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WASH for WORMS: A Cluster-Randomized Controlled Trial of the Impact of a Community Integrated Water, Sanitation, and Hygiene and Deworming Intervention on Soil-Transmitted Helminth Infections

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Abstract. Water, sanitation, and hygiene (WASH) interventions have been proposed as an important complement to deworming programs for sustainable control of soil-transmitted helminth (STH) infections. We aimed to determine whether a community-based WASH program had additional benefits in reducing STH infections compared with community deworming alone. We conducted the WASH for WORMS cluster-randomized controlled trial in 18 rural communities in Timor-Leste. Intervention communities received a WASH intervention that provided access to an improved water source, promoted improved household sanitation, and encouraged handwashing with soap. All eligible community members in intervention and control arms received albendazole every 6 months for 2 years. The primary outcomes were infection with each STH, measured using multiplex real-time quantitative polymerase chain reaction. We compared outcomes between study arms using generalized linear mixed models, accounting for clustering at community, household, and individual levels. At study completion, the integrated WASH and deworming intervention did not have an effect on infection with *Ascaris* spp. (relative risk [RR] 2.87, 95% confidence interval [CI]: 0.66–12.48, $P = 0.159$) or *Necator americanus* (RR 0.99, 95% CI: 0.52–1.89, $P = 0.987$), compared with deworming alone. At the last follow-up, open defecation was practiced by 66.1% (95% CI: 54.2–80.2) of respondents in the control arm versus 40.2% (95% CI: 25.3–52.6) of respondents in the intervention arm ($P = 0.005$). We found no evidence that the WASH intervention resulted in additional reductions in STH infections beyond that achieved with deworming alone over the 2-year trial period. The role of WASH on STH infections over a longer period of time and in the absence of deworming remains to be determined.

INTRODUCTION

Soil-transmitted helminths (STHs)—comprising *Ascaris lumbricoides*, hookworm (*Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum*), *Trichuris trichiura*, and *Strongyloides stercoralis*—are intestinal parasites that infect more than 1.45 billion people worldwide,[1] with a burden of more than three million disability-adjusted life years.[2] Soil-transmitted helminths are transmitted through the fecal–oral route, or by direct skin penetration in the case of hookworm and *S. stercoralis*. Soil-transmitted helminth infections are therefore more common in poor countries and communities where sanitation is lacking, water access deficient, and hygiene poor.[3] Chronic and high-intensity STH infections have been associated with significant morbidity, including malnutrition, and in the case of hookworm infections, iron-deficiency anemia that may be associated with poor maternal and cognitive outcomes.[4]

Present World Health Organization (WHO) guidelines advocate for large-scale regular deworming campaigns with anthelmintic drugs (albendazole or mebendazole) that are safe and highly effective against *A. lumbricoides* and moderately effective against hookworm infections.[5,6] Deworming campaigns for STH control have mainly targeted school-aged children because the adverse health effects of STH infection disproportionately affect children, and school-based delivery of anthelmintic drugs has operational advantages.[6] However, there is an emerging body of evidence suggesting that expanding deworming campaigns to include entire communities has the potential to achieve interruption of transmission, possibly leading to elimination,[7] is cost-effective,[8] and may be more beneficial for children.[9]

Although deworming programs are effective at killing adult worms in infected individuals, in the short term, they have limited impact on transmission, especially if they only target children. Poor hygiene practices coupled with environmental contamination with parasite infective stages can result in rapid reinfection, and consequently, treatment needs to be repeated periodically.[10] Therefore, water, sanitation, and hygiene (WASH) interventions have been proposed as an important complementary intervention to deworming for sustainable STH control, given that these interventions can effectively separate humans from their feces, thereby reducing transmission.[11] Although there are several observational studies suggesting an association between individual WASH components and decreased STH infection,[12] there have been few intervention studies demonstrating the benefits of WASH on STH infections, particularly when delivered at the community level. The impact of individual and combined WASH components implemented in schools has been reported to reduce STH infections.[13–16] However, the two trials on community-based sanitation (in the context of the Indian

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Total Sanitation Campaign) published so far did not detect reductions in STH infections arising from the sanitation intervention, possibly because of low latrine coverage and usage in intervention communities.[17,18]

Here, we report the results of WASH for WORMS, the first cluster-randomized controlled trial (RCT) aiming to determine whether a community-based WASH program has additional benefits in reducing STH infections when compared with community deworming alone, in the context of a highly endemic country.[19]

MATERIAL AND METHODS

Full description of the trial setting and methods, including additional details regarding the intervention, sample size calculation, and randomization, can be found in the previously published protocol.[19]

Setting, study design, and participants. We conducted a two-arm cluster RCT in 18 communities in Manufahi municipality, Timor-Leste, where WaterAid Australia, an international nongovernmental organization (NGO) implements its WASH projects in partnership with local NGOs. At the time the study was implemented, there was no ongoing deworming program in Timor-Leste—the "*Lumbriga. . .Mak Lae Duni*" (Worms, no way!) program initiated in 2005 was discontinued in 2008 because of the lack of funding and was planned to restart in selected municipalities in 2015. Our initial cross-sectional surveys found that the prevalence of *N. americanus* in study communities was 60% and that of *Ascaris* spp. was 24%.[20] Briefly, the WASH intervention consisted of the following components:

1. Improving water supply and working with residents over a period of up to 10 months, usually culminating in building of several tap stands per community, with the maximum distance between each dwelling and collection point of 200 m (or less than 5 minutes round trip walking time). Most of the water supply systems built were gravity fed, with groundwater supply systems built when there were no elevated water sources available. Microbiological tests were performed to guarantee water quality.[21]
2. Promoting improved household sanitation by increasing demand. Improved sanitation options are as per the Joint Monitoring Program definitions. This used a strategy based on the community-led total sanitation (CLTS) process, whereby following a 1–2 day "triggering" meeting, residents committed to ending open defecation in their community by constructing and using household latrines.[22] The most common types of latrine that residents built, with explanations provided by WaterAid and partners, were simple direct pit latrines and offset pit pour-flush latrines. Squat slabs were either precast or made from local timber or compacted earth. Usually, a shelter made of local materials was also constructed.
3. Encouraging handwashing with soap at critical times: before preparing food, before feeding children, before eating, after using the toilet, and after cleaning a child's bottom. Hygiene promotion activities were conducted by community hygiene promoters from local partner NGOs, using a variety of information, education, and communication materials such as flip charts, games, songs, and posters. This was conducted through community meetings, smaller group meetings for women and children, and household visits. The community hygiene promoters visited communities approximately three times a month for 4–6 months, initiating just after the "triggering" meeting.

All clusters in both study arms received the deworming intervention as follows: 400 mg albendazole was delivered to all eligible members of a community (residents older than 1 year of age, excluding pregnant women in the first trimester), and taken under direct observation, every 6 months for a period of 2 years, for a total of five deworming rounds. In the intervention arm, the first distribution occurred shortly after 80% of the households had built a latrine, as assessed by the local NGOs monitoring the WASH intervention. This happened 2–6 months after the triggering meeting. In the control arm, we waited a similar amount of time between the baseline survey and the first albendazole distribution. A 2-year follow-up period was selected by taking into account the following: 1) average life expectancy of STH eggs and larvae[3] and 2) logistical difficulties of following communities for longer time periods, given expected overall improvements in WASH conditions due to economic development and migration from rural to urban centers. Furthermore, a 2-year time frame was considered policy relevant, given that the impact of deworming on STH infections is detectable in such time frames[9]; this trial aimed to assess whether there would be an additional benefit from WASH while deworming was taking place. The control clusters received the WASH intervention at the end of the trial.

