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 diarrhoea 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-randomised 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 randomised 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 enrol newborn children (N=5760 in Bangladesh and N=8000 in Kenya) and measure outcomes at 12 and 24 months after intervention delivery. Primary outcomes include child length-for-age Z-scores and caregiver-reported diarrhoea. Secondary outcomes include stunting prevalence, markers of environmental enteropathy and child development scores (verbal, motor and personal/social). We will estimate unadjusted and adjusted intention-to-treat effects using semiparametric 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, poor drinking water quality, sanitation, hygiene (WASH) and nutrition are leading risk factors for morbidity and mortality among children <5 years.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 believed to have profound negative consequences on health, cognitive development and human capital that span the life course.2–4

The WASH Benefits study includes cluster randomised 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 2 years of life. The second is whether greater reductions in diarrhoea can be achieved by combining individual WASH interventions compared to delivering them in isolation. The third is whether the combined WASH and nutritional interventions jointly reduce diarrhoea or improve linear growth more than each component alone. Below, we briefly summarise the rationale for the conduct of randomised 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.\(^8\)\(^9\)\(^10\) 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.\(^14\)\(^15\)\(^16\) 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% CI +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 three to four episodes of acute diarrhoea 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 diarrhoea 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 diarrhoea, greater nutrient losses from persistent diarrhoea (eg, zinc) or intestinal bleeding (eg, 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 aetiology of EE remains unknown, but the condition is generally characterised by a set of physiological changes to the small intestine’s epithelial layer, which include villous 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 believed to have impaired growth through two mechanisms: (1) reduced nutrient absorption due to decreased surface area in the small (upper) intestine and (2) 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 impair a child’s ability to effectively utilise nutrients in the 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 (1) strengthening epithelial barrier integrity and the immune response; (2) compensating for malabsorption, reallocation or losses of key nutrients during infection; (3) accelerating gut repair following infection; and (4) favouring 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,\(^43\) with some evidence for improvements in gut function following vitamin A,\(^43\) alanyl-glutamine...
supplementation\textsuperscript{44} and zinc supplementation\textsuperscript{45, 46} but there is no evidence for gut function improvement in trials that delivered probiotics\textsuperscript{47}, glutamine supplementation\textsuperscript{48}, omega-3 fatty acids\textsuperscript{49} or richly fortified complementary foods.\textsuperscript{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 independent of nutritional interventions.

Unlike the large literature on child nutritional interventions, we are aware of only 10 studies that measure the effect of WASH interventions on child growth; a forthcoming systematic review\textsuperscript{51} may perhaps identify more. Four studies have found no improvement in linear growth as a result of WASH interventions, despite demonstrating reductions in caregiver-reported diarrhoea in most cases.\textsuperscript{9, 52–56} A small randomised trial that enrolled children <12 months and delivered handwashing promotion in Kathmandu slums additionally found no improvements in EE biomarkers.\textsuperscript{53} The authors hypothesised that handwashing alone was inadequate as sufficient protection from the slum environment to change 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.\textsuperscript{26, 57, 58} A prospective birth cohort study in periurban Peru found that children living in households with home water supply and sewerage connections were 1 cm taller by age 24 months compared with children in households without them, and the effects of water supply and sewerage conditions were not mediated entirely by reductions in diarrhoea.\textsuperscript{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.\textsuperscript{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.\textsuperscript{63} A trial to assess the impact of rural sanitation on diarrhoea includes length for age as a secondary outcome but is still underway.\textsuperscript{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\textsuperscript{26, 58, 65–66} and theoretical modelling of pathogen transmission pathways.\textsuperscript{67, 68} However, the limited available evidence from randomised trials does not support this approach. In the only randomised 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 diarrhoea (51\% and 64\% reduction), but there was no additional reduction in diarrhoea among children exposed to both interventions (55\% reduction).\textsuperscript{54} These findings are consistent with the results of a meta-analysis of published interventions to improve WASH, which found that combined interventions led to no greater reduction in diarrhoeal disease than single interventions.\textsuperscript{69}

For WASH programmes, single interventions are less expensive and easier to scale than combined interventions. By complicating communication and behaviour change, combined interventions can potentially diminish the overall effect achievable from a single intervention.\textsuperscript{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 (1) when deciding overall budgets for sanitation, water and handwashing; and (2) when weighing the trade-offs 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.

