

1 **Integrating water, sanitation, handwashing, and nutrition interventions to reduce child soil-**  
2 **transmitted helminth and *Giardia* infections: a cluster-randomized controlled trial in rural**  
3 **Kenya**

4

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26 **Short title: Integrated WASH and child parasite infections**

27 **Trial registration:** ClinicalTrials.gov NCT01704105,

28 <https://clinicaltrials.gov/ct2/show/NCT01704105>

29 **Abstract**

30 **Background.** Helminth and protozoan infections affect >1 billion children globally. Improved  
31 water, sanitation, handwashing, and nutrition could be more sustainable control strategies for  
32 parasite infections than mass drug administration (MDA), while providing other quality of life  
33 benefits.

34 **Methods and Findings.** We enrolled geographic clusters of pregnant women into a cluster-  
35 randomized controlled trial that tested six interventions: disinfecting drinking water(W),  
36 improved sanitation(S), handwashing with soap(H), combined WSH, improved nutrition(N), and  
37 combined WSHN. We assessed intervention effects on parasite infections by measuring *Ascaris*  
38 *lumbricoides*, *Trichuris trichiura*, hookworm, and *Giardia duodenalis* among individual children  
39 born to enrolled mothers and their older siblings (ClinicalTrials.gov NCT01704105). We collected  
40 stool specimens from 9077 total children in 622 clusters, including 2346 children in control,  
41 1117 in water, 1160 in sanitation, 1141 in handwashing, 1064 in WSH, 1072 in nutrition, and  
42 1177 in WSHN. In the control group, 23% of children were infected with *Ascaris lumbricoides*,  
43 1% with *Trichuris trichuria*, 2% with hookworm and 39% with *Giardia duodenalis*. After two  
44 years of intervention exposure, *Ascaris* infection prevalence was 18% lower in the water  
45 treatment arm (95% confidence interval (CI) 0%, 33%), 22% lower in the WSH arm (CI 4%, 37%),  
46 and 22% lower in the WSHN arm (CI 4%, 36%) compared to control. Individual sanitation,  
47 handwashing, and nutrition did not significantly reduce *Ascaris* infection on their own, and  
48 integrating nutrition with WSH did not provide additional benefit. *Trichuris* and hookworm were  
49 rarely detected, resulting in imprecise effect estimates. No intervention reduced *Giardia*.  
50 Reanalysis of stool samples by quantitative polymerase chain reaction (qPCR) confirmed the  
51 reductions in *Ascaris* infections measured by microscopy in the WSH and WSHN groups. Lab  
52 technicians and data analysts were blinded to treatment assignment, but participants and

53 sample collectors were not blinded. The trial was funded by the Bill & Melinda Gates Foundation  
54 and USAID.

55 **Conclusions.** Our results suggest integration of improved water quality, sanitation, and  
56 handwashing could contribute to sustainable control strategies for *Ascaris* infections,  
57 particularly in similar settings with recent or ongoing deworming programs. Water treatment  
58 alone was similarly effective to integrated WSH, providing new evidence that drinking water  
59 should be given increased attention as a transmission pathway for *Ascaris*.

60

61 **Key words:** water, sanitation, handwashing, nutrition, intestinal worms, protozoa, low-income  
62 countries

63

64

65 **Introduction**

66 Intestinal soil-transmitted helminth (STH) infections, including *Ascaris lumbricoides*, *Trichuris*  
67 *trichiura*, and hookworm, and the protozoa *Giardia duodenalis* are common parasitic infections  
68 among children in low-resource settings and neglected tropical diseases. Globally, STH are  
69 estimated to affect 1.45 billion people(1), while *Giardia* has been cited as the most common  
70 enteropathogen in low-income countries(2). STH and *Giardia* infections can result in poor  
71 absorption of nutrients and weight loss(3,4). There is some evidence that STH and *Giardia*  
72 infections, even when asymptomatic, may contribute to growth faltering and impaired cognitive  
73 development(5-8). Longitudinal cohort studies in Bangladesh and Brazil have identified early  
74 infection with *Giardia* as a risk factor for stunting among children(7,9). In Peru, children with  
75 multiple *Giardia* infections per year during the first two years of life had lower cognitive function  
76 scores at age 9 than children with one or fewer *Giardia* infections(10). The effect of child STH  
77 infections on child growth, cognitive development, and school performance has been mixed and  
78 strongly debated by experts, with some suggesting additional evidence is needed(5,6,11).

79

80 School-based mass drug administration (MDA) campaigns have been the cornerstone of the  
81 global strategy to control STH infections; however, high reinfection rates limit the ability of MDA  
82 to achieve sustained reduction in STH infection prevalence(12). *Ascaris*, *Trichuris*, *Giardia*, and  
83 *Ancylostoma duodenale* are primarily transmitted through the fecal-oral ingestion route,  
84 although *Ancylostoma duodenale* as well as *Necator americanus* can be transmitted  
85 transdermally. A meta-analysis of studies from settings with medium-to-high endemic STH  
86 prevalence identified an average reinfection rate for *Ascaris* at 12 months at 94% of baseline  
87 prevalence, while the average 12-month reinfection rates for *Trichuris* and hookworm were 82%

88 and 57%, respectively(13). To achieve elimination of STH transmission, it has been suggested  
89 that MDA control efforts may need to be integrated with improved water, sanitation, and  
90 handwashing(14). Control of *Giardiasis* has historically relied on drug treatment after diagnosis  
91 as well as exposure prevention by water treatment and improved sanitation, but zoonotic  
92 transmission can complicate exposure prevention(15). Recent systematic reviews suggest that  
93 improved water, sanitation, and handwashing can reduce the odds of STH and *Giardia*  
94 infections, though the quality of the evidence base remains poor and consists almost exclusively  
95 of observational analyses(16,17).

96  
97 An individual's susceptibility to STH and *Giardia* infection is influenced by exposure and immune  
98 response. A recent systematic review concluded that there was some evidence that nutritional  
99 supplementation decreases the risk of infection or reinfection with STH, but studies have been  
100 of low quality(18). Plausible mechanisms by which nutrition might reduce STH or *Giardia*  
101 infection are through improvements in effective immune response including repair of cell  
102 damage caused by parasite infection, and through changes to the gut microbiome(19,20).