Ethics statement. This study was approved by the Human Research Ethics Committees at the University of Queensland (2011000734), Australian National University (2014/311), and the Timorese Ministry of Health (2011/51). The trial is registered with the Australian and New Zealand Clinical Trials Registry (registration number 12614000680662). Because of logistical and human resource constraints, it was registered after the baseline surveys were conducted (but before the measurement of study outcomes). The same primary outcomes were specified in the registration as in the ethics protocols that were approved before study commencement. The study was managed throughout according to protocols developed before data collection. General information about the trial was given to the community during a community meeting that took place after random allocation to intervention and control arms and before baseline data collection. Detailed verbal and written information was provided to individual participants during subsequent house-to-house visits. Written informed consent was obtained from all participants aged 18 years or older and from parents or guardians for those younger than 18 years. Participants aged 12–17 years provided written assent.

Randomization and masking. Informed by our sample size requirements, WaterAid provided a list of 24 eligible clusters to be enrolled in the study, which were randomly allocated to intervention and control arms by A. C. A. C. and S. V. N. using a computer random number generator.[19] Inclusion criteria were as follows: having a suitable water source (e.g., a spring with capacity to provide water for the entire community) and having poor access to clean water and sanitation as determined by the Timorese municipality water and infrastructure office, and therefore being eligible for assistance from WaterAid. Five of these communities (two intervention and three control) had to

be replaced during the enrollment process because of not meeting the necessary criteria: unsuitable water source (completely or partially dried out), proximity to intervention clusters (control), unwillingness to comply with the 2-year waiting period to receive the WASH intervention (control) or with building the water system (intervention), and small size. Replacement of each cluster was performed sequentially, one by one, as soon as they were deemed ineligible, using a list of replacement communities. Therefore, this process did not allow for random allocation to a study arm. WaterAid selected which cluster (community) to include as needed, accounting for geographical location and suitability of water source. One intervention community was subsequently lost to follow-up because the identified water source was no longer suitable for the water intervention, leaving 18 communities that followed the randomization protocol—nine intervention and nine control communities. Considering the five replacement clusters that were not randomly allocated, 23 communities in total completed the study.

Because of the nature of the intervention, masking of clusters was not possible, and both participants and the research team were aware of the allocation. Contamination was minimized by making sure that communities were geographically well separated. However, by the third follow-up visit (18 months after baseline), three control clusters had been exposed to government-led sanitation promotion interventions.

Procedures. In each of the communities, baseline parasitological, clinical, and sociodemographic surveys were conducted no longer than 4 weeks after the initial community meeting, before any component of the WASH intervention was in place. Similar surveys were repeated at each 6 monthly follow-up for 2 years, except for the clinical surveys, which were repeated annually. Each survey was completed before albendazole administration.

All residents of the participating clusters who were older than 1 year at the time of each visit were eligible to participate in the study and were recruited during house-to-house visits. A fecal sample was obtained from each participant in a plastic container distributed the previous day, and processed by the research team no longer than 4 hours after collection.[19] For preservation, stool aliquots were mixed with 5 mL of 5% potassium dichromate and sent to the QIMR Berghofer Medical Research Institute (Brisbane, Australia) for molecular diagnosis by multiplex real-time quantitative polymerase chain reaction (qPCR) to identify and quantify infections with each STH (*Ascaris* spp., *N. americanus*, *Ancylostoma* spp., *T. trichiura*, and *S. stercoralis*).[23]

During the clinical surveys, we measured height and weight of participants younger than 18 years, to calculate anthropometric indices used as proxies for malnutrition. These were computed as *Z*-scores and included weight-for-age for participants aged 1–10 years (to measure underweight), height-for-age (stunting) and body mass index (BMI)-for-age (thinness) for individuals aged 1–18 years, and weight-for-height (wasting) for participants aged 1–5 years.[24] The 2006 WHO database for child growth standards was used to calculate *Z*-scores, defined as the number of standard deviations (SDs) in relation to the mean of the standard population, with *Z*-scores less than two defined as malnutrition.[25,26] We also tested for anemia by measuring hemoglobin (Hb) concentration in all age groups, using a finger-prick blood sample and a portable analyzer. Hemoglobin values were adjusted for altitude, and anemia was diagnosed based on WHO cutoffs for age, gender, and pregnancy status.[27]

Individual participants (or caregivers for young children), heads of household, and community leaders were interviewed to collect sociodemographic characteristics including age, gender, education, employment, income, and assets, as well as history of diarrhea and deworming. Questionnaires also included self-reported WASH-related practices (ownership and use of latrines, defecation practices, availability of water, and hygiene behaviors), to assess changes related to the WASH intervention. When a household latrine was reported, study field-workers directly observed the latrine and assessed its cleanliness.[19]

Outcomes. The primary outcomes were infection with each STH (*Ascaris* spp., *N. americanus*, *Ancylostoma* spp., *T. trichiura*, and *S. stercoralis*), measured every 6 months at the 4 follow-up surveys. Secondary outcomes, also measured at each 6 monthly follow-up, included *Ascaris* spp. and *N. americanus* infection intensity as determined by qPCR, and intensity category (higher intensity, lower intensity, or no infection). Intensity of infection was categorized based on the cycle threshold (Ct) values obtained by qPCR using the following approach: 1) Ct values were converted to qPCR intensity using the equation provided by the RotorGene Q software (qPCR intensity = $10^{-0.298*Ct + 9.81}$); 2) the median intensity for all positive samples at baseline was calculated; and 3) individuals having qPCR intensity values higher than the baseline median were classified as "higher intensity" infections, whereas individuals with PCR intensity values lower than the baseline median were classified as "lower intensity" infections. This method allowed us to assess relative changes in higher versus lower intensity infections at each follow-up, compared with the baseline distribution in a population that had not been exposed to mass deworming in the previous 5 years. Other secondary outcomes, measured at 12 monthly intervals (second and fourth follow-ups), were as follows: adjusted Hb concentration and presence of anemia; weight-for-age, height-for-age, BMI-for-age, and weight-for-height *Z*-scores; and presence of underweight, stunting, thinness, and wasting.

Statistical analysis. Initial sample size calculations determined the requirement for 12 clusters in each study arm, corresponding to 2,880 participants, assuming an intra-cluster correlation coefficient (ICC) of 0.19,[28] 120 participants per cluster, and a 10% loss to follow-up, to detect a 50% reduction in prevalence of each STH in the intervention arm compared with the control arm, with a power of 80% and $\alpha = 0.05$. We chose 50% as the estimate of impact because we believed that WASH interventions would only be attractive as tools specifically for STH control if there is a sufficiently large benefit compared with deworming alone. Analysis of the baseline data indicated that our a priori sample size calculations overestimated the ICC for *N. americanus* but underestimated the ICC for *Ascaris* spp. (0.15 and 0.47, respectively). Power calculations described in the protocol paper confirmed that with a sample size of 18 communities (nine in each arm), for *N. americanus*, we still had the necessary power to detect a 50% reduction in the follow-up prevalence in the intervention arm compared with the control arm.[19]

Data were entered in duplicate using a Microsoft Access database[29] and subsequently imported into Stata version 14.1 (College Station, TX) for data cleaning and analysis.

All analyses were conducted using the 18 communities that were randomly allocated to study arms. Descriptive analyses were conducted at each of the five study time points to examine participation; demographic, socioeconomic, and clinical characteristics; WASH access and use; STH prevalence and infection intensity by qPCR; anthropometric indices; and anemia. Standard deviations and 95% confidence intervals (CI) were obtained for means and proportions in each study arm. When comparing proportions between the two arms at each time point, CI and *P* values were calculated using logistic regression models accounting for community-level clustering.

