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Title:
Cluster-randomized controlled trials of individual and combined water, sanitation, hygiene, and nutritional interventions in rural Bangladesh and Kenya: The WASH Benefits Study design and rationale

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ABSTRACT

Introduction: Enteric infections are common during the first years of life in low-income countries and contribute to growth faltering with long-term impairment of health and development. Water quality, sanitation, handwashing, and nutritional interventions can independently reduce enteric infections and growth faltering. There is little evidence that directly compares the effects of these individual- and combined- interventions on diarrhea and growth when delivered to infants and young children. The objective of the WASH Benefits study is to help fill this knowledge gap.

Methods and Analysis: WASH Benefits includes two cluster-randomized trials to assess improvements in water quality, sanitation, handwashing, and child nutrition – alone and in combination – to rural households with pregnant women in Kenya and Bangladesh. Geographically matched clusters (groups of household compounds in Bangladesh and villages in Kenya) will be randomized to one of six intervention arms or control. Intervention arms include: water quality, sanitation, handwashing, nutrition, combined water+sanitation+handwashing (WSH), and WSH+nutrition. The studies will enroll newborn children (N= 5,760 in Bangladesh, N=8,000 in Kenya) and measure outcomes at 12-months and 24-months after intervention delivery. Primary outcomes include child length-for-age Z-scores and caregiver-reported diarrhea. Secondary outcomes include stunting prevalence, markers of environmental enteropathy, and child development scores (verbal, motor, personal/social). We will estimate unadjusted and adjusted intention-to-treat effects using semi-parametric estimators and permutation tests.

Ethics and Dissemination: Study protocols have been reviewed and approved by human subjects review boards at the University of California, Berkeley, Stanford University, The International Centre for Diarrheal Disease Research, Bangladesh, the Kenya Medical Research Institute, and Innovations for Poverty Action. Independent data safety monitoring boards in each

country oversee the trials. This study is funded by a grant from the Bill & Melinda Gates Foundation to the University of California, Berkeley.

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INTRODUCTION

Together, inadequate drinking water quality, sanitation, hygiene (WASH), and nutrition are leading risk factors for morbidity and mortality among children < 5 y.[1] Despite substantive progress spurred by the Millennium Development Goals to reduce these poverty-related risks, millions of children are born each year into environmental conditions that hinder their ability to achieve their full potential. Repeated insults from infection and undernutrition in the first years of life are thought to have profound negative consequences for health, cognitive development, and human capital that span the life course.[2–4]

The WASH Benefits study includes cluster randomized trials in Bangladesh and Kenya to address three important research questions related to the early life impacts of WASH and nutritional interventions. The first question is whether WASH and nutritional interventions can prevent linear growth faltering in the first two years of life. The second question is whether greater reductions in diarrhea can be achieved by combining individual WASH interventions compared to delivering them in isolation. The third question is whether the combined WASH and nutritional interventions jointly reduce diarrhea or improve linear growth more than each component alone. Below, we briefly summarize the rationale for the conduct of randomized trials to address each of these areas of scientific uncertainty.

Question 1: Can WASH and nutritional interventions prevent early life linear growth faltering?

Children in low-income countries experience severe linear growth faltering in the first 18-24 months of life that is thought to be preventable, at least in part, by postnatal interventions.[5,6] Interventions designed to improve nutrition among very young children measure length-for-age because it is a reliable, objective measure associated with subsequent child development at older ages.[7] During this early window, undernutrition and infection likely influence child

development and human capital through additional pathways besides linear growth.[8–10] Unfortunately, measuring child development at very young ages is difficult,[11] and documenting the full range of intervention impact thus requires longer term follow-up.[4]

In the first years of life, intervention trials and observational studies have implicated poor diet and infectious diseases as likely causes for a large share of child undernutrition.[8,12,13] Interventions to promote breastfeeding, improve complementary feeding practices, or provide nutritional supplements can lead to small improvements in nutritional indicators and length-for-age,[14–16] particularly among children who are at highest risk for severe stunting.[17,18] Nevertheless, effects of nutritional interventions on linear growth (upper bound of 95% confidence interval = +0.79 Z-scores)[19] fall far short of the median growth deficits observed in Sub-Saharan Africa and Southeast Asia, which are on the order of –2.0 Z-scores.[6]

One hypothesis for the inability of nutritional interventions alone to prevent a large share of growth faltering by age 24 months is that symptomatic and asymptomatic infections are important contributors to undernutrition. Symptomatic infection is common during the first years of life in low-income countries: on average, children under 24 months suffer from 3 to 4 episodes of acute diarrhea each year;[20] respiratory infections and other infectious diseases, such as malaria, are also common in many settings. Observational studies show that repeated episodes of diarrhea or parasitic infection are associated with increased risk of stunting [8,21–27] and subsequent cognitive deficits in childhood and later in life.[4,28,29] Possible mechanisms for enteric infections leading to growth faltering include reduced nutrient absorption through lower intestinal contact time during episodes of acute diarrhea, greater nutrient losses from persistent diarrhea (e.g. zinc) or intestinal bleeding (e.g. hookworm infection), reduced appetite, and diversion of energy and nutrients from growth to the immune system to fight the infection.

In addition to symptomatic infection, a subclinical condition called environmental enteropathy (EE), also known as tropical enteropathy, may also contribute to early life growth faltering.[30–32] The etiology of EE remains unknown, but the condition is generally characterized

by a set of physiologic changes to the small intestine's epithelial layer, which include villus atrophy, crypt hyperplasia, reduced absorptive capacity, increased permeability and inflammatory cell infiltration.[33] The causes are most likely related to repeated ingestion of pathogenic bacteria and an altered composition of the intestinal microbiota, which together lead to chronic enteric inflammation.[32] Children with EE are thought to have impaired growth through two mechanisms: (i) reduced nutrient absorption due to decreased surface area in the small (upper) intestine and (ii) elevated intestinal permeability, which increases translocation of antigenic molecules that stimulate the immune system and divert energy from growth. The combined effect of these two processes may harm a child's ability to effectively utilize nutrients in her existing diet for growth and development. EE is thought to be highly prevalent in low-income countries [34] and develops early in life: by age 8 months, 95% of a birth cohort in The Gambia showed signs of EE, and on average children in the cohort exhibited signs of EE during 75% of their first year of life.[31] Studies of Peace Corps volunteers and immigrant populations have demonstrated that intestinal malabsorption and permeability typically return to normal levels within 1-2 years after individuals move from highly contaminated environments to cleaner environments.[35,36] Since community-based studies that measure intestinal structure through biopsies would be extremely difficult, investigators typically rely on biomarkers of intestinal permeability, inflammation, and immune system stimulation as measures of subclinical EE.[31,37,38]

It is possible that improved nutrition alone can reduce the negative effects of a limited number of episodes of infection on growth due to the improved ability of better-nourished children to fight off enteric infections and exhibit catch-up growth during the convalescent period.[21,28,39–42] Effective nutritional interventions may be able to prevent or shorten the duration of EE via several mechanisms, such as a) strengthening epithelial barrier integrity and the immune response, b) compensating for malabsorption, reallocation or losses of key nutrients during infection, c) accelerating gut repair following infection, and d) favoring the growth of

beneficial gut microorganisms.[39] While it is possible that nutritional interventions alone may prevent or shorten the duration of EE, the limited evidence to date has been mixed,[33] with some evidence for improvements in gut function following vitamin A,[43] alanyl-glutamine supplementation [44] and zinc supplementation, [45,46] but no evidence for gut function improvement in trials that delivered probiotics,[47] glutamine supplementation,[48] omega-3 fatty acids [49] or richly fortified complementary foods.[50] As noted above, in many studies nutritional interventions have been insufficient to completely prevent growth faltering in low-income populations, and in the context of repeated or chronic infection, improved nutrition may only be able to mitigate – but not necessarily overcome – some of the effects of enteric infection on growth. If acute infections and subclinical EE contribute significantly to growth faltering, then interventions to reduce enteric infections during the first years of life would be expected to improve linear growth, perhaps independently of nutritional interventions.

Unlike the large literature on child nutritional interventions, we are aware of just 10 studies to measure the effect of WASH interventions on child growth; a forthcoming systematic review [51] may identify more. Four studies have found no improvement in linear growth as a result of WASH interventions, despite demonstrating reductions in caregiver-reported diarrhea in most cases.[9,52–56] A small randomized trial that enrolled children < 12 months and delivered handwashing promotion in Kathmandu slums additionally found no improvements in EE biomarkers.[53] The authors hypothesized that handwashing alone was inadequate to clean up the slum environment sufficiently to change the intestinal physiology, and suggested that more comprehensive environmental improvements may be necessary to reduce EE and improve growth.

Six studies have found positive associations between improved WASH conditions and child growth. Multiple cross-sectional or case-control studies found that young children living in households with improved sanitation and water supply had better linear growth.[57,26,58] A prospective birth cohort study in peri-urban Peru found that children living in households with

home water supply and sewerage connections were 1cm taller by age 24 months compared to children in households without them, and the effects of water supply and sewerage conditions were not mediated entirely by reductions in diarrhea.[59] A water quality intervention trial in rural Kenya found an average linear growth increase of 0.8 cm among children <5 years old after 1 year of exposure.[60–62] A prospective cohort from rural Bangladesh enrolled in a pilot for this study found that children raised in households with improved sanitation, hygiene, and water quality conditions had lower levels of parasite infection, better growth, and improved EE biomarkers compared to children raised in households without such access.[63] A trial to assess the impact of rural sanitation on diarrhea includes length-for-age as a secondary outcome but is still underway.[64] Taken together, the mixed evidence to date does not conclusively link improved WASH conditions with improved child growth, and the field would benefit from additional efficacy studies.

Question 2: Are combined WASH interventions more effective than single interventions?

In addition to quantifying the independent effects of WASH interventions, an important question is whether and how to combine sanitation, water quality, and handwashing promotion interventions to cost-effectively achieve health gains. Many implementing groups have publicly embraced the notion that combining interventions to improve water quantity, water quality, sanitation, and hygiene results in added benefits. This claim is based, in part, on observational studies [26,58,65,66] and theoretical modeling of pathogen transmission pathways.[67,68] However, the limited available evidence from randomized trials does not support this approach. In the only randomized controlled trial specifically designed to evaluate combined interventions, the two interventions evaluated were point-of-use water treatment and handwashing promotion with soap; individually, each intervention reduced child diarrhea (51% and 64% reduction), but there was no additional reduction in diarrhea among children exposed to both interventions (55% reduction).[54] These findings are consistent with results of a meta analysis of published

interventions to improve water quality, sanitation and hygiene, which found that combined interventions led to no greater reduction in diarrheal disease than single interventions.[69]

For WASH programs, single interventions are less expensive and easier to scale than combined interventions. By complicating communication and behavior change, combined interventions can potentially diminish the overall effect achievable from a single intervention.[70] Understanding the marginal benefits of sanitation, water treatment, and handwashing in the absence and presence of each of the other interventions will therefore be important for policy makers (i) when deciding overall budgets for sanitation, water, and handwashing, and (ii) when weighing the tradeoffs between allocating resources to an intense, expensive approach combining multiple interventions in a single site, or choosing the most cost-effective interventions and rolling them out at scale. This same reasoning applies to our third research question, below.