103  
104 We conducted a cluster-randomized controlled trial in rural Kenya to assess the effects of water,  
105 sanitation, handwashing, and nutrition interventions delivered alone and in combination on  
106 child parasite infections. STH and *Giardia* infections were pre-specified as trial outcomes before  
107 the trial began(21). In a separate paper, we reported the effects of the interventions on child  
108 growth and diarrhea(22). The trial's nutrition intervention was the only component that  
109 improved child growth, but none of the interventions reduced diarrhea(22). Here, we report  
110 intervention effects on *Ascaris*, *Trichuris*, hookworm, and *Giardia* infections measured after two  
111 years of intervention exposure.

112

113 **Materials and Methods**

114

115 *Study design*

116 The trial protocol and detailed methods are published(21). The trial was registered at  
117 ClinicalTrials.gov, identification number: NCT01704105. The study protocol was approved by the  
118 Committee for the Protection of Human Subjects at the University of California, Berkeley  
119 (protocol number 2011-09-3654), the Institutional Review Board at Stanford University (IRB-  
120 23310), and the Scientific and Ethics Review Unit at the Kenya Medical Research Institute  
121 (protocol number SSC-2271). Innovations for Poverty Action (IPA) enrolled participants,  
122 implemented the intervention delivery, and collected the data. Mothers provided written  
123 informed consent for themselves and their children.

124

125 Clusters of eligible pregnant women each were randomized by geographic proximal blocks into  
126 one of eight study arms: chlorine treatment of drinking water (W); improved sanitation  
127 including provision of toilets with plastic slabs and hardware to manage child feces (S);  
128 handwashing with soap (H); combined WSH; infant and young child feeding counseling plus  
129 small-quantity lipid-based nutrient supplements (N); combined WSHN; a double-sized active  
130 control, and a passive control arm. Children in the passive control arm were purposively  
131 excluded from parasitology measurement (Figure 1).

132

133 We conducted a cluster-randomized trial because components of the intervention promotion  
134 activities were at the community level and there could have been behavior and infectious  
135 disease interactions between neighboring households. Villages were eligible for selection into

136 the study if they were rural, the majority of the population lacked access to piped water  
137 supplies, and there were no other ongoing WSH or nutrition programs. Within selected villages,  
138 a census was conducted to identify eligible pregnant women in their second or third trimester  
139 that planned to continue to live at their current residence for the next year. Since interventions  
140 were designed to reduce child exposure to pathogens through a cleaner environment and  
141 exclusive breastfeeding, we enrolled pregnant women to allow time for intervention delivery to  
142 occur prior to or as close to birth as possible. Clusters were formed from 1-3 neighboring villages  
143 and had a minimum of six pregnant women per cluster after the enrollment survey. Children  
144 born to enrolled pregnant mothers were considered “index” children. Outcomes were assessed  
145 after two years of intervention exposure among index children, including twins, as well as  
146 among one older child in the index child’s compound to understand the effect of the  
147 interventions on both preschool aged and school aged children. The older child was selected by  
148 enrolling the youngest available child within the age range of 3-15 years old, with priority for a  
149 sibling in the index child’s household.

150

#### 151 *Baseline survey*

152 A survey at enrollment measured household socioeconomic characteristics and demographics  
153 (maternal age, maternal education, electricity access, type of floor, number of people in the  
154 household), as well as water, sanitation, and handwashing infrastructure and behaviors (type of  
155 water source, reported water treatment, defecation location, type of toilet, presence of water  
156 and soap at a handwashing station). In addition, at study enrollment we measured *Giardia*,  
157 *Entamoeba histolytica* and *Cryptosporidium spp.* among children residing in study compounds  
158 between 18 and 27 months of age (the projected age range for index children at the end of the  
159 study) to assess baseline prevalence of these pathogens. STH were not measured at enrollment

160 among these proxy children because it was not logistically feasible to deworm infected children  
161 at baseline. We also collected 100ml samples from primary drinking water sources accessed by  
162 study households and household stored drinking water (if available). We transported the  
163 samples on ice to field labs and enumerated *Escherichia coli* in each sample by membrane  
164 filtration followed by culture on MI media.

165

#### 166 *Randomization and blinding*

167 A few weeks after enrollment, clusters were randomly assigned to intervention arms at the  
168 University of California, Berkeley by an investigator independent of the field research team  
169 (BFA) using a random number generator. Groups of nine, geographically adjacent clusters were  
170 block-randomized into the six intervention arms, the double-sized active control arm, and the  
171 passive control arm (the passive control arm was not included in the parasite assessment).  
172 Participants and other community members were informed of their intervention group  
173 assignment after the baseline survey. Blinding (masking) participants was not possible given the  
174 nature of the interventions. Data and stool sample collectors were not informed of the cluster  
175 intervention assignment, but could have inferred treatment status by observing intervention  
176 hardware. Lab technicians were blinded to intervention status. Two authors (AJP and JS)  
177 independently replicated the statistical analyses while blinded to intervention status.

178

#### 179 *Intervention delivery*

180 Intervention delivery began <3 months after enrollment. In the water intervention arms (W,  
181 WSH, WSHN), community health promoters encouraged drinking water treatment with chlorine  
182 (liquid sodium hypochlorite) using either manual dispensers installed at the point-of-collection  
183 (community water source) in study villages or using bottled chlorine provided directly to



184 households every 6 months. In the sanitation arms (S, WSH, WSHN), households received new  
185 latrines or existing latrines were upgraded and improved by installing a plastic slab that included  
186 a lid. All households in sanitation arm study compounds were provided with a child potty for  
187 each child <3 years as well as a “sani-scoop” to remove animal and human feces from the  
188 compound. In the handwashing arms (H, WSH, WSHN), households were provided with two  
189 handwashing stations—near the latrine and the cooking area. Stations included dual foot-pedal  
190 operated jerry cans that could be tipped to dispense either soapy water or rinse water.  
191 Households were responsible for keeping the stations stocked with rinse water, and community  
192 health promoters refilled soap regularly. In the nutrition arms (N, WSHN), small quantity lipid-  
193 based nutrient supplements (LNS) were provided to children from 6-24 months of age. Children  
194 received monthly rations of LNS for addition to complementary foods twice per day. Nutrition  
195 messaging included promoting dietary diversity during pregnancy and lactation, early initiation  
196 of breastfeeding, exclusive breastfeeding from 0-6 months, continued breastfeeding through 24  
197 months, timely introduction of complementary foods, dietary diversity for child feeding, and  
198 child feeding during illness.