The primary analysis was an available case analysis comparing the two study arms, and included all participants for whom outcome data (stool samples) were available at one or more follow-up time points. Generalized linear mixed models accounting for village-, household-, and individual-level clustering (i.e., to account for multiple measurements on the same individuals over time, with individuals nested within households and villages) were used to calculate relative risk (RR) for the primary and secondary outcomes in the intervention compared with the control arm, as a measure of the impact of the integrated intervention. We used Poisson regression to model RR for binary outcomes, ordinal logistic regression for categorical outcomes, and linear regression for continuous outcomes. Data from all follow-up time points were analyzed, with an interaction term between study arm and follow-up time point included in the fixed part of the model. To calculate a RR and CI for the study intervention (versus control) at each study time point, a post-estimation linear combination of coefficients and standard errors was calculated, using Wald-type methods. All models were adjusted for age and gender, entered as covariates in the models, and for village, household, and individual clustering, entered as random effects. For the infection-related outcomes (STH prevalence and intensity), models were only run for *Ascaris* spp. and *N. americanus* because baseline prevalence of the other species was very low. Additional models adjusting for baseline prevalence were also run; these models decreased the number of included observations relative to the original models because of missing data.

A sensitivity analysis was performed by repeating the aforementioned generalized linear mixed models, with all 23 clusters that finished the trial, including the five clusters that were not randomly allocated, and observing whether this significantly impacted study results.

RESULTS

Figure 1 shows the trial profile. At baseline, between May 2012 and October 2013, in the 18 clusters that remained in the trial, we registered 2,306 residents in 493 households, of whom 2,100 were present at the time and 1947 participated in data collection (1,046 in the control arm and 901 in the intervention arm). Fieldwork was completed in April 2016.

Baseline sociodemographic, clinical, and WASH characteristics as well as STH infections were mostly balanced across study arms and are shown in Table 1. Approximately half of the participants were aged 18 years or older, with more than 40% of adults having never attended school.

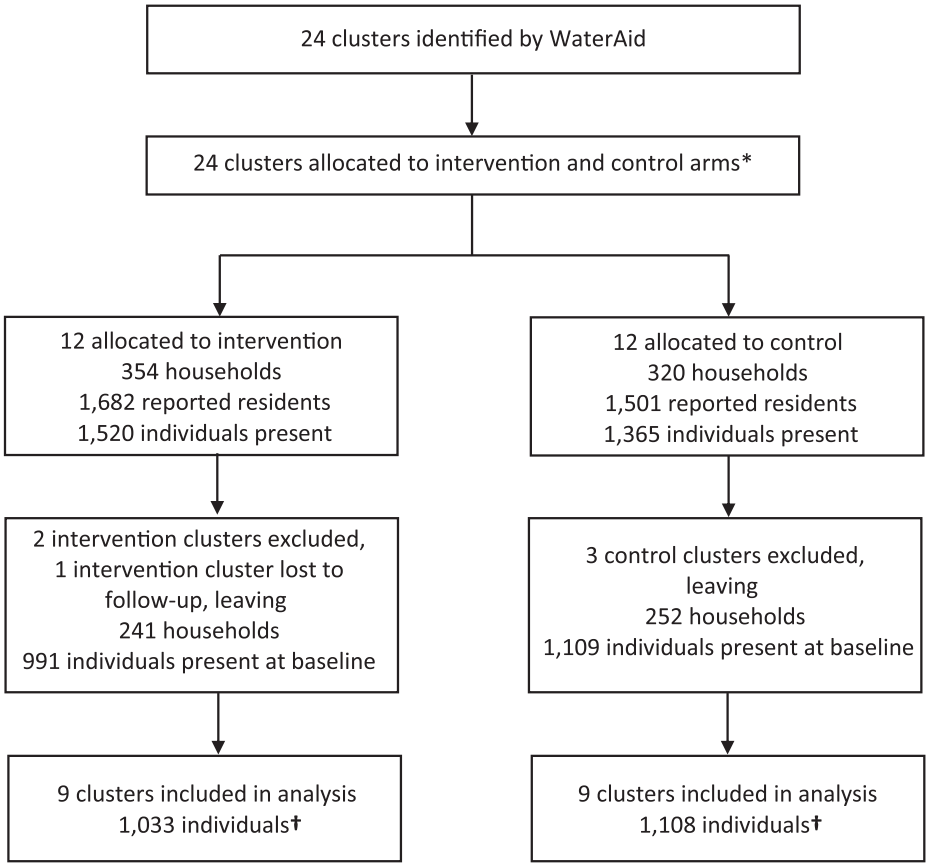

FIGURE 1. Trial profile. *In three blocks. †Individuals who provided a stool sample or questionnaire for at least one follow-up time point.

TABLE 1
Baseline characteristics of study participants* and households

	Control arm	Intervention arm
Individual variables		
Demographics	*n* = 1,046	*n* = 901
Female	514 (49.1%)	485 (53.8%)
Mean (standard deviation) age in years	27.01 (21.6)	26.02 (21.5)
Less than 5 years of age	146 (14.0%)	166 (18.4%)
Between 5 and 18 years of age	358 (34.3%)	284 (31.6%)
Aged 18 years and older	539 (51.7%)	450 (50.0%)
For children aged 6–17 years	*n* = 358	*n* = 270
Attends school	300 (83.8%)	246 (91.1%)
For adults older than 18 years	*n* = 539	*n* = 450
Has never been to school	222 (43.7%)	170 (42.2%)
Employed†	473 (90.1%)	367 (82.7%)
Clinical information	*n* = 1,046	*n* = 901
Reported deworming in the last year	**3 (0.3%)‡**	**78 (9.0%)‡**
Diarrhea§	83 (8.0%)	133 (15.7%)
Shoe-wearing practices	*n* = 1,046	*n* = 901
Always wear shoes indoors	561 (53.6%)	304 (33.7%)
Always wear shoes outdoors	713 (68.2%)	462 (51.3%)
Always wear shoes while toileting	731 (69.9%)	498 (55.3%)
Soil-transmitted helminth infections	*n* = 891	*n* = 711
Ascaris spp. infections	125 (14.0%)	156 (21.9%)
Ascaris spp. higher intensity infections	48 (5.4%)	79 (11.1%)
Necator americanus infections	533 (59.8%)	430 (60.5%)
Necator americanus higher intensity infections	288 (32.3%)	223 (31.4%)
Ancylostoma spp. infections	43 (4.8%)	23 (3.2%)
Trichuris trichiura infections	1 (0.1%)	5 (0.7%)
Strongyloides stercoralis infections	0	1 (0.1%)
Household variables	*n* = 244	*n* = 219
Has household toilet	51 (20.9%)	48 (21.9%)
Main water source is unprotected	210 (86.1%)	157 (71.7%)
Has earth floor	160 (65.6%)	101 (46.3%)
Lives on < 1 USD/day	108 (44.4%)	108 (51.7%)
Owns a motor vehicle	19 (7.8%)	22 (10.0%)
Has electricity	210 (89.0%)	129 (63.2%)
Owns any electrical appliance	116 (47.5%)	75 (34.3%)

* Study participants are defined as residents who were present at the time of the visit and provided questionnaires or stool samples.
† Being employed includes all work carried out outside the house.
‡ Significant difference $P < 0.05$ between control and intervention arms, adjusted for community-level clustering.
§ Participants who had diarrhea at the time of questionnaire, or within the previous 2 weeks.

Detailed characterization of participants at baseline, including environmental and WASH risk factors for STH infection, and intensity of infection are described elsewhere.[20,30–32] Participation rates at each study time point were similar in the intervention and control arms and are shown in Supplemental Table 1. In total, 2,141 individuals (1,033 in the intervention arm and 1,108 in the control arm) participated in at least one follow-up time point, by completing a questionnaire and/or providing stool samples. Of these, 1,878 individuals (977 in the intervention arm and 901 in the control arm) provided stool samples at one or more follow-up time points and were included in the primary analysis.

