1   Full Title

2   Effects of an urban sanitation intervention on childhood enteric infection and diarrhea in Maputo,

3   Mozambique: a controlled before-and-after trial

4   Author list

5   Jackie Knee, PhD;[1,2] Trent Sumner, BS;[2] Zaida Adriano, BA;[3] Claire Anderson, BS;[2] Farran

6   Bush, BS;[4] Drew Capone, PhD;[2,6] Veronica Casmo, MSc;[5] David Holcomb, PhD;[6,7] Pete Kolsky,

7   PhD;[6] Amy Macdougall, PhD;[8] Evgeniya Molotkova, BS;[9] Judite Monteiro Braga, MSc;[5] Celina

8   Russo, BS;[2] Wolf Peter Schmidt, PhD;[1] Jill Stewart, PhD;[6] Winnie Zambrana, BS;[2] Valentina

9   Zuin, PhD;[10] Rassul Nalá, MPH;[5] Oliver Cumming, MSc;[1] Joe Brown, PhD[6,2]

10   [1] London School of Hygiene & Tropical Medicine, Faculty of Infectious Tropical Diseases,
11   Disease Control Department, Keppel Street, Bloomsbury, London WC1E 7HT, United Kingdom

12   [2] Georgia Institute of Technology, School of Civil and Environmental Engineering, 311 Ferst
13   Drive, Atlanta, GA, USA 30332, USA

14   [3] WE Consult ltd, Rua Tomás Ribeiro 177, RC, Postal code 3248, Maputo, Moçambique

15   [4] Georgia Institute of Technology, School of Chemical and Biomolecular Engineering, 311 Ferst
16   Drive, Atlanta, GA 30332, USA

17   [5] Instituto Nacional de Saúde, Estrada Nacional N°1, Bairro da Vila, Distrito de Marracuene,
18   Província de Maputo, Moçambique

19   [6] University of North Carolina at Chapel Hill, Gillings School of Global Public Health,
20   Department of Environmental Sciences and Engineering, 135 Dauer Drive, Chapel Hill, NC,
21   27599, USA

22   [7] University of North Carolina at Chapel Hill, Gillings School of Global Public Health,
23   Department of Epidemiology, 135 Dauer Drive, Chapel Hill, NC, 27599, USA

24   [8] London School of Hygiene & Tropical Medicine, Faculty of Epidemiology and Population
25   Health, Department of Medical Statistics, Keppel Street, Bloomsbury, London WC1E 7HT,
26   United Kingdom

27   [9] Georgia Institute of Technology, School of Biological Sciences, 310 Ferst Drive NW, Atlanta,
28   GA 30332, USA

29   [10] Yale-NUS College, Division of Social Science, 28 College Ave West, 138527, Singapore

30

31   Correspondence to:

32   Joe Brown (0000-0002-5200-4148)
33   University of North Carolina at Chapel Hill, Gillings School of Global Public Health,
34   Department of Environmental Sciences and Engineering, 135 Dauer Drive, Chapel Hill, NC,
35   27599, USA, Email: Joebrown@unc.edu Phone: +1 919 966 7892

36

Abstract

38   We conducted a controlled before-and-after trial to evaluate the impact of an onsite urban

39   sanitation intervention on the prevalence of enteric infection, soil transmitted helminth re-

40   infection, and diarrhea among children in Maputo, Mozambique. A non-governmental

41   organization replaced existing poor-quality latrines with pour-flush toilets with septic tanks

42   serving household clusters. We enrolled children aged 1-48 months at baseline and measured

43   outcomes before and 12 and 24 months after the intervention, with concurrent measurement

44   among children in a comparable control arm. Despite nearly exclusive use, we found no evidence

45   that intervention affected the prevalence of any measured outcome after 12 or 24 months of

46   exposure. Among children born into study sites after intervention, we observed a reduced

47   prevalence of *Trichuris* and *Shigella* infection relative to the same age group at baseline (<2

48   years old). Protection from birth may be important to reduce exposure to and infection with

49   enteric pathogens in this setting.

Introduction

51   Rapid urbanization has led to the expansion of informal settlements in many low- and middle-

52   income countries (LMICs). Such settlements often have very limited sanitation infrastructure

53   (UN-Habitat, 2016). Separation of human waste from human contact can prevent exposure to

54   enteric pathogens that cause infection, diarrhea (Liu et al., 2016), and potentially long-term

55   health effects such as environmental enteric dysfunction (EED) (Kosek et al., 2017), linear

56   growth deficits (Rogawski et al., 2018), impaired cognitive development (MAL-ED Network

57   Investigators, 2018), and reduced oral vaccine immunogenicity (Parker et al., 2018). Children

58   living in densely populated slum areas where fecal contamination is pervasive and sanitation

3

59   infrastructure is limited may be at an increased risk of adverse health effects due to frequent

60   exposure to enteric pathogens (Ezeh et al., 2017; Fink, Günther, & Hill, 2014).

61   Household-level sewerage has demonstrated health benefits (Barreto et al., 2010, 2007; Norman,

62   Pedley, & Takkouche, 2010) and remains an important long-term goal for many urban settings

63   despite limited evidence from controlled trials (Norman et al., 2010; Wolf et al., 2018). Such

64   systems may not be feasible short-term solutions due to cost, space, and logistical constraints,

65   challenges that have also impeded their evaluation via randomized trials (Norman et al., 2010).

66   Further, in densely populated areas, there may not be space for household-level sanitation of any

67   type. Shared sanitation is a subject of considerable debate but may represent the only near-term

68   sanitation option in some settings (Evans et al., 2017; Heijnen et al., 2014; Tidwell et al., 2020).

69   Yet, while shared, onsite systems may fill the growing need for safe sanitation in rapidly

70   expanding urban areas in LMICs, to date, there has been little evidence of their health impacts in

71   these settings. Recent large-scale, rigorous evaluations of onsite sanitation interventions and

72   combined water, sanitation, and hygiene interventions have demonstrated mixed effects on

73   health (Clasen et al., 2014; Humphrey et al., 2019; Luby et al., 2018; Null et al., 2018; Patil et

74   al., 2014; Pickering, Djebbari, Lopez, Coulibaly, & Alzua, 2015) but all were conducted in rural

75   areas with household-level interventions, and their findings may have limited generalizability to

76   urban areas. A recent meta-analysis estimated that non-sewered interventions reduced the risk of

77   self-reported diarrhea by 16% but did not estimate effects on objective health outcomes, such as

78   enteric infection (Brown & Cumming, 2019), and could not stratify estimates by rural versus

79   urban setting given the lack of evidence in urban areas (Wolf et al., 2018). To-date, no controlled

4

80   trials of urban onsite sanitation have been conducted despite over 740 million urban residents

81   relying on such technologies (Berendes, Sumner, & Brown, 2017).