199

200 Community health promoters were nominated by mothers in the community and trained to  
201 provide intervention-specific behavior change activities and instructions on hardware use or  
202 provision of nutrition supplements. They were also trained to measure child mid-upper arm  
203 circumference to identify and provide referrals for potential cases of severe acute malnutrition.  
204 Each intervention consisted of a comprehensive behavior change package of key messages;  
205 visual aids in the form of flip charts, posters, and reminder cue cards; interactive activities with  
206 songs, games, or pledges to commit to practice target behaviors; and the distribution of arm-  
207 specific hardware, products, or supplements. Households in the active control group received

208 visits from promoters to measure child mid-upper arm circumference and provide malnutrition  
209 referrals, but did not receive any intervention related hardware or messaging. Promoters were  
210 instructed to visit households monthly. Key messages and promoter materials are available at  
211 <https://osf.io/fs23x/>.

212

213 Adherence to the interventions was measured during unannounced household visits after one  
214 year and two years of intervention exposure (see SI).

215

#### 216 *Measurement of parasite infections*

217 We measured parasite infections approximately 27 months post-enrollment (which equates to a  
218 minimum of 24 months of intervention exposure since intervention hardware was delivered <3  
219 months of enrollment). Stool samples were collected from index children and older children in  
220 sterile containers and transported on ice to the closer of two central field labs located in  
221 Kakamega or Bungoma. Field staff revisited households up to 3 times to collect stool samples.  
222 *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm eggs were immediately enumerated  
223 (same day) by double-slide Kato Katz microscopy with 41.7 mg templates. Both slides created  
224 from each stool sample were counted by a trained parasitologist, and two different  
225 parasitologists counted each slide from the same sample. A supervisor with expertise in STH egg  
226 identification reviewed 10% of all slides and any discrepancies were corrected. STH egg counts  
227 were averaged for analysis if both slides from one stool sample were positive. Two aliquots of  
228 stool (one mixed with ethanol) were transported on dry ice to the Eastern and Southern Africa  
229 Centre of International Parasite Control laboratory at KEMRI in Nairobi, Kenya for further  
230 analysis.

231

232 One aliquot was analyzed by monoclonal enzyme-linked immunosorbent assay (ELISA) assay  
233 (*Giardia* II<sup>TM</sup>, Alere International Limited, Galway, Ireland) for the presence or absence of *Giardia*  
234 *duodenalis* cysts. Samples were measured by ELISA in duplicate; if there was a discrepancy  
235 between duplicates, the sample was re-run. DNA was extracted from the other aliquot  
236 (preserved in ethanol) for stool samples collected from children in the control, WSH, and WSHN  
237 groups. Four qPCR assays were run in duplicate on each sample to detect the following targets:  
238 *Necator americanus*, *Ancylostoma duodenale*, *Trichuris trichiura*, and *Ascaris lumbricoides* (see  
239 SI for further details)(23).

240

#### 241 *Outcomes*

242 STH and *Giardia* infections were pre-specified outcomes in the parent WASH Benefits trial prior  
243 to the start of data collection; see Figure 3 in Arnold and others (21). Parasite infections were  
244 measured two years after the start of intervention activities. The main indicators of parasite  
245 infections were prevalence of each individual STH infection, any STH infection, and the  
246 prevalence of *Giardia* infection among index and older children from the same compound.  
247 Additional indicators of parasite infections included intensity of *Ascaris*, *Trichuris*, and  
248 hookworm measured in eggs per gram (epg) of feces; intensity binary category of *Ascaris*  
249 infection measured as low intensity (1-5000 epg) or moderate/high intensity (>5000 epg)  
250 infection following World Health Organization (WHO) cutoffs; prevalence of co-infection with  
251 two or three STH; and prevalence of co-infection with *Giardia* and any STH. The trial's original  
252 protocol included *Entamoeba histolytica* and *Cryptosporidium spp.* as additional protozoan  
253 endpoints. At enrollment, *Giardia* prevalence was 40% among 535 children 18-27 months old in  
254 study compounds, while *Cryptosporidium Spp.* prevalence was 1% and *E. histolytica* prevalence  
255 was 0%. We determined the extremely low prevalence made these trial endpoints futile due to

256 limited statistical power, and since each required a separate assay on the ELISA platform, the  
257 study's steering committee decided to not test for them at follow-up.

258

### 259 *Sample size calculations*

260 All households in all clusters enrolled into the main trial were invited to participate in the  
261 measurement of parasite infections. The main trial was powered for a minimum detectable  
262 effect of 0.15 in length-for-age Z score and a relative risk of diarrhea of 0.7 or smaller for a  
263 comparison of any intervention with the double-sized control group, assuming a type I error ( $\alpha$ )  
264 of 0.05 and power ( $1-\beta$ ) of 0.8, a one-sided test for a two-sample comparison of means, and  
265 10% loss to follow-up. This led to a planned design of 100 clusters per arm and 10 index children  
266 per cluster. Given this design and a single, post-intervention measure, we estimated that the  
267 trial's sample size would be sufficient at 80% power with a two-sided  $\alpha$  of 0.05 to detect a  
268 relative reduction of 18% in infection prevalence of any parasite. Our minimum detectable  
269 effect calculations assumed 50% prevalence in the control arm, a village intraclass correlation  
270 (ICC) of 0.14, two children measured per enrolled household (index child plus an older sibling),  
271 and 70% successful stool collection and analysis.