At baseline, the prevalence of *Ascaris* spp. was 14.0% (95% CI: 8.1–37.8) in the control arm versus 21.9% (7.6–36.6) in the intervention arm, whereas the prevalence of *N. americanus* was 59.8% (51.9–74.0) versus 60.5% (51.3–73.4). *Ancylostoma* spp., *T. trichiura*, and *S. stercoralis* were all much less prevalent. In terms of intensity of infection, 5.4% (1.9–14.6) of all samples were categorized as higher intensity *Ascaris* spp. infections in the control arm versus 11.1% (4.0–27.2) in the intervention arm, whereas 32.3% (24.1–38.2) versus 31.4% (26.6–36.6) were higher intensity *N. americanus* infections in control and intervention arms, respectively (Table 1).

Aggregated WASH-related characteristics are shown in Table 1, Figure 2, and Supplemental Table 2. At baseline, study arms were mostly balanced and characterized by low levels of sanitation and piped water access. Individual household toilet use was 20.3% (5.9–30.9) in the control arm versus 19.9% (6.3–31.3) in the intervention arm. Open defecation (defined as any nonuse of toilet, irrespective of toilet ownership) was practiced by 82.1% (72.8–95.6) of participants in the control arm versus 82.8% (70.9–94.7) in the intervention arm. The majority of the households used an unprotected water source: 86.1% (81.7–90.4) in the control arm versus 71.7% (65.8–77.7) in the intervention arm. No households in the control arm had access to piped water (tap stand in the community or their own plot), compared with 21.5% (16.0–26.9) in the intervention arm.

In the intervention arm, household latrine use peaked at the first follow-up at 74.9% (62.4–89.9). Similarly, open defecation was lowest in the intervention arm at the first follow-up at 26.1% (12.0–39.1). Over the subsequent three follow-ups, latrine use in the intervention arm decreased and open defecation increased, whereas there were some improvements in sanitation in the control arm that were most evident at the last follow-up. Nevertheless, at the end of the trial, there remained

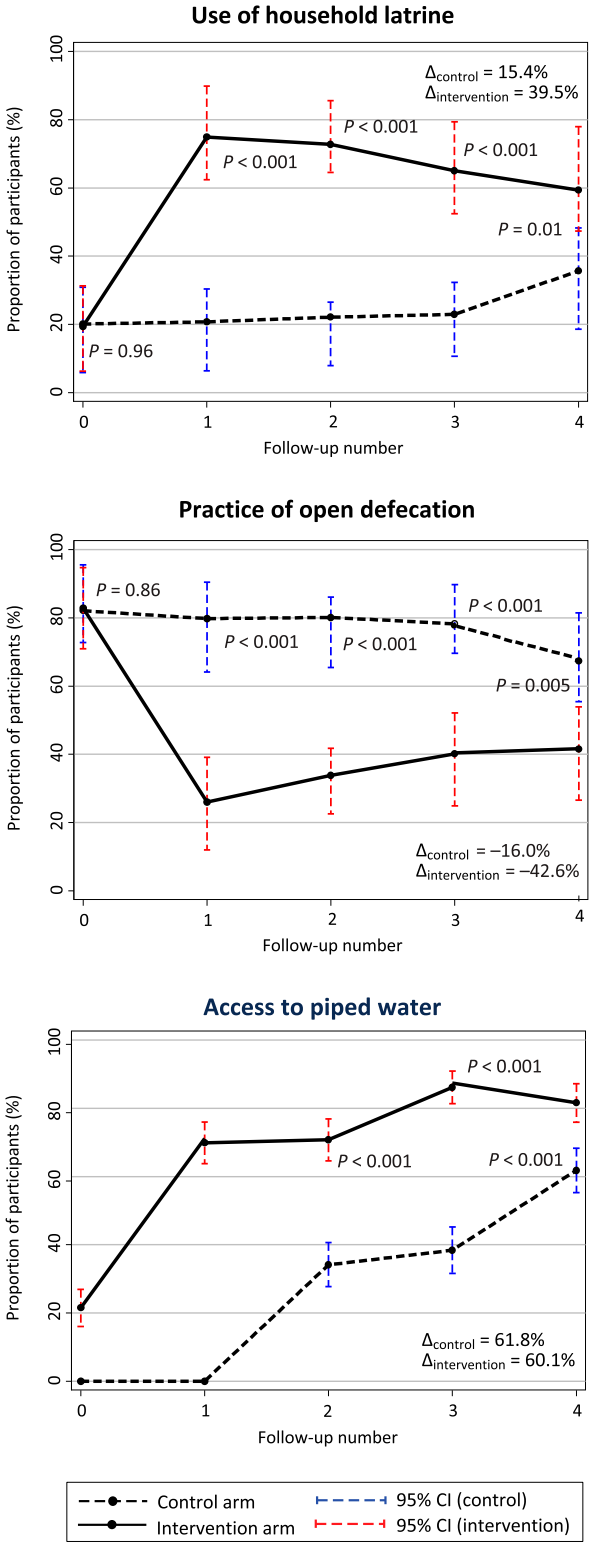

FIGURE 2. Use of household latrines, practice of open defecation, and piped water as main water source in the two study arms over time. *P*-values and 95% CI calculated using logistic regression models accounting for community-level clustering. Δ = absolute change in proportion = Proportion [Follow-up 4]–Proportion [Baseline]. CI = confidence interval. This figure appears in color at www.ajtmh.org.

a significant difference in sanitation practices between study arms. Household latrine use was 35.7% (18.6–48.3) in the control arm versus 59.4% (47.4–78.0) in the intervention arm (P = 0.010). Open defecation was practiced by 66.1% (54.2–80.2) of respondents in the control arm versus 40.2% (25.3–52.6) in the intervention arm (P = 0.005) (Figure 2, Supplemental Table 2). Of note is the fact that children less than 5 years of age were the main open defecators in the presence of a household latrine: at the final follow-up, 29.7% (11.3–47.9) of children less than 5 years of age in the control arm and 35.2% (19.4–57.8) in the intervention arm practiced open defecation, despite having a household latrine. During the final visit, we asked heads of households who had never built a latrine, or failed to rebuild a nonfunctional latrine, about their reasons for not building or failing to rebuild a latrine (Supplemental Table 3). The two most common reasons were lack of time, and lack of money or access to materials.

Access to piped water increased over time in both arms. At the first follow-up, no households in the control arm had access to piped water, compared with 71.2% (65.0–77.3) in the intervention arm. At the end of the study, 61.8% (55.3–68.3) of participants in the control arm versus 81.6% (75.9–87.2) in the intervention arm (P < 0.001) had access to piped water (Figure 2, Supplemental Table 2). With regard to handwashing behaviors, at baseline, 77.2% (63.5–91.6) of the respondents in the control arm reported using soap to wash hands versus 77.4% (56.5–87.7) in the intervention arm. Improvements in reported handwashing practices over time were modest, with no difference between study arms at any of the follow-up visits (Supplemental Table 2).

Results of the generalized linear mixed models showed that the integrated WASH and deworming intervention had no effect on infection with *Ascaris* spp. (RR 2.87, 95% CI: 0.66–12.48, P = 0.159) or *N. americanus* (RR 0.99, 95% CI: 0.52–1.89, P = 0.987), relative to deworming alone (Table 2, Supplemental Table 4). The intervention also had no detectable effect on the RR of higher intensity infection (Table 2, Supplemental Table 4), or on infection intensity as a continuous measure (Supplemental Table 5), for either STH. Similar results were observed when running the models also adjusting for baseline infection status or intensity group (Supplemental Table 6).