Question 3: Are there larger effects on diarrhea or linear growth from combining a) nutritional interventions with b) a combined water, sanitation and handwashing intervention compared to each component alone?

In the 1960s Scrimshaw et al. proposed a theory that repeated infections interact with poor nutrition to cause a cycle of infection and malnutrition.[71] Consistent with this earlier work, McDade [72] outlined a life history theory of immune function in which he posited that infants face a resource allocation tradeoff between maintenance (fighting infection, physiologic repair) and growth. During infection, the immune system diverts energy and nutrients away from growth; a developing infant prioritizes survival and maintenance over growth. When resources are limited, the absolute level of energy or nutrients available to infants can be a major determinant of growth and physiologic repair. An impaired gut in a child without access to sufficient energy or nutrients will further suffer from impaired healing, with subsequent decline in gut function and nutrient absorption for growth; thus begins a vicious cycle between infection and

malnutrition.[71,73,74] The potential contribution of infection to malnutrition and mortality risk was recently illustrated in a dramatic 35% reduction in all cause mortality among severely malnourished Malawian children after the provision of prophylactic antibiotics.[75]

Dewey and Mayers [39] reviewed the evidence for the potential interaction between nutrition and infection on early child growth. The review identified just one study that suggested that infections could reduce the effectiveness of nutritional interventions and four trials that demonstrated that improved nutrition could limit the negative consequences of infection. The authors concluded that the potential interaction between nutrition and infection control should be a priority for research, which echoes earlier calls for additional research in this area.[33,34] The only study to date that we are aware of that was explicitly designed to test for interaction between infection control and improved nutrition was the Narangwal nutrition project, conducted in Punjab, India between 1968 and 1973.[10,76–78] The 10-village study (2,900 newborns) was a factorial trial that randomized villages to control, improved medical services, improved nutrition, or their combination. The nutrition intervention included growth monitoring, food supplementation for children who were not growing well, and nutrition education. The medical care intervention improved access to vaccines and morbidity surveillance for acute illness. Both nutritional and medical service villages also received prenatal care for pregnant mothers, which included iron and folic acid supplements as well as food supplements for mothers who were underweight. The study found that the medical services intervention improved height and weight compared to control, and that the nutritional services intervention improved height and weight even more. The study found no additional benefit to combining nutrition and medical services above the nutritional services alone with respect to height and weight. Although international guidelines for infant and young child feeding practices published by Unicef, WHO, and the Alive and Thrive initiative all include handwashing recommendations,[79–81] the degree to which additional infection control measures could complement nutrition programs remains an important knowledge gap.

Objectives of the WASH Benefits Study

Given the likely long-term negative consequences of undernutrition and infection during a child's first years, the global development community would benefit from rigorous evidence about the effects of single- and combined WASH and nutritional interventions on child illness and growth. As outlined above, there remains substantial uncertainty about which interventions or combination of interventions are most effective. The WASH Benefits study includes two highly comparable cluster randomized trials in rural Bangladesh and Kenya to help fill these knowledge gaps. The intervention trials include single and combined interventions in sanitation, water quality, handwashing, and nutrition. Each intervention has been developed over multiple years of formative research. The two trials share the following scientific objectives, which will contribute evidence toward the identified evidence gaps.

Primary scientific objectives:

1. Measure the impact of sanitation, water quality, handwashing, and nutrition interventions on child diarrhea and linear growth after 2 years of exposure.
2. Determine whether there are larger reductions in child diarrhea when providing a combined water, sanitation and handwashing intervention compared to each component alone.
3. Determine whether there are larger effects on child diarrhea and linear growth from combining a) a comprehensive child nutrition intervention with b) a combined water, sanitation and handwashing intervention compared to each component alone.

Secondary scientific objectives:

4. Measure the impact of a child nutritional intervention and household environmental interventions on environmental enteropathy biomarkers, and more clearly elucidate this potential pathway between environmental interventions and child growth and development.

5. Measure the impact of sanitation, water quality, handwashing and nutritional interventions on intestinal parasitic infection prevalence and intensity.

6. Measure the association between parasitic infection and other measures of enteric health, including acute diarrhea and environmental enteropathy biomarkers.

To achieve these objectives, the studies will enroll pregnant women and their children who are born within approximately 6 months of the baseline survey. The study will measure linear growth and caregiver-reported diarrhea, biological markers of EE, intestinal parasite infections and child development in the cohort over the first 24 months of exposure to the intervention.

METHODS AND ANALYSIS

Overview of the design

The Bangladesh trial is led by the International Center for Diarrheal Disease Research, Bangladesh (icddr,b); the Kenya trial is led by Innovations for Poverty Action (IPA) and the Kenya Medical Research Institute (KEMRI). Both trials include 6 intervention arms and a double-sized control arm (Figure 1). In Bangladesh, the unit of randomization is a group of compounds visited by a single local promoter and separated by at least a 15-minute walk. Bangladesh clusters consist of 8 proximate household compounds that meet our eligibility criteria within a village. In Kenya, clusters consist of one or two adjoining administrative villages with at least 6 eligible pregnant women. The studies enroll pregnant women and their children who are born within approximately 6 months of the baseline survey. We will follow the closed cohort longitudinally and measure primary outcomes at 12 months and 24 months after initiating the intervention.

The design includes a large number of clusters per arm with a small number of children per cluster, which was motivated by three, inter-related considerations: (i) WASH interventions need

to be delivered at the cluster level because the promotion activities are inherently community level; (ii) there are potential interactions between adjacent households with respect to behavior and infectious disease and we wish to maintain independent units for randomization; and (iii) at the time our study enrolls a cluster and initiates an intervention, pregnant women are relatively scarce. The large study population spread over a wide geographic area means that we will measure intervention effects over heterogeneous environmental conditions.[82] The design is optimized to measure group-level differences in our primary outcomes. The infrequent measurements in WASH Benefits will mean that we will not characterize infectious outcomes (e.g., diarrhea, parasitic infections) well for individual children if the outcomes vary temporally within children.[83]

Participant eligibility criteria, study setting, and enrollment strategy

Participant eligibility criteria

In both countries, the trials enroll pregnant women identified in community-based surveys who expect to deliver in the 6 months following enrollment based on date of last menstruation. The study will enroll all children born in study clusters in the 6 months following the baseline survey (some target children will be born after 6 months due to inaccuracies in gestational age using reported date of last menstruation). Our target sample size of pregnant women at enrollment is 5,760 in Bangladesh and 8,000 in Kenya. The Kenya cohort will be larger because we expect to find more variation in child length-for-age than in Bangladesh (sample size details below). Within study compounds, the study enrolls all children < 36 months at baseline to measure diarrhea outcomes over the study period; the study measures diarrhea outcomes in a wider age group because older children are still at high risk for diarrheal disease.[20]

In both countries, compounds consist of multiple households (typically 3-10 in Bangladesh, 1-4 in Kenya), usually comprising blood relatives, who share a common courtyard. Compounds are eligible to participate if (i) they have a pregnant woman and (ii) the woman plans to stay in

the village for the next 12 months. The study excludes households who do not own their home to help mitigate attrition during follow-up. The Kenya trial excludes villages that have chlorine dispensers at water sources installed by programs separate from the present study. In Bangladesh, the study excludes households who report high iron in their drinking water most of the year because pilot studies showed it was difficult to maintain the appropriate chlorine residual for continued disinfection in high-iron water. In cases where the respondent is unsure about iron content, field staff check the water's chlorine demand using Aquatabs® and a digital Hach Pocket Colorimeter II; if residual chlorine is below 0.2 mg/L after 30 minutes staff exclude the household. Within a study compound, the studies enroll pregnant women and children from the following age groups:

1) Children in utero at enrollment (target children): All children born to enrolled mothers within approximately 6 months of the baseline survey.

2) Children 18 – 27 months old at enrollment (specimen collection): Older children living in the compound and aged 18-27 months at enrollment will be eligible for stool and blood specimen collection. This age window reflects the age window of the target children at the final study measurement, and serves as a baseline measure for the study population.

3) Children < 36 months at enrollment (diarrhea): All children < 36 months living in the compound are eligible for caregiver reported diarrhea measurement.

4) Additional children born into study compounds after 6 months: We will enroll children born into study compounds who are too young to meet our enrollment criteria (group 1, above), deliver interventions to them according to randomized assignment, and measure anthropometry and diarrhea at follow-up surveys. These additional enrollees will not be included in the primary analysis because very young children may not be exposed to intervention for sufficient amount of time to expect to see impact on our primary outcomes (particularly length-for-age). However the additional young children will provide information (in exploratory analyses) about the effect of established interventions on very young infants.

Field staff discuss the prospect for participation in the study with adults in each compound, including the mother/caregiver of the target infants. After providing time for discussion among the compound residents, a member of the field team seeks formal informed consent from pregnant women.

Bangladesh setting and enrollment

The Bangladesh trial is located in Gazipur, Mymensingh, and Tangail districts. These 3 districts are located in the floodplain of central Bangladesh where the majority of the rural population is engaged in agriculture. The majority of the population uses shallow tubewells for drinking water, which are known to be frequently contaminated with fecal indicator bacteria.[84] Enrollment commenced in June 2012. The study has enrolled compounds in communities that meet the following criteria:

- Located in a rural area
- Drinking water with low levels of iron (<1milligrams/L on average) and arsenic (<50 micrograms/L on average) as documented in the collaborative assessments by the Government of Bangladesh and the British Geological Survey. Water chemistry eligibility criteria were used because pilot studies indicated that when iron or arsenic levels were high the chlorine demand for household water treatment was unpredictable.
- The Government of Bangladesh, international non-government organizations working in Bangladesh and local government authorities report that no major water, sanitation, or focused nutrition programs are currently operating or planned in the area in the next 2 years
- Not located in haor areas (areas completely submerged during the monsoon season)

Each study cluster includes a group of compounds with 8 eligible pregnant women. The compounds within a cluster are located closely enough together so that a single promoter can

reach each of the participating compounds by walking. If the compounds were too dispersed for a promoter to reach all of them on foot, then they will not be enrolled in the study. More than one cluster could be enrolled in a single village but clusters within the same village need to be separated from each other by a minimum of 15 minutes walking distance.