272

### 273 *Statistical analysis*

274 All statistical analyses and comparisons between arms (W, S, H, WSH, N, WSHN compared to  
275 active-control) were pre-specified prior to unblinding of investigators and published with a time-  
276 stamp on the Open Science Framework (OSF) (<https://osf.io/372xf/>). Replication scripts and  
277 data are also provided at the same link. Our alternative hypothesis for all comparisons was that  
278 group means were not equal (two-sided tests). We estimated unadjusted and adjusted  
279 intention-to-treat effects between study arms using targeted maximum likelihood estimation

280 (TMLE) with influence curve-based standard errors that treated clusters as independent units  
281 and allowed for outcome correlation within clusters(24,25). Our parameters of interest for  
282 dichotomous outcomes were prevalence ratios. Our parameter of interest for helminth intensity  
283 was the relative fecal egg count reduction. We calculated the relative reduction using both  
284 geometric and arithmetic means. We did not perform statistical adjustments for multiple  
285 outcomes to preserve interpretation of effects and because many of our outcomes were  
286 correlated(26). We estimated adjusted parameters by including variables that were associated  
287 with the outcome to potentially improve the precision of our estimates. We pre-screened  
288 covariates (see SI for full list) to assess whether they are associated (P-value <0.2) with each  
289 outcome prior to including them in adjusted statistical models. We conducted subgroup  
290 analyses to explore effect modification on *Ascaris* and *Giardia* infection presence for the  
291 following factors: index child status, consumed deworming medicine in past 6 months (*Ascaris*  
292 only), consumed soil in past week (index children only), >8 people in compound, and if  
293 defecation occurred on the same day as stool collection. Statistical analyses were conducted  
294 using R version 3.3.2 ([www.r-project.org](http://www.r-project.org)).

295

## 296 **Results**

297

### 298 *Enrollment*

299 Pregnant women were enrolled into the cluster-randomised controlled trial from Kakamega,  
300 Bungoma, and Vihiga counties in Kenya's western region. Enrollment occurred between  
301 November 2012 - May 2014; 8246 pregnant women were enrolled. Clusters with an average of  
302 12 eligible pregnant women each were randomized by geographic proximal blocks into one of  
303 eight study arms: chlorine treatment of drinking water (W); improved sanitation including

304 provision of toilets with plastic slabs and hardware to manage child feces (S); handwashing with  
305 soap (H); combined WSH; infant and young child feeding counseling plus small-quantity lipid-  
306 based nutrient supplements (N); combined WSHN; a double-sized active control, and a passive  
307 control arm. Children in the passive control arm were purposively excluded from parasitology  
308 measurement (Figure 1). Parasite infections were measured among children born to enrolled  
309 pregnant mothers (index children) as well as their older siblings.

310

311 Enrollment characteristics of the study population were similar between arms (Table S1). Most  
312 households accessed springs or wells as their primary drinking water source. In the control  
313 group, 24% of households accessed unprotected water sources, such as springs, dug wells, and  
314 surface water. The microbial quality of drinking water was very poor, as has been reported  
315 previously for this study area(27); 96% (n=1829) of source water samples and 94% (n=5959) of  
316 stored drinking water samples contained *Escherichia coli* contamination. Most (82%) households  
317 owned a latrine, but only 15% had access to a latrine with a slab or ventilation pipe (Table S1).  
318 Soap and water availability for handwashing at a designated handwashing location was low  
319 (<10%).

320

### 321 *Indicators of intervention uptake*

322 After one year of intervention, 89-90% of households that received the sanitation intervention  
323 had access to an improved latrine (compared to 18% in active-control arm) and 79-82% of these  
324 had access to an improved latrine after two years of intervention. In the water intervention  
325 arms, 40-44% of households had a detectable chlorine residual in their stored drinking water at  
326 the one-year follow up (compared to 3% of control households) and 19-23% had chlorine  
327 detected after two years. 76-78% of households that received the handwashing intervention

328 had soap and water available at a handwashing station (compared to 12% in the control arm)  
329 after one year and this decreased to 19-23% at year two. Consumption of LNS sachets by  
330 children in the nutrition arms was 95-96% of the expected two sachets per day at the one-year  
331 follow up and 114-116% of expected at the 2-year follow up (>100% is possible because  
332 additional LNS packets were delivered in case of future delivery delays) (Tables S2 & S3).

333

### 334 *Infection prevalence*

335 Soil-transmitted helminth and *Giardia* infections were measured after two years of exposure to  
336 the interventions. We collected stool specimens from 9077 children aged 2-15 years old at the  
337 two-year survey during January 2015 – July 2016; including 4928 index children (median age in  
338 years: 2.0, interquartile range (IQR): 1.9, 2.1) and 4149 older children (median age in years: 5.0,  
339 IQR: 4.2, 6.4) residing in an index child's compound (Figure 1). A total of 2346 children in 158  
340 control clusters, 1117 children in 77 water clusters, 1160 children in 77 sanitation clusters, 1141  
341 children in 77 handwashing clusters, 1064 children in 76 WSH clusters, 1072 children in 78  
342 nutrition clusters, and 1177 children in 79 WSHN clusters provided stool specimens. Stool  
343 specimens were successfully collected from 95% (4928 of 5202) of available index children and  
344 from 93% (4149 of 4484) of available older children two years after intervention delivery (Figure  
345 1 shows number of children not available due to no live birth, death, refusal, or absent; Table S7  
346 shows characteristics of children lost to follow up). In the control group 22.6% of children were  
347 infected with *Ascaris* (ICC: 0.10), 2.2% with hookworm (ICC: 0.04), 1.2% with *Trichuris* (ICC: 0.07)  
348 (measured by Kato-Katz microscopy), and 39% with *Giardia* (measured by enzyme-linked  
349 immunosorbent assay)(Table S4). *Ascaris* infection prevalence was similar for index children  
350 (22.8%) and older children (22.3%) in the control group (Table S6). Caregivers reported that 39%  
351 of index children and 10% of older children had consumed soil in the past 7 days.

352

353 *Effect of interventions on parasite infection prevalence*

354 Infection prevalence of each STH, any STH, and *Giardia* was compared between each  
355 intervention group (W, S, H, WSH, WSHN) and the double-sized active control group (C); see  
356 methods for further details of the analysis. Compared to the control group, *Ascaris* infection  
357 prevalence was 18% lower in the water arm (Prevalence Ratio [PR]: 0.82, 95% Confidence  
358 Interval [CI] 0.67, 1.00), 22% lower in the combined WSH arm (PR: 0.78, 95% CI 0.63, 0.96), and  
359 22% lower in the WSHN arm (PR: 0.78, 95% CI 0.64, 0.96) (Figure 2, Table S4). Sanitation,  
360 handwashing, and nutrition did not significantly reduce *Ascaris* infection on their own (Figure 2).  
361 The combined WSH intervention reduced infection with any STH by 23% (PR: 0.77, 95% CI 0.63,  
362 0.95) and the combined WSHN intervention reduced infection with any STH by 19% (PR: 0.81,  
363 95% CI 0.66, 0.98) (Table S4). No interventions significantly reduced the prevalence of  
364 hookworm and *Trichuris*, though the low prevalence in the control arm meant that any  
365 reduction due to intervention would be difficult to detect in the trial (Table S4). No interventions  
366 reduced *Giardia* prevalence (Figure 2).