Infection-related outcomes over time are shown in Figure 3 and Supplemental Table 7. At the end of the trial, prevalence of *Ascaris* spp. decreased to 4.5% (0.0–13.3) in the control arm and 14.3% (2.9–30.3) in the intervention arm. The prevalence of *N. americanus* decreased to 16.9% (11.6–28.2) in the control arm and 15.4% (9.6–24.6) in the intervention arm. Higher intensity *Ascaris* spp. infections decreased to 1.6% (0.2–11.1) in the control arm versus 5.6% (1.5–19.0) in the intervention arm, and higher intensity *N. americanus* infections to 6.3% (2.7–13.8) versus 4.0% (2.3–6.8). There were no significant differences at any time point in the prevalence, mean intensity of infection as determined by qPCR, or proportion of higher intensity infections between the control and intervention arms (Supplemental Table 7).

Generalized linear mixed models for morbidity outcomes showed that by the end of the trial, the WASH and deworming intervention did not have any additional impact on anemia, stunting, thinness, wasting, or underweight, compared with deworming alone (Table 3, Supplemental Table 8). A similar lack of effect of the intervention was observed when looking at

TABLE 2
Effect of the study intervention on soil-transmitted helminth prevalence and intensity group

			Infection prevalence			Infection intensity group		
		N	Prevalence (95% CI)	Adjusted RR* (95% CI)	*P*-value	Prevalence† (95% CI)	Adjusted RR* (95% CI)	*P*-value
Ascaris spp.								
Follow-up 1	Intervention	584	17.3 (4.3–30.7)	1.38 (0.37–5.11)	0.632	10.8 (2.9–32.5)	1.59 (0.21–11.77)	0.650
	Control	689	12.8 (2.1–24.5)			8.0 (2.0–27.0)		
Follow-up 2	Intervention	552	13.6 (1.9–29.6)	1.44 (0.35–5.87)	0.607	5.3 (1.0–23.2)	1.58 (0.23–10.89)	0.643
	Control	624	10.6 (0.0–21.8)			4.6 (1.0–18.5)		
Follow-up 3	Intervention	531	12.4 (1.0–24.7)	1.49 (0.39–5.79)	0.560	4.5 (1.6–12.4)	1.46 (0.24–8.85)	0.684
	Control	609	7.9 (0–20.0)			3.8 (0.7–17.2)		
Follow-up 4	Intervention	553	14.3 (2.9–30.3)	2.87 (0.66–12.48)	0.159	5.6 (1.5–19.0)	4.91 (0.77–31.37)	0.093
	Control	623	4.5 (0.0–13.3)			1.6 (0.2–11.1)		
Necator americanus								
Follow-up 1	Intervention	584	33.6 (24.1–44.2)	1.06 (0.68–1.64)	0.795	14.7 (6.5–30.1)	0.94 (0.26–3.35)	0.921
	Control	689	35.3 (26.8–47.6)			17.6 (8.9–31.8)		
Follow-up 2	Intervention	552	22.3 (15.3–31.7)	1.10 (0.66–1.85)	0.715	11.1 (5.2–21.9)	1.07 (0.37–3.14)	0.896
	Control	624	22.4 (15.5–32.1)			8.3 (5.1–13.2)		
Follow-up 3	Intervention	531	22.0 (15.2–30.7)	1.26 (0.72–2.20)	0.416	5.3 (3.4–8.0)	1.94 (0.74–5.07)	0.178
	Control	609	19.5 (13.4–28.0)			3.4 (2.1–5.5)		
Follow-up 4	Intervention	553	15.4 (9.6–24.6)	0.99 (0.52–1.89)	0.987	4.0 (2.3–6.8)	0.92 (0.29–2.95)	0.893
	Control	623	16.9 (11.6–28.2)			6.3 (2.7–13.8)		

CI = confidence interval; RR = relative risk.
* Adjusted RR obtained from generalized linear mixed models, adjusted for age and gender (fixed effects) and clustering at the community, household, and individual levels (random effects). Models included 1,878 participants in 456 households in 18 communities.
† Intensity group was run as an ordinal model, with the following categories: no infection, lower intensity infection, and higher intensity infection. Prevalence shown here is that of higher intensity infection.

each of these outcomes as continuous variables, except for height-for-age, where being in the intervention arm was associated with a lower *Z*-score (Supplemental Table 9).

Morbidity indicators over time are shown in Supplemental Table 10. At baseline, 15.4% (11.7–20.5) of participants who provided a finger-prick blood sample in the control arm were anemic versus 21.1% (15.3–25.4) in the intervention arm. There were no significant differences between study arms at most of the time points, except at baseline when participants in the intervention clusters had lower Hb, and at the last follow-up, when anemia was less prevalent in the intervention arm. In terms of proxy indicators for malnutrition, from the total number of eligible participants who provided height and weight measurements at baseline, 51.9% (41.4–60.4) were stunted in the control arm versus 64.7% (55.3–72.7) in the intervention arm, 22.5% (16.1–29.5) versus 17.8% (11.9–23.3) were thin, 13.8% (5.6–24.3) versus 15.1% (5.2–22.5) were wasted, and 52.0% (42.3–61.2) versus 60.4% (48.3–66.7) were underweight (Table 3, Supplemental Table 10). The only significant differences between study arms in nutrition-related morbidity indicators were on mean height-for-age *Z*-score that was lower in the intervention arm at baseline and the second follow-up and stunting that was higher at the same time points (Table 3, Supplemental Table 10).

The results of the generalized linear mixed models including participants in all 23 clusters who completed the study, including the five that were not randomly allocated, are shown in Supplemental Tables 11 and 12, and show that study results remained similar.

DISCUSSION

This is the first cluster RCT investigating the additional benefit of a community WASH intervention on STH infections, relative to that achieved by community deworming alone.

When looking at STH infection and intensity, for both *Ascaris* spp. *and N. americanus*, we found no effect of the integrated WASH and deworming intervention compared with deworming alone, after 2 years of follow-up. Over time, there was a substantial decrease in both study arms in prevalence and proportion of higher intensity infections of both STHs, which can be attributed to the regular biannual community deworming in all participating communities. Of note is that the impact of deworming was more pronounced for *N. americanus* than for *Ascaris* spp., despite albendazole being more efficacious against *Ascaris* spp. and the baseline prevalence of *N. americanus* being higher. This is likely due to greater environmental persistence of *Ascaris* spp. compared with that of *N. americanus*, resulting in more intense reinfection with the former.[33,34] In terms of morbidity outcomes, we did not detect any impact of the WASH and deworming intervention relative to deworming alone, except on height-for-age *Z*-score, where the intervention arm was more likely to have a lower score; however, this may be explained by the fact that participants in the intervention arm had lower *Z*-scores at baseline. Furthermore, the trial was not powered to detect differences in these morbidity outcomes.

