Using Child Health Outcomes to Identify Effective Measures of Handwashing

Stephen P. Luby,* Amal K. Halder, Tarique M. N. Huda, Leanne Unicomb, and Richard B. Johnston

Infectious Diseases and Vaccine Sciences, ICDDR,B, Dhaka, Bangladesh; Department of Water and Sanitation in Developing Countries, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

Abstract. We assessed which practical handwashing indicators were independently associated with reduced child diarrhea or respiratory disease. Fieldworkers collected 33 indicators of handwashing at baseline in 498 households in 50 villages in rural Bangladesh. Community monitors visited households monthly and asked standard questions about diarrhea and symptoms of respiratory illness among children under 5 years of age. In multivariate analysis, three handwashing indicators were independently associated with less child diarrhea—mothers reporting usually washing hands with soap before feeding a child, mothers using soap when asked to show how they usually washed their hands after defecation, and children having visibly clean finger pads. Two indicators were independently associated with fewer respiratory infections—mothers allowing their hands to air dry after the handwashing demonstration and the presence of water where the respondents usually wash hands after defecation. These rapid handwashing indicators should be considered for inclusion in handwashing assessments.

INTRODUCTION

Diarrhea and respiratory illness are leading causes of child mortality globally.¹ In several small-scale studies, people who received focused intensive handwashing promotion interventions reported less diarrhea and respiratory disease compared with people who did not receive such an intervention.^{2–5} Large-scale handwashing promotion interventions have the potential to markedly improve community health, but the intensive interpersonal communication used to promote handwashing in small-scale studies is prohibitively expensive when targeting populations with millions of people at risk. Evaluating whether large-scale handwashing promotion interventions that use mass media or other less labor-intensive approaches are effective in changing habits requires assessing the effect of these interventions on handwashing behavior. An important barrier to such evaluations is the paucity of evidence that any of the measures of handwashing behavior are a valid measure of habitual practice.

Asking people about their handwashing behavior consistently generates prevalence of handwashing practice that are much higher than observed behavior.^{6–8} Directly observing handwashing behavior is intrusive, expensive, and generates higher prevalence of handwashing practices compared with when people do not know they are being observed.^{9–13} Microbiological assessments of hand contamination are expensive and highly variable.^{14–16} Proxy measures of handwashing (for example, the presence of soap or a place to wash hands) are easy to collect but are strongly associated with health,^{17–19} and they may not be valid measures of actual behavior.

Several scientists have appreciated the importance of developing a valid, low-cost practical measure of handwashing behavior. The most common approach is to assume that structured observation provides a gold-standard assessment of handwashing behavior, and then researchers assess how well other indicators singly or in combination are associated with structured observation.^{6,7,20,21} This approach has failed to identify a valid, low-cost robust alternative to structured observation. Even when some measures are associated with structured

observation, sensitivity and specificity are poor.²⁰ Moreover, because structured observation exaggerates actual handwashing practice, the relevance of proxy measures of structured observation is unclear.

We propose a fundamentally different approach to evaluating indicators of handwashing behavior. Rather than evaluating whether candidate indicators are associated with a not-so-golden standard, we propose evaluating whether candidate handwashing indicators are associated with improvement in child health. Previous researchers developed composite indices comprised of 3–39 indicators of household hygiene (the majority unrelated to handwashing) and assessed the associations of these indices with child diarrhea.^{18,22–24} The resulting hygiene indices were offered as a guide to frame behavior change interventions and as a tool to assess hygiene status and the impact of interventions.

In contrast to efforts to improve household hygiene along the many dimensions included in these composite indices, specific interventions to promote handwashing are based on the contention that handwashing is the key hygiene behavior to interrupt pathogen transmission and improve health. This contention is based on controlled trials of handwashing in several contexts that have shown marked improvements in objective measures of health, including reduced maternal mortality,²⁵ improved school attendance,²⁶ and reduced incidence of laboratory-confirmed vancomycin-resistant enterococci²⁷ and influenza infection,²⁸ as well as randomized controlled trials in low-income communities where handwashing promotion has been consistently associated with reduced reports of child illness.^{2,3} Because behavior change interventions that focus on a few specific behaviors are more effective than those changes that have numerous targets,²⁹ many current interventions focus on handwashing promotion rather than attempting to affect diverse aspects of household hygiene.

Different handwashing indicators assess different aspects of handwashing. There is considerable uncertainty regarding the most appropriate times and techniques to wash hands.^{30–32} For example, is it so important for caregivers to wash hands after cleaning an infant who has defecated when the infant, who is the primary target of diarrhea prevention, has already been exposed to the organisms in his or her own feces? Similarly, respiratory illnesses cause higher childhood mortality than diarrhea, and the key times and handwashing techniques to interrupt respiratory pathogen transmission may differ from

* Address correspondence to Stephen P. Luby, Infectious Diseases and Vaccine Sciences, ICDDR,B, GPO Box 128, Dhaka-1000, Bangladesh. E-mail: sluby@icddr.org

interrupting gastrointestinal pathogen transmission. With these uncertainties, it is unclear which specific handwashing behaviors are most appropriate to measure. Assessing the relationship between various handwashing indicators and health outcome permits identifying indicators associated with effective handwashing (that is, handwashing that improves child health).

To implement this evaluation, we reviewed baseline handwashing measures collected from control households from an evaluation of a large-scale handwashing, water supply, and sanitation intervention program in Bangladesh and evaluated the subsequent prevalence of childhood diarrhea and respiratory disease in these households over the next 2 years. Our objective was to identify handwashing indicators that were independently associated with reduced diarrhea and respiratory disease.

METHODS

Evaluation context. In 2007, the Government of Bangladesh Department of Public Health Engineering in collaboration with The United Nations Children's Fund (UNICEF) and with support from the Department for International Development (DFID) of the British Government launched a program—Sanitation, Hygiene Education, and Water Supply–Bangladesh (SHEWA-B)—which is among the largest intensive handwashing, hygiene/sanitation, and water-quality improvement programs ever attempted in a low-income country. The intervention initially targeted 19.6 million people in rural Bangladesh. To assess the program's impact, fieldworkers evaluated households' characteristics, practices, and illnesses in 50 randomly selected villages that served as non-intervention control households to compare with outcomes in communities receiving the SHEWA-B program. The selection of the study population and the procedures used for data collection have been reported previously,^{8,33} and therefore, we only summarize them here.

Study population. SHEWA-B targeted 68 sub-districts (upazilas) in 19 districts. We randomly selected 50 intervention communities.³³ For each SHEWA-B intervention upazila where a village was chosen for evaluation, we selected a control upazila that had similar geography, hydrogeology, infrastructure, agricultural productivity, and household construction and where the government confirmed that no other major water, sanitation, or hygiene programs were ongoing. We randomly selected villages for evaluation in the control areas.³³ Because the intervention was designed to affect both handwashing behavior and health, we included only households from control communities in this analysis.

Fieldworkers identified the household closest to the village center that had a child < 3 years of age and sought consent for participation. Fieldworkers looked for the next closest household with a child < 3 years of age. Fieldworkers repeated the process for enrolling additional households until 10 households in each selected village were enrolled for monthly disease surveillance.