82   The Maputo Sanitation (MapSan) trial was the first controlled trial to evaluate an onsite, shared

83   sanitation intervention in an urban setting and the first to use the prevalence of enteric infection,

84   as detected by molecular methods, as the primary study outcome (Brown et al., 2015). The study

85   was located in densely populated, low-income, informal neighborhoods of Maputo, Mozambique

86   where the sanitary conditions are poor and disease burden high (Knee et al., 2018). As of 2017,

87   only half of urban residents in Mozambique had access to at least basic sanitation infrastructure,

88   3% had access to sewerage, and 9% shared sanitation with multiple households, often in poor

89   neighborhoods where space and resources are limited (UNICEF/WHO, 2019). We investigated

90   whether an engineered, onsite, shared sanitation intervention could reduce enteric infection and

91   diarrhea in young children living in these low-income, densely populated neighborhoods in

92   Maputo, Mozambique.

Results

94   The MapSan trial was a controlled before-and-after trial designed to evaluate the impact of an

95   onsite sanitation intervention on child health after 12 and 24 months of follow-up. The

96   intervention consisted of pour-flush toilets to septic tanks with soakaway pits to discharge the

97   liquid portion of the waste. A non-governmental organization (NGO) delivered the intervention

98   to clusters of households known as compounds, replacing the existing poor-condition shared

99   facilities. Control compounds did not receive the intervention and continued to use their poor-

100   condition sanitation for the duration of the study. We assessed several measures of child health,

101   including enteric infection measured via stool-based molecular methods, soil-transmitted

102   helminth (STH) re-infection measured via Kato-Katz, and diarrhea measured via caregiver report

103   in both intervention and control children during three phases: baseline (pre-intervention), 12-

104   month follow-up, and 24-month follow-up. Children were eligible for baseline enrollment if they

105   were less than four years old (1-48 months old). At follow-up, children were eligible for

106   enrollment if they were less than four years old or if they would have been less than four years

107   old during baseline.

108   We enrolled 987 children in 495 compounds during the baseline phase (February 2015 –

109   February 2016) and collected stool samples (whole stool or diaper samples containing liquid

110   diarrhea) from 765 children (78%) (Figure 1). During the 12-month follow-up phase (March

111   2016 – April 2017), we enrolled or revisited 939 children in 438 compounds and collected 805

112   stool samples (86%). During the 24-month follow-up phase (April 2017 – August 2018), we

113   enrolled or revisited 1001 children in 408 compounds and collected stool samples from 922

114   (90%). To improve the success rate of stool sample collection during the 12- and 24-month

115   follow-up visits, we collected rectal swabs from children who did not provide a whole stool

116   sample after multiple collection attempts. The proportion of each type of sample (whole stool,

117   diaper sample, and rectal swab) was similar between arms at each phase (Supplemental Figure

118   1). Fewer than 5% of all samples were diapers and approximately 7% of 12-month samples and

119   25% of 24-month samples were rectal swabs (Supplemental Table 1). The NGO delivered

120   interventions to 15 control compounds after baseline and children in those compounds were

121   censored at the time of intervention receipt (Figure 1). Children living in control compounds that

122   independently upgraded their latrines were included in the main analyses. However, as inclusion

123   of these control children may have diluted the intervention effect, they were excluded from

6

124 sensitivity analyses designed to understand the impact of the intervention when compared with

125 controls with poor-condition sanitation throughout the study. Children in intervention and control

126 compounds were enrolled at similar rates during each phase (Supplemental Figure 2). Due to

127 migration out of the compound, we collected longitudinal data from 62% of children (59%

128 controls, 67% interventions) between baseline and 12-month and 51% of children (46% controls,

129 58% interventions) between baseline and 24-month.

130 At baseline enrollment, intervention compounds had more residents, households, and on-premise

131 water taps than controls, though the number of shared latrines was similar (Table 1). Animals

132 were observed in over half of all compounds. Intervention and control households had similar

133 wealth scores, though intervention households had more members and were more crowded while

134 control households more often had walls made of sturdy materials. All households used a

135 municipal water tap as their primary drinking water source with 78% reporting use of a tap on

136 the compound grounds. At baseline, latrines used by intervention households more often had

137 pedestals or slabs, drop-hole covers, and sturdy walls compared with controls. Consistent with

138 previous estimates in urban Maputo (Satterthwaite, Beard, Mitlin, & Du, 2019), open defecation

139 was rare in our study population with only one control household reporting open defecation at

140 baseline. Baseline characteristics of intervention and control children were similar: the average

141 age at enrollment was 23 months (SD = 13), 51% were female, and 32% were still breastfeeding

142 (Table 1). The age distributions of intervention and control children were similar at baseline and

143 both follow-up phases (Supplemental Figure 3).

144 We used the Luminex Gastrointestinal Pathogen Panel (GPP), a qualitative multiplex molecular

145 assay, to simultaneously test for 15 enteric pathogens in stool samples, including nine bacteria,

146   three protozoa, and three viruses. We detected ≥1 bacterial or protozoan enteric infection, our

147   pre-defined primary outcome, in 78% (591/753) of children with stools available at baseline. We

148   measured our pre-defined secondary outcome, ≥1 STH re-infection, using the Kato-Katz

149   microscope method and detected ≥1 STH in 45% (308/698) of stools at baseline. The

150   prevalences of pre-defined outcomes, individual pathogens, and pathogen types were similar

151   between the intervention and control arms at baseline (Table 2). The prevalence of most

152   bacterial, protozoan, and STH infections increased with age while the prevalence of enteric

153   viruses decreased with age (Supplemental Table 2 and Supplemental Figure 4).

154   The characteristics of children with repeated observations (including baseline) were similar to

155   characteristics of children measured at baseline only and to characteristics of children measured

156   at 12-month and/or 24-month only with the exception of age-related characteristics

157   (Supplemental Tables 3-6). Over half of the children enrolled after baseline were born into study

158   sites (336/622 [54%], Figure 1).

159   Our main analyses included observations from all eligible children enrolled at baseline (mean

160   sampling age 664 days, SD=393) and the 12-month (940 days, SD=498) and 24-month (1137

161   days, SD=603) follow-up visits (Table 2). We used a difference-in-difference (DID) analysis to

162   estimate the intervention effect and adjust for baseline differences between intervention and

163   control compounds. We present effect estimates from the DID analyses as prevalence ratios

164   (ratio of ratios). To assess the validity of the parallel trend assumption, a key assumption of DID

165   analyses, we ran "placebo tests" by replacing outcomes with variables unrelated to the

166   intervention, such as child age, respondent role, and presence of animals. Placebo tests showed

167   no effect of the intervention on these variables, suggesting the parallel trend assumption was

8

168   valid. We found no evidence that the intervention had an effect on the prevalence of any

169   bacterial or protozoan infection (adjusted PR 1.04, 95% CI [0.94 – 1.15]), or any STH re-

170   infection (1.11 [0.89 – 1.38]) 12 months after implementation (Table 2) despite household

171   respondents reporting almost exclusive use of the intervention latrine (97%, 404/417). The

172   prevalence of diarrhea remained fairly constant in both arms in all three phases with the

173   exception of the 12-month measure in the control arm which was lower, resulting in a larger

174   effect estimate with low precision (1.69 [0.89-3.21]).