367

368 We re-analyzed all stool samples collected from children enrolled in the control, combined WSH,  
369 and WSHN arms by quantitative polymerase chain reaction (qPCR) to validate our estimates  
370 based on microscopy measurements. These three arms were selected for the qPCR subset  
371 analysis prior to unblinding of investigators to results and were chosen based on the hypothesis  
372 that these arms would be the most likely to have low-intensity STH infections if any of the  
373 interventions were effective. qPCR analyses resulted in almost identical intervention effect  
374 estimates to those based on microscopy (Figure 3, Table S8). Compared to the control group,  
375 *Ascaris* infection prevalence was 21% lower (PR: 0.79, 95% CI 0.64, 0.97) in the WSN group and



376 23% lower (PR: 0.77, 95%CI 0.64, 0.93) in the WSHN group. We also did not detect any  
377 significant effects of the interventions on *Trichuris* or hookworm infections using qPCR data  
378 (Table S8).

379

#### 380 *Effect of interventions on infection intensity*

381 *Ascaris* infection intensity was lower in children in the water arm (fecal egg count reduction with  
382 geometric means [FECR]: -16%, 95% CI -32%, -1%), the WSH arm (FECR: -19%, 95% CI -33%, -5%),  
383 and the WSHN arm (FECR: -18%, 95% CI -32%, -4%) compared to the control arm; FECR with  
384 arithmetic means showed similar results (Table 1). The prevalence of heavy/moderate intensity  
385 *Ascaris* infections was 10.0% in the water arm, 10.9% in WSH, and 10.3% in WSHN compared to  
386 12.7% in the control arm; these differences were not statistically significant at the 95%  
387 confidence level (Table S4).

388

389 The FECR with arithmetic means indicated that children in the WSH arm had lower intensity  
390 infections with hookworm (3 eggs per gram [epg] vs. 11 epg in control) (Table 1). In addition, the  
391 FECR with arithmetic means indicated lower *Trichuris* infection intensity in the WSH (0 epg vs. 6  
392 epg in control), nutrition (2 epg), and the WSHN (1 epg) arms. Children that received the WSHN  
393 intervention had 27% lower prevalence of coinfection with *STH* and *Giardia* compared to the  
394 control group (PR: 0.73, 95% CI 0.56, 0.97)(Table S4). *STH* coinfection was rare (<2% in control  
395 arm) and at similarly low levels in interventions arms (Table S4).

396

#### 397 *Adjusted models and subgroup analyses*

398 Adjusted effect estimates were similar to unadjusted effects (Table S4). Subgroup analyses of  
399 intervention effects stratified by child age, reported soil consumption (index children only),

400 number of people living in the compound, deworming (*Ascaris* only), and time since defecation  
401 did not show any strong effect modification (Table S6).

402

### 403 **Discussion**

404

405 This study provides new evidence on the effect of improved water, sanitation, handwashing in  
406 the household, and nutrition interventions, alone and in combination, on the prevalence of  
407 infection with STH and *Giardia*. Our findings demonstrate that an integrated water, sanitation,  
408 and handwashing intervention targeting the household environment in rural Kenya reduced  
409 *Ascaris* infection prevalence by 22%, while a water treatment intervention reduced *Ascaris*  
410 infection by 18%. Almost identical effect estimates generated by analyzing stool samples with  
411 microscopy and qPCR in a subset of arms lent additional credibility to the overall results (Figure  
412 3). In addition, we found that improved nutrition did not enhance the effectiveness of the WSH  
413 intervention. *Trichuris* and hookworm prevalence were too low to precisely assess intervention  
414 impact in this setting, and *Giardia* was unaffected by the interventions. Although the integrated  
415 WSH intervention did not succeed in improving child growth or reducing symptomatic diarrhea  
416 in this trial(22), our findings confirm that WSH can effectively interrupt environmental helminth  
417 transmission.

418

419 A limited number of randomized controlled trials (RCTs) have previously analyzed the effect of  
420 WSH interventions on STH infection. Two RCTs in rural India found no impact of community  
421 sanitation interventions on helminth infections; however, both studies reported low usage rates  
422 of toilets among intervention households(28,29). Several school-based RCTs combining  
423 deworming with handwashing promotion have reported significant reductions in *Ascaris*

424 reinfection prevalence in China, Ethiopia, and Peru(17,30). A school-based integrated WSH  
425 intervention combined with deworming in rural Kenya also reduced the odds of *Ascaris*  
426 reinfection(31). While previous RCTs demonstrate the success of school-based deworming  
427 combined with hygiene promotion, our results contribute new evidence from a large, cluster-  
428 randomized trial that improving WSH in the household environment can reduce *Ascaris*  
429 infections in a rural, low-income setting.

430

431 We did not detect an effect of the single sanitation intervention on STH infection prevalence.  
432 One potential explanation for the lack of impact may be that transitioning households from  
433 using traditional pit latrines to pit latrines with slabs may not have a measurable impact on STH  
434 transmission. A shift from households practicing open defecation to using latrines might be  
435 more likely to reduce STH transmission, with little additional benefit from improving latrine  
436 quality. A recent trial in Cote d'Ivoire reported greater reduction in hookworm infection  
437 prevalence among communities that received a community-led total sanitation intervention  
438 (designed to reduce open defecation levels) integrated with community-wide MDA compared to  
439 community-wide MDA alone (32). A second explanation may be that sanitation interventions are  
440 more effective at interrupting environmental transmission of pathogens when they are  
441 implemented at the community level(33), whereas our intervention only improved sanitation  
442 access in compounds with enrolled pregnant women.