Study personnel. Fieldworkers who administered the cross-sectional survey and observed the handwashing demonstrations were men and women with at least 14 years of formal education, most of whom had several years experience collecting survey data. All fieldworkers participated in 3 weeks of formal training on the data collection instruments. The

training included classroom teaching, where each of the items was discussed in detail, and field testing, where teachers and supervisors observed and provided feedback.

Data collection. *Cross-sectional survey.* Fieldworkers conducted a baseline survey that included a structured interview and observational spot checks. The interview collected household demographics and composition, details of house construction, and possessions to measure wealth and included both open- and close-ended questions on handwashing behavior.

Fieldworkers asked the respondent to "show me where you usually wash your hands after you use the toilet." The fieldworker noted the location of the toilet and whether water and soap were available at the identified location.

Fieldworkers assessed hand cleanliness of mothers/caregivers of the youngest children of all sampled households and all available children under the age of 5 years. Fieldworkers inspected the fingernails, palm, and finger pads of both hands; they coded them as unclean if they saw any visible dirt and clean if they saw no visible dirt. If fieldworkers identified no dirt on the fingernail, palms, or finger pads of any of the children under age 5 years in the household, then we classified the household as a household where children's fingernail, palms, or finger pads were clean.

To minimize the variability among fieldworker's assessments, managers trained fieldworkers and their supervisors by using role-playing exercises. Fieldworkers then pre-tested all instruments in pilot communities and received feedback from peers and supervisors before collecting any data for the evaluation.

Handwashing demonstrations. Fieldworkers asked one person in each household to show how they usually washed their hands after defecation. If the household had a child between ages 3 and 5 and the child was willing, the child showed his/her normal handwashing. If there was no child in this age group or if the child refused, then the fieldworker requested that the mother show her normal handwashing behavior after defecation. For this analysis, we only analyzed observations from mothers' handwashing demonstrations and ignored households where we had data on child's demonstrated handwashing but not the mothers. The fieldworker noted whether the mother used soap or other handwashing materials, whether she washed one or both hands, and how she dried her hands.

Monthly surveillance. Fieldworkers recruited one female resident who had at least 10 years of formal education and lived in or near the village enrolled in the evaluation to visit participating households monthly and administer a brief questionnaire. These community monitors participated in a formal training program to learn how to administer the questionnaire. The initial 3-day training included 2 days of classroom instruction with role playing followed by 1 day of field testing. After 12 months, the community monitors participated in a 1-day refresher training session. During each monthly visit, community monitors asked whether the child had diarrhea, fever, cough, or difficulty breathing during the preceding 2 days. This surveillance continued for 24 months after the baseline survey. We included households in the analysis who participated both in the baseline survey and the monthly surveillance.

Data analysis. *Variable definitions.* We assessed all 33 handwashing indicators that were included in the baseline cross-sectional survey. We used principal component analysis of household assets to evaluate household wealth.³⁴ We excluded hygiene and sanitary infrastructure from the wealth index, because we wanted to analyze the impact of wealth independent

of the specific facilities that might contribute to handwashing. We classified children as having diarrhea if the caregiver reported that the child had three or more loose stools (the Bengali phrase *patla paykhana*) within the last 2 days. We classified children as having an acute respiratory illness if their caregiver reported they had either cough and fever or difficulty breathing and fever within the last 2 days.

Bivariate analysis. We calculated odds ratios to evaluate the association between the exposure variables—household characteristics and handwashing indicators—and outcome variables—diarrhea and acute respiratory infection. To account for the repeated observations for the outcomes in single households and the clustering of observations in villages, we used generalized estimated equations to calculate these adjusted odds ratios and 95% confidence intervals.³⁵

Multivariate analysis. We developed multivariate models for handwashing indicators included in the baseline survey. Because fieldworkers collected handwashing demonstration data from mothers in only a subset of households, we developed separate multivariate models for handwashing indicators included as part of the handwashing demonstrations. We also constructed separate multivariate models for diarrhea as an outcome and acute respiratory illness as an outcome. For each multivariate model, we began with an initial bivariate model with the handwashing indicator that had the strongest association and the outcome variable (i.e., diarrhea or acute respiratory illness). We then added additional handwashing indicators that were associated with the outcome on bivariate analysis with a P value < 0.05 . We retained variables if they both significantly improved fit ($P < 0.05$) of the model and were independently associated with the outcome variable ($P < 0.05$). We next added potential confounders to the model in order of the strength of association of these variables on bivariate analysis. The final multivariate model retained all those variables that both significantly improved fit of the model ($P < 0.05$) and were independently associated with diarrhea ($P < 0.05$).

We used a nested correlation structure for all general estimated equations analyses to account for, at the first level, the clustering of measures within the same village and at the second level, the repeated observations within households. For the majority of households that had only one child under the age of 5 years, the second-level clustering captured the repeated measurements of that single child. In those households where there were multiple children, the second level of clustering captured the repeated measurements of all children within the household as well as the intrahousehold transmission between these children. We used SAS for Windows (PROC GENMOD) Version 9.1 (SAS Institute, Cary, NC) for the generalized estimated equations modeling.

Ethics. All households provided written informed consent. The plan for the evaluation was reviewed by UNICEF and the Government of Bangladesh Department of Public Health Engineering. Because the primary purpose of the evaluation was evaluating the government program and not producing generalizable scientific information, the evaluation plan was not reviewed by a human subjects research committee.³²

RESULTS

The evaluation team completed baseline surveys and enrolled 500 households who had children under the age of

3 years in monthly surveillance; 498 households participated in at least the first 3 months of surveillance. These 498 households were the primary focus of the analysis and are referred to as the followed households. In 360 of the followed households, the mother (rather than a child) showed her normal handwashing behavior after defecation.

At enrollment in 2007, the followed households had a mean of 5.4 residents (Table 1); 35% of the fathers and 27% of the mothers lacked formal education. Their primary source of drinking water was a shallow tube well (80%); 50% owned a toilet; 23% owned a mobile phone. Fifty percent of households had an electrical connection. The characteristics of the 360 households where the mother showed her handwashing behavior were similar to the 138 households where mothers did not show their handwashing practices, although the mothers who demonstrated handwashing had slightly smaller households (Table 1).

The mean age of the 540 children under the age of 5 years present in the household at enrollment was 18.5 months. Eighty-three children were born into households under surveillance; another 11 children moved into surveillance households. Five children aged out; 19 children moved out, and 5 children died. The mean age of children at 24 months of follow-up was 37.5 months. Among 14,105 potential follow-up monthly child assessments, the community monitors completed 13,932 (99%).

Diarrhea. During 24 months of follow-up, the mean proportion of children with diarrhea during the monthly follow-up was 10.0%. In the bivariate analysis, children who lived in wealthier households, who were over the age of 2 years, and whose father had more than 7 years of education had less diarrhea (Table 2). The prevalence of diarrhea was lower in the second year of surveillance (7.6%) than in the first year (12.6%).

Three handwashing indicators were significantly associated with reduced subsequent child diarrhea in the bivariate analysis (Table 2). Children who lived in households whose mother, when asked the open-ended question, “when do you usually wash your hands with soap?,” replied without prompting “before feeding a child” had less diarrhea than children whose mother did not mention washing hands before feeding a child. Children who lived in households where fieldworkers judged the finger pads of all the evaluated children as clean subsequently experienced significantly less diarrhea compared with children living in households where at least one child’s finger pads had visible dirt. Among the subset of households where mothers participated in the demonstration of their usual handwashing practices after defecation, children who lived in households where mothers washed their hands with soap during the handwashing demonstration subsequently had significantly less diarrhea compared with children living in households where mothers did not wash with soap during the demonstration. There was no association between any closed-ended handwashing question, the frequency of reported soap purchase, or the presence of soap or water at the most convenient place to wash hands and subsequent child diarrhea.