Question 3: Are there larger effects on diarrhoea 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 \textit{et al.}\textsuperscript{24} proposed a theory that repeated infections interact with poor nutrition to cause a cycle of infection and malnutrition. Consistent with this earlier work, McDade\textsuperscript{72} outlined a life history theory of immune function in which he posited that infants face a resource allocation trade-off between maintenance (fighting infection and physiological repair) and growth. During infection, the immune system diverts energy and nutrients away from growth; a developing infant prioritises 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 physiological 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.\textsuperscript{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.\textsuperscript{75}

Dewey and Mayers\textsuperscript{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.\textsuperscript{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.\textsuperscript{10, 76–78} The 10-village study (2900 newborns) was a factorial trial that randomised 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 in 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,\textsuperscript{79–81} the degree to which additional infection control measures could complement nutrition programmes 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 randomised 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 towards the identified evidence gaps.

**Primary scientific objectives**

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

1. Measure the impact of a child nutritional intervention and household environmental interventions on EE biomarkers, and more clearly elucidate this potential pathway between environmental interventions and child growth and development.
2. Measure the impact of sanitation, water quality, handwashing and nutritional interventions on intestinal parasitic infection prevalence and intensity.
3. Measure the association between parasitic infection and other measures of enteric health, including acute diarrhoea and EE biomarkers.

To achieve these objectives, the studies will enroll pregnant women and their children born within approximately 6 months of the baseline survey. The study will measure linear growth and caregiver-reported diarrhoea, biological markers of EE, intestinal parasite infections and child development in the cohort for 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 Diarrhoeal 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 six intervention arms and a double-sized control arm (figure 1). In Bangladesh, the unit of randomisation is a group of compounds visited by a single local promoter and separated by at least a 15 min walk. Bangladesh clusters consist of eight 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 six eligible pregnant women. The studies enrol 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 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: (1) WASH interventions need to be delivered at the cluster level because the promotion activities are inherently community level, (2) there are potential interactions between adjacent households with respect to behaviour and infectious disease and we wish to maintain independent units for randomisation, and (3) at the time our study enrols 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. The design is optimised to measure group-level differences in our primary outcomes. The infrequent measurements in WASH Benefits will mean that we will not characterise infectious outcomes (eg, diarrhoea and parasitic infections) well for individual children if the outcomes vary temporally within children.

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

Participant eligibility criteria, study setting and enrolment strategy

Participant eligibility criteria

In both countries, the trials enrol pregnant women identified in community-based surveys who expect to deliver in the 6 months following enrolment based on date of last menstruation. The study will enrol 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 enrolment is 5760 in Bangladesh and 8000 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 enrols all children <36 months at baseline to measure diarrhoea outcomes over the study period; the study measures diarrhoea outcomes in a wider age group because older children are still at high risk for diarrhoeal disease.

In both countries, compounds consist of multiple households (typically 3–10 in Bangladesh and 1–4 in Kenya), usually comprising blood relatives, who share a common courtyard. Compounds are eligible to participate if (1) they have a pregnant woman and (2) 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 programmes 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 in which 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 min staff exclude the household. Within a study compound, the studies enrol pregnant women and children from the following age groups

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

2. **Children 18–27 months old at enrolment (specimen collection):** older children living in the compound and aged 18–27 months at enrolment 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 aged <36 months at enrolment (diarrhoea):** All children aged <36 months living in the compound are eligible for caregiver-reported diarrhoea measurement.