443

444 The reductions in *Ascaris* prevalence in the combined arms could have resulted from improved  
445 water quality alone; *Ascaris* prevalence was 18% lower in the single water intervention arm than  
446 the control, a similar magnitude to the 22% reduction in the integrated intervention arms. Near  
447 identical reductions in *Ascaris* infection across all three water intervention arms suggests that

448 water could have been an important transmission pathway in this population, which was  
449 interrupted by chlorine treatment. However, we cannot rule out contribution to reductions from  
450 other interventions in the combined arms; *Ascaris* prevalence was lower (20%) in each of the  
451 single sanitation, handwashing, and nutrition intervention arms, compared to 23% prevalence in  
452 the control arm. Chlorine is not known to inactivate *Ascaris* eggs, but one experimental study  
453 did find that chlorine can delay egg development and infectivity(34); it's possible that delayed  
454 egg infectivity could reduce the risk of consuming an infective egg through drinking water. The  
455 proportion of households using jerry cans (a plastic water container with a narrow capped  
456 opening) to safely store drinking water was slightly higher in the water intervention arms than  
457 other arms (Tables S2 & S3). Our findings indicate that water is an understudied transmission  
458 pathway for *Ascaris*. We believe drinking water treatment should be further investigated as an  
459 STH control strategy, and that chlorine should be further explored as a method for inhibiting  
460 *Ascaris* egg development in drinking water supplies.

461

462 The combined WSHN intervention was similarly effective to WSH in reducing *Ascaris* prevalence,  
463 and improved nutrition did not reduce STH or *Giardia* infection on its own. Together, these  
464 results suggest that improved nutrition intervention did not reduce parasite infection in this  
465 population. Trials investigating the impact of micronutrient supplementation on STH infection or  
466 reinfection have reported mixed results(18). Our results are consistent with a Kenyan trial that  
467 found no effect of school-based micronutrient supplementation on reinfection with *Ascaris*(35).  
468 Considering interventions in this trial did not include treatment with antiparasitic drugs, further  
469 research would be valuable to understand if LNS supplementation could prevent parasite  
470 infections after drug treatment.

471

472 *Giardia* prevalence was unaffected by any of the interventions in this trial. Our results stand in  
473 contrast to results from the parallel WASH Benefits trial conducted in Bangladesh(36), which  
474 detected reductions in *Giardia* infection prevalence in the handwashing, sanitation, combined  
475 WSH, and combined WSHN arms(37). One potential explanation for lack of intervention effects  
476 in this trial is that water could be the primary transmission pathway for *Giardia* in this study  
477 setting, and *Giardia* is highly resistant to chlorination. The majority of households in the WASH  
478 Benefits Bangladesh trial accessed protected tubewells providing water with lower levels of  
479 fecal contamination compared to the springs and shallow wells accessed by households in this  
480 trial(27,38). Another potential explanation is that handwashing rates with soap were not high  
481 enough at the time of measurement to interrupt *Giardia* transmission; presence of soap and  
482 water at a handwashing station decreased from 78% at year one to 19% at year two among  
483 households in the WSH arm (Tables S2 & S3). *Giardia* is also zoonotic(4); exposure to avian and  
484 ruminant fecal contamination in the household environment could mitigate the effect of  
485 improved sanitation on transmission. Animal feces management was not a targeted behavior of  
486 the intervention packages.

487

488 This trial had some limitations. Chlorination does not inactivate protozoa, but was selected as  
489 the most appropriate water treatment intervention for the study context considering previous  
490 local acceptability, affordability, and effectiveness against bacterial and viral enteric pathogens.  
491 We measured parasite infections two years after intervention delivery; measurement among  
492 the study population at one year could have produced different results because of higher  
493 intervention adherence at that time (Table S2) and different child age-related exposures (e.g.  
494 younger children may be more likely to consume soil). We were unable to blind study  
495 participants due to the nature of the interventions; however, our outcomes were objective

496 indicators of infection analyzed by blinded laboratory technicians, and blinded analysts  
497 replicated the data analysis.  
498  
499 During our trial, Kenya implemented a national school-based mass drug administration (MDA)  
500 program to reduce STH prevalence(39); and 43% of study children reported consuming  
501 deworming medication in the past 6 months (Table S6). Reported consumption of deworming  
502 medicine was similar across study arms, suggesting no systematic differences in program  
503 coverage or intensity between arms (Table S10). We observed similar *Ascaris* prevalence among  
504 study index children (23%, median age 2 years) and older children (22%, median age 5 years),  
505 suggesting that school-based MDA could be missing a key reservoir of infection among young,  
506 preschool aged children. Moreover, an environmental survey conducted during the national  
507 deworming program in our study region reported common detection of STH eggs in soil  
508 collected from the entrance to homes, with *Ascaris* eggs detected in soil in 19% of  
509 households(40). Taken together, these findings suggest additional control strategies beyond  
510 school-based deworming might be necessary to fully interrupt environmental STH transmission.  
511  
512 In contrast to most previous trials evaluating the effect of WSH or nutrition on STH infection,  
513 administering deworming medication was not included with our intervention. Our findings  
514 represent the potential impact of WSH and nutrition interventions in the context of exposure to  
515 a deworming program implemented at national scale. Although the magnitude of *Ascaris*  
516 prevalence reduction observed in the WSH and water intervention arms may be lower than  
517 what could be achieved by drug treatment in the short term, reduced STH infection after two  
518 years of intervention exposure indicates sustained impact. Our results support the proposal that  
519 improved WSH complement chemotherapy in the global effort to eliminate STH transmission.