Kenya setting and enrollment

The Kenya trial is located in rural areas of 10 districts in Bungoma, Kakamega, and Vihiga counties in the western part of the country. The region is populated mainly by subsistence farmers. Unimproved latrine coverage is high (at least 85%), and our pilot study in the region estimated that among children < 27 months old 11% had diarrhea in the preceding two days. Very few (<5%) households have piped water, and the majority of households report obtaining drinking water from sources such as protected springs, where chlorination has previously been shown effective.[85] Enrollment commenced in November 2012. The study region contains over 2,000 villages, from which study villages were selected to form clusters using the following criteria:

- Located in a rural area (defined as villages with <25% residents living in rental houses, <2 gas/petrol stations, and <10 shops)
- Not enrolled in ongoing WASH or nutrition programs
- Majority (>80%) of households do not have access to piped water into the home
- At least 6 eligible pregnant women in the cluster at baseline

Description of the Interventions

Overview of the intervention approach and assumptions

The WASH Benefits study has focused on identifying and testing water, sanitation, handwashing and nutritional interventions that have strong potential to reduce infection and

malnutrition during the first years of life. WASH Benefits is designed to measure intervention effects under conditions of high uptake in our target populations since our central hypotheses have not been tested rigorously in randomized studies. The enabling technologies and behavioral intervention packages were developed in the target populations over a two-year period before the start of the trials. Details of the behavior change theoretical frameworks and methods used in each country will be published in separate, forthcoming articles. Local promoters that are residents of the study villages deliver the interventions at the cluster level; each promoter completes at least 5 days of training with refresher courses periodically through the study period. Promoters visit and counsel study compounds weekly in the early phase of intervention, with visits declining in frequency over time; we anticipate visits as infrequent as one per month after one year of intervention.

The environmental interventions in this study focus on modifying the compound environment to reduce infant exposure to enteric pathogens. The interventions focus on compound-level modifications because we assume that the dominant transmission pathways for the infants in our study will be within the compound. Since we expect on average 8-10 household compounds with eligible children per study cluster, we expect to intervene in a small fraction of each community. While point-of-use water quality, hygiene and nutrition interventions operate at a household level, some sanitation interventions may require wider coverage in a neighborhood, community or other larger environment in order to effectively mitigate personal exposure. However, cost and logistical limitations prevented us extending implementation beyond the compound. Furthermore, a pilot study suggested that the compound was a relevant unit of intervention for modifying infant exposure to environmental conditions.[63]

Control

It is possible that the simple act of regular visits by intervention promoters could lead to improvements in the primary outcomes through unknown channels that are independent of

WASH or nutrition interventions. The WASH Benefits team discussed this possibility extensively in the year preceding the trials, and the teams agreed to pursue slightly different strategies in the two countries. The Bangladesh team concluded that their intervention behavior change model is so tightly integrated into the enabling technology components that the effect of a visit is inseparable from the WASH and nutrition interventions themselves; moreover, it is fairly common for mothers in the study area to be visited by community promoters associated with other programs. The control arm in Bangladesh will be a “passive” control, meaning there is no promotion or intervention activity during the study.

The Kenya team was more concerned about the possibility of the promotion visits leading to changes in behaviors not related to WASH or nutrition that could nonetheless affect the primary outcomes since promoter visits are atypical in the Kenyan study area. For this reason, the Kenya team decided to include promoters in their control arm and to add a simple activity across all arms of the study: monthly measurement of mid-upper arm circumference (MUAC) or measuring the pregnant woman’s belly circumference prior to the birth. The key assumption for the Kenya design is that whatever non-WASH- or –nutrition-related behavior changes occur in the intervention arms will also occur in the control arm. The Kenya control arm promoters do not promote any water, sanitation, hygiene, or nutrition messages, and strictly engage in measuring child MUAC and mother belly circumference. In all arms, children >6 months old with MUAC <115mm are classified as severely malnourished and are referred to treatment (details below in Referral Guidelines).

Water quality

The Bangladesh study delivers a 10-liter, insulated water storage vessel and a free supply of chlorine tablets (Aquatabs® brand, sodium dichloroisocyanurate) to enrolled households to improve the microbiological quality of their drinking water.[86] The Kenya study installs chlorine dispensers within the cluster boundary at public water sources used by study participants. All

community members will be able to use the dispensers. After filling their water collection container (typically a 20L plastic jerry can) at the source, users can place the container under the dispenser, and turn a knob to release 3 mL of 1.25% sodium hypochlorite, an amount designed to yield 2.0ml/L of free chlorine residual after 30 minutes for 20L of water.[87] The Kenya study also includes community level promotion of dispenser use, and all households in the study compound receive bottles of sodium hypochlorite (6 months' supply) to facilitate householders' water treatment during periods when they rely on rainwater harvesting (common during the rainy season) or if they use a water source where a dispenser has not been installed. In both countries, the behavior change strategies target the consistent provision of treated water to all children living in the household.

Sanitation

Both the Bangladesh and Kenya studies include three enabling technologies in the compound-level sanitation intervention with the goals of reducing children's exposure to feces in the household environment and increasing latrine use: 1) a locally developed sani-scoop dedicated to the removal of child and animal feces from the compound,[88] 2) plastic child potties for children ages 6 months and older until they use the latrine, and 3) a new or upgraded latrine for each household in the compound. In Bangladesh, latrines are upgraded to a dual pit latrine with a water seal and super structure. In Kenya, plastic latrine slabs that include a tightly fitting hole-cover are installed to improve existing latrines that have a mud or wood floor. Simple pit latrines (unlined pits with a earthen superstructure and the plastic slab) are constructed in the compounds of study participants who do not have access to a latrine. The behavior change strategies in both countries target the use of the latrine for defecation and the safe disposal of feces by all households in the compound to prevent contact by young children.

Handwashing

Both country studies install two handwashing stations for enrolled households: one near the latrine and one near the cooking area. In Bangladesh, handwashing stations include a locally made bucket with a tap fitting (40 L near the latrine, 16 L near the cooking area), a stool, a bowl, and a bottle to dispense soapy water. In Kenya, handwashing stations are constructed from locally available materials and include a dual tippy-tap design with independent pedals attached to two 5-liter jerry cans of clean water and soapy water.[89] In both countries the studies provide soap to families free of charge to replenish the handwashing stations. The behavior change strategies of the intervention target handwashing with soapy water messaging at two critical times for caregivers: after defecation/cleaning the child's anus and before food preparation.[90] Promoters frame the concept of handwashing as a nurturing behavior facilitated by the ease and convenience of a nearby handwashing station.[91]

Combined WSH

In both countries, the combined WSH intervention integrates all intervention components from the water quality, sanitation, and handwashing arms. Intervention promoters sequence the interventions so that they are not introduced at the same time. In Bangladesh, the interventions are delivered sequentially in the following order: sanitation, handwashing and water treatment, with a minimum of 21 days between each start date. In Kenya, all intervention technologies aside from latrine construction are provided at the same time but the behavior change counseling is rolled out in the following sequence approximately spaced approximately two weeks apart: handwashing and basic water treatment, sanitation, in-depth water treatment. The provision of latrines can range from one to several weeks after the commencement of work in a cluster in Kenya. The behavior change strategy emphasizes the interconnected aspect of WASH and the need to practice all behaviors in order to benefit from them.

Nutrition

In both countries, the nutrition intervention strategy targets age appropriate behaviors (pregnancy to 24 months) including use of and lipid-based nutrient supplements (ages 6 – 24 months). The behavior change counseling is modeled after the Guiding Principles for Complementary Feeding of the Breastfed Child,[80] the UNICEF Program Guide for Infant and Young Child Feeding Practices,[81] and the Alive and Thrive initiative.[79] Target behaviors include: 1) practice exclusive breastfeeding from birth to 6 months of age and introduce complementary foods at 6 months of age while continuing to breastfeed; 2) continue breast feeding as you did before receiving study-provided nutritional supplements; 3) provide your child micronutrient-rich foods such as meat, fish, eggs, and vitamin A rich fruits and vegetables (adapted to locally available food examples); and 4) feed your child complementary foods at least 2-3 times per day when 6-8 months old and 3-4 times per day when 9-24 months old.

When target children are between 6 and 24 months old, intervention promoters will deliver monthly supplies of Lipid-based Nutrient Supplements (LNS). The LNS used in the study is a next generation version of Nutributter®.[92] Appendix 1 includes the specific LNS formulation. LNS is administered daily using 10 gram sachets that can be mixed into pre-prepared meals (e.g., porridge) or consumed directly from the sachet; a child eats two sachets per day. LNS is intended to supplement – and not replace – breastfeeding and locally available complementary foods, by providing 118 kcal/day and including a broad suite of essential fatty acids and micronutrients at dosages appropriate for children in this age group.[92] It has an 18-month shelf life, does not spoil at high temperatures and costs as little as $0.08 per day. Reported adherence has been 88% of days in controlled trials,[14] in part due to the ease of incorporating it into existing feeding routines. Breastfeeding is highly prevalent in both populations based on pilot studies, and so we have focused on supplements that would not replace this essential source of nutrition.[93,94] In Kenya, the trial will provide LNS to older, age-eligible siblings (6-24 months) living in study households to prevent potential sharing of LNS with older siblings. The

Bangladesh trial will deliver LNS only to target children because older, age-eligible siblings are rare in the study population.

Nutrition + Combined WSH

In both countries the Nutrition + combined WSH arm will include the interventions delivered in the nutrition and combined WSH arms. The nutrition intervention is delivered in parallel with the WSH interventions according to the stage of pregnancy and age of the target child.

Intervention monitoring

Given the importance of good uptake (also called take-up or compliance) for the success of the trial, it is essential for the team to have early and frequent feedback on intervention uptake. If an intervention has poor uptake, then the team needs to consider modifying or redoubling implementation efforts in that arm. To preserve external validity, each country team will document any adaptive changes used to modify the intervention. Investigators will be blinded to outcomes from the trial, so any adaptation to intervention will be based solely on information about intervention implementation and uptake.

Both country teams have in place a detailed implementation monitoring system. One of the outputs from the monitoring system is a summary of whether the implementation has achieved a limited set of critical benchmarks (Appendix 2); benchmarks are intended to flag serious problems in implementation. If any of the uptake measures falls below its critical benchmark, then a qualitative team will review the monitoring and process documentation in the low performing area, visit the site of the low uptake, meet with intervention promoters, supervisors and study subjects, and troubleshoot the cause of the low uptake. Because the interventions have each been piloted and the pilots achieved these benchmarks of uptake, we expect that uptake below the benchmark will indicate a problem where the intervention was not

implemented as planned, and the investigation will identify what additional training or other support is required to achieve high intervention uptake.