175   The intervention had no meaningful effect at 12 months on the prevalence of infection with any

176   of the three pathogen types measured by the GPP (bacterial, protozoan, viral), pathogen

177   coinfection, or on any individual pathogen (Table 2). There was poor precision in the effect

178   estimates for infrequently detected pathogens, evident from their wide confidence intervals.

179   Therefore, some estimates suggestive of a large protective or detrimental effect (*Campylobacter*,

180   *C. difficile*, *E. coli* O157, STEC, Norovirus GI/GII, Adenovirus 40/41) may have arisen by

181   chance. While the National Deworming Campaign (NDC) provided albendazole to all compound

182   members following baseline, during 12-month visitation only 58% of caregivers (56% control,

183   60% intervention) confirmed that their child was dewormed during these visits. A sensitivity

184   analysis restricted to children confirmed to have been dewormed produced similar results to the

185   main analysis (Supplemental Table 7). By the 12-month visit, 19 control compounds (19/240

186   [8.0%]) had independently upgraded their facilities to pour-flush toilets. Results from sensitivity

187   analyses excluding children living in control compounds with independently upgraded facilities

188   were consistent with the main results (Supplemental Table 8).

189   There was no evidence that the intervention had an effect on the prevalence of any bacterial or

190   protozoan infection, any STH re-infection, or diarrhea after 24 months among all enrolled

191   children (Table 2). We also found limited evidence of effect on the prevalence of any pathogen

192   type or coinfection with ≥2 GPP pathogens 24 months after intervention. Results for several

193   individual outcomes were suggestive of a protective (STEC, *E. coli* O157, *Cryptosporidium*,

194   STH coinfection) or adverse (*Campylobacter*, *C. difficile*) effect, but evidence was weak as

195   estimates were accompanied by wide confidence intervals and chance discoveries were possible

196   given multiple comparisons. At the 24 month visits, caregivers confirmed baseline and/or 12-

197   month deworming more frequently for intervention children (339/502 [68%]) than for control

198   children (286/499 [57%]). Adjustment for deworming status or time since deworming had no

199   impact on effect estimates (Supplemental Table 7). Excluding children from control compounds

200   which independently upgraded their facilities by the 24-month visit (35/211 compounds, [17%])

201   did not impact the results (Supplemental Table 8).

202   Point estimates of effect and associated confidence intervals were largely similar in unadjusted

203   and adjusted models with few exceptions (e.g. ETEC at 24-month) (Table 2). Multivariable

204   models for GPP outcomes and STH outcomes were adjusted for covariates selected *a priori*

205   (child age, sex, caregiver education, and household wealth index). No other variables met our

206   inclusion criteria for multivariable models, which included being imbalanced between

207   intervention and control at baseline and meaningfully changing 12-month effect estimates (>10%

208   change in prevalence ratios) (Supplemental Table 9). While the relationship between age and

209   pathogen prevalence appeared to be non-linear for many pathogens (Supplemental Figure 4), the

210   inclusion of a higher order age term (age squared) did not meaningfully change effect estimates

10

211   in the main or sub-group analyses (Supplemental Table 10). Three measures of seasonality were

212   considered for inclusion in multivariable models to adjust for any difference in seasonal

213   distributions of data collection: (1) a binary variable defining the 'rainy' (November – April) and

214   'dry' seasons (May – October) in Maputo, (2) a measure of cumulative rainfall (mm) in the 30

215   days prior to data collection, and (3) sine and cosine terms representing dates of sample

216   collection. While there was some imbalance between arms in data collected during the wet and

217   dry seasons at baseline (Supplemental Table 9), no measure of seasonality meaningfully changed

218   effect estimates in the 12- and 24-month analyses and seasonality was excluded from final

219   multivariable models (Supplemental Table 9 and 11). For diarrhea, two variables in addition to

220   variables selected *a priori* met our inclusion criteria and were included in adjusted models:

221   presence of a latrine drop-hole cover at baseline and reported use of a water tap located within

222   the compound grounds at baseline (Supplemental Table 9). The magnitude of effect estimates

223   were larger and confidence intervals wider for diarrhea in adjusted versus unadjusted models in

224   the 12-month and 24-month analyses (Table 2). In addition to the main analyses which included

225   all enrolled children, we also performed two sub-group analyses. The first included children who

226   were born after the intervention was implemented (or after baseline in control compounds) and

227   present at the 12- and/or 24-month follow-up visit. This analysis allowed us to evaluate the

228   impact of the intervention on young children who were never exposed to the poor sanitation at

229   baseline. The second sub-group analysis included only children with repeated measures at

230   baseline and 12- and/or 24-month follow-up.

231   In sub-group analyses comparing children born into study compounds before the 24-month visit

232   with children of similar ages at baseline (<2 years old), there was suggestive evidence that the

11

233   intervention reduced the prevalence of infection with any STH by half (n=522; adjusted

234   prevalence ratio 0.51, [95% CI 0.27 - 0.95]), *Trichuris* by 76% (n=522; 0.24, [0.10 - 0.60]), and

235   *Shigella* by 51% (n=630; 0.49, [0.28 - 0.85]) (Table 3). These effects were attenuated in sub-

236   group analyses restricted to older children (>24 months) who were born before the intervention

237   was implemented and present at the 24-month phase (Supplemental Table 12). We did not

238   observe intervention effects among children born into the study by the 12-month visit, but the

239   sample size was small, resulting in high uncertainty in effect estimates (Supplemental Table 13).

240   Longitudinal sub-group analyses explored the effect of the intervention on children with repeated

241   measures at baseline and 12-month (for unadjusted analyses: n=870 data points [435 children

242   with repeat measures] for GPP outcomes, n=572 [286] for Kato-Katz outcomes, and n=1112

243   [556] for diarrhea) and at baseline and 24-month (n=716 (358), n=402 (201), n=834 (417)).

244   Effect estimates were consistent with results from the main analyses (Supplemental Tables 14-

245   15) but less precise due to the reduced sample numbers.

12

Discussion

246   We found no evidence that this urban, onsite shared sanitation intervention was protective

247   against our pre-specified child health outcomes of enteric infection, STH re-infection, or

248

249   diarrhea. We also found no strong evidence that the intervention affected prevalence of any

250   individual pathogen, pathogen type, or coinfection with ≥2 enteric pathogens or STH. In

251   exploratory sub-group analyses, we found suggestive evidence that the intervention reduced the

252   prevalence of any STH, *Trichuris,* and *Shigella* infections among children born into the study by

253   the 24-month follow-up visit. Studying children born into intervention sites after implementation

254   allowed us to examine the effect of the intervention from birth through the first two years of life.