520

521

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531

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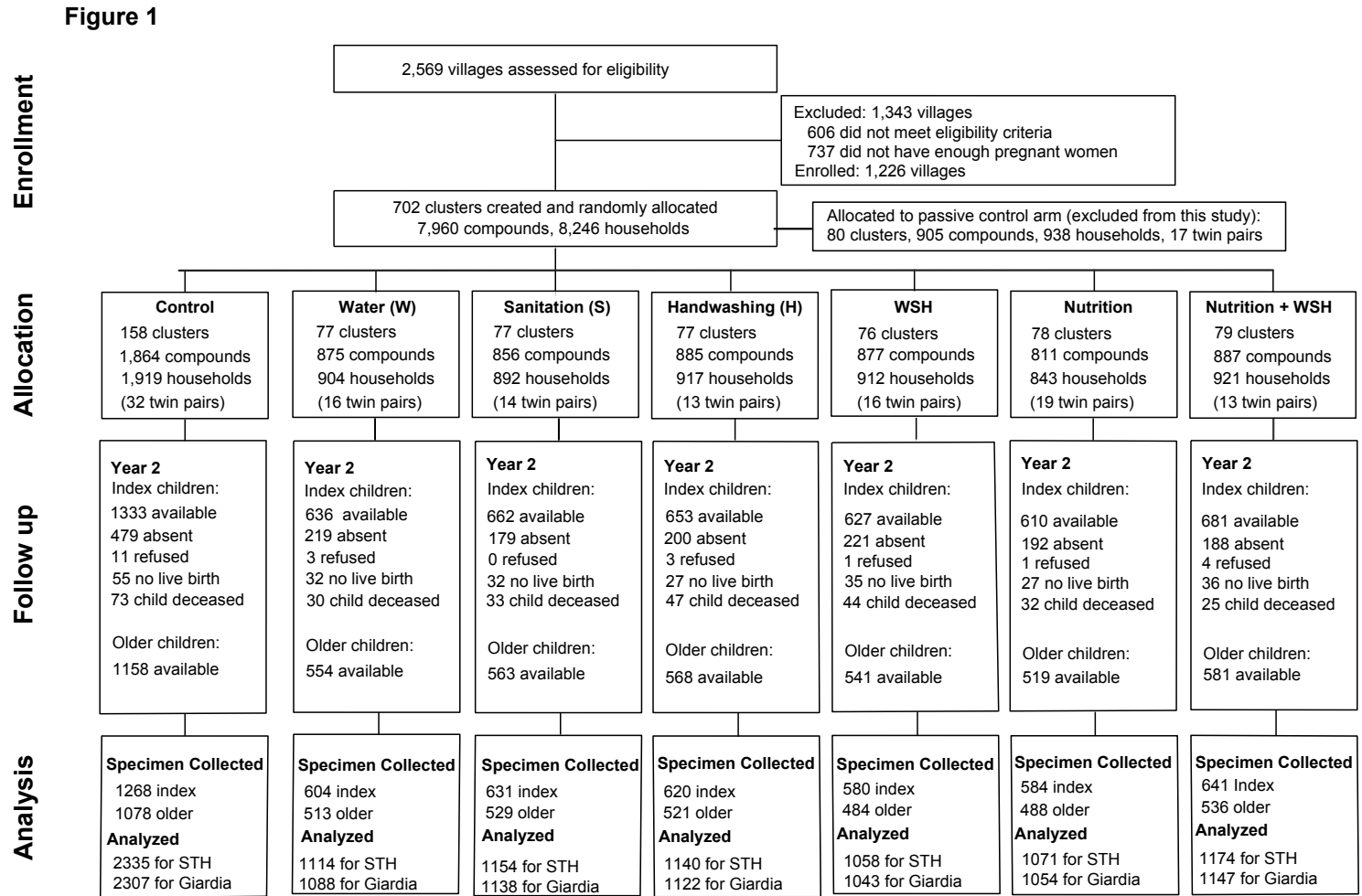
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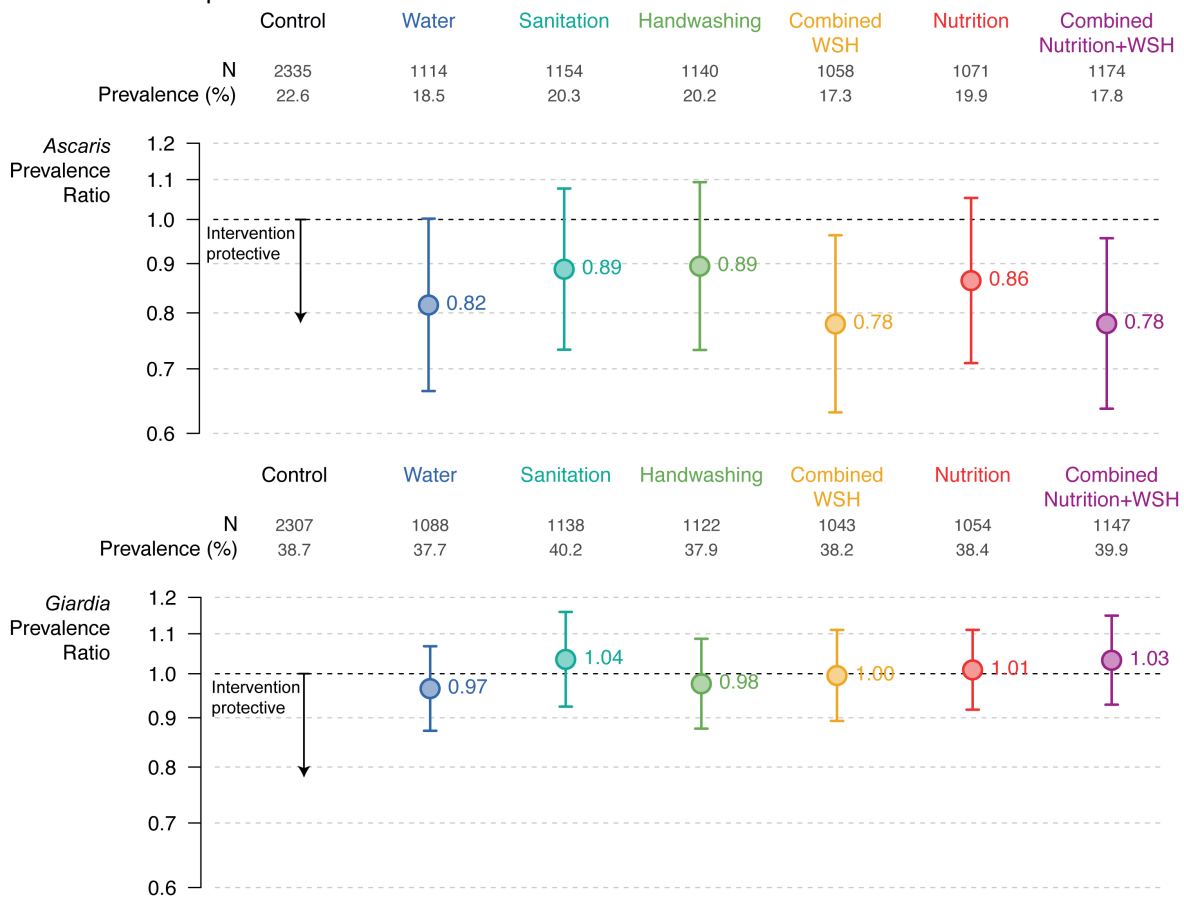
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**FIGURES**