Additional principles that we will follow with respect to adapting the interventions include:

1. If we identify easily fixable problems in an intervention that we expect will improve uptake, then we will make the change uniformly in the study population.
2. If we identify a problem in an intervention arm and devise a solution, the solution must be implemented in all clusters assigned to that intervention to ensure that we do not differentially modify the intervention on a subsample of the population.
3. Since WASH Benefits is an efficacy trial, we will replace broken hardware in our study population.
4. We will maintain a detailed record of the timing and scope of any changes to the interventions (if any).

Outcomes

Primary outcomes

Primary outcomes include length-for-age Z-scores (LAZ) measured 24 months after intervention initiation in target children and diarrhea prevalence in compound children < 36 months old at enrollment. Child age will be determined using birthdates verified when possible using vaccination cards. Following standard protocols for anthropometric outcomes measurement,[95,96] pairs of trained anthropometrists will measure recumbent length (accurate to 0.1 cm) and weight without clothing (accurate to 0.1 kg) in triplicate. The median of the three measurements will be used in the analysis.[97] We will measure diarrhea at baseline among children <36 months old and again 12- and 24-months after intervention initiation using a definition of ≥ 3 loose or watery stools in 24 hours or ≥ 1 stool with blood based on caregiver reported symptoms;[98] we will use a 7-day recall period unless we find differential recall errors by randomized group, in which case we will use a 2-day recall period.[99,100]

Secondary outcomes

Secondary outcomes include two additional measures of linear growth, child development measures, and measures of EE. We will calculate differences between groups in LAZ at the 12-month measurement, and stunting prevalence (LAZ < –2) at the 24-month measurement. At the 24-month visit, we will measure child development in communication, gross motor, and personal/social domains using the Extended Ages and Stages Questionnaire;[11,101] the instrument has been adapted to each study population, relies on caregiver-report, and has been used in many low-income countries.[102] We will compare groups for each domain independently, and overall by summing scores across domains. In a subsample of up to 1,500 children across four arms of each trial, we will measure EE biomarkers at 3, 12, and 24 months following intervention initiation (Figure 2); assays planned include: urinary lactulose mannitol ratio,[103] fecal myeloperoxidase,[104] fecal alpha-1-antitrypsin,[105] fecal neopterin,[106] and plasma total IgG.[37]

Additional outcomes

The study will collect stool specimens from 7 target children per cluster at the 24 month visit and from an older child living in the compound (Figure 3), and will test specimens for soil transmitted helminths (*Ascaris lumbricoides, Trichuris trichiura*, hookworm) using the Kato-Katz method [107] and protozoans (*Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica*) using PCR methods (Bangladesh) and commercial enzyme-linked immunosorbent assay kits (Kenya). Appendix 3 includes a full list of tertiary outcomes. In a subsample of households in which the study measures EE biomarkers, we will also measure markers of environmental fecal contamination to help trace the causal path between the interventions and outcomes. Environmental contamination measures will include enumeration of fecal indicator bacteria (*Escherichia coli)* in household stored drinking water, on child toy balls, and child hand rinses. In

addition, the study will collect quantitative measures of fly density at the latrine and the food preparation area.

Referral guidelines

The study will refer participants for treatment at appropriate local government health care providers if we observe any of the three following outcomes: soy or nut allergies related to LNS, acute malnutrition, and intestinal parasite infection (described below).

Soy or nut allergies related to LNS

In the LNS arms, intervention promoters will recommend that caregivers stop using the LNS and notify one of the study staff immediately should their child have any adverse reactions shortly after ingesting the supplement (such as vomiting, stomach pain, rash, breathing problems with wheezing). In the event of an adverse reaction, study staff will assess the child's condition and, if necessary, provide transport to the closest medical facility for treatment.

Acute malnutrition:

In the anthropometry and enteropathy assessment survey, children who are found to be acutely malnourished based on WHO/Unicef criteria (severely wasted [WLZ < -3] and/or bipedal edema) will be referred to the appropriate existing treatment programs in each country. In Kenya, where promoters measure MUAC each month for all target children, children >6 months with MUAC <115mm will be considered severely malnourished and will be referred to treatment.

Intestinal parasites:

All children who provide a stool specimen in the 24-month survey will be offered deworming medication, which is consistent with national standards in both countries.

Randomization and Blinding

The trials will randomly allocate clusters to each intervention arm of the study in equal proportion along with a double-sized control arm. The randomization is pair-matched by geography, with adjacent clusters randomized in blocks. The rationale for using geography to match the randomization is that it is logistically feasible, it may add efficiency to our effect estimation if geography is strongly correlated with our outcomes, and it will help ensure that the different arms are balanced with respect to characteristics and events that are spatially clustered. In Bangladesh, the trial will randomize groups of 8 geographically proximate clusters to one of the 6 intervention arms or the double-sized control arm with allocation probabilities of 2/8 for control and 1/8 for each intervention arm. In Kenya, the randomization is identical but includes 9 proximate clusters in each block with allocation probabilities of 2/9 for active control, 1/9 for each intervention arm, and 1/9 for a potential passive control (not yet funded). Clusters allocated to a passive control arm in Kenya will enable the study to measure the effect of regular visits to the study's active control arm, if any, pending future funding.

The randomization sequence generation and allocation for both trials will be conducted by the coordinating team at the University of California, Berkeley using a random number generator in Stata v12 (StataCorp, College Station, TX) with a reproducible seed. Due to the nature of the interventions, participants are not blinded to their treatment assignment. Principal investigators and primary analysts for the trial will remain blinded to the randomized group assignments until the primary analysis is complete. Cluster level assignments will be under control of each country's lead data manager in separate data files that are independent from the main datasets of the study. Access to the treatment assignment information (even if blinded), will be limited to the core analysis team in each country until the primary results are published.

Sample Size

The sample size calculations were based on the two primary outcomes: LAZ and caregiver-reported diarrhea. We calculated the minimum detectable effect for LAZ measured at two years using a standard equation,[108] and for diarrhea using a simulation-based approach to accommodate two levels of correlation in the outcome (within-child and within-cluster).[109] To inform our sample size calculations we used existing datasets from relevant populations. In Bangladesh, we used diarrhea and anthropometric measurements from 982 children < 36 months, collected from 100 rural villages between 2007-2009.[110] In Kenya, we informed the sample size calculations using diarrhea data collected from 1,704 children in 95 control villages enrolled in a cluster-randomized trial of spring protection conducted in Western Province between 2005 and 2007;[85] we also informed the sample size calculation with LAZ measurements from 310 children 4 – 30 months old in a pilot study in our study region. We selected final designs in each country to detect differences of +0.15 in LAZ and a relative risk of diarrhea of 0.7 or smaller for a comparison of any intervention with the double-sized control arm. We chose the effect size for LAZ based on our team's expert opinion of the smallest effect that would be biologically meaningful and measurable given measurement error in field conditions (+0.15 Z equals 0.48 cm in a 24 month old girl). We chose the effect size for diarrhea based on earlier WASH efficacy studies.[111] The control arm is double-sized because it will be used in multiple hypothesis tests and, given available information, a 2:1 allocation ratio is close to the optimal allocation that minimizes the variance for the six tests planned under our first hypothesis, below.[112,113] Appendix 4 includes the detailed assumptions used in the calculations.

Analysis Plan

General analysis approach

Each study team will develop its own analysis plan, but both teams will include in their analyses unadjusted means and SDs by randomized group, along with unadjusted comparisons between groups for the primary hypotheses.[114,115] We will also re-estimate our parameters of

interest in adjusted analyses (details below). We will produce public replication files for our primary analyses in both countries. We will analyze participants according to their randomized assignment (intention to treat; ITT).

Parameters of interest

This section discusses parameters of interest for the primary analyses. Let Y be an outcome of interest and let T index the randomized group assignment, where $T \in$ (*C, W, S, H, WSH, N, NWSH*). There are seven arms: *C*=control; *W*=water; *S*=sanitation; *H*=handwashing; *WSH*= combined water, sanitation and handwashing; *N*=nutrition supplement; and *NWSH*= nutrition plus combined *WSH*. Let Z be a set of indicators for matched blocks used in the randomization. Finally, let ψ denote parameters of interest. In each comparison below, we define ψ as a difference between various randomized groups. For dichotomous outcomes like diarrhea, this implies a risk difference. We will additionally report risk ratios for dichotomous outcomes as recommended by CONSORT.[114]

H1: Water, sanitation, handwashing, nutrition and their combination reduce child diarrhea and improve linear growth.

The mean outcomes in each active intervention arm (*W, S, H, WSH, N, NWSH*) will be compared to the mean outcomes in the control arm (6 comparisons per outcome). The null hypothesis is that there is no difference between intervention and control. The same control group (double sized) will be used in every comparison. The parameters of interest are the difference in means between the intervention groups and the control group. For $t \in$ (*W, S, H, WSH, N, NWSH*):

$$\psi_{1,t} = E_Z(E[Y \mid T = t, Z] - E[Y \mid T = C, Z])$$

H2: When delivered in combination, water, sanitation and handwashing interventions reduce child diarrhea more than when delivered individually.

The combined arm (*WSH*) treatment effect for diarrhea will be compared to individual WASH treatment effects to determine whether the combined effect is larger than the individual effects. The parameters of interest are the difference in means between the combined group and the individual intervention groups. For $t \in$ (*W, S, H*):

$$\psi_{2,t} = E_Z(E[Y \mid T = WSH, Z] - E[Y \mid T = t, Z])$$

Note that this parameter and associated test differs from a test for interaction (departure from additive effects). We expect this study to have limited power to detect interactions between interventions, but describe tests in Appendix 5.

H3: Combined Nutrition and WASH interventions reduce diarrhea and improve linear growth more than each component alone

We will compare the combined Nutrition+WASH arm (*NWSH*) treatment effects for growth to the nutrition arm (*N*) and the combined WASH arm (*WSH*). The null hypothesis is that the treatment effect in the combined arm is equal to the single arms, and the parameter of interest is the difference in means between groups. For $t \in$ (*WSH, N*):

$$\psi_{3,t} = E_Z(E[Y \mid T = NWSH, Z] - E[Y \mid T = t, Z])$$

As with H2, this hypothesis is not a hypothesis of interaction or synergy. Rather, it is a test to determine if one intervention is better than another (additive interaction would test whether the combined arm is greater than the sum of the independent intervention arms). If the interaction were of equal magnitude to the overall treatment effect, a roughly four-fold increase in the sample size would be required,[116] which would be logistically infeasible given the already large size of the trial.