255   These results suggest that the intervention delayed pathogen exposure and the accumulation of

256   enteric infections during early childhood, but it needs to be treated with caution as this was an

257   exploratory subgroup analysis.

258   The trial was neither designed nor powered to detect differences in sub-groups of children such

259   as those born after the intervention was implemented, potentially limiting our ability to detect

260   small effects in such analyses. Further, all exploratory sub-group analyses included multiple

261   comparisons, increasing the likelihood of chance discoveries. However, the magnitude of the

262   effect estimates for the outcomes of any STH, *Trichuris*, and *Shigella* observed among children

263   born into the study by the 24-month visit, and the directional consistency of effect estimates

264   among most other outcomes in this sub-group analysis, strengthens the plausibility of these

265   findings.

266   There are several reasons we observed suggestive evidence of an effect for some outcomes

267   among this sub-group of young children but not among older children or in the main analyses.

268 Children's exposures vary by age, particularly as they become mobile and begin independent

269 exploration of their environment. It is possible that the intervention reduced exposure via

270 pathways that are important for very young children but may represent just minor pathways of

271 exposure among older children (Kwong et al., 2020) Additionally, young children may

272 experience fewer exposures outside of the compound. Reductions in exposure and subsequent

273 infection early in life may delay or prevent the development of environmental enteric

274 dysfunction (EED), a subclinical condition that affects the structure and function of the gut and

275 may increase susceptibility to future infection (Keusch et al., 2014; Prendergast & Kelly, 2016).

276 Results from the EED sub-study of the WASH Benefits cluster randomized controlled trial

277 (cRCT) in Bangladesh suggest that the intervention delayed but did not prevent the onset of EED

278 (Lin et al., 2019). If this intervention similarly delayed the development of EED among children

279 born into intervention sites, they may have been less susceptible to infection than children of a

280 similar age at baseline. Finally, some pathogens, like *Giardia* and certain STH, can cause

281 persistent infections that can remain active for months or years if not treated (Else et al., 2020;

282 Rogawski et al., 2017). The intervention would have no effect on such infections, highlighting

283 the potentially important role of protection from birth.

284 Notably, both *Shigella* and *Trichuris* are primarily anthroponotic, and infection was strongly age-

285 dependent in this study population (Knee et al., 2018). These factors may help explain the

286 differing intervention effects observed both among pathogens and age groups. The intervention

287 was unlikely to limit exposure to animal feces, reducing the likelihood that it would impact

288 infection prevalence of zoonotic pathogens like *Campylobacter* or *Giardia*. The strong positive

289 associations between age and prevalence for *Shigella* and *Trichuris* suggest that exposure

14

290   increases with age. This supports the hypothesis that the intervention may have reduced the

291   overall frequency or intensity of exposure enough to impact *Shigella* and *Trichuris* infection

292   among young children but not older children.

293   Rapid urbanization is expanding informal settlements and out-pacing the expansion of sanitation

294   services in many cities, widening the gap in sanitation access between the urban rich and poor

295   (UNICEF/WHO, 2019). To our knowledge, MapSan was the first trial to estimate the health

296   impact of an urban, onsite shared sanitation intervention and the first to use enteric infection as

297   the primary trial outcome. Most of the urban sanitation literature published to date has evaluated

298   the expansion of sewerage, an important and ambitious goal that is out of reach for many cities in

299   the near-term (Norman et al., 2010). Access to sewerage is associated with a 30-60% reduction

300   of diarrheal disease depending on starting conditions, and an approximately 30% reduction in

301   enteric parasite detection, though most studies are observational and few controlled trials exist

302   (Barreto et al., 2010; Norman et al., 2010; Wolf et al., 2018).

303   Most studies of onsite sanitation interventions have occurred in rural areas. Despite good

304   evidence that onsite sanitation is associated with reductions in diarrheal disease (M. C. Freeman

305   et al., 2017; Wolf et al., 2018), several recent rural trials of basic sanitation and combined

306   WASH interventions with good uptake and use reported mixed effects on child health outcomes

307   including diarrhea, linear growth, and more recently, enteric infection (Ercumen et al., 2019;

308   Grembi et al., 2020; Humphrey et al., 2019; Lin et al., 2018; Luby et al., 2018; Null et al., 2018;

309   Pickering et al., 2019; Rogawski McQuade, Platts-Mills, et al., 2020).

310   The Sanitation, Hygiene, Infant Nutrition Efficacy (SHINE) trial in rural Zimbabwe found no

311   impact of a combined WASH intervention on diarrhea, growth, or the prevalence of a suite of

15

312   enteric pathogens among children aged <12 months old but did report a small reduction in the

313   number of parasitic pathogens detected.(Humphrey et al., 2019; Rogawski McQuade, Platts-

314   Mills et al., 2020)

315   While the WASH Benefits Bangladesh cRCT reported no effect of any WASH intervention on

316   child growth, the sanitation, hygiene, and combined WASH study arms reduced the prevalence

317   of diarrheal disease from 5.7% to 3.5% (Luby et al., 2018), accompanied by absolute reductions

318   in *Giardia* prevalence of 6-9% among children aged 2-3 years in the same arms (Lin et al.,

319   2018). The sanitation arm also reduced the prevalence of *T. trichiura* among children 2-3 years

320   old (from 5.2% to 3.2%) but had no impact on *A. lumbricoides* or hookworm, the only other

321   parasites detected frequently enough to estimate effects in that study (Ercumen et al., 2019). In a

322   parallel analysis, only the water treatment and combined WASH interventions of the WASH

323   Benefits Kenya cRCT reduced the prevalence *A. lumbricoides*, suggesting that the reduction in

324   prevalence in the combined WASH arm may be attributable to the water treatment intervention

325   (Pickering et al., 2019). The sanitation-only arm had no impact on any parasite measured, though

326   *T. trichiura* was too infrequently detected to estimate effects (Pickering et al., 2019). An

327   evaluation of a comprehensive suite of 34 enteric pathogens reported reduced prevalence and

328   quantity of enteric viruses, but not bacteria or parasites, among children aged 14 months old in

329   the combined WASH arms in the Bangladesh trial (Grembi et al., 2020). Together with our

330   findings, these results suggest that sanitation and combined WASH interventions can reduce the

331   prevalence of enteric infection in some settings but that effects may vary by pathogen, child age,

332   intervention, and setting.