Several factors, including study limitations, may explain the absence of an additional impact of the WASH program on infection outcomes beyond the benefit achieved by deworming. Importantly, although the WASH intervention considerably increased the number of participants who reported using a household latrine and households with access to piped water, it failed to achieve "open defecation-free" status, which is the ultimate goal of this type of intervention. At the end of the trial, more than a third of participants in the intervention arm were still practicing open defecation. Furthermore, the CLTS-inspired sanitation promotion was successful in motivating people to build latrines, with a peak at the first follow-up, but was unable to prevent slippage of latrine coverage. Therefore, it remains to be determined whether WASH would have a detectable impact if open defecation was eliminated. In addition, although the intervention arm was apparently superior to the control arm in terms of both sanitation and

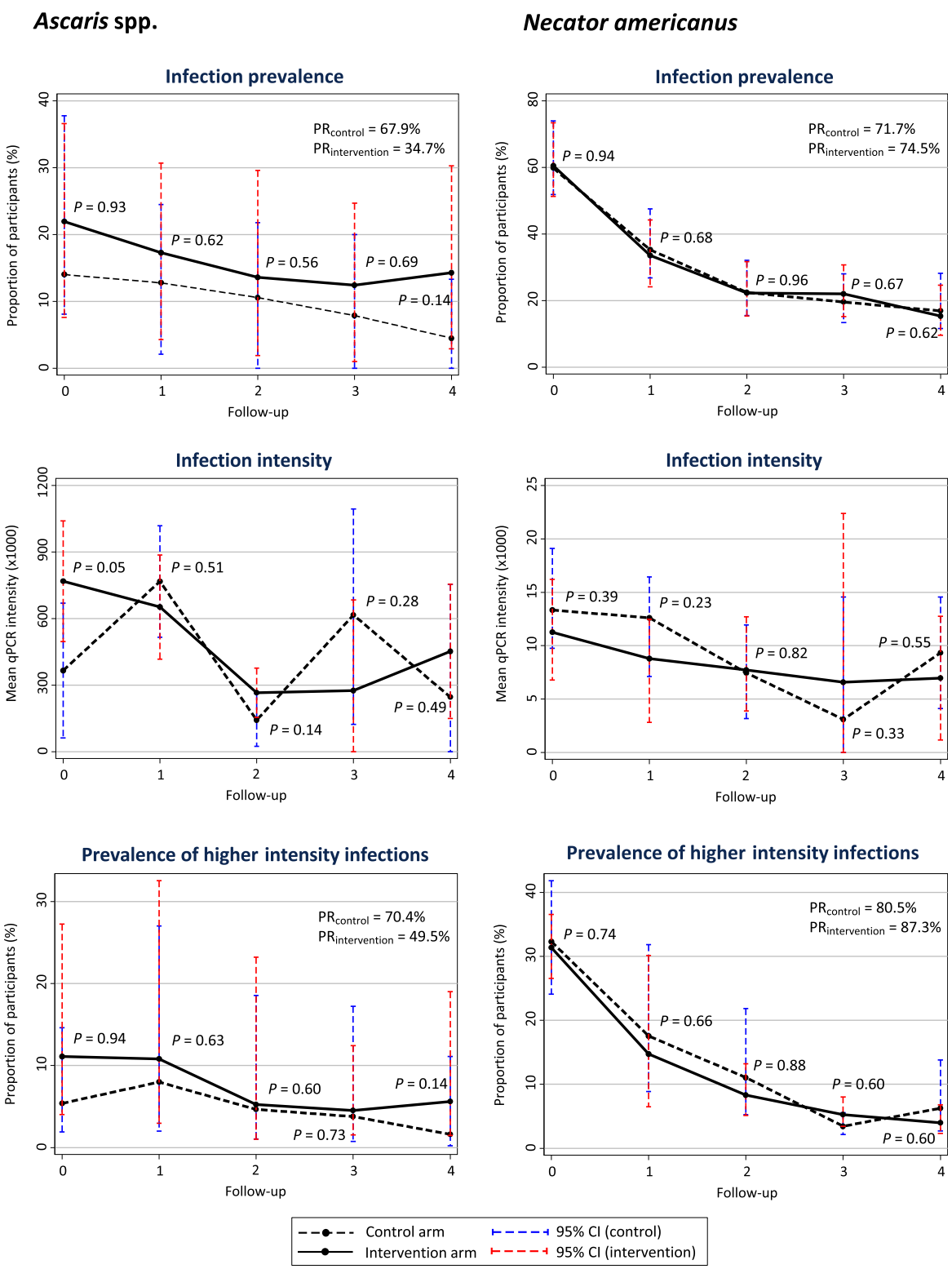

FIGURE 3. Infection prevalence, intensity, and prevalence of high-intensity infections in the two study arms over time. *P*-values and 95% CI calculated using logistic regression models accounting for community-level clustering. PR = prevalence reduction, calculated as: (Prevalence [Baseline]–Prevalence [Follow-up 4])/Prevalence [Baseline]. CI = confidence interval. This figure appears in color at www.ajtmh.org.

water access at all time points, there was an improvement in WASH conditions in the control arm that could have masked the impact of WASH intervention. Those improvements were either due to government initiatives, or because control clusters were aware that they would receive the WaterAid intervention at the end of the trial and that it required building latrines.

We followed the participating communities for 2 years after the first round of deworming. It is known that *Ascaris* spp. eggs can survive for up to 5–10 years in the environment under

TABLE 3
Effect of the study intervention on anemia and growth parameters

		N	Prevalence (95% CI)	Adjusted RR* (95% CI)	*P* value
Anemia					
Follow-up 2	Intervention	607	12.7 (8.2–18.6)	0.75 (0.43–1.31)	0.317
	Control	634	15.9 (9.2–20.3)		
Follow-up 4	Intervention	521	16.3 (8.9–20.1)	0.63 (0.34–1.14)	0.126
	Control	652	23.9 (17.8–33.6)		
Stunting†					
Follow-up 2	Intervention	338	63.9 (56.1–72.2)	1.28 (1.03–1.60)	**0.026**
	Control	319	52.7 (41.0–59.2)		
Follow-up 4	Intervention	303	59.9 (49.4–69.3)	1.18 (0.92–1.51)	0.198
	Control	294	56.0 (43.2–64.1)		
Thinness†					
Follow-up 2	Intervention	338	23.9 (18.3–37.5)	0.76 (0.47–1.22)	0.256
	Control	319	40.4 (24.6–46.1)		
Follow-up 4	Intervention	303	21.6 (14.8–29.4)	0.75 (0.51–1.11)	0.151
	Control	294	37.4 (22.7–40.9)		
Wasting†					
Follow-up 2	Intervention	99	22.4 (13.1–40.8)	0.86 (0.40–1.87)	0.711
	Control	84	29.8 (15.3–44.6)		
Follow-up 4	Intervention	86	21.2 (12.0–31.1)	0.79 (0.45–1.38)	0.413
	Control	68	30.9 (18.5–42.3)		
Underweight†					
Follow-up 2	Intervention	215	49.8 (41.4–67.3)	0.85 (0.58–1.23)	0.393
	Control	209	63.2 (48.4–73.6)		
Follow-up 4	Intervention	197	59.2 (48.7–69.0)	1.06 (0.84–1.34)	0.614
	Control	177	61.9 (50.4–71.4)		

BMI = body mass index; CI = confidence interval; RR = relative risk. **Bold** text indicates statistically significant *P* value (< 0.05).

* Adjusted RR obtained from generalized linear mixed models, adjusted for age and gender (fixed effects) and clustering at the community, household, and individual levels (random effects). Models included the following numbers of participants in 18 communities: for anemia, 1,598 participants in 428 households; for stunting, 789 participants in 304 households; for thinness, 781 participants in 301 households; for wasting, 231 participants in 157 households; and for underweight 511 participants in 249 households.

† Anthropometric indices is defined as < −2 standard deviation below the mean of a standard population for the following indicators: stunting = weight-for-age; thinness = BMI-for-age, where BMI is calculated as weight (kg)/height^2 (cm); wasting = weight-for-height; and underweight = weight-for-age.

favorable conditions.[34] Therefore, for *Ascaris* spp., a 2-year follow-up may not be sufficient for the impact of WASH to become apparent, given that the existing eggs contaminating the environment may be sufficient to continue reinfection. On the other hand, hookworm larvae only survive for a couple of months,[33] so one would expect to see reduced infections if the WASH intervention was successful at separating humans from their excreta. An additional limitation of this trial is the fact that we were only able to randomly allocate and follow 18 clusters, instead of the 24 initially recruited; however, sensitivity analysis indicated no differences in impact measures, and therefore it is unlikely that we would have found an effect with the larger sample. Also, of note is the fact that randomization achieved balance in the two arms for most variables analyzed at baseline, except for piped water access and deworming in the previous year; we believe the imbalance arose by chance. Finally, for *Ascaris* spp., given the underestimation of ICC, we were underpowered to detect a 50% reduction in infection in the intervention arm compared with the deworming alone clusters.