The three handwashing indicators that were significantly associated with subsequent child diarrhea on bivariate analysis had nearly identical odds ratios and remained significantly associated with subsequent child diarrhea on multivariate analysis (Table 3).

TABLE 1
Characteristics of participating households in rural Bangladesh in 2007

Characteristic	Households with handwashing demonstration (N = 360)		Households without handwashing demonstration (N = 138)		All followed households (N = 498)	
	n	Percent/mean	n	Percent/mean	n	Percent/mean
General						
Number household residents	1,858	5.2	821	5.9	2,679	5.4
Number of children age < 5 years	397	1.1	258	1.9	655	1.3
Father of the youngest child lacks formal education	120	34	53	38	173	35
Mother of the youngest child lacks formal education	94	26	42	30	136	27
Drinking water source						
Shallow tube well	289	80	107	78	396	80
Deep tube well	30	8	15	11	45	9
Tara pump	19	5	4	3	23	5
Piped water	7	2	6	4	13	3
Protected well	7	2	4	3	11	2
Other	8	2	2	1	10	2
Owns source of drinking water	96	27	40	29	136	27
Owns toilet	179	50	72	52	251	50
Uses improved latrine	279	78	102	74	381	77
Characteristics used in constructing wealth index						
Proportion who own the item						
House	335	93	127	92	462	93
Wardrobe	95	26	51	37	146	29
Bicycle	104	29	30	22	134	27
Mobile phone	86	24	28	20	114	23
Television (black and white)	64	18	26	19	90	18
Television (color)	34	9	19	14	53	11
Sewing machine	23	6	13	9	36	7
Refrigerator	7	2	7	5	14	3
Motorcycle	5	1	3	2	8	2
Mean number of items owned						
Tables	360	1.0	138	1.0	498	1.0
Chairs	360	2.0	138	2.5	498	2.2
Watches/clocks	360	1.3	138	1.6	498	1.4
Beds	360	0.8	138	1.0	498	0.9
Inexpensive sleeping cots	360	1.2	138	1.3	498	1.2
House construction						
Tin roof	320	89	127	92	447	90
Cement floor	30	8	13	9	43	9
Brick/cement walls	28	8	17	12	45	9
Mean number of rooms	360	2.2	138	2.2	498	2.2
Electrical connection	176	49	71	51	247	50
Cooking fuel						
Crop residue/grass/dung	212	59	77	58	289	58
Wood	90	25	33	24	123	25
Dung	57	16	28	20	85	17
Median amount of homestead land (acre)	360	0.08	138	0.08	498	0.08
Median amount of other land (acre)	360	0.08	138	0.10	498	0.08

Acute respiratory infections. During 24 months of follow-up, the mean proportion of children with acute respiratory infection during the monthly follow-up was 13.2%. In the bivariate analysis, children whose father or mother had more than 7 years of education, who lived in wealthier households, whose household owned a mobile phone, who were female, and who were over the age of 2 years had less acute respiratory infection (Table 4). The prevalence of acute respiratory infection was lower in the second year of surveillance (9.3%) than in the first year (17.3%).

Two handwashing indicators were significantly associated with less subsequent acute respiratory infection in the bivariate analysis (Table 4). Children who lived in households where water was present at the most convenient place to wash hands had less acute respiratory infection than children who lived in households where water was absent at the most convenient place to wash hands. Among the subset of households where mothers participated in the demonstration of their usual handwashing practices after defecation,

children who lived in households where mothers air-dried their hands had significantly less acute respiratory infection compared with children living in households where mothers dried their hands on their clothing. There was no association between any handwashing question, the frequency of reported soap purchase, or the presence of soap at the most convenient place to wash hands and subsequent child acute respiratory infection.

The two handwashing indicators significantly associated with subsequent child acute respiratory infection on bivariate analysis had nearly identical odds ratios and remained significantly associated with subsequent child acute respiratory infection on multivariate analysis (Table 5).

DISCUSSION

This analysis identified a set of five rapid, easy to collect handwashing indicators that were independently associated with less diarrhea or fewer respiratory infections among

TABLE 2
Bivariate relationship between baseline characteristics and subsequent diarrhea among children under age 5 years in the ensuing 24 months