333   We previously published two baseline risk factor analyses to identify demographic,

334   environmental, and WASH-related predictors of infection and environmental fecal contamination

335   in our study setting prior to the intervention implementation (Holcomb et al., 2020; Knee et al.,

336   2018). Age was an important predictor of infection, though the direction of its effect varied by

337   pathogen type. Increasing age was associated with increased risk of bacterial and protozoan

338   infections and decreased risk of viral infections (Knee et al., 2018). Other socio-demographic

339   predictors of infection included breastfeeding, which was associated with a decreased risk of any

340   infection (driven by its strong association with protozoan infection), and female sex which was

341   associated with an increased risk of viral infection. Few sanitation-related or environmental

342   variables were associated with infection at baseline and the magnitude of associations were often

343   small. The presence of a latrine superstructure and drop-hole cover were associated with small

344   reductions in risk of bacterial or protozoan infection, often only in unadjusted analyses, but other

345   latrine features (e.g. presence of a cleanable slab) were not. The observation of feces or used

346   diapers around the compound grounds was associated with increased risk of bacterial and

347   protozoan infection but most other environmental and sanitary hazards were not (Knee et al.,

348   2018).

349   Fecal contamination was common among all environmental reservoirs tested (water, soil, food

350   preparation surfaces) at baseline. We detected one or more microbial markers of contamination

351   in over 95% of environmental samples (Holcomb et al., 2020). *E. coli* was the most frequently

352   detected and abundant marker of contamination among all sample types, and human-associated

353   markers were most frequently detected in soil (59%) and stored drinking water (17%) samples.

354   Measures of latrine quality that were associated with small reductions in infection risk (e.g. drop-

355   hole covers, latrine superstructures) were not associated with decreased odds of fecal

356   contamination in this setting. Overall, we found few consistent relationships between markers of

357   fecal contamination and environmental, WASH-related, and demographic characteristics at

358   baseline (Holcomb et al., 2020).

359   While these results suggest WASH-related and environmental risk factors may be poor

360   determinants of child health in this setting, the lack of heterogeneity in WASH conditions at

361   baseline, given the selection criterion that compounds must share sanitation in "poor condition,"

362   may have limited our ability to identify strong WASH-related predictors of infection or

363   environmental fecal contamination. Results from a forthcoming companion study suggests the

364   intervention had mixed effects on environmental fecal contamination. The intervention may have

365   reduced the concentration of *E. coli* by an order of magnitude in soil collected from latrine

366   entrances after 12 months, however, there was no effect on the prevalence or concentration of

367   indicators of fecal contamination in any other environmental compartment sampled at that time

368   (Holcomb et al., 2021). It is unlikely that the observed reductions in fecal contamination in soils

369   alone would be sufficient to impact health outcomes in this setting. Other studies that have

370   evaluated the impact of sanitation interventions on fecal contamination of the surrounding

371   environment have found limited evidence of effect (Clasen et al., 2014; Ercumen, Mertens, et al.,

372   2018; Ercumen, Pickering, et al., 2018; Fuhrmeister et al., 2020; Patil et al., 2014; Pickering et

373   al., 2015; Gloria D. Sclar et al., 2016; Steinbaum et al., 2019).

374   In this setting, where fecal contamination was pervasive and burden of infection high, even

375   considerable reductions in contamination and exposure may have been insufficient to realize

376   measurable health gains as the intervention did not address all potential transmission pathways

18

377   (Briscoe, 1984; Julian, 2016; Robb et al., 2017). For example, the intervention did not address

378   child feces disposal practices or handwashing behaviors and it is unlikely that the intervention

379   infrastructure would have changed these (Majorin, Torondel, Chan, & Clasen, 2019). Previous

380   studies of sanitation interventions have found no reduction in hand contamination (Ercumen,

381   Pickering, et al., 2018), which has been associated with increased incident diarrheal disease in

382   young children (Pickering et al., 2018). The intervention may not have reduced exposure via

383   consumption of contaminated food – particularly foods contaminated prior to arrival in the

384   compound – likely an important source of enteric pathogen transmission in some settings (Julian,

385   2016; Kwong et al., 2020). Children's exposure to animal feces has been documented in rural,

386   peri-urban, and urban settings and could be an important, unmitigated source of exposure to

387   enteric pathogens in both intervention and control arms where animals were frequently observed

388   (Delahoy et al., 2018; Kwong et al., 2020; Penakalapati et al., 2017). Observation of animals in

389   compounds was examined as a potential confounder but did not change effect estimates.

390   The intervention was delivered at the compound level, not the community level, and was not

391   designed to achieve any specified threshold of sanitation coverage in the study neighborhoods.

392   Previous studies have suggested that achieving a certain level of community sanitation coverage

393   may be necessary to reduce disease burdens (Barreto et al., 2007; Fuller & Eisenberg, 2016;

394   Fuller, Villamor, Cevallos, Trostle, & Eisenberg, 2016; Harris, Alzua, Osbert, & Pickering,

395   2017; Jung, Lou, & Cheng, 2017; Spears, Ghosh, & Cumming, 2013; Wolf et al., 2018). For

396   example, a study of a large-scale sewerage expansion in urban Brazil found that the intervention

397   reduced diarrheal disease by 22%, with neighborhood coverage level being the single most

398   important explanatory variable (Barreto et al., 2007). We did not measure neighborhood-level

399   sanitation coverage, but previous estimates show that while coverage is high and open defecation

400   is limited (1%), only 9% of sanitation systems are safely managed (Satterthwaite et al., 2019).

401   Further, in the Nhlamankulu district where many of our study sites are located, the majority of

402   households (56%) rely on pit latrines serving individual households, most of which are in poor

403   condition (Devamani, Norman, & Schmidt, 2014; Satterthwaite et al., 2019). Together with our

404   results, this suggests that both the extent and quality of community coverage are likely important

405   to reducing overall transmission. Sanitation coverage and quality may be especially important in

406   urban areas given the proximity of compounds and the opportunity for person-to-person contact,

407   neighborhood-level exposure, and for external sources of contamination (e.g. a neighbor's

408   flooded pit latrine) to influence compound-level exposures (Barreto et al., 2007). We did not

409   measure neighborhood-level exposures, which may be important for young children in slum

410   settings (Ezeh et al., 2017; Medgyesi et al., 2019), and their impact on our health outcomes is

411   unclear. In addition to neighborhood-level exposures, the transience of the study population

412   meant that trips to and from provinces outside of Maputo, where exposures were varied and

413   unmeasured, were common.

414   It is unlikely that our findings are due to poor intervention fidelity or use, a challenge

415   encountered in some trials of rural sanitation interventions (Clasen et al., 2014; Patil et al., 2014).

416   The use of the intervention required minimal behavior change as compound members switched

417   from using their existing latrine in poor condition, which was removed following construction of

418   the intervention latrine, to using the new hygienic latrine. The results of a forthcoming process

419   evaluation demonstrate that 96% of intervention latrines were well-maintained two or more years

420   after construction, suggesting continued use by compound members (Bick et al., 2021). Further,

20

421   only 3% of intervention compounds (8/270) had a secondary, non-intervention latrine in use after

422   two or more years, indicating that members of most intervention compounds exclusively used the

423   intervention latrines (Bick et al., 2021). It is possible that development in the study

424   neighborhoods, including changes to sanitation facilities in control compounds, contributed to

425   the limited effect of the intervention. However, results from sensitivity analyses that excluded

426   control compounds with upgraded sanitation were consistent with results from the main analyses.