Testing and estimation

One strength of a randomized trial is that it allows investigators to draw inference non-parametrically, relying only on randomization.[117] One approach to test for statistical significance is a permutation test based on randomly permuting randomized assignments in the data (following the original randomization strategy, i.e., permuting *T* within strata Z), and re-estimating a test statistic.[117–121] We plan to use a rank-based test statistic, which has been shown to have good power against alternatives,[122] and estimate it on un-weighted cluster means.[118,119] We will use one-sided tests because we would only expect the interventions to be beneficial.[123] Due to the relatively small number of tests involved, we do not plan to adjust the *P*-values for multiple testing.[124]

The permutation test is a test for statistical independence with good power against alternatives but does not estimate a specific parameter of interest (and thus will not provide standard errors and confidence intervals for our parameters). Since the trials depart from an individually randomized design, we will bootstrap the dataset, resampling clusters in matched blocks with replacement, and re-estimate our parameters of interest. Resampling matched blocks preserves the correlation structure in the data and retains any efficiency gains from the matched randomization. Since we will have a large number of units to resample, the asymptotic assumptions will be reasonable, the bootstrap distribution will be smooth, and percentile-based confidence intervals will be accurate for all parameters of interest. We will examine the bootstrap estimate of the sampling distribution to confirm these assumptions. The SDs of the bootstrap distributions will provide estimates of the standard error.

We will complement our unadjusted analyses with a second set of estimates that are conditional on baseline covariates to potentially increase the efficiency of our analysis and reduce bias from any chance imbalances in prognostic covariates despite randomization.[125] It is straight-forward to extend permutation tests to include covariate adjustment while still taking advantage of the exact distribution theory provided by randomized inference.[118,120] For

example, let Y_{ijk} be the outcome of interest for individual *i* in village *j* and randomization stratum *k*; let T_{jk} be the randomized intervention indicator, and X_{ijk} be a vector of adjustment covariates. Models are fit of the form: $E[Y_{ijk} \mid X_{ijk}] = m(X_{ijk})$, where $m(.)$ is some function of the covariates X. For example, $m(X_{ijk}) = \alpha_k + \beta \times X_{ijk} + \varepsilon_{ijk}$ for a linear regression, but it could be a more sophisticated prediction function. The residuals are then calculated using predicted values of Y_{ijk} from the model: $r_{ijk} = Y_{ijk} - \hat{Y}_{ijk}$, and the permutation test is conducted on the residuals. The test has nominal size for the null hypothesis even if the model $m(.)$ is mis-specified and if the covariates are measured with error.[118,120] There is no stochastic model for $m(.)$, just a reduced algorithmic fit; the approach increases statistical efficiency because the residuals are less variable than the original outcomes, assuming the covariates are strongly associated with the outcome or heterogeneous within strata.[118]

Following CONSORT guidelines, [114,115] we pre-specify a repeatable, objective approach that we will use to identify adjustment covariates. We plan to consider the following covariates in adjusted models:

- Administrative Union (Bangladesh) or Location (Kenya)
- Field staff team member who recorded the measurement
- Time between intervention delivery and measurement
- Month of measurement, to account for seasonal variation
- Household food insecurity
- Child age
- Child sex
- Mother's age
- Mother's height
- Mother's education level and literacy
- Number of children < 15 y in the household

- Number of individuals living in the compound
- Distance (in minutes) to the primary water source
- Housing materials (floor, walls, roof) and household assets

We will use a repeatable data-adaptive algorithm to control for the covariates flexibly and semi-parametrically that will be chosen before the analysis (e.g., [126]). We will calculate adjusted *P*-values using the permutation test described above based on predicted residuals from the algorithm. We will estimate SEs, and confidence intervals for our parameters of interest using the bootstrap described in the unadjusted analysis section. Appendix 5 includes the details of additional, pre-specified analyses, including tests of interactions between interventions, subgroup analyses, and tests for between-cluster spillover effects.

Differential attrition (loss to follow-up): detection and effect bounds calculation

The study will track enrolled participants carefully to help minimize attrition in the study. We will compare attrition rates across randomized arms, and we will compare the characteristics of those lost to follow-up versus those that remain to determine whether attrition is random. If we find systematic attrition that is not balanced across arms, then we will conduct sensitivity analyses using "worst-case" imputation bounds for our effect estimates (proposed by Horowitz and Manski,[127] and summarized by Duflo et al.,[108] and we will also calculate bounds proposed by Lee.[128] If overall levels of attrition approach 20%, we will attempt to locate individuals who left the study area to measure outcomes at the 2-year measurement and include them in our analyses; if attrition is high we will also consider the use of semi-parametric weighting using baseline characteristics.[129]

Interim analyses and stopping rules

Interim Analyses: Except for monitoring uptake of the interventions described above, the WASH Benefits study team does not plan to conduct interim outcome analyses that include

information about randomized assignment until all of the data from the 2-year measurement are collected.[125,130,131]

Negative Stopping Rule: There is always a risk that interventions will have unintended consequences. Although we would not conduct the trial if we anticipated such harm, the interventions are complex and there is always the chance for unanticipated outcomes. If one of the country Data and Safety Monitoring Boards were to find clear evidence of harm based on adverse events, then the study will halt the harmful intervention arm under international ethical guidelines for medical research.[132]

Positive Stopping Rule: Since this is an efficacy study designed to identify proof of principle, even if a marked early benefit is identified with one or more of the interventions, neither the study implementers nor the Governments of Bangladesh or Kenya will be in a position to immediately scale up effective interventions. Thus, the social benefit of early stoppage is limited. However, we will provide 1-year anthropometry measurements to each country's DSMB. If at the 1-year measurement, child length for age Z- score in any of the intervention arms is more than 2.0 standard deviations above the control arm we will look to the country DSMB to decide on the appropriateness of continuing the trial.

Additional analyses

WASH Benefits is a large study with many collaborators, and the research will be able to answer scientific questions beyond those posed in this protocol. Indeed, the study team expects to conduct and publish analyses that extend beyond those specified in this protocol. For example, Objective 5 of the study is to explore the association between multiple enteric infection measures collected in the study. Yet, many promising multiplex antigen assays for parasitic infection are still in development and so the study plans to archive samples for future analyses.

ETHICS AND DISSEMINATION

Study protocols have been reviewed and approved by institutional review boards at the University of California, Berkeley, Stanford University, the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), the Kenya Medical Research Institute (KEMRI), and Innovations for Poverty Action (IPA). Each trial is overseen by an independent Data and Safety Monitoring Board, which review the study protocols and monitor severe adverse events. All study communities, compounds, and caregivers provide informed consent. The data collected in the study will be publically distributed along with metadata and critical documents (i.e., protocols and questionnaires) following the publication of the primary results from the trials, which is expected to be within 24 months of the final data collection date.

FIGURE LEGENDS

Figure 1.
Summary of the overall study design in both countries, including cluster and target child enrollment in each arm. Growth and diarrhea measurements will take place at 15- and 27-months following enrollment, which corresponds to 12- and 24- months following initial intervention delivery due to a 3-month lag between enrollment and intervention implementation. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

Figure 2.
Summary of the environmental enteropathy (EE) subsample in both countries, including cluster and target child enrollment in each arm. The EE subsample includes an equal number of clusters and target children from 4 arms of the study. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

Figure 3.
Summary of enteric parasite measurement in both countries, including cluster and target child enrollment in each arm. At enrollment stool specimens will be collected from an older sibling

aged 18-27 months if present and will be tested for protozoan infections. At the final measurement, specimens will be collected from the same older siblings plus 7 target children per cluster in each country, and analyzed for protozoan infections and soil transmitted helminth infections. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

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Description of each author's contribution to writing the protocol

BFA, CN, SPL, LU, CPS, SA, GC, AEH, AL, AJP, and JMC drafted the protocol. KGD, TA, TC, HD, LHF, RH, PK, EL, SMN, PKR, FT, and PJW reviewed and provided critical input to the protocol.

Registration

Trial registration identifiers (www.clinicaltrials.gov): NCT01590095 (Bangladesh), NCT01704105 (Kenya).

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Competing interests statement

The authors have no competing interests.

Data Sharing Statement

The data collected in the study will be publically distributed along with metadata and critical documents (i.e., protocols and questionnaires) following the publication of the primary results from the trials, which is expected to be within 24 months of the final data collection date.

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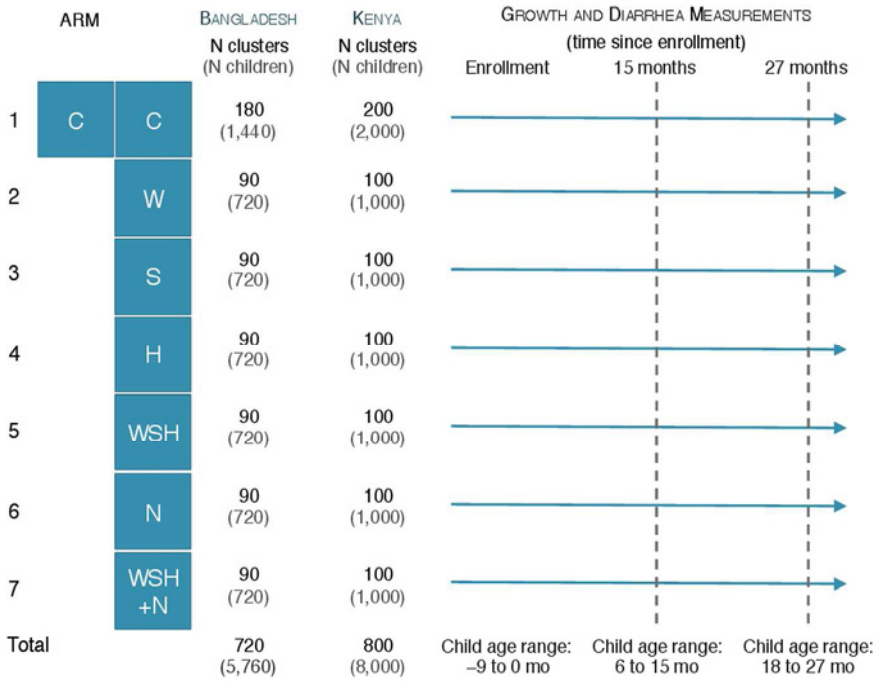

Figure 1.
Summary of the overall study design in both countries, including cluster and target child enrollment in each arm. Growth and diarrhea measurements will take place at 15- and 27-months following enrollment, which corresponds to 12- and 24- months following initial intervention delivery due to a 3-month lag between enrollment and intervention implementation. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