Characteristic	Monthly observation	Monthly visits with this exposure (%)	Monthly visits with diarrhea (%)	Adjusted OR*	95% CI*	P value*
Household characteristics						
Mother's education ≥ 7 years	13,932	4,852 (35)	384 (7.9)	0.83	0.68, 1.10	0.059
Father's education ≥ 7 years	13,860	4,432 (32)	336 (7.6)	0.76	0.60, 0.96	0.020
PCA quintile						
1 (reference)	13,932	2,684 (19)	316 (11.8)	—	—	—
2	13,932	2,223 (18)	267 (10.7)	0.85	0.64, 1.14	0.284
3	13,932	2,777 (20)	299 (10.8)	0.97	0.77, 1.24	0.828
4	13,932	3,140 (23)	259 (8.3)	0.74	0.57, 0.97	0.031
5	13,932	2,841 (20)	251 (8.8)	0.77	0.58, 1.02	0.067
Own radio	13,932	3,139 (23)	264 (8.4)	0.87	0.71, 1.06	0.175
Own television	13,932	3,790 (27)	322 (8.5)	0.89	0.72, 1.10	0.273
Own radio or television	13,932	5,708 (41)	479 (8.4)	0.85	0.70, 1.04	0.119
Household owns water source	13,932	3,884 (28)	366 (9.4)	0.97	0.82, 1.14	0.692
Household owns toilet	13,932	7,130 (51)	734 (10.0)	0.97	0.83, 1.14	0.719
Household owns mobile phone	13,932	3,127 (22)	283 (9.1)	0.84	0.68, 1.05	0.133
Child characteristic						
Male child	13,932	6,987 (50)	701 (10.2)	1.09	0.94, 1.27	0.242
Age < 2 years	13,932	5,291 (38)	648 (12.2)	1.48	1.19, 1.83	< 0.001
Year 1 surveillance (vs. year 2)	13,932	6,725 (48)	845 (12.6)	1.81	1.40, 2.33	< 0.001
Month since initiation of surveillance†	13,932			0.96	0.94, 0.98	< 0.001
Exclusive breastfeeding in the last 24 hours (children age < 2 years)	4,562	454 (10)	52 (11.5)	0.84	0.57, 1.23	0.361
Handwashing indicators						
Response to handwashing questions						
Reported usually washing hands with soap (open-ended)						
Before preparing food	13,932	780 (5.6)	73 (9.4)	1.15	0.96, 1.43	0.203
Before eating	13,932	1,918 (14)	153 (8.0)	0.76	0.56, 1.04	0.088
Before feeding a child	13,932	544 (3.9)	32 (5.9)	0.60	0.43, 0.84	0.003
After eating	13,932	1,050 (7.5)	57 (5.4)	0.71	0.48, 1.05	0.083
After defecation	13,932	11,355 (82)	1,104 (9.7)	0.92	0.073, 1.15	0.454
After cleaning a child's anus	13,932	5,364 (39)	549 (10.2)	1.14	0.97, 1.35	0.114
After disposal of child feces	13,932	1,029 (7.4)	91 (8.8)	0.81	0.57, 1.15	0.247
After handling cow dung	13,932	3,497 (25)	353 (10.1)	1.03	0.86, 1.24	0.723
After returning from outside	13,932	496 (3.6)	53 (10.7)	1.13	0.65, 1.95	0.672
Closed-ended handwashing question:						
“The last time you ____, did you wash your hands with soap?”						
Prepared food	13,932	2,772 (20)	272 (9.8)	0.87	0.69, 1.09	0.221
Ate with your hands	13,932	1,912 (14)	215 (11.2)	1.06	0.80, 1.40	0.693
Fed your child	13,932	1,753 (13)	169 (9.6)	0.87	0.63, 1.20	0.397
Defecated	13,932	8,654 (62)	808 (9.3)	0.89	0.76, 1.05	0.161
Cleaned your child's anus	13,932	8,069 (58)	728 (9.0)	0.88	0.77, 1.02	0.098
Used soap today or yesterday	13,932	13,571 (97)	1,354 (10.0)	0.92	0.62, 1.37	0.692
Reported washing hands > 18 times on the preceding day‡	13,932	6,627 (48)	592 (8.9)	0.93	0.76, 1.14	0.498
Has separate soap for handwashing	13,932	3,106 (22)	325 (10.5)	1.05	0.87, 1.26	0.605
Has spare soap in the household	13,932	4,682 (34)	396 (8.5)	0.82	0.64, 1.04	0.103
Frequency of purchasing soap	13,932					
Weekly		4,779 (34)	452 (9.5)	1.00	0.77, 1.29	0.994
Every 1–2 weeks		4,791 (34)	523 (10.9)	0.98	0.73, 1.31	0.887
Every 2–4 weeks		2,210 (16)	220 (10.0)	0.87	0.68, 1.11	0.258
> 1 month (reference)		2,152 (15)	197 (9.2)			
Observations						
Water present at the most convenient place to wash hands	13,932	10,054 (72)	1,030 (10.2)	0.99	0.84, 1.16	0.873
Soap available at the most convenient place to wash hands	13,932	8,355 (60)	663 (9.8)	0.84	0.67, 1.05	0.130
Handwashing location	13,520					
Inside or near toilet (reference)		4,446 (32)	381 (8.6)			
Inside or near kitchen		1,364 (9.8)	110 (8.1)	0.91	0.69, 1.20	0.524
Outside in the yard (within 10 ft of the latrine)		3,824 (27)	480 (12.6)	1.21	0.97, 1.51	0.099
Outside of the yard (> 10 ft from latrine)		3,886 (28)	392 (10.1)	1.15	0.93, 1.42	0.197
Observed visibly clean						
Mother's fingernails	13,932	8,185 (59)	813 (9.9)	0.96	0.79, 1.17	0.704
Mother's palms	13,932	12,888 (93)	1,293 (10.0)	0.97	0.71, 1.32	0.826
Mother's finger pads	13,932	12,887 (92)	1,299 (10.1)	1.03	0.74, 1.44	0.839
Child's fingernails	13,406	5,355 (40)	583 (10.9)	1.10	0.92, 1.32	0.309
Child's palms	13,406	9,688 (72)	938 (9.7)	0.85	0.71, 1.01	0.071

(Continued)

TABLE 2
Continued

Characteristic	Monthly observation	Monthly visits with this exposure (%)	Monthly visits with diarrhea (%)	Adjusted OR*	95% CI*	P value*
Child's finger pads	13,406	9,852 (73)	945 (9.6)	0.83	0.69, 0.99	0.042
Handwashing demonstration						
Handwashing materials	9,954					
Water only (reference)		1,551 (16)	169 (10.9)			
Ash		694 (7)	63 (9.1)	0.98	0.69, 1.40	0.927
Mud		1,127 (11)	164 (14.6)	1.17	0.86, 1.60	0.328
Soap		6,582 (66)	522 (7.9)	0.71	0.56, 0.90	0.005
Washed both hands	9,954	6,571 (66)	622 (9.5)	0.98	0.79, 1.22	0.859
Washed with soap	9,954	6,582 (66)	522 (7.9)	0.67	0.55, 0.82	< 0.001
> 15 seconds	6,606	2,864 (43)	258 (9.0)	1.26	1.00, 1.60	0.052
Hand drying	9,954					
Dried on clothes (reference)		8,465 (85)	758 (9.0)			
Dried on dirty cloth		553 (6)	70 (12.7)	1.05	0.62, 1.77	0.850
Dried on clean cloth		403 (4)	44 (10.9)	0.90	0.53, 1.54	0.708
Air dried		533 (5)	46 (8.6)	1.09	0.63, 1.89	0.709

CI = confidence interval; OR = odds ratio.

* Adjusted for repeated measures and village clustering.

† Because reported diarrhea prevalence decreased during the course of evaluation, we assessed the decline in diarrhea per month.

‡ Asked as an open-ended question: "how many times did you wash hands yesterday?" The median response was 18.

children under the age of 5 years in rural Bangladesh. The central question is whether these indicators are valid measures of effective handwashing behavior that, when practiced, reduce childhood illness, or if they are simply proxies for socioeconomic status?

Several lines of evidence suggest that these indicators are a valid measure of handwashing practice. Controlled trials of handwashing promotion have consistently concluded that handwashing promotion reduces the incidence of caregiver-reported child diarrhea² and respiratory disease.^{3,36,37} These trials are consistent with hospital- and school-based prospective trials that have shown that handwashing promotion reduces objective assessments of disease.^{25–28}

A causal relationship between these identified handwashing indicators and disease is consistent with proposed biological mechanisms of how handwashing interrupts pathogen transmission. Rural Bangladeshis eat food directly with their hands. Finger pads that are visibly dirty suggest the presence of foreign organic material on the part of a hand that directly contacts food and water. The visible dirt is both an indicator of environmental contamination and provides a microenvironment for the survival of bacteria, including pathogens. Handwashing with soap removes microorganisms from hands, and therefore, it can remove pathogens before they contaminate water and food or are directly ingested.

After defecation, residents of rural Bangladesh most commonly splash water on their anus using their left hand to rinse away adhering external anal and perianal feces. Hands contaminated with feces are an efficient pathway for transmission of enteric pathogens to food, water, and others' hands. In a separate analysis of a subgroup of SHEWA-B control households who were observed during 5 hours of structured observation at baseline, children living in households where fieldworkers observed residents wash their hands with soap after defecation had less diarrhea than children living in households where persons did not wash their hands after defecation.³² In the present analysis, when mothers were asked to show how they usually washed hands after defecation (a much easier indicator to collect than 5 hours of structured observation), children who lived in households where mothers washed their hands with soap had less diarrhea than children in households where mothers did not use soap. We know that the handwashing behavior that the mother showed at the fieldworker's request does not precisely replicate her everyday practice. Although 66% of mothers used soap for washing their hands in the handwashing demonstration, during 5-hour structured observation, only 34% of adult caregivers washed both hands with soap after defecation.⁸ Nevertheless, the handwashing demonstration apparently identified a group of mothers who better understood the value of handwashing