Finally, although this would not have affected results of the primary analysis, an additional limitation of this trial was that WASH-related behaviors—particularly latrine use and handwashing practices—were self-reported. It was logistically not feasible to observe these behaviors because of the financial cost of doing so. This makes it difficult to appropriately monitor behavior change and uptake of the intervention, particularly in relation to handwashing behaviors, use of latrines, and persistence of open defecation. Self-reporting may result in overreporting of "desirable" behaviors (courtesy bias),[35] and structured observations can lead to a modification of the participants' behavior ("Hawthorne" effect),[36] even in the case of rapidly collected spot check measurements.[37] Although techniques have been developed to assess latrine use and handwashing that do not rely on observation, including sensor systems[38] and testing for the presence of fecal bacteria in participants' hands,[17] additional research should prioritize examining soil contamination with STH infective forms that would quantify the extent to which WASH interventions, particularly the sanitation component, are effective.[39–41]

So far, the only WASH intervention studies that reported an impact on STH infections are school-based interventions with a strong focus on promoting individual hygiene behavior.[13–16] The two previous RCTs investigating the impact of community sanitation intervention, which were conducted in the context of the Indian Total Sanitation Campaign, failed to detect a reduction in STH infections as a result of the sanitation intervention.[17,18] Short follow-up time, suboptimal coverage and use of latrines in the intervention arm, and contamination in the control arm have also been proposed to explain those results. This raises a question that must be addressed by the WASH sector and implementers of sanitation programs: What threshold of sanitation coverage is required for WASH interventions to effectively decrease STH reinfection and eventually interrupt transmission? Greater emphasis may need to be placed on achieving "open defecation–free" status.[42] Furthermore, given that most of the participants who reported practicing open defecation were children aged 5 years and younger, tailored approaches targeting this age group and their parents should be emphasized.

Current debates about the best approach to achieve lasting behavior changes and sustainable latrine use have divided the field between proponents of CLTS-based approaches and

proponents of subsidized approaches. A recent review and meta-analysis reported similar and modest (lower than 20%) increases in latrine coverage and use for both approaches.[43] Only one RCT has directly compared the uptake of different sanitation interventions. In this study, a community motivation approach did not increase latrine coverage, whereas subsidies increased coverage modestly.[44]

A related issue is latrine sustainability.[45] Implementers of sanitation programs have reported that motivating people to build a latrine is less challenging than to sustain their use, especially if reconstruction is needed on latrine failure.[46] We found that in the intervention arm, a quarter of the residents did not build a latrine, and of those who did, around 10% failed to rebuild a latrine that became nonfunctional. Supporters of subsidized sanitation approaches argue that one of the advantages of subsidies is higher quality latrines, leading to greater durability and long-lasting changes in defecation practices.[47] In Timor-Leste, other innovative strategies for sustaining latrine coverage and use include marketing approaches introducing new affordable plastic products to upgrade latrines and vouchers for vulnerable households (A. Grumbley, personal communication).

The present WHO guidelines recommend that deworming programs are stopped when the prevalence of high-intensity STH infections is less than one percent.[6] In this context, WASH may be able to prevent infection levels from returning to pre-deworming levels and contribute to sustainable STH control with eventual elimination. Future research is needed to test this hypothesis. The WASH Benefits RCT, comparing the effect of individual and combined WASH interventions on diarrhea, growth, and enteric infections including STH, in Kenya and Bangladesh, may be able to contribute evidence to fill this knowledge gap.[48] Whereas experimental studies may be necessary to generate evidence to inform guidelines and policies, mathematical modeling can also robustly test such hypotheses. Modeling can also shed light on the level of latrine uptake necessary to effectively reduce STH transmission.

The recent fourth WHO report on neglected tropical diseases (NTDs) gives additional emphasis to WASH relative to its previous editions, following the release of the joint NTD-WASH strategy in 2015.[49,50] The findings of our trial suggest that WASH interventions may not deliver immediate health benefits in terms of STH control and that deworming will decrease infections more rapidly. Program managers in both NTD control and WASH programs must be aware of the long-term investment that WASH interventions require before measurable indicators of health impact may be realized, and WASH interventions should focus on not only promoting initial latrine building but also achieving "open defecation–free" status and durable latrines able to sustain lasting change in behavior.

CONCLUSION

In the context of high endemicity and over a 2-year period, we found no evidence that an integrated community WASH intervention resulted in an additional reduction in STH infections beyond that achieved with deworming alone. Additional research is needed to determine the role of WASH on STH infections over a longer period of time and in the absence of deworming.

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REFERENCES

1. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ, 2014. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors 7:* 37.
2. GBD 2015 DALYs and HALE Collaborators, 2016. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet 388:* 1603–1658.
3. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ, 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet 367:* 1521–1532.
4. Campbell SJ, Nery SV, Doi SA, Gray DJ, Magalhaes RJS, McCarthy JS, Traub RJ, Andrews RM, Clements AC, 2016. Complexities and perplexities: a critical appraisal of the evidence for soil-transmitted helminth infection-related morbidity. *PLoS Negl Trop Dis 10:* e0004566.
5. Vercruysse J et al., 2011. Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. *PLoS Negl Trop Dis 5:* e948.
6. WHO, 2012. *Eliminating Soil-Transmitted Helminthiasis as a Public Health Problem in Children: Progress Report 2001–2010 and Strategic Plan 2011–2020*. Geneva, Switzerland: World Health Organization.
7. Anderson RM, Turner HC, Truscott JE, Hollingsworth TD, Brooker SJ, 2015. Should the goal for the treatment of soil transmitted helminth (STH) infections be changed from morbidity control in