4. **Additional children born into study compounds after 6 months:** We will enrol children born into study compounds who are too young to meet our enrolment criteria (group 1, above), deliver interventions to them according to randomised assignment and measure anthropometry and diarrhoea 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 enrolment**

The Bangladesh trial is located in Gazipur, Mymensingh and Tangail districts. These three 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 faecal indicator bacteria. Enrolment 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 (<1 mg/L on average) and arsenic (<50 mg/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 organisations working in Bangladesh and local government authorities report that no major water, sanitation or focused nutrition programmes 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 eight eligible pregnant women. The compounds within a cluster are located sufficiently closely 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, they will then 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 min walking distance.

**Kenya setting and enrolment**

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 diarrhoea in the preceding 2 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 to be effective. Enrolment commenced in November 2012. The study region contains over 2000 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 programmes;
- Majority (>80%) of households do not have access to piped water into the home;
- At least six 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 randomised studies. The enabling technologies and behavioural intervention packages were developed in the target populations over a 2-year period before the start of the trials. Details of the behaviour change theoretical frameworks and methods used in each country will be published in separate, forthcoming articles. Local promoters who are residents of the study villages deliver the interventions at the cluster level; each promoter completes at least 5 days of training and also attends refresher courses periodically throughout 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 1 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 neighbourhood, community or other larger environment in order to effectively mitigate personal exposure. However, cost and logistical limitations prevented us from 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.

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 behaviour 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 programmes. 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 behaviours 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-related or nutrition-related behaviour changes occur in the intervention arms will also occur in the control arm. The Kenya control arm promoters do not promote any WASH, or nutrition messages, and strictly engage in measuring child MUAC and mother belly circumference. In all arms, children >6 months old with MUAC <115 mm are classified as severely malnourished and are referred to treatment (details mentioned below in Referral guidelines).

Water quality

The Bangladesh study delivers a 10 L, 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. 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 20 L 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 mL/L of free chlorine residual after 30 min for 20 L of water. 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 households’ water treatment during periods when they rely on rainwater harvesting (common during the rainy season) or if they use a water source in which a dispenser has not been installed. In both countries, the behaviour 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 faeces in the household environment and increasing latrine use: (1) a locally developed sani-scoop dedicated to the removal of child and animal faeces...
from the compound, (2) plastic child potties for children aged 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 superstructure. 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 an earthen superstructure and the plastic slab) are constructed in the compounds of study participants who do not have access to a latrine. The behaviour change strategies in both countries target the use of the latrine for defaecation and the safe disposal of faeces 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 and 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 L jerry cans of clean water and soapy water. In both countries the studies provide soap to families free of charge to replenish the handwashing stations. The behaviour change strategies of the intervention target handwashing with soapy water messaging at two critical times for caregivers: after defaecation/cleaning the handlining and basic water treatment, sanitation, in-depth water treatment. The provision of latrines can range from one to several weeks after the start of work in a cluster in Kenya. The behaviour change strategy emphasises the interconnected aspect of WASH and the need to practice all behaviours in order to benefit from them.

**Nutrition**

In both countries, the nutrition intervention strategy targets age-appropriate behaviours (pregnancy to 24 months) including use of lipid-based nutrient supplements (LNSs; aged 6–24 months). The behaviour change counselling is modelled after the Guiding Principles for Complementary Feeding of the Breastfed Child, the UNICEF Program Guide for Infant and Young Child Feeding Practices and the Alive and Thrive initiative. Target behaviours 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 LNS. The LNS used in the study is a next generation version of Nutributter. Online supplementary appendix 1 includes the specific LNS formulation. LNS is administered daily using 10 g sachets that can be mixed into 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. It has an 18-month shelf life, does not spoil at high temperatures and costs as little as US$0.08/day. Reported adherence has been 88% of days in controlled trials, 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.