TABLE 3
Multivariate analysis of handwashing indicators and diarrhea prevalence

Characteristic	Bivariate OR (95% CL)	Multivariate OR* (95% CL)	P value†
Cross-sectional survey (N = 13,334)			
Reported usually washing hands before feeding child in open-ended question	0.60 (0.43, 0.84)	0.60 (0.42, 0.84)	0.003
Child's finger pads visibly clean	0.83 (0.69, 0.99)	0.82 (0.68, 0.99)	0.040
Month since initiation of surveillance	0.96 (0.94, 0.98)	0.96 (0.94, 0.98)	< 0.001
Child aged less than 24 months	1.48 (1.19, 1.83)	1.23 (1.03, 1.48)	0.026
Father's education above primary	0.76 (0.60, 0.96)	0.77 (0.60, 0.98)	0.035
Handwashing demonstration (N = 9,882)			
Used soap during handwashing demonstration	0.71 (0.56, 0.90)	0.69 (0.57, 0.83)	< 0.001
Month since initiation of surveillance	0.96 (0.94, 0.98)	0.96 (0.94, 0.98)	< 0.001
Father's education above primary	0.71 (0.54, 0.94)	0.73 (0.56, 0.95)	0.016

CL = confidence limit; OR = odds ratio.

* OR was calculated using a generalized estimated equations model that accounted for neighborhood clustering and repeated household sampling using a nested correlation structure.

† For the multivariate analysis.

TABLE 4

Bivariate relationship between baseline characteristics and subsequent acute respiratory illness among children under age 5 years in the ensuing 24 months

Characteristic	Monthly observations	Monthly visits with this exposure (%)	Monthly visits with acute respiratory illness (%)	Adjusted OR*	95% CI*	P value*
Household characteristics						
Mother's education ≥ 7 years	13,932	4,852 (35)	497 (10.2)	0.78	0.65, 0.93	0.005
Father's education ≥ 7 years	13,860	4,432 (32)	446 (10.1)	0.75	0.63, 0.90	0.002
PCA quintile						
1 (reference)	13,932	2,684 (19)	429 (16.0)			
2	13,932	2,223 (18)	406 (16.3)	0.78	0.62, 0.98	0.033
3	13,932	2,777 (20)	307 (11.1)	0.64	0.50, 0.82	< 0.001
4	13,932	3,140 (23)	360 (11.5)	0.69	0.54, 0.90	0.001
5	13,932	2,841 (20)	334 (11.8)	0.70	0.54, 0.89	0.004
Own radio	13,932	3,139 (23)	382 (12.2)	1.05	0.84, 1.30	0.673
Own television	13,932	3,790 (27)	423 (11.2)	0.91	0.76, 1.10	0.330
Own radio or television	13,932	5,708 (41)	657 (11.5)	0.95	0.79, 1.15	0.632
Household owns water source	13,932	3,884 (28)	491 (12.6)	0.97	0.83, 1.13	0.707
Household owns toilet	13,932	7,130 (51)	977 (13.7)	1.02	0.89, 1.18	0.758
Household owns mobile phone	13,932	3,127 (22)	345 (11.0)	0.76	0.59, 0.99	0.043
Child characteristic						
Male child	13,932	6,987 (50)	930 (13.5)	1.15	1.01, 1.31	0.032
Age < 2 years	13,932	5,291 (38)	901 (17.0)	1.74	1.53, 1.98	< 0.001
Year 1 surveillance (vs. year 2)	13,932	6,725 (48)	1,164 (17.3)	2.11	1.69, 2.63	< 0.001
Month since initiation of surveillance†	13,932			0.95	0.93, 0.96	< 0.001
Exclusive breastfeeding in the last 24 hours (children aged < 2 years)	4,562	454 (10)	84 (18.5)	1.03	0.76, 1.39	0.862
Handwashing indicators						
Response to handwashing questions						
Reported usually washing hands with soap (open-ended)						
Before preparing food	13,932	780 (5.6)	77 (9.9)	0.95	0.71, 1.27	0.720
Before eating	13,932	1,918 (14)	229 (11.9)	0.94	0.76, 1.16	0.560
Before feeding a child	13,932	544 (3.9)	51 (9.4)	0.81	0.58, 1.13	0.221
After eating	13,932	1,050 (7.5)	95 (9.1)	0.90	0.76, 1.06	0.212
After defecation	13,932	11,355 (82)	1,490 (13.1)	1.10	0.89, 1.36	0.378
After cleaning a child's anus	13,932	5,364 (39)	715 (13.3)	1.12	0.97, 1.29	0.118
After disposal of child feces	13,932	1,029 (7.4)	145 (14.1)	1.06	0.82, 1.37	0.658
After handling cow dung	13,932	3,497 (25)	427 (12.2)	0.94	0.76, 1.17	0.574
After returning from outside	13,932	496 (3.6)	74 (14.9)	0.98	0.62, 1.55	0.943
Closed-ended handwashing question: "the last time you ____, did you wash your hands with soap?"						
Prepared food	13,932	2,772 (20)	437 (15.8)	1.07	0.88, 1.30	0.490
Ate with your hands	13,932	1,912 (14)	280 (14.6)	0.92	0.71, 1.19	0.514
Fed your child	13,932	1,753 (13)	255 (14.5)	0.99	0.80, 1.22	0.921
Defecated	13,932	8,654 (62)	1,115 (12.9)	0.98	0.84, 1.15	0.821
Cleaned your child's anus	13,932	8,069 (58)	1,008 (12.5)	0.98	0.88, 1.09	0.700
Used soap today or yesterday	13,932	13,571 (97)	1,769 (13.0)	0.80	0.60, 1.06	0.116
Reported washing hands > 18 times on the preceding day‡	13,932	6,627 (48)	800 (12.1)	1.00	0.86, 1.16	0.971
Has separate soap for handwashing	13,932	3,106 (22)	402 (12.9)	0.90	0.73, 1.12	0.364
Has spare soap in the household	13,932	4,682 (34)	523 (11.2)	0.87	0.72, 1.04	0.128
Frequency of purchasing soap	13,932					
Weekly		4,779 (34)	640 (13.4)	1.15	0.90, 1.46	0.263
Every 1–2 weeks		4,791 (34)	667 (13.9)	1.04	0.79, 1.37	0.795
Every 2–4 weeks		2,210 (16)	271 (12.3)	0.92	0.71, 1.19	0.518
> 1 month (reference)		2,152 (15)	258 (12.0)			
Observations						
Water present at the most convenient place to wash hands	13,932	10,054 (72)	1,296 (12.9)	0.82	0.70, 0.97	0.021
Soap available at the most convenient place to wash hands	13,932	8,355 (60)	1,073 (12.8)	0.93	0.78, 1.10	0.393
Handwashing location	13,520					
Inside or near toilet		4,446 (32)	513 (11.6)	1.04	0.85, 1.28	0.710
Inside or near kitchen		1,364 (9.8)	183 (13.4)	0.97	0.78, 1.20	0.765
Outside in the yard (within 10 ft of the latrine)		3,824 (27)	595 (15.6)	0.96	0.82, 1.13	0.633
Outside of the yard (> 10 ft from latrine; reference)		3,886 (28)	501 (12.9)	1.19	0.71, 2.00	0.514
Observed visibly clean						
Mother's fingernails	13,932	8,185 (59)	990 (12.1)	0.89	0.75, 1.06	0.200
Mother's palms	13,932	12,888 (93)	1,728 (13.4)	1.37	0.98, 1.92	0.067
Mother's finger pads	13,932	12,887 (92)	1,730 (13.4)	1.26	0.93, 1.72	0.135
Child's fingernails	13,406	5,355 (40)	669 (12.5)	0.91	0.79, 1.06	0.227
Child's palms	13,406	9,688 (72)	1,277 (13.2)	0.94	0.78, 1.14	0.541