children to community-wide transmission elimination? *PLoS Negl Trop Dis 9:* e0003897.
8. Turner HC, Truscott JE, Bettis AA, Shuford KV, Dunn JC, Hollingsworth TD, Brooker SJ, Anderson RM, 2015. An economic evaluation of expanding hookworm control strategies to target the whole community. *Parasit Vectors 8:* 1–11.
9. Clarke NE, Clements AC, Doi SA, Wang D, Campbell SJ, Gray D, Nery SV, 2017. Differential effect of mass deworming and targeted deworming campaigns for soil-transmitted helminth control in children: a systematic review and meta-analysis. *Lancet 389:* 287–297.
10. Jia TW, Melville S, Utzinger J, King CH, Zhou XN, 2012. Soil-transmitted helminth reinfection after drug treatment: a systematic review and meta-analysis. *PLoS Negl Trop Dis 6:* e1621.
11. Campbell SJ et al., 2014. Water, sanitation, and hygiene (WASH): a critical component for sustainable soil-transmitted helminth and schistosomiasis control. *PLoS Negl Trop Dis 8:* e2651.
12. Strunz EC, Addiss DG, Stocks ME, Ogden S, Utzinger J, Freeman MC, 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. *PLoS Med 11:* e1001620.
13. Freeman MC, Clasen T, Brooker SJ, Akoko DO, Rheingans R, 2013. The impact of a school-based hygiene, water quality and sanitation intervention on soil-transmitted helminth reinfection: a cluster-randomized trial. *Am J Trop Med Hyg 89:* 875–883.
14. Bieri FA et al., 2013. Health-education package to prevent worm infections in Chinese schoolchildren. *N Engl J Med 368:* 1603–1612.
15. Gyorkos TW, Maheu-Giroux M, Blouin B, Casapia M, 2013. Impact of health education on soil-transmitted helminth infections in schoolchildren of the Peruvian Amazon: a cluster-randomized controlled trial. *PLoS Negl Trop Dis 7:* e2397.
16. Mahmud MA, Spigt M, Bezabih AM, Pavon IL, Dinant G-J, Velasco RB, 2015. Efficacy of handwashing with soap and nail clipping on intestinal parasitic infections in school-aged children: a factorial cluster randomized controlled trial. *PLoS Med 12:* e1001837.
17. Clasen T et al., 2014. Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial. *Lancet Glob Health 2:* e645–e653.
18. Patil SR, Arnold BF, Salvatore AL, Briceno B, Ganguly S, Colford JM Jr., Gertler PJ, 2014. The effect of India's total sanitation campaign on defecation behaviors and child health in rural Madhya Pradesh: a cluster randomized controlled trial. *PLoS Med 11:* e1001709.
19. Nery SV et al., 2015. A cluster-randomised controlled trial integrating a community-based water, sanitation and hygiene programme, with mass distribution of albendazole to reduce intestinal parasites in Timor-Leste: the WASH for WORMS research protocol. *BMJ Open 5:* e009293.
20. Campbell SJ et al., 2016. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and Giardia duodenalis infections in rural communities in Timor-Leste. *Int J Parasitol 46:* 771–779.
21. WaterAid, 2011. *Training Manual—Water Resource Management: Integrated Planning and Management at Community Level.* Kathmandu, Nepal: WaterAid.
22. Kar K, 2005. *Practical Guide to Triggering Community-Led Total Sanitation (CLTS)*. Brighton, England: Institute of Development Studies.
23. Llewellyn S, Inpankaew T, Nery S, Gray D, Verweij J, Clements A, Gomes S, Traub R, McCarthy J, 2016. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. *PLoS Negl Trop Dis 10:* e0004380.
24. WHO Multicentre Growth Reference Study Group, 2006. *WHO Child Growth Standards: Length/Height-for-Age, Weight-for-Age, Weight-for-Length, Weight-for-Height and Body Mass Index-for-Age. Methods and Development*. Geneva, Switzerland: World Health Organization.
25. WHO, 2007. *WHO AnthroPlus Software*. Geneva, Switzerland: World Health Organization.
26. WHO, 2011. *WHO Anthro (Version 3.2.2, January 2011) and Macros*. Geneva, Switzerland: World Health Organization.
27. WHO, 2011. *Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity*. Geneva, Switzerland: World Health Organization.
28. Leonardo L et al., 2012. A national baseline prevalence survey of schistosomiasis in the Philippines using stratified two-step systematic cluster sampling design. *J Trop Med 2012:* 936128.
29. Gray DJ, Forsyth SJ, Li RS, McManus DP, Li Y, Chen H, Zheng F, Williams GM, 2009. An innovative database for epidemiological field studies of neglected tropical diseases. *PLoS Negl Trop Dis 3:* e413.
30. Campbell SJ et al., 2017. Water, sanitation and hygiene (WASH) and environmental risk factors for soil-transmitted helminth intensity of infection in Timor-Leste, using real time PCR. *PLoS Negl Trop Dis 11:* e0005393.
31. Wardell R, Clements ACA, Lal A, Summers D, Llewellyn S, Campbell SJ, McCarthy J, Gray DJ, Nery SV, 2017. An environmental assessment and risk map of *Ascaris lumbricoides* and *Necator americanus* distributions in Manufahi District, Timor-Leste. *PLoS Negl Trop Dis 11:* e0005565.
32. Campbell SJ et al., 2017. Investigations into the association between soil-transmitted helminth infections, haemoglobin and child development indices in Manufahi District, Timor-Leste. *Parasit Vectors 10:* 192.
33. Udonsi J, Atata G, 1987. *Necator americanus*: temperature, pH, light, and larval development, longevity, and desiccation tolerance. *Exp Parasitol 63:* 136–142.
34. Muller R, 2002. Chapter 5: the nematodes. Muller R, ed. *Worms and Human Disease*. London, United Kingdom: CABI.
35. Manun'Ebo M, Cousens S, Haggerty P, Kalengaie M, Ashworth A, Kirkwood B, 1997. Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. *Trop Med Int Health 2:* 1015–1021.
36. Ram PK et al., 2010. Is structured observation a valid technique to measure handwashing behavior? Use of acceleration sensors embedded in soap to assess reactivity to structured observation. *Am J Trop Med Hyg 83:* 1070–1076.
37. Arnold BF, Khush RS, Ramaswamy P, Rajkumar P, Durairaj N, Ramaprabha P, Balakrishnan K, Colford JM Jr., 2015. Reactivity in rapidly collected hygiene and toilet spot check measurements: a cautionary note for longitudinal studies. *Am J Trop Med Hyg 92:* 159–162.
38. Clasen T et al., 2012. Making sanitation count: developing and testing a device for assessing latrine use in low-income settings. *Environ Sci Technol 46:* 3295–3303.
39. Collender PA, Kirby AE, Addiss DG, Freeman MC, Remais JV, 2015. Methods for quantification of soil-transmitted helminths in environmental media: current techniques and recent advances. *Trends Parasitol 31:* 625–639.
40. Steinbaum L, Kwong LH, Ercumen A, Negash MS, Lovely AJ, Njenga SM, Boehm AB, Pickering AJ, Nelson KL, 2017. Detecting and enumerating soil-transmitted helminth eggs in soil: new method development and results from field testing in Kenya and Bangladesh. *PLoS Negl Trop Dis 11:* e0005522.
41. Gyawali P, Ahmed W, Sidhu JP, Nery SV, Clements AC, Traub R, McCarthy JS, Llewellyn S, Jagals P, Toze S, 2016. Quantitative detection of viable helminth ova from raw wastewater, human feces, and environmental soil samples using novel PMA-qPCR methods. *Environ Sci Pollut Res Int 23:* 18639–18648.
42. Sigler R, Mahmoudi L, Graham JP, 2015. Analysis of behavioral change techniques in community-led total sanitation programs. *Health Promot Int 30:* 16–28.
43. Garn JV, Sclar GD, Freeman MC, Penakalapati G, Alexander KT, Brooks P, Rehfuess EA, Boisson S, Medlicott KO, Clasen TF, 2017. The impact of sanitation interventions on latrine coverage and latrine use: a systematic review and meta-analysis. *Int J Hyg Environ Health 220:* 329–340.
44. Guiteras R, Levinsohn J, Mobarak AM, 2015. Encouraging sanitation investment in the developing world: a cluster-randomized trial. *Science 348:* 903–906.
45. Montgomery MA, Bartram J, Elimelech M, 2009. Increasing functional sustainability of water and sanitation supplies in rural sub-Saharan Africa. *Environ Eng Sci 26:* 1017–1023.
46. Partnership for Human Development Australia Timor-Leste, 2017. *ODF Sustainability in Timor-Leste*. Dili, Timor-Leste: PHD.

47. Perez E et al., 2012. *Working Paper: What Does it Take to Scale up Rural Sanitation?* Washington, DC: Water and Sanitation Program.
48. Arnold BF et al., 2013. 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. *BMJ Open 3:* e003476.
49. WHO, 2017. *Integrating Neglected Tropical Diseases in Global Health and Development: Fourth WHO Report on Neglected Tropical Diseases*. Geneva, Switzerland: World Health Organization.
50. WHO, 2015. *Water Sanitation & Hygiene for Accelerating and Sustaining Progress on Neglected Tropical Diseases: A Global Strategy 2015–2020*. Geneva, Switzerland: World Health Organization.