427   The two intervention designs we evaluated in this study – communal sanitation blocks and

428   shared latrines – utilized the same basic sanitation technology but differed in the number of

429   cabins and amenities available. While it is possible that this heterogeneity in design may have

430   modified the effect of the intervention, this study was not powered to test this. Moreover, all

431   intervention compounds were encouraged to independently upgrade their facilities by adding

432   features like electricity and handwashing stations, or by connecting existing handwashing

433   stations to the water supply, resulting in heterogeneity even within the two broad categories of

434   intervention type.

435   While the NDC dewormed every study compound annually during the study period, it is possible

436   that not all study participants received, or took, the medication and that the time between

437   deworming and subsequent measurement of STH re-infection varied among children.

438   Additionally, single-dose albendazole can have limited effectiveness against certain STH,

439   notably *Trichuris* (Moser, Schindler, & Keiser, 2017). Inadequate or ineffective deworming

440   could have limited our ability to detect an effect on STH outcomes. Sensitivity analyses

441   adjusting for caregiver-confirmed deworming and for estimated time between deworming and re-

442   infection measurement produced similar results to the main analysis.

443   There are several important limitations of this study. As the intervention was pre-planned and not

444   implemented by the study team, we could not randomize its allocation, increasing the risk of

445   confounding. We assessed potential confounding variables at baseline and used a DID analysis,

446   which accounts for baseline outcome measures, to limit the effect of unmeasured, residual

447   confounding. While we attempted to enroll intervention and control compounds with comparable

448   numbers of residents, the NGO which identified and implemented the intervention selected most

449   of the largest eligible compounds for intervention. This resulted in intervention compounds

450   having a slightly higher mean number of residents than control compounds (Table 1). Crowding

451   has been identified as a risk factor for pathogen transmission and poor health outcomes in other

452   studies, (Halpenny, Koski, Valdés, & Scott, 2012; Rahman, Wojtyniak, Mujibur Rahaman, &

453   Aziz, 1985; Rogawski McQuade, Shaheen, et al., 2020) though we found limited evidence of this

454   in our study population at baseline (Knee et al., 2018). Further, we assessed the number of

455   compound residents as a potential confounder but found that it did not meaningfully change the

456   DID estimates for our pre-defined outcomes (Supplemental Table 9). We consider our analysis to

457   be robust to small differences in study arms at baseline, however, we cannot exclude the

458   possibility of residual confounding due to such differences, a limitation of non-randomized

459   designs.

460   It was not possible to mask participants to their intervention status, and our measure of caregiver-

461   reported diarrhea could be subject to respondent and recall biases. To reduce the risk of

462   respondent bias, the MapSan field enumerator team and implementation team were different, and

463   respondents were not informed explicitly that the MapSan team was evaluating the health effect

464   of the intervention. To limit recall bias, we used a 7-day recall period (Arnold et al., 2013). Our

465   other pre-specified outcomes were objective measures of pathogen infection and not subject to

466   the same biases (Brown & Cumming, 2019).

467   Due to the greater than expected losses to follow-up in both study arms, we were not able to

468   follow all children enrolled at baseline through time as expected, but we still achieved our target

469   enrollment numbers due to migration and births into study compounds. We conducted the

470   originally planned longitudinal analysis as a sub-group analysis. It also served as a sensitivity

471   analysis to estimate the impact of migration on our effect estimates. Results from this sub-group

472   analysis were largely similar to results of the main analysis which treated measures as repeated

473   cross-sections, though the reduction in sample size led to wider confidence intervals

474   (Supplemental Tables 14-15). Measures of outcomes and covariates in children with and without

475   repeated measures were mostly similar, further limiting the likelihood that changes in the study

476   population biased our results.

477   While molecular detection of enteric pathogens in stool is evidence of pathogen exposure, it is

478   not necessarily evidence of active infection, making its clinical significance less clear (Brown &

479   Cumming, 2019). We assumed pathogen detection by the GPP indicated infection because the

480   assay's limits of detection exceeded the median infectious dose of most pathogens. While the

481   GPP detects many enteric pathogens recognized as important causes of childhood diarrhea in

482   LMICs, (Liu et al., 2016) it does not detect all enteric pathogens of importance. Further,

483   qualitative, cross-sectional analysis of stools does not provide information on the duration or

484   intensity of infection or pathogen carriage. Quantitative results, like those produced by multiplex

485   quantitative PCR panels, can be used to aid identification of etiologic agents of diarrhea,

486   especially in cases of coinfection, and to differentiate between low-level enteric pathogen

23

487   detection of unknown clinical relevance and higher concentration shedding which is more clearly

488   associated with disease (Liu et al., 2014, 2016; Platts-Mills, Liu, & Houpt, 2013). Some studies

489   have demonstrated overall good performance of the GPP but observed elevated false positive

490   detection rates for the *Salmonella* targets (Duong et al., 2016; Kellner et al., 2019). For this

491   reason, we removed *Salmonella* results from our pre-specified outcome definition. Results from

492   analyses including and excluding *Salmonella* were similar. In addition, some studies have

493   observed reduced sensitivity or specificity for some GPP targets compared with qPCR-based

494   methods, including norovirus, adenovirus, *Campylobacter*, *Yersinia enterocolitica*, ETEC, and

495   *Salmonella*, though inconsistencies between studies exist and are likely due to differences in

496   comparator assays or sample storage and processing (Chhabra et al., 2017; Deng et al., 2015;

497   Duong et al., 2016; Huang et al., 2016; Zhan et al., 2020; Zhuo et al., 2017). Further, the lack of

498   an adequate reference standard in most comparative studies complicates interpretation (K.

499   Freeman et al., 2017).

500   Our ability to detect an effect on our primary outcome, the prevalence of ≥1 bacterial or

501   protozoan infection, may have been limited by (1) the extended duration of shedding of some

502   pathogens following active infection; (2) the overall high burden of disease in our study

503   population, particularly among older children; and (3) residual confounding by age given the

504   strong observed relationship between age and infection status (particularly for protozoan

505   pathogens), all of which may have biased our results toward the null. Further, the intervention

506   may have impacted the concentration of pathogens shed (Grembi et al., 2020; Lin et al., 2019),

507   but our binary outcome was not sensitive to such differences The qualitative nature of the GPP

508   did not allow us to interrogate this question.