(Continued)

TABLE 4
Continued

Characteristic	Monthly observations	Monthly visits with this exposure (%)	Monthly visits with acute respiratory illness (%)	Adjusted OR*	95% CI*	P value*
Child's finger pads	13,406	9,852 (73)	1,285 (13.0)	0.92	0.76, 1.11	0.401
Handwashing demonstration						
Handwashing materials	9,954					
Water only (reference)		1,551 (16)	240 (15.5)			
Ash		694 (7)	106 (15.3)	1.15	0.75, 1.75	0.518
Mud		1,127 (11)	138 (12.2)	0.73	0.42, 1.26	0.252
Soap		6,582 (66)	825 (12.5)	0.86	0.66, 1.12	0.265
Washed both hands	9,954	6,571 (66)	876 (13.3)	1.06	0.85, 1.32	0.621
Washed with soap	9,954	6,582 (66)	825 (12.5)	0.92	0.78, 1.08	0.315
> 15 seconds	6,606	2,864 (43)	360 (12.6)	0.93	0.76, 1.13	0.448
Hand drying	9,954					
Dried on clothes (reference)		8,465 (85)	1,107 (13.1)			
Dried on dirty cloth		553 (6)	106 (19.2)	1.30	0.91, 1.86	0.154
Dried on clean cloth		403 (4)	68 (16.9)	1.05	0.73, 1.49	0.807
Air dried		533 (5)	28 (5.3)	0.40	0.27, 0.60	< 0.001

CI = confidence interval; OR = odds ratio.

* Adjusted for repeated measures and village clustering.

† Because the reported prevalence of respiratory infections decreased during the course of evaluation, we assessed the decline in respiratory infections per month.

‡ Asked as an open-ended question: "how many times did you wash hands yesterday?" The median response was 18.

with soap and likely practiced it more commonly than mothers who, even during the demonstration, did not use soap.

Mothers in rural Bangladesh feed their children directly with their hands, hands that often reflect the high level of fecal contamination in the environment.¹⁶ In our previously published analysis of prolonged structured observation of 1,000 households across rural Bangladesh, only 1% of adult caregivers were observed to wash their hands with soap before feeding a child.⁸ In the present analysis, when mothers were asked when they usually washed their hands with soap, 3.9% of mothers reported washing their hands with soap before feeding a child, and these children had less diarrhea in the subsequent 2 years compared with the children living with the 96% of mothers who did not mention handwashing with soap before feeding a child. Again, the indicator seems to overreport actual behavior; however, it also seems to identify a subset of mothers who at least occasionally remove pathogens from their hands by washing them with soap before feeding their children.

Many respiratory pathogens are transmitted through saliva and fomites.^{38,39} Handwashing can remove saliva and its associated pathogens from hands and therefore, reduce the risk for respiratory pathogen transmission. A number of studies on human behavior note the importance of an environment

that is conducive to supporting desirable behavior.⁴⁰ An earlier analysis of the 5-hour structured observation in rural Bangladeshi households confirmed that persons living in households that had water available at the handwashing station were more likely to wash their hands with soap and water compared with persons living in households where water was not conveniently available.³³ The findings in the present analysis (that the children living in households where water was present at the most convenient place to wash hands experienced less respiratory infections compared with children living in households where water was absent) are consistent with the observation that hand hygiene can reduce respiratory disease transmission⁴¹ and separate data from urban Bangladesh that also found fewer respiratory infections among children living in households where water was available at the most convenient place to wash hands.¹⁷

Rural Bangladeshi residents live in an environment with considerable mud, dirt, and environmental contamination, and therefore, their clothing becomes contaminated with organic matter and microbiological flora from the environment. In one study of structured observation in 80 Bangladeshi households, fieldworkers observed an average of one episode where a household resident coughed or sneezed into their clothing

TABLE 5
Multivariate analysis of handwashing indicators and acute respiratory illness prevalence

Characteristic	Bivariate odds ratio* (95% confidence limit)	Multivariate odds ratio* (95% confidence limit)	P value†
Cross-sectional survey (<i>N</i> = 13,334)			
Water present at the most convenient place to wash hands	0.82 (0.70, 0.97)	0.84 (0.70, 0.99)	0.040
Child aged less than 24 months	1.75 (1.53, 2.00)	1.34 (1.19, 1.51)	< 0.001
Month since initiation of surveillance	0.95 (0.93, 0.96)	0.95 (0.94, 0.97)	< 0.001
Mother's education above primary	0.78 (0.65, 0.93)	0.78 (0.65, 0.94)	0.009
Handwashing demonstration (<i>N</i> = 9,946)			
Air-dried hands during handwashing demonstration	0.40 (0.27, 0.60)	0.41 (0.26, 0.65)	< 0.001
Child aged less than 24 months	1.85 (1.59, 2.16)	1.41 (1.22, 1.62)	< 0.001
Month since initiation of surveillance	0.94 (0.93, 0.96)	0.95 (0.93, 0.97)	< 0.001
Mother's education above primary	0.77 (0.63, 0.95)	0.76 (0.60, 0.96)	0.024

* Odds ratio was calculated using a generalized estimated equations model that accounted for neighborhood clustering and repeated household sampling using a nested correlation structure.

† For the multivariate analysis.

per every 3-hour visit.⁴² Pathogens that commonly cause child respiratory illnesses typically survive on cloth for a few hours to several days.^{39,43} In the present analysis, children living in households where mothers allowed their hands to air dry as part of the handwashing demonstration had fewer subsequent respiratory infections compared with children living in households where mothers dried their hands on their clothes. Air drying would reduce the risk of reinoculating hands with pathogens on clothing.

Although there are credible biological mechanisms linking these handwashing indicators to child diarrhea and respiratory illness, it remains possible that this relationship is not causal. Measures of handwashing are associated with wealth and education.^{17–19} Increased wealth and education are strongly associated with less childhood illness.^{44,45} Thus, these indicators could be associated with better child health because of their association with wealth and education and not because of actually reflecting an independent contribution of handwashing to improved child health. More educated households where mothers are more hygiene-aware may engage in a number of practices other than handwashing with soap that reduce childhood risk for respiratory and diarrheal disease.

However, there are difficulties in ascribing these associations to confounding. There was no evidence of confounding in the multivariate analysis. The measured associations of these indicators were unchanged between the bivariate and multivariate analyses, which accounted for maternal education and a number of other strong associations between household and child characteristics in diarrhea and respiratory disease. None of the many measures of wealth, when placed in the model, improved overall fit and were significantly associated with the outcome. Moreover, if the association between handwashing indicators and child diarrhea and respiratory disease were primarily a result of confounding by socioeconomic status, we would expect to see a consistent association across most indicators of handwashing, especially those indicators reflecting messages most frequently stressed in handwashing promotion interventions (for example, handwashing after defecation or cleaning a child who defecated) or those times closely linked to social acceptability (for example, handwashing before eating). However, the data did not show this pattern.