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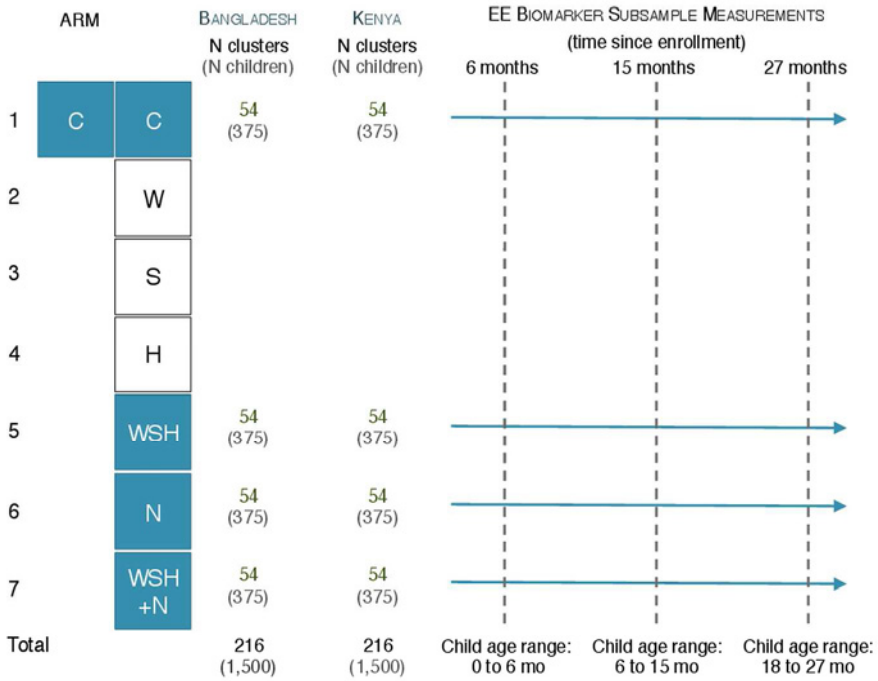

Figure 2.
Summary of the environmental enteropathy (EE) subsample in both countries, including cluster and target child enrollment in each arm. The EE subsample includes an equal number of clusters and target children from 4 arms of the study. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

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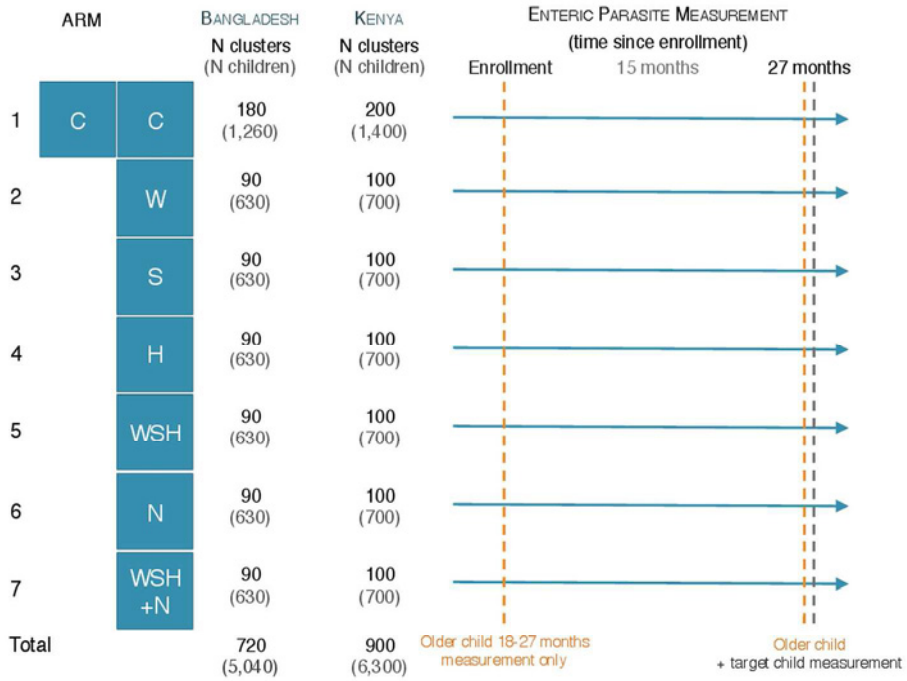

Figure 3.
Summary of enteric parasite measurement in both countries, including cluster and target child enrollment in each arm. At enrollment stool specimens will be collected from an older sibling aged 18-27 months if present and will be tested for protozoan infections. At the final measurement, specimens will be collected from the same older siblings plus 7 target children per cluster in each country, and analyzed for protozoan infections and soil transmitted helminth infections. Abbreviations for intervention arms: C = control; W = improved water quality; S = improved sanitation; H = improved handwashing; WSH = combined improvements in water quality, sanitation, and handwashing; N = improved nutrition; WSH+N = combined improvements in water quality, sanitation, handwashing, and nutrition.

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Cluster-randomized controlled trials of individual and combined water, sanitation, hygiene, and nutritional interventions in rural Bangladesh and Kenya: The WASH Benefits Study design and rationale

Appendix 1. Nutrient Content of the Lipid-based Nutrient Supplement (LNS) used in WASH Benefits compared to the WHO/FAO Recommended Nutrient Intakes (RNI) [1] for children 1-3 years

Nutrient	Unit	WHO/FAO RNIs for children 1-3 y*	LNS nutrient content		
			Content	% RNI	Chemical form
Daily Dose‡	g		20		
Energy	kcal		118		
Fat	g		9.6		
Linoleic acid	g		4.46		
Alpha-linolenic acid	g		0.58		
Ratio of LA to ALA			7.7		
Protein	g		2.6		
Vitamins					
Vitamin A	µg	400	400	100%	Retyinyl acetate
Vitamin D	µg	5	5	100%	Cholecalciferol (D3)
Vitamin E	mg	5	6	120%	DL-alpha-tocopherol acetate
Vitamin K	µg	15	30	200%	Phylloquinone 5%
Vitamin C	mg	30	30	100%	L-ascorbic acid
Biotin	µg	8	NA		
Folic acid	µg	150	150	100%	Pteroyl monoglutamic acid
Thiamine (B1)	mg	0.5	0.5	100%	Thiamin hydrochloride
Riboflavin (B2)	mg	0.5	0.5	100%	Riboflavin
Niacin	mg	6	6	100%	Niacinamide
Pantothenic acid (B5)	mg	2	2	100%	Calcium pantothenate
Vitamin B6	mg	0.5	0.5	100%	Pyridoxine hydrochloride
Vitamin B12	µg	0.9	0.9	100%	Cyanocobalamin (0.1%)

Nutrient	Unit	WHO/FAO RNIs for children 1-3 y*	LNS nutrient content		
			Content	% RNI	Chemical form
Minerals					
Calcium§	mg	500	280	56%	Tri-calcium phosphate
Copper¶	mg	0.34	0.34	100%	Encapsulated copper sulfate
Iodine	µg	90	90	100%	Potassium iodate
Iron**	mg	11.6	9	78%	Encapsulated ferrous sulfate (Bangladesh) Ferrous fumarate (Kenya) ‡‡
Magnesium§	mg	60	40	67%	Magnesium citrate
Manganese	mg	1.2	1.2	100%	Manganeze sulfate
Phosphorous§	mg	460	190	41%	Tri-calcium phosphate & Di-potassium phosphate
Potassium	mg		200		Di-potassium phosphate & potassium chloride
Selenium	µg	17	20	118%	Sodium selenite 1.5%
Zinc**	mg	8.3	8	96%	Zinc sulfate

*RNI=Recommended Nutrient Intake; LNS=Lipid-based nutrient supplement; RDA=Recommended Dietary Allowance; WHO = World Health Organization; FAO = Food and Agriculture Organization of the United Nations
‡ In malaria endemic areas, it is recommended that the supplement be split into two 10 g servings in one day to reduce the iron consumed in a single bolus dose. Although malaria is less common in Bangladesh, we recommend children consume two 10 g sachets per day in both trials.
§ The calcium, phosphorus, and magnesium content of LNS do not meet 100% of the RNI for technical reasons
¶ The Institute of Medicine RDA level for copper for infants 1-3 y is shown here [2].
** The RNI for iron and zinc is that assumed under a diet of low bioavailability.
‡‡ Bangladesh will use encapsulated ferrous sulfate, similar to other LNS products on the market. Ferrous fumarate will be used in Kenya due to an interaction between ferrous sulfate and polyphenols in the commonly consumed millet flour.

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Cluster-randomized controlled trials of individual and combined water, sanitation, hygiene, and nutritional interventions in rural Bangladesh and Kenya: The WASH Benefits Study design and rationale

Appendix 2. Critical Benchmarks for Intervention Monitoring

The principal and co-principal investigators will carefully review the intervention fidelity assessments and identify any areas of low uptake of interventions. Critical benchmarks for uptake based on unannounced visits are summarized below for each country.

While unlikely, it is also possible that the study promoters will be implementing the intervention precisely as planned, but uptake is lower than expected. If uptake is below the benchmark in the setting where implementation followed the prescribed approach, the qualitative team in each country will conduct more in-depth evaluation will be framed around the behavior change models guiding the intervention design.

Bangladesh critical benchmarks

Intervention	Indicator	Benchmark
Overall implementation	Participant reports a promoter visit in the past 28 days to deliver messages about the intervention	90%
Water quality	Households with children 6 – 24 months of age have stored chlorinated drinking water (measured by residual chlorine)	65%
Sanitation	Among participants with a child under 36 months, the participant reports that the youngest child's most recent defecation was either directly into the latrine or the feces were disposed of into the latrine (based on open-ended questions about where the child defecated and what was done with the feces)	65%
	Sani-scoop easily accessible to mother	80%
	Households in the bari have a latrine with a functional water seal	80%
Handwashing	Households have at least one handwashing station with soap and water present	65%
Nutrition	Within households with targeted children > 6 months of age, the stock of LNS sachets is consistent with the daily use of two sachets per day based on records of the last distribution and the number of sachets currently observed in the home.	70%
	Report hearing any messages on infant/child nutrition and or Sonamoni (lipid based nutrient supplement)	80%

Kenya critical benchmarks

Intervention	Indicator	Benchmark
Overall implementation	Participant reports a promoter visit in the past 28 days to deliver messages about the intervention Mid-upper arm circumference recorded in the past 28 days based on caregiver's tracking booklet	90% 90%
Water quality	Drinking water stored in the participant's home has residual chlorine	65%
Sanitation	Latrine cover observed over the hole in the primary latrine used by the participant Among participants with a child under 36 months, the participant reports that the youngest child's most recent defecation was either directly into the latrine or the feces were disposed of into the latrine (based on open-ended questions about where the child defecated and what was done with the feces).	65% 65%
Handwashing	Soapy water and rinse water are observed at one or more tippy taps in participant's compound	65%
Nutrition	Within households with targeted children > 6 months of age, the stock of LNS sachets is consistent with the daily use of two sachets per day based on records of the last distribution and the number of sachets currently observed in the home.	70%

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Appendix 3. Tertiary outcomes.

