kerrison IM, et al. Simple and effective measures for control of enteric cross-infection in a children's hospital. Lancet 1979;1:865-7.
21. Koopman JS. Diarrhea and school toilet hygiene in Cali, Colombia. Am J Epidemiol 1978; 107:412-19.
22. Hodges RG, McCorkle LP, Badger GF, et al. A study of illness in a group of Cleveland families. XI. The occurrence of gastrointestinal symptoms. Am J Hyg 1956;64:349-56.
23. Dingle JH, McCorkle LP, Badger GF, et al. A study of illness in a group of Cleveland families. XIII. Clinical description of acute nonbacterial gastroenteritis. Am J Hyg 1956;64:368-75.
24. Moffet HL, Shulenberger HK, Burkholder ER. Epidemiology and etiology of severe infantile diarrhea. J Pediatr 1968;72:1-14.
25. Flewett TH, Bryden AS, Davies H. Virus particles in gastroenteritis. Lancet 1973;2:1497.
26. Whitelaw A, Davies H, Parry J. Electron microscopy of fatal adenovirus gastroenteritis. Lancet 1977;1:361.
27. Richmond SJ, Caul EO, Dunn SM, et al. An outbreak of gastroenteritis in young children caused by adenoviruses. Lancet 1979;1:1178-80.