A second non-causal explanation of the observed relationship between these handwashing indicators and child diarrhea and respiratory infection is that the associations occurred by chance. This analysis is an exploratory analysis of data collected within a program evaluation. We evaluated 33 separate handwashing indicators and evaluated their association with two different health outcomes. By chance, we would expect three indicators to be associated with a P value ≤ 0.05 . Although chance is a plausible explanation for the association of finger pad contamination with child diarrhea ($P = 0.04$) and the association of water present at the most convenient place to wash hands with child respiratory infection ($P = 0.04$), three of the associations were so highly statistically significant that random association is an implausible explanation. These associations include mothers responding that they usually wash hands with soap before feeding their child in an open-ended question ($P = 0.003$), mothers using soap in the handwashing demonstration with child diarrhea ($P < 0.001$), and mothers air-drying hands after the handwashing demonstration with childhood respiratory infection ($P < 0.001$). In addition, as

outlined above, each of these associations has a sound biological basis. There is also evidence external to this analysis that identifies some of the same indicators. In a separate study in an urban setting in Bangladesh, the presence of water at the most convenient place to wash hands was associated with a lower level of childhood respiratory illness after accounting for measures of socioeconomic status.¹⁷ In an earlier analysis of baseline data from both intervention and control households in the SHEWA-B evaluation, the presence of visible dirt on either the palm or finger pads was associated with the presence of water at the most convenient place to wash hands.⁸ This finding again suggests that there is a physical basis for the association between the handwashing indicator visible dirt and the regular practice of handwashing, and therefore, there is a plausible link between the indicator and child health.

The handwashing indicators associated with a reduced prevalence of diarrhea were different than the indicators associated with a reduced prevalence of respiratory infection. Different indicators of handwashing are likely associated with different handwashing behaviors, including use of different handwashing agents (e.g., water alone, ash, or soap) and use in different circumstances (e.g., after defecation, after returning home from the market, or before preparing food). Different handwashing behaviors, in turn, may differentially interrupt respiratory pathogens compared with diarrheal pathogen transmission. For example, handwashing with soap after defecation may be quite effective in interrupting gastrointestinal pathogen transmission but have little effect on interrupting respiratory pathogen transmission.

There are important limitations to the conclusions that can be drawn from this analysis. Because this evaluation was conducted in rural Bangladesh, the observations, questions, and their response may reflect the physical and cultural environment for handwashing and infectious disease transmission specific to rural Bangladesh. These indicators may perform quite differently in other contexts. However, the data were collected from households located in 50 villages, 43 sub-districts, and 26 districts across rural Bangladesh where diarrhea and respiratory disease are common major health problems, and therefore, these results are representative of a large at-risk population. The most meaningful assessment of whether the associations in this analysis identified robust indicators of effective handwashing behavior or resulted from either chance or factors that were unique to these assessment households in this particular time is to conduct similar evaluations in other settings.

A second limitation is that the list of handwashing indicators was not optimized for indicators to prevent respiratory disease transmission. There may be other indicators that would better capture handwashing during the critical times for respiratory disease transmission.⁴²

Standardizing assessment of the presence of dirt on finger pads or palms is difficult. There is a risk that some evaluators would subjectively judge hands to be less dirty in households with higher socioeconomic status. However, fieldworkers received formal training, including fieldwork to align assessments, and supervisors reevaluated assessments in a subset of households. Moreover, the association between visible dirt on palms and child diarrhea was independent of measures of socioeconomic status in the multivariate analysis.

Although some of these indicators were associated with subsequent child diarrhea, they may perform differently in the

context of a handwashing promotion intervention designed to change the attitudes and practice of the community to handwashing. Indeed, with more promotion of handwashing, more courtesy bias would be expected. This finding would not affect the relationships identified within this evaluation, because none of the study communities received handwashing promotion interventions; however, it may affect the validity if these indicators are used to evaluate handwashing promotion interventions.

This analysis implemented an alternative approach to assess the validity of handwashing indicators. It escapes the circular logic of comparing various imperfect indicators with each other by directly comparing indicators with child health outcomes. By using this approach, we identified five rapid handwashing indicators in rural Bangladesh that were subsequently associated with child diarrhea or respiratory illness. We recommend additional assessments of handwashing indicators using this approach in various settings to work to a robust set of meaningful handwashing indicators that can be readily implemented as part of national demographic surveys and program evaluations. In the meantime, we offer these indicators as the rapid handwashing indicators that have the best evidence base for inclusion in rapid handwashing assessments.

Received March 11, 2011. Accepted for publication July 27, 2011.

Acknowledgments: The SHEWA-B program evaluation was funded by the United Kingdom Department for International Development (DFID) through The United Nations Children's Fund (UNICEF) Bangladesh. The Centers for Disease Control and Prevention (CDC) contributed salary support for Dr. Luby's participation. ICDDR,B acknowledges, with gratitude, the commitment of DFID, UNICEF, and CDC to the Centre's research efforts.

Authors' addresses: Stephen P. Luby, Amal K. Halder, Tarique M. N. Huda, and Leanne Unicomb, Infectious Diseases and Vaccine Sciences, ICDDR,B, Dhaka, Bangladesh, E-mails: sluby@icddr.org, amalk@icddr.org, tariqueh@icddr.org, and leanne@icddr.org. Richard B. Johnston, Department of Water and Sanitation in Developing Countries, Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland, E-mail: richard.johnston@eawag.ch.