509   We analyzed a smaller number of stool samples for STH than for other enteric pathogens due to

510   requirements of the Kato-Katz method used for STH detection. The Kato-Katz method can only

511   be performed on whole, solid stool. Diarrheal samples and rectal swabs, the latter of which were

512   introduced during the 12-month follow-up phase, were not eligible for STH analysis by Kato-

513   Katz. Further, when limited stool material was collected, we prioritized the molecular analysis

514   used for the primary outcome. While the smaller sample size available for the STH analyses may

515   have reduced our ability to detect small effects, the proportions of whole stool, diarrheal diaper

516   samples, and rectal swabs were similar between arms at each phase (Supplemental Table 1). This

517   limited the potential impact that sample type could have on our results.

518   While the Kato-Katz method performs similarly to other microscope-based and molecular

519   methods for detection of moderate to high intensity infections, it may be less sensitive than

520   molecular methods in detecting low intensity infections (Benjamin-Chung et al., 2020; Cools et

521   al., 2019). A recent study has also suggested reduced specificity of the Kato-Katz method for

522   detection of low-intensity *A. lumbricoides* infections (Benjamin-Chung et al., 2020). In settings

523   where low-intensity infections are common, or where STH may be targeted for elimination,

524   methods with better diagnostic accuracy, like qPCR, may be considered.

525   We had limited ability to evaluate the impact of seasonality or weather-related trends on our

526   effect estimates due to drought conditions during the 2015/2016 rainy season. We adjusted

527   models for cumulative 30-day rainfall, a binary indicator of wet/dry season, and sine/cosine

528   terms of sample collection date (Stolwijk, Straatman, & Zielhuis, 1999) but excluded all

529   seasonality terms from final multivariable models because they did not meaningfully change

530   effect estimates.

25

531   Our results demonstrate that access to hygienic, shared onsite sanitation systems was not

532   sufficient to reduce enteric infection or diarrhea in children aged 6 years or younger (≤4 at

533   baseline) 12-24 months after implementation. Results from our sub-group analysis of children

534   born into intervention sites showed a substantial reduction in the prevalence of any STH,

535   *Trichuris*, and *Shigella* infection, suggesting that children may require protection from birth to

536   reduce or delay infection burdens. Our results do not suggest that shared sanitation is inadvisable

537   in this setting, as we did not compare against household-level sanitation improvements, nor do

538   they account for the many non-health related benefits associated with this intervention or

539   upgraded sanitation generally (Caruso et al., 2018; G.D. Sclar et al., 2018; Shiras et al., 2018).

540   The need for effective sanitation solutions may be most urgent in densely populated, low-

541   income, informal communities like our study setting where ubiquitous fecal contamination drives

542   high infection burdens. Disease transmission in these settings may be driven by multiple

543   interrelated pathways, complicated by frequent migration and the diversity of circulating

544   pathogens, and therefore difficult to interrupt. While decades of research have demonstrated

545   meaningful health gains following sanitation improvements, the results of this study, and other

546   rigorous trials of sanitation interventions, suggest that the relationship between sanitation and

547   health is complex, difficult to measure, and may not be generalizable across diverse settings and

548   populations.

Methods

549

550   *Study design and intervention*

551   MapSan was a controlled before-and-after trial, and details of the study design and analysis plan

552   have been published previously (Brown et al., 2015). We conducted the study in 17 densely

553   populated, low-income, informal neighborhoods in Maputo, Mozambique. The intervention was

554   delivered to compounds, typically groups of three to five households (though larger and smaller

555   compounds exist) often delineated by a wall or barrier, that shared sanitation and outdoor living

556   space. Shared compound sanitation facilities are not considered public facilities. We collected

557   data in an open cohort of children in intervention and control compounds at three time-points:

558   baseline (pre-intervention), 12 months post-intervention, and 24 months post-intervention.

559   The NGO Water and Sanitation for the Urban Poor selected intervention compounds and

560   designed and built 300 intervention facilities - pour-flush toilets discharging to septic tanks, the

561   liquid effluent of which flows to the soil through soakaway pits (Supplemental Figures 5a and

562   5b). There were two intervention designs with the same basic sanitation technology: communal

563   sanitation blocks (CSBs) and shared latrines (SLs) (Supplemental Figure 6a and 6b). The

564   primary difference between CSBs and SLs was size. CSBs (n=50) included multiple stalls with

565   toilets and served compounds of 21 or more people with one stall allocated per 20 residents.

566   CSBs also included rainwater harvesting systems, a municipal shared water connection, elevated

567   water tanks for storage of municipal water, a handwashing basin, a laundry facility, and a well-

568   drained area for bathing. Shared piped water connections were part of the municipal water

569   system and could be used for drinking in addition to other domestic purposes. Rainwater was

570   intended for cleaning and flushing but not drinking. Shared latrines (n=250) were single-stall

571   facilities serving fewer than 21 people. All septic tanks were sized to require emptying after

572   approximately two years.

573   Intervention compounds were located in 11 neighborhoods of the Nhlamankulu and KaMaxakeni

574   districts of Maputo (Supplemental Figure 7). The NGO selected intervention compounds using

27

575 the following criteria: (1) residents shared sanitation in poor condition as determined by an

576 engineer; (2) the compound was located in the pre-defined implementation neighborhoods; (3)

577 there were no fewer than 12 residents; (4) residents were willing to contribute financially to

578 construction costs; (5) sufficient space was available for construction of the new facility; (6) the

579 compound was accessible for transportation of construction materials and tank-emptying

580 activities; (7) the compound had access to a legal piped water supply; and (8) the groundwater

581 level was deep enough for construction of a septic tank. Intervention compounds were expected

582 to pay approximately 10-15% of the construction costs (~$64 for shared latrines and ~$97 for

583 CSBs) within one year of construction, with 25% of the total due upfront. Presence of a child

584 was not a selection criterion and therefore not all intervention sites were included in the study.

585 Opening of newly constructed intervention latrines occurred between February 2015 and

586 February 2016. The study team used criteria 1, 3, 4, and 7 to select control sites that had at least

587 one child younger than 48 months old in residence.　We enrolled intervention and control

588 compounds concurrently to limit any differential effects of seasonality or other secular trends on

589 the outcomes (Supplemental Figure 2). Additionally, we attempted to enroll control compounds

590 with similar numbers of residents as intervention compounds. Willingness to pay for facilities

591 among controls was assessed using hypothetical versions of questions posed to interventions.

592 Control compounds were located within the 11 intervention neighborhoods and six adjacent but

593 similar neighborhoods due to the limited availability of eligible compounds remaining within

594 intervention neighborhoods (Supplemental Figure 7). Intervention selection criteria (5), (6), and

595 (8) were not used to select control sites as they were deemed to be related to intervention

596 construction and maintenance and unlikely to influence our outcomes. It was not possible to

597 blind participants or enumerators to intervention status.