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, the team then 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 (see online supplementary 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 participants 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 diarrhoea prevalence in compound children <36 months old at enrolment. Child age will be determined using birthdates verified when possible using vaccination cards. Following standard protocols for anthropometric outcomes measurement,55 56 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.57 We will measure diarrhoea 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 h or ≥1 stool with blood based on caregiver-reported symptoms58, we will use a 7-day recall period unless we find differential recall errors by the randomised group, in which case we will use a 2-day recall period.59 100

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 Questionnaire13 101; the instrument has been adapted to each study population, relies on caregiver’s 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 1500 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-to-mannitol ratio,103 faecal myeloperoxidase,104 faecal α-1-antitrypsin,105 faecal neopterin106 and plasma total IgG.37

Additional outcomes
The study will collect stool specimens from seven 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 method107 and protozoans (Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica) using PCR methods (Bangladesh) and commercial ELISA kits (Kenya). Online supplementary 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 faecal contamination to help trace the causal path between the interventions and outcomes. Environmental contamination measures will include enumeration of faecal indicator bacteria (Escherichia coli) in household-stored drinking water, on child toy balls and child hand rines. 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 healthcare 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 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 and 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 [weight for length Z-score $<-3$] and/or bipedal oedema) will be referred to the appropriate existing treatment programmes in each country. In Kenya, where promoters measure MUAC each month for all target children, children $>6$ months with MUAC $<115$ mm 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.

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

### Randomisation 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 randomisation is pair-matched by geography, with adjacent clusters randomised in blocks. The rationale for using geography to match the randomisation 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 randomise groups of eight geographically proximate clusters to one of the six 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 randomisation is identical but includes nine 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.
College Station, Texas, USA) with a reproducible seed. Owing 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 randomised 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 diarrhoea. We calculated the minimum detectable effect for LAZ measured at 2 years using a standard equation and for diarrhoea using a simulation-based approach to accommodate two levels of correlation in the outcome (within child and within cluster). To inform our sample size calculations we used existing datasets from relevant populations. In Bangladesh, we used diarrhoea and anthropometric measurements from 982 children <36 months, collected from 100 rural villages between 2007 and 2009. In Kenya, we conducted the sample size calculations using diarrhoea data, collected from 1704 children in 95 control villages enrolled in a cluster-randomised trial of spring protection conducted in Western Province between 2005 and 2007; we also conducted 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 diarrhoea 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 diarrhoea based on earlier WASH efficacy studies. 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 minimises the variance for the six tests planned under our first hypothesis, below. Online supplementary 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

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**Figure 3** Summary of enteric parasite measurement in both countries, including cluster and target child enrolment in each arm. At enrolment 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 seven target children per cluster in each country, and analysed for protozoan infections and soil-transmitted helminth infections. C, control; EE, environmental enteropathy; H, improved handwashing; N, improved nutrition; S, improved sanitation; W, improved water quality; WSH, combined improvements in water quality, sanitation and handwashing; WSH+N, combined improvements in water quality, sanitation, handwashing and nutrition.
means and SDs by randomised groups, along with unadjusted comparisons between groups for the primary hypotheses.\textsuperscript{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 analyse participants according to their randomised assignment (intention to treat).

**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 randomised 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; N nutrition supplement; and NWSH nutrition plus combined WSH. Let $Z$ be a set of indicators for matched blocks used in the randomisation. Finally, let $\psi$ denote parameters of interest. In each comparison below, we define $\psi$ as a difference between various randomised groups. For dichotomous outcomes like diarrhoea, this implies a risk difference. We will additionally report risk ratios for dichotomous outcomes as recommended by CONSORT.\textsuperscript{114}

H1: water, sanitation, handwashing, nutrition and their combination reduce child diarrhoea and improve linear growth.