	Tertiary Outcome	Population	Definition	Measurement	Citation
1	Weight-for-age at 1 year and 2 years	Target children	Child's weight standardized to Z-scores using the WHO 2006 growth standards	Weight measured after 1 and 2 years of intervention.	[1–4]
2	Weight-for-height at 1 year and 2 years	Target children	Child's weight and length standardized to Z-scores using the WHO 2006 growth standards	Weight and length measured after 1 and 2 years of intervention.	[1–3]
3	Underweight at 2 years	Target children	Weight-for-age Z < – 2 at the year-2 measurement.	Weight measured after 2 years of intervention.	[1–3]
4	Wasted at 2 years	Target children	Weight-for-height < – 2 at the year-2 measurement.	Weight and length measured after 2 years of intervention.	[1–3]
5	Severely stunted at 2 years	Target children	Length-for-age Z < – 3 at the year-2 measurement.	Severe stunting classification is based on the WHO 2006 standard.	[1–3]
6	Head circumference-for-age at 1 year and 2 years	Target children	Child's weight standardized to Z-scores using the WHO 2006 growth standards, measured after 1 and 2 years of intervention.	Head circumference measured after 1 and 2 years of intervention.	[1–3]
7	Soil transmitted helminth infection at 2 years	Target children	*Ascaris*, *Trichuris*, and Hookworm eggs present in a single stool sample.	Kato-Katz microscopy on preserved stool samples.	[5]
8	Protozoan infection at 2 years	Target children	*Giardia*, *Cryptosporidium*, and *Entamoeba histolytica* present in a single stool sample.	*Giardia*, *Cryptosporidium*, and *E. histolytica* TechLab ELISA test (Kenya) or real time qPCR assay (Bangladesh)	[6]

	Tertiary Outcome	Population	Definition	Measurement	Citation
9	Soil transmitted helminth infection at 2 years	Children 18 – 27 months at enrollment	*Ascaris*, *Trichuris*, and Hookworm eggs present in a single stool sample.	Kato-Katz microscopy on preserved stool samples.	[5]
10	Protozoan infection at 2 years	Children 18 – 27 months at enrollment	*Giardia*, *Cryptosporidium*, and *Entamoeba histolytica* present in a single stool sample.	*Giardia*, *Cryptosporidium*, and *E. histolytica* TechLab ELISA test (Kenya) or real time qPCR assay (Bangladesh)	[6]
11	Verbal Communicative Development Inventory at 1 year	Target children	CDI score	CDI measured using linguistically adapted instruments that are rely on caregiver report (Bangladesh only).	[7]
12	WHO motor milestones at 1 year	Target children	Six milestones: sitting without support, hands-and-knees crawling, standing with assistance, walking with assistance, standing alone, walking alone	Measured using caregiver report and demonstration to fieldworker.	[8]
13	Acute upper respiratory illness	Children < 36 months at enrollment	Constant cough or difficulty breathing	Caregiver-reported symptoms with 2 day and 7 day recall, measured after 1 year and 2 years of intervention.	[9]
14	All cause mortality	Target children	Mortality during follow-up	Mortality confirmed by the caregiver and head of household between enrollment and 2 years of intervention.	

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Appendix 4. Assumptions used to calculate minimum detectable effects

All of the calculations assume a Type I error (α) of 0.05, power (1-β) of 0.8, a one-sided test for a two-sample comparison of means, and 10% dropout after baseline. The length-for-age Z-score (LAZ) calculations used a standard equation assuming a single, post-treatment measurement at 2 years.[1] Since the diarrhea outcome measurement includes a partial baseline (target children will be in utero at baseline, but their older siblings will be present) and multiple levels of correlation (within-child, within-cluster), we used a simulation-based approach.[2 3]

Appendix References

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Bangladesh

We used the following assumptions to calculate minimum detectable effects (MDEs) for length-for-age and diarrhea in the Bangladesh trial:

	Assumption	Source / rationale
Overall Design		
Clusters in the control arm	180	Double-sized control arm
Clusters in each treatment arm	90	
Length-for-age Z-score (LAZ)		
Baseline measurements	0	Target children in utero at baseline
Post intervention measurements	1	Primary outcome, measured at 2 years post-intervention (ages 18 - 27 mo)
Children per cluster	7	Enrolling 8 children per cluster, but have conservatively assumed 7.
SD	1.243	SHEWA-B cohort,[4] children < 36 months
Cluster-level ICC	0.008	SHEWA-B cohort,[4] children < 36 months
Diarrhea		
Baseline measurements	1	Note: simulations assume no baseline for target children in the cohort.
Post intervention measurements	2	
Children per cluster	10	SHEWA-B cohort [4] 1.45 children < 36 months, conditional on 1 child – 6 to 0 months in the household. 7*1.4=10
Prevalence in control	12%	SHEWA-B cohort [4] 2-day period prevalence for children < 36 months at enrollment = 12.5%
Prevalence in single treatment arms (for WSH vs. W | S | H)	8%	33% relative reduction from 12% in control
Child-level standard deviation	0.618	SHEWA-B cohort [4]
Cluster-level standard deviation	0.776	SHEWA-B cohort [4]

Under these assumptions in Bangladesh, we calculated the LAZ MDE for a treatment versus control comparison equal to +0.15, and for a treatment versus treatment comparison equal to +0.18. The diarrhea MDE for a treatment versus control arm is equal to –3.1% (RR=0.74), and for the combined versus single intervention arms is equal to –2.4% (RR=0.70).

Kenya

We used the following assumptions to calculate minimum detectable effects (MDEs) for length-for-age and diarrhea in the Kenya trial:

	Assumption	Source / rationale
Overall Design		
Clusters in the control arm	200	Double-sized control arm
Clusters in each treatment arm	100	
Length-for-age Z score (LAZ)		
Baseline measurements	0	Target children in utero at baseline
Post intervention measurements	1	Primary outcome, measured at 2 years post-intervention (ages 18 - 27 mo)
Children per cluster	10	
SD	1.218	WASH Benefits Kenya pilot study
Cluster-level ICC	0.07	WASH Benefits Kenya pilot study
Diarrhea		
Baseline measurements	1	Note: simulations assume no baseline for target children in the cohort.
Post intervention measurements	2	
Children per cluster	14	Kenya 2008-9 DHS [5] 1.48 children < 36 months, conditional on 1 child – 6 to 3 months in the household. Used 1.4 because the DHS estimate is a slight over-estimate: it does not include women who have an eligible target child as their first birth. 10*1.4=14
Prevalence in control	12%	Rural Water Project control group [6] 1 day prevalence = 9.9%. Estimates of 2-day prevalence using standard methods [7] range from 12.2% - 13.7%.
Prevalence in single treatment arms (for WSH vs. W | S | H)	8%	33% relative reduction from 12% in control
Child-level standard deviation	0.617	Rural Water Project control group [6]
Cluster-level standard deviation	0.378	Rural Water Project control group [6]

Under these assumptions in Kenya, we calculated the LAZ MDE for a treatment versus control comparison equal to +0.15, and for a treatment versus treatment comparison equal to +0.18. The diarrhea MDE for a treatment versus control arm is equal to –2.2% (RR=0.82), and for the combined versus single intervention arms is equal to –1.8% (RR=0.78).

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Appendix 5. Pre-specified secondary analyses: treatment interactions, cross-cluster externalities, subgroup analyses.

Tests of treatment interaction

The study is powered for the tests described in the main text. We chose to design the study around main effects and not these interaction tests because we expect the interactions, if present, to be small and thus difficult to detect in feasible designs. However, the design will enable us to test for large interactions between treatments (related to H2 and H3 in the main text). The rationale for including the interaction tests in our analysis plan is that if the interactions are large, they will be both detectable and scientifically important. Nonetheless, we recognize that the study will not have power to detect these interactions unless they are at least 2 times larger than the main effects.[1] This is because the interaction tests will rely on variance terms from more than 2 arms (in contrast to the parameters described in the main text). The interactions we describe below are on the additive scale.

The first interaction test is whether combined water quality, handwashing, and sanitation interventions improve our primary outcomes more (or less) than the additive effect of the components delivered separately. The The null hypothesis is:

$$Ho:\ E_Z(\,[Y \mid T = WSH, Z\,]\,) = E_Z\,(\,\mathrm{E}[Y \mid T = W, Z\,] + \mathrm{E}[Y \mid T = S, Z\,] + \mathrm{E}[Y \mid T = H, Z\,]\,)$$

There are theoretical [2–4] and observational [5,6] studies to support this hypothesis, but the only randomized trial to date found no positive interaction between water treatment and handwashing [7] (and, if anything, antagonism, where the effect of the combined treatment is less than the additive effect of water treatment + handwashing).

The second interaction test is whether combined WASH and Nutrition interventions improve our primary outcomes more (or less) than the additive effect of the components delivered separately. The null hypothesis is:

$$Ho:\ E_Z(\,\mathrm{E}[Y \mid T = NWSH, Z\,]\,) = E_Z\,(\,\mathrm{E}[Y \mid T = WSH, Z\,] + \mathrm{E}[Y \mid T = N, Z\,]\,)$$

While there is biologic plausibility for this interaction, there is scant empirical evidence to support or refute the hypothesis.[8]

Testing for- and estimating cross-cluster spillovers/externalities

A fundamental assumption for unbiased causal inference in a randomized trial is that the units of randomization are independent.[9] In this study, clusters are the unit of randomization. Cross-cluster spillover effects occur when the treatment assignment of one cluster influences outcomes in another cluster. The mechanism for spillover could be through disease transmission or through information diffusion. Although we expect a priori that cross-cluster spillovers are likely to be quite small, we plan to test this assumption. We plan to test for spillovers over geographic distance and through shared

school membership and market attendance. In the notation below we use distance as an example, but the same parameter and notation applies to spillovers through other channels, which we will test for separately.