REFERENCES

1. Bryce J, Boschi-Pinto C, Shibuya K, Black RE, 2005. WHO estimates of the causes of death in children. *Lancet* 365: 1147–1152.
2. Ejemot RI, Ehiri JE, Meremikwu MM, Critchley JA, 2008. Hand washing for preventing diarrhoea. *Cochrane Database Syst Rev* 1: CD004265.
3. Luby SP, Agboatwalla M, Feikin DR, Painter J, Billhimer W, Altaf A, Hoekstra RM, 2005. Effect of handwashing on child health: a randomised controlled trial. *Lancet* 366: 225–233.
4. Master D, Hess Longe SH, Dickson H, 1997. Scheduled hand washing in an elementary school population. *Fam Med* 29: 336–339.
5. Ryan MA, Christian RS, Wohlrahe J, 2001. Handwashing and respiratory illness among young adults in military training. *Am J Prev Med* 21: 79–83.
6. Manun'Ebo M, Cousens S, Haggerty P, Kalengaie M, Ashworth A, Kirkwood B, 1997. Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. *Trop Med Int Health* 2: 1015–1021.
7. Curtis V, Cousens S, Mertens T, Traore E, Kanki B, Diallo I, 1993. Structured observations of hygiene behaviours in Burkina Faso: validity, variability, and utility. *Bull World Health Organ* 71: 23–32.
8. Halder AK, Tronchet C, Akhter S, Bhuiya A, Johnston R, Luby SP, 2010. Observed hand cleanliness and other measures of handwashing behavior in rural Bangladesh. *BMC Public Health* 10: 545.
9. Drankiewicz D, Dundes L, 2003. Handwashing among female college students. *Am J Infect Control* 31: 67–71.
10. Pedersen DM, Keithly S, Brady K, 1986. Effects of an observer on conformity to handwashing norm. *Percept Mot Skills* 62: 169–170.
11. Munger K, Harris SJ, 1989. Effects of an observer on handwashing in a public restroom. *Percept Mot Skills* 69: 733–734.
12. Pittet D, Simon A, Hugonnet S, Pessoa-Silva CL, Sauvan V, Perneger TV, 2004. Hand hygiene among physicians: performance, beliefs, and perceptions. *Ann Intern Med* 141: 1–8.
13. Ram PK, Halder AK, Granger SP, Jones T, Hall P, Hitchcock D, Wright R, Nygren B, Islam MS, Molyneaux JW, Luby SP, 2010. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. *Am J Trop Med Hyg* 83: 1070–1076.
14. Luby SP, Agboatwalla M, Billhimer W, Hoekstra RM, 2007. Field trial of a low cost method to evaluate hand cleanliness. *Trop Med Int Health* 12: 765–771.
15. Kaltenthaler EC, Pinfold JV, 1995. Microbiological methods for assessing handwashing practice in hygiene behaviour studies. *J Trop Med Hyg* 98: 101–106.
16. Ram PK, Jahid I, Halder AK, Nygren B, Islam MS, Granger SP, Molyneaux JW, Luby SP, 2011. Variability in hand contamination based on serial measurements: implications for assessment of hand-cleansing behavior and disease risk. *Am J Trop Med Hyg* 84: 510–516.
17. Luby SP, Halder AK, 2008. Associations among handwashing indicators, wealth, and symptoms of childhood respiratory illness in urban Bangladesh. *Trop Med Int Health* 13: 835–844.
18. Gorter AC, Sandiford P, Pauw J, Morales P, Perez RM, Alberts H, 1998. Hygiene behaviour in rural Nicaragua in relation to diarrhoea. *Int J Epidemiol* 27: 1090–1100.
19. Bhuiya A, Streatfield PK, Meyer P, 1990. Mother's hygienic awareness, behaviour, and knowledge of major childhood diseases in Matlab, Bangladesh. Caldwell J, ed. *What We Know About Health Transition: The Cultural, Social and Behavioural Determinants of Health*. Canberra, Australia: Health Transition Centre, 462–477.
20. Biran A, Rabie T, Schmidt W, Juvekar S, Hirve S, Curtis V, 2008. Comparing the performance of indicators of hand-washing practices in rural Indian households. *Trop Med Int Health* 13: 278–285.
21. Larson EL, Aiello AE, Cimiotti JP, 2004. Assessing nurses' hand hygiene practices by direct observation or self-report. *J Nurs Meas* 12: 77–85.
22. Strina A, Cairncross S, Barreto ML, Larrea C, Prado MS, 2003. Childhood diarrhea and observed hygiene behavior in Salvador, Brazil. *Am J Epidemiol* 157: 1032–1038.
23. Webb AL, Stein AD, Ramakrishnan U, Hertzberg VS, Urizar M, Martorell R, 2006. A simple index to measure hygiene behaviours. *Int J Epidemiol* 35: 1469–1477.
24. Ruel MT, Arimond M, 2002. Spot-check observational method for assessing hygiene practices: review of experience and implications for programmes. *J Health Popul Nutr* 20: 65–76.
25. Semmelweis I, 1983. *Etiology, Concept and Prophylaxis of Childbed Fever*. Madison, WI: University of Wisconsin Press.
26. Bowen A, Ma H, Ou J, Billhimer W, Long T, Mintz E, Hoekstra RM, Luby S, 2007. A cluster-randomized controlled trial evaluating the effect of a handwashing-promotion program in Chinese primary schools. *Am J Trop Med Hyg* 76: 1166–1173.
27. Larson EL, Early E, Cloonan P, Sugrue S, Parides M, 2000. An organizational climate intervention associated with increased handwashing and decreased nosocomial infections. *Behav Med* 26: 14–22.
28. Talaat M, Afifi S, Dueger E, El-Ashry N, Marfin A, Kandeel A, Mohareb E, El-Sayed N, 2011. Effects of hand hygiene campaigns on incidence of laboratory-confirmed influenza and absenteeism in schoolchildren, Cairo, Egypt. *Emerg Infect Dis* 17: 619–625.
29. Loevinsohn BP, 1990. Health education interventions in developing countries: a methodological review of published articles. *Int J Epidemiol* 19: 788–794.

30. Iyer P, Sara J, Curtis V, Scott B, Cardosi J, 2005. *The Handwashing Handbook. A Guide for Developing a Hygiene Promotion Program to Increase Handwashing with Soap*. Washington, DC: World Bank.
31. Centers for Disease Control and Prevention, 2010. *Handwashing: Clean Hands Save Lives*. Atlanta, GA: Centers for Disease Control and Prevention.
32. Luby SP, Halder AK, Huda T, Unicomb L, Johnston RB, 2011. The effect of handwashing at recommended times with water alone and with soap on child diarrhea in rural Bangladesh: an observational study. *PLoS Med* 8: e1001052.
33. Luby SP, Halder AK, Tronchet C, Akhter S, Bhuiya A, Johnston RB, 2009. Household characteristics associated with handwashing with soap in rural Bangladesh. *Am J Trop Med Hyg* 81: 882–887.
34. Vyas S, Kumaranayake L, 2006. Constructing socio-economic status indices: how to use principal components analysis. *Health Policy Plan* 21: 459–468.
35. Hanley JA, Negassa A, Edwardes MD, Forrester JE, 2003. Statistical analysis of correlated data using generalized estimating equations: an orientation. *Am J Epidemiol* 157: 364–375.
36. Roberts L, Smith W, Jorm L, Patel M, Douglas RM, McGilchrist C, 2000. Effect of infection control measures on the frequency of upper respiratory infection in child care: a randomized, controlled trial. *Pediatrics* 105: 738–742.
37. Niffenegger JP, 1997. Proper handwashing promotes wellness in child care. *J Pediatr Health Care* 11: 26–31.
38. Barker J, Stevens D, Bloomfield SF, 2001. Spread and prevention of some common viral infections in community facilities and domestic homes. *J Appl Microbiol* 91: 7–21.
39. Boone SA, Gerba CP, 2007. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl Environ Microbiol* 73: 1687–1696.
40. Sallis JF, Owen N, Fisher EB, 2008. Ecological models of health behavior. Glanz K, Rimer BK, Viswanath K, eds. *Health Behavior and Health Education*. San Francisco, CA: Josey-Bass, 465–485.
41. Rabie T, Curtis V, 2006. Handwashing and risk of respiratory infections: a quantitative systematic review. *Trop Med Int Health* 11: 258–267.
42. Nasreen S, Azziz-Baumgartner E, Gurley ES, Winch PJ, Unicomb L, Sharkar MA, Southern D, Luby SP, 2010. Prevalent high-risk respiratory hygiene practices in urban and rural Bangladesh. *Trop Med Int Health* 15: 762–771.
43. Walther BA, Ewald PW, 2004. Pathogen survival in the external environment and the evolution of virulence. *Biol Rev Camb Philos Soc* 79: 849–869.
44. NIPORT, 2007. *Bangladesh Demographic and Health Survey 2007*. Dhaka, Bangladesh: National Institute of Population Research and Training, Mitra and Associates.
45. Schell CO, Reilly M, Rosling H, Peterson S, Ekstrom AM, 2007. Socioeconomic determinants of infant mortality: a worldwide study of 152 low-, middle-, and high-income countries. *Scand J Public Health* 35: 288–297.