The mean outcomes in each active intervention arm 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 $t \in \{W, S, H, WSH, N, NWSH\}$:

$$\psi_{1,t} = E[Z,Y = t,Z] - E[Y|T = C,Z]$$

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

The combined arm (WSH) treatment effect for diarrhoea 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 $t \in \{W, S, H\}$:

$$\psi_{2,t} = E[Z,Y = WSH,Z] - E[Y|T = t,Z]$$

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 online supplementary appendix 5.

H3: combined nutrition and WASH interventions reduce diarrhoea 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 $t \in \{WSH, N\}$:

$$\psi_{3,t} = E[Z,Y = WSH,Z] - E[Y|T = t,Z]$$

As with H2, this hypothesis is not a hypothesis of interaction or synergy. Rather, it is a test to determine whether 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 fourfold increase in the sample size would be required,\textsuperscript{116} which would be logistically infeasible given the already large size of the trial.

**Testing and estimation**

One strength of a randomised trial is that it allows investigators to draw inference non-parametrically, relying only on randomisation.\textsuperscript{117} One approach to test for statistical significance is a permutation test based on randomly permuting randomised assignments in the data (following the original randomisation strategy, ie, permuting T within strata Z) and re-estimating a test statistic.\textsuperscript{117-121} We plan to use a rank-based test statistic, which has been shown to have good power against alternatives,\textsuperscript{122} and estimate it on unweighted cluster means.\textsuperscript{118, 119} We will use one-sided tests because we would only expect the interventions to be beneficial.\textsuperscript{123} Owing to the relatively small number of tests involved, we do not plan to adjust the p values for multiple testing.\textsuperscript{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 SEs and CIs for our parameters). Since the trials depart from an individually randomised 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 randomisation. 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 CIs 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 SE.
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 randomisation.\textsuperscript{125} It is straightforward to extend permutation tests to include covariate adjustment while still taking advantage of the exact distribution theory provided by randomised inference.\textsuperscript{118 120} For example, let $Y_{ijk}$ be the outcome of interest for individual $i$ in village $j$ and randomisation stratum $k$; let $T_{jk}$ be the randomised intervention indicator and $X_{ijk}$ be a vector of adjustment covariates. Models are fit of the form: $E[Y_{ijk}|X_{ijk}]=m(X_{ijk})$, where $m(.)$ is some function of the covariates $X$. For example, $m(X_{ijk}) = \alpha + \beta X_{ijk} + \gamma_{ijk}$ 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: $\hat{r}_{ijk} = Y_{ijk} - \hat{Y}_{ijk}$ and the permutation test is conducted on the residuals. The test has nominal size for the null hypothesis even if the model $m(.)$ is misspecified and if the covariates are measured with error.\textsuperscript{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 the strata.\textsuperscript{118}

Following CONSORT guidelines,\textsuperscript{114 115} we prespecify 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 years in the household;
- Number of individuals living in the compound;
- Distance (in minutes) to the primary water source;
- Housing materials (floor, walls and roof) and household assets.

We will use a repeatable data-adaptive algorithm to control for the covariates flexibly and semiparametrically that will be chosen before the analysis.\textsuperscript{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 CIs for our parameters of interest using the bootstrap described in the unadjusted analysis section. Online supplementary appendix 5 includes the details of additional, prespecified 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 minimise attrition. We will compare attrition rates across randomised arms and also 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,\textsuperscript{127} and summarised by Duflo \textit{et al},\textsuperscript{108}) and we will also calculate bounds proposed by Lee.\textsuperscript{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 semiparametric weighting using baseline characteristics.\textsuperscript{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 randomised assignment until all of the data from the 2-year measurement are collected.\textsuperscript{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’s Data and Safety Monitoring Boards (DSMBs) 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.\textsuperscript{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 SDs above the control arm we will look to the country’s DSMB to decide on the appropriateness of continuing the trial.
ETHICS AND DISSEMINATION

Each trial is overseen by an independent DSMB, 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 publicly distributed along with metadata and critical documents (ie, 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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Cluster-randomised 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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