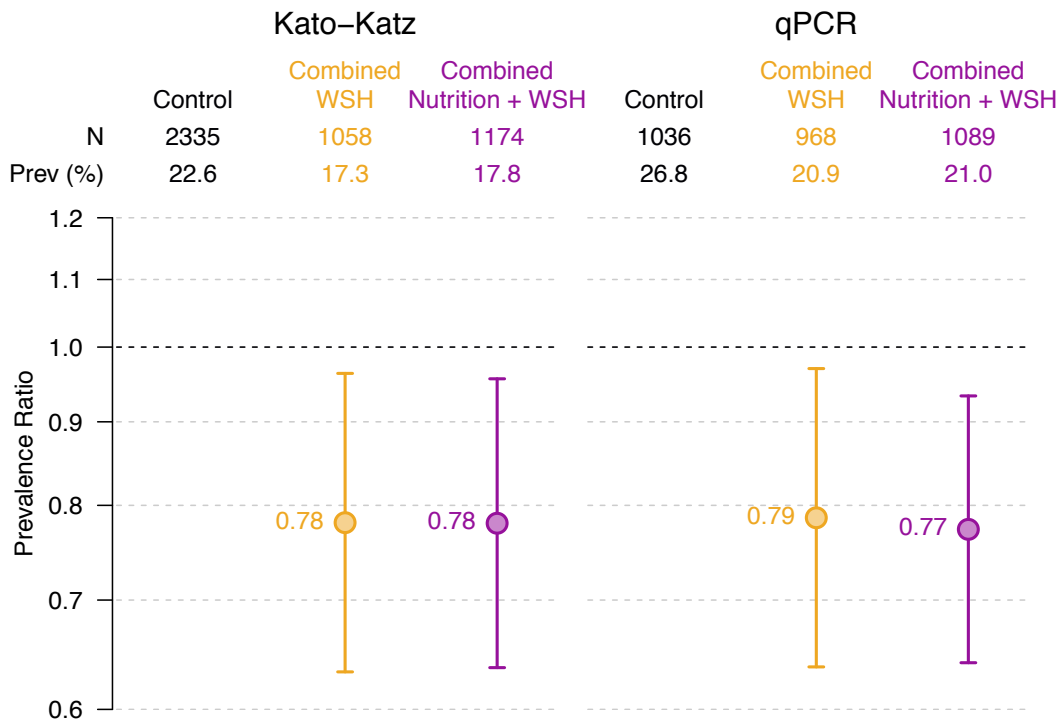
**Figure 1. Trial profile.**



**Figure 2.** Effect of the interventions on infection with *Ascaris* and *Giardia*. Prevalence ratios estimated by targeted maximum likelihood estimation. Error bars show 95% confidence intervals for the prevalence ratios.



**Figure 3.** Effect of the combined interventions on infection with *Ascaris* estimated with Kato-Katz microscopy (left) and by qPCR (right). Prevalence ratios estimated by targeted maximum likelihood estimation. Error bars show 95% confidence intervals for the prevalence ratios.



**Table 1.** Effect of the interventions on infection intensity, measured by fecal egg count reduction (FECR) with arithmetic and geometric means in eggs per gram (epg). FECR estimated by targeted maximum likelihood estimation. Bold indicates  $p < 0.05$ . \*Values of 0.5 epg substituted for samples below the detection limit to calculate log-transformed mean

Intervention	N	Arithmetic mean					Log10 mean*, epg	Geometric mean			
		Arithmetic mean, epg	FECR	95% CI	P-value	FECR		95% CI	P-value		
<b>Ascaris FECR</b>											
Control	2335	3641					0.60				
Water	1114	2682	<b>-0.26</b>	<b>-0.52</b>	<b>-0.01</b>	<b>0.04</b>	0.40	<b>-0.16</b>	<b>-0.32</b>	<b>-0.01</b>	<b>0.04</b>
Sanitation	1154	3443	-0.04	-0.32	0.23	0.75	0.50	-0.09	-0.25	0.07	0.27
Handwashing	1140	3386	-0.03	-0.34	0.28	0.85	0.50	-0.08	-0.25	0.08	0.31
WSH	1058	2571	<b>-0.27</b>	<b>-0.52</b>	<b>-0.02</b>	<b>0.03</b>	0.40	<b>-0.19</b>	<b>-0.33</b>	<b>-0.05</b>	<b>0.01</b>
Nutrition	1071	3303	-0.11	-0.34	0.12	0.35	0.50	-0.10	-0.25	0.04	0.16
Nutrition + WSH	1174	2927	-0.21	-0.46	0.03	0.09	0.40	<b>-0.18</b>	<b>-0.32</b>	<b>-0.04</b>	<b>0.01</b>
<b>Hookworm FECR</b>											
Control	2335	12					-0.25				
Water	1114	10	-0.20	-0.84	0.44	0.54	-0.23	0.02	-0.02	0.05	0.37
Sanitation	1154	10	-0.16	-0.90	0.57	0.67	-0.24	0.01	-0.02	0.04	0.42
Handwashing	1140	23	0.93	-1.39	3.25	0.43	-0.21	0.03	0.00	0.07	0.08
WSH	1058	3	<b>-0.74</b>	<b>-0.91</b>	<b>-0.58</b>	<b>0.00</b>	-0.26	-0.02	-0.04	0.01	0.18
Nutrition	1071	12	0.16	-1.17	1.50	0.81	-0.23	0.03	-0.01	0.06	0.14
Nutrition + WSH	1174	24	1.02	-1.87	3.91	0.49	-0.23	0.02	-0.01	0.06	0.22
<b>Trichuris FECR</b>											
Control	2335	6					-0.27				
Water	1114	6	0.04	-1.91	1.98	0.97	-0.27	0.00	-0.03	0.03	0.92
Sanitation	1154	4	-0.19	-1.49	1.11	0.78	-0.27	0.00	-0.03	0.02	0.77
Handwashing	1140	6	0.03	-1.40	1.46	0.97	-0.26	0.01	-0.02	0.04	0.46
WSH	1058	0	<b>-0.91</b>	<b>-1.07</b>	<b>-0.75</b>	<b>0.00</b>	-0.29	-0.02	-0.04	0.00	0.06
Nutrition	1071	2	<b>-0.64</b>	<b>-1.22</b>	<b>-0.05</b>	<b>0.03</b>	-0.28	-0.02	-0.04	0.01	0.18
Nutrition + WSH	1174	1	<b>-0.81</b>	<b>-1.15</b>	<b>-0.47</b>	<b>0.00</b>	-0.29	-0.02	-0.05	0.00	0.10