Let N_d^T be the number of treated compounds with treatment *T* in some distance *d*, defined as straight-line, geographic distance from the cluster perimeter. We do not control N_d^T by design, but we expect that there will be random variation created by our design. Define a new parameter among the control clusters (*T*=*c*), which includes the effect of adjacent treated compounds (N_d^T) as a measure of the spillover effect, controlling for cluster-level covariates *X*:

$$\theta = E_{X,N^T}\left(E[Y|T=c,X,N_d^T] - E[Y|T=c,X,N_d^T=0]\right)$$

To estimate this parameter, we will need to model E[*Y* | *T, X,* N_d^T]. The linear model used by Miguel and Kremer [10] is a sensible choice, but we may consider less parametric prediction algorithms.[11] To test for cross-cluster spillovers, we will restrict the analysis to the control clusters to simplify the test. The first term is the empirical distribution of *Y* in the control group, including observed spillovers (N_d^T). The second term is estimated from the predicted values of *Y* from the algorithmic fit under conditions of no spillover effects (N_d^T =0). (Note: if there are no clusters without spillover effects, the model would need to extrapolate beyond the observed data.) Under the null hypothesis of no spillovers, the parameter equals zero. The null hypothesis is:

$$Ho: Y \perp N_d^T \mid T, X$$

We can test the null hypothesis with a clustered permutation test for each treatment, *T*. This involves permuting the cluster IDs in the control group, re-fitting the algorithm, and re-estimating *θ* for a large number of permutations. This will generate a null distribution of *θ*. We can then obtain a *P*-value for the test by comparing the observed *θ* to its null distribution.

If we cannot reject the null hypothesis, then we will proceed with the standard Intention-To-Treat (ITT) analysis (parameters described in the main text). If we reject the null hypothesis, then *θ* will provide an estimate of the magnitude of spillover effects for each treatment *T*. In the presence of spillovers the ITT estimates will be a lower bound of the estimate of the total effect of treatment under the assumption that spillover effects are positive.

Scope: We plan to test for spillovers in behavior change uptake indicators (Appendix 2) and our primary outcomes. We will repeat the test for each outcome and treatment. We do not expect spillover effects from the nutrition intervention treatment and will not test for them. We will test for spillovers through three main channels:

1. Geographic proximity, with bands (*d*) similar to Miguel and Kremer [10] defined after the baseline survey (not using outcomes) when we have a sense for relevant geographic distances between clusters in each country
2. School attendance
3. Market attendance

To help improve the estimation in all cases, we will attempt collect some measure of total population or compounds in each institution as a variable in *X* to control for differences in density.

Pre-specified subgroup analyses

We recognize that the study is powered to detect main effects on our primary outcomes, and so we will be unlikely to detect subgroup-specific effects unless they are larger than the overall ITT effect.[1] However, we feel that some of the subgroup-specific effects are highly relevant to interpreting the study and to informing intervention targeting in the future. This type of analysis extends the interaction tests between treatments described above by looking at treatment interactions with baseline covariates. For example, the most relevant effect of a water quality intervention is among households who have poor water quality at baseline; it is less likely that a water quality intervention would improve health among children who live in households with microbiologically clean drinking water at baseline.

For all of the subgroup-specific effects that that we plan estimate *a priori* in this study, we will first screen the variables to ensure that there is sufficient variation for the tests to make sense. We will estimate different ITT effects for the different subgroups by interacting subgroup variables with the treatment indicators of interest.[1,12] Within each category of baseline covariates, the country teams have selected characteristics that they will include in subgroup analyses.

Household water treatment and quality, source water access and water quality

Rationale: The effect of our drinking water quality intervention may be smaller among households with good baseline drinking water quality. The effect of our other WASH interventions may be greater or smaller, depending on baseline drinking water quality and water source availability. In Kenya, we expect that the majority of our study population will have received a Lifestraw family filter as part of a Vestergaard Frandsen (VF) distribution program throughout Western Province. If the filters are in regular use, we would expect smaller impacts from the chlorine dispenser intervention among those households.

Both countries
- Drinking water source (surface water vs. other)
- Household reports regularly treating their drinking water
- Free residual chlorine in stored drinking water

Kenya
- Detectable *E. coli* in source water (> 0 CFU / 100 ml)
- Detectable *E. coli* in drinking water (> 0 CFU / 100 ml)
- Field staff observe a VF water filter hanging in the household and household members report frequent use
- Observed VF water filter has visible moisture in it.
- Walking distance in minutes to primary drinking water source

Handwashing practices

Rationale: The effect of our handwashing intervention may be smaller among households with good baseline handwashing practices. The effect of our other WASH interventions may be greater or smaller, depending on baseline handwashing practices.

Both countries
- Mother has clean palms, finger pads, and finger nails

Kenya
- Mother was observed to use soap during a handwashing demonstration
- Mother lists (unprompted) as critical times for handwashing: before preparing food, eating, or feeding a child and after defecating or cleaning a child who has defecated.

Bangladesh
- Presence of a handwashing station with water and soap

Sanitation conditions

Rationale: The effect of our sanitation intervention may be smaller among households with high levels of baseline sanitation. The effect of our other WASH interventions may be greater or smaller, depending on baseline sanitation conditions. For example, an observational study using DHS data documented larger effects of improved source water only in the presence of improved sanitation conditions.[6]

Both countries
- Household latrine status (none, unimproved, JMP improved)

Kenya
- Stool visible on floor of the latrine
- Any person in household reported to not always use latrine
- Most recent feces of child under 36 months were disposed of in latrine
- Latrine is located in another compound
- Household already owns potty
- Cover observed over latrine drop hole

Food security

Rationale: The effect of our Nutrition intervention or combined Nutrition+WSH intervention may be greater among households with low food security at baseline.

Both countries
- Questions will be adapted from the Household Food Insecurity and Access Scale (HFIAS), with modifications for the local language, cultural context, and food availability patterns.

Child age

Rationale: All target children will be enrolled in the study while in utero, but their experience of the intervention will differ slightly depending on their relative age within the cohort, which will span approximately 6 months of age. Our outcome measurements will

take place at a fixed calendar time – not child age. It is possible that younger children will benefit more from being born into more mature intervention conditions. A competing hypothesis is that the younger children will benefit less from intervention because they will have had less post-natal exposure compared to older children.

Both countries
- Stratify the results by age in 3-month brackets at the endline survey: [18,21), [21, 24), [24, 27)

Child sex

Rationale: Biologic differences, cultural practices, or behavioral practices may modify the effect of the interventions with respect to boys or girls.

Both countries
- Stratify the results by sex

Number of older children living in the compound

Rationale: Children living in compounds with older children may be at higher risk for pathogen transmission into the compound. Older children have greater exposure through schools and social networks, and if they do not use latrines they may have greater pathogen shedding in the compound through open defecation.

Both countries
- Stratify the results by the number of older children (<15 years old) in the compound.

Cluster density and cluster size

Rationale: The positive or negative effects of proximate neighbors may modify the protective effects of the intervention. For negative spillover effects, like disease transmission, we would expect the interventions to be less efficacious in densely populated environments than in more sparsely populated environments. In Kenya, where cluster sizes vary, is possible that the intervention effects will be heterogeneous with cluster size because the number of treated households per intervention promoter may change the nature of the promotion.

Both countries
- Stratify the results into clusters of high compound density and low compound density.

Kenya only
- Stratify the results by the number of households per promoter in the cluster.

Maternal intelligence and education

Rationale: mothers who are better educated and/or perform better on literacy tests may be more capable of adapting to new information and technology. They may have greater ability to optimize their behavior to take advantage of the messages and materials that the study provides.

Both countries

- Mothers that score in the top 25th percentile of the study population on at least one of the maternal intelligence tests that we administer at the 1-year follow-up survey
- Maternal schooling attainment

Kenya

- Maternal self-reported literacy

Appendix References

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CONSORT Checklist for the protocol (excluding Results and Discussion)

BMJ 2012;345:e5661 doi: 10.1136/bmj.e5661

ITEM	DESCRIPTION	REPORTED IN SECTION
Title and Abstract		
1a	Identification as a cluster randomised trial in the title	Title
1b	Structured summary of trial design, methods, results, and conclusions	Abstract
Introduction		
Background and Objectives		
2a	Scientific background and explanation of rationale; Rationale for using a cluster design	Introduction, Methods and Analysis
2b	Specific objectives or hypotheses; Whether objectives pertain to the cluster level, the individual participant level, or both	Introduction, Methods and Analysis: Overview of the design
Methods		
Trial design:		
3a	Description of trial design (such as parallel, factorial) including allocation ratio; definition of cluster and description of how the design features apply to the clusters	Methods and Analysis: Overview of the design
3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants		
4a	Eligibility criteria for participants; Eligibility criteria for clusters	Methods and Analysis: Participant eligibility criteria, study setting, and enrollment strategy
4b	Settings and locations where the data were collected	Methods and Analysis: Participant eligibility criteria, study setting, and enrollment strategy
Interventions		
5	The interventions for each group with	Methods and Analysis:

ITEM	DESCRIPTION	REPORTED IN SECTION
	sufficient details to allow replication, including how and when they were actually administered; Whether interventions pertain to the cluster level, the individual participant level, or both	Description of the interventions Appendix 1
Outcomes		
6a	Completely defined prespecified primary and secondary outcome measures, including how and when they were assessed; whether outcome measures pertain to the cluster level, the individual participant level, or both	Methods and Analysis: Outcomes Appendix 2 Appendix 3
6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample Size		
7a	How sample size was determined	Methods and Analysis: Sample Size Appendix 4
7b	When applicable, explanation of any interim analyses and stopping guidelines	Methods and Analysis: Analysis Plan, Interim analyses
Randomisation		
Sequence generation		
8a	Method used to generate the random allocation sequence	Methods and Analysis: Randomization and Blinding
8b	Type of randomisation; details of any restriction (such as blocking and block size); Details of stratification or matching if used	Methods and Analysis: Randomization and Blinding
Allocation concealment mechanism:		
9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned; specification that allocation was based on clusters rather than individuals and whether allocation concealment (if any) was at the cluster level, the individual participant level, or both	Methods and Analysis: Randomization and Blinding
Implementation:		
10a	Who generated the random allocation	Methods and Analysis:

ITEM	DESCRIPTION	REPORTED IN SECTION
	sequence, who enrolled clusters, and who assigned clusters to interventions	Randomization and Blinding
10b	Mechanism by which individual participants were included in clusters for the purposes of the trial (such as complete enumeration, random sampling)	Methods and Analysis: Participant eligibility criteria, study setting, and enrollment strategy
10c	From whom consent was sought (representatives of the cluster, or individual cluster members, or both) and whether consent was sought before or after randomisation	Methods and Analysis: Participant eligibility criteria, study setting, and enrollment strategy
Blinding		
11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Methods and Analysis: Randomization and Blinding
11b	if relevant, description of the similarity of interventions	N/A
Statistical methods:		
12a	Statistical methods used to compare groups for primary and secondary outcomes; How clustering was taken into account	Methods and Analysis: Analysis Plan
12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	Methods and Analysis: Analysis Plan Appendix 5