Participants

We enrolled eligible children at three time points: baseline (0 months), 12 months post-intervention, and 24 months post-intervention. Children aged 1- 48 months old were eligible for baseline enrollment if we received written informed consent from a parent or guardian and if the head of the compound provided verbal assent for the compound to be included in the study. Children were eligible for enrollment at 12- and 24-month visits if they were aged 1-48 months or if they were eligible for enrollment at baseline but absent during that study visit. Children who moved into the compound fewer than six months before the 12-month or 24-month visit were not eligible for enrollment during that phase given their limited exposure to their new compound.

Ethics

The study protocol was approved by the Comité Nacional de Bioética para a Saúde (CNBS), Ministério da Saúde (333/CNBS/14), the Research Ethics Committee of the London School of Hygiene & Tropical Medicine (reference # 8345), and the Institutional Review Board of the Georgia Institute of Technology (protocol # H15160).

Procedures

Trained field enumerators completed consent procedures and surveys in the participant's preferred language (Portuguese or Changana) and collected biological specimens from enrolled children (Supplemental Methods). At baseline, we aimed to visit intervention compounds two weeks prior to the opening of the new latrines. We scheduled follow-up visits to be 12 months (±2 weeks) and 24 months (±2 weeks) from the date compound members began using their new latrines, with visits to control compounds made concurrently (±2 weeks).

29

619   We collected stool samples independently of reported symptomology. If we were unable to

620   collect a stool sample after multiple attempts, a registered nurse collected a rectal swab after

621   obtaining written consent for the procedure from a parent or guardian. Stool samples were kept

622   cold and delivered to the Laboratory of Molecular Parasitology at the Instituto Nacional de

623   Saúde (INS) within six hours of collection for analysis and storage at -80°C.

624   Samples were shipped frozen with temperatures monitors to the Georgia Institute of Technology

625   (Atlanta, USA) where we used the xTAG Gastrointestinal Pathogen Panel (Luminex Corp,

626   Austin, USA), a qualitative multiplex molecular assay, to detect 15 enteric pathogens in stool

627   samples: *Campylobacter jejuni/coli/lari*; *Clostridium difficile*, toxin A/B; enterotoxigenic

628   *Escherichia coli* (ETEC) LT/ST; Shiga-like toxin producing *E. coli* (STEC) stx1/stx2; *E. coli*

629   O157; *Salmonella*; *Shigella boydii/sonnei/flexneri/dysenteriae*; *Vibrio cholerae*; *Yersinia*

630   *enterocolitica*; *Giardia lamblia*; *Cryptosporidium parvum/hominis*; *Entamoeba histolytica;*

631   adenovirus 40/41; norovirus GI/GII; and rotavirus. The GPP has been rigorously tested and

632   extensively used for stool-based enteric pathogen detection (Chisenga et al., 2018; Claas,

633   Burnham, Mazzulli, Templeton, & Topin, 2013; Deng et al., 2015; Duong et al., 2016; Huang et

634   al., 2016; Kellner et al., 2019; Khare et al., 2014; Navidad, Griswold, Gradus, & Bhattacharyya,

635   2013; Patel, Navidad, & Bhattacharyya, 2014). We analyzed samples according to manufacturer

636   instructions with the addition of elution steps for the pretreatment of rectal swabs and diaper

637   material saturated with liquid stool (Supplemental Methods). Technicians at INS assessed stool

638   samples for the presence of soil-transmitted helminths (STH) using the single-slide Kato-Katz

639   microscope method (Vestergaard Frandsen, Lausanne, Switzerland).

30

640   Representatives of the National Deworming Campaign (NDC) at the Mozambican Ministério da

641   Saúde (MISAU) offered single-dose albendazole (400 mg, 200 mg for children aged six to 12

642   months) to all eligible members of intervention and control compounds following sample

643   collection activities of each phase. Eligibility was defined by the NDC and included compound

644   members older than six months who were not pregnant.

645   *Outcomes*

646   For the 12-month analysis, we pre-specified the primary outcome as infection with one or more

647   of the 12 bacterial or protozoan enteric pathogens detected by the GPP and secondary outcomes

648   as re-infection with one or more STH as detected by Kato-Katz (following albendazole treatment

649   at baseline), and seven-day period prevalence of caregiver-reported diarrhea. All three outcomes

650   were considered secondary outcomes in the 24-month analysis. We defined diarrhea as the

651   passage of three or more loose or liquid stools in a 24-hour period or any stool with blood

652   (Arnold et al., 2013; Baqui et al., 1991). We excluded viral enteric pathogens from the primary

653   outcome definition. The intervention may not have interrupted virus transmission due to their

654   low infectious doses, high concentration shed in feces and extended period of shedding,

655   environmental persistence, and capability for direct person-to-person transmission (Julian, 2016).

656   Following reported specificity issues with the *Salmonella* target of the GPP, we removed it from

657   our GPP-based outcome definitions (Duong et al., 2016; Kellner et al., 2019). In addition to the

658   pre-specified outcomes, we evaluated the effect of the intervention on specific pathogen types

659   (bacterial, protozoan, viral) and on individual pathogens. The results for other secondary

660   outcomes listed in the trial registration (growth and environmental enteric dysfunction) will be

661   published separately.

31

662   *Statistical analysis*

663   Our sample size calculation has been described previously (Brown et al., 2015). We included all

664   enrolled children at each visit and analysed data as repeated cross-sectional observations. We

665   examined the effect of the intervention at the 12-month and 24-month phases separately. We

666   conducted two sets of exploratory sub-group analyses. The first assessed the effect of the

667   intervention on children with repeat observations at baseline and 12-months and at baseline and

668   24-months visits. These longitudinal analyses also served as sensitivity analyses of the impact of

669   participant migration on effect estimates. The second sub-group analysis compared children who

670   were born into study sites after the intervention (or after baseline in controls) but before the 12-

671   month or 24-month visit with children of a similar age group at baseline. For example, children

672   born after baseline but before the 24-month visit were compared with children aged two years

673   old or younger at baseline. These analyses allowed us to explore whether exposure to the

674   intervention from birth would reduce enteric pathogen infection during the first 1-2 years of life.

675   We used a DID approach to assess the impact of the intervention on all outcomes at the 12- and

676   24-month visits. We used generalized estimating equations (GEE) to fit Poisson regression

677   models with robust standard errors. Our GEE models accounted for clustering at the compound

678   level because it was the highest level of nested data and the level of the intervention allocation

679   (Bottomley, Kirby, Lindsay, & Alexander, 2016). We estimated the effect of the intervention as

680   the interaction of variables representing treatment status (intervention versus control) and phase

681   (pre- or post-intervention). Therefore, effect estimates from our DID analysis are presented as

682   ratio measures (ratio of prevalence ratios) instead of absolute differences. Multivariable models

683   were adjusted for covariates determined *a priori* as potentially predictive of our outcomes,

684   including child age and sex, caregiver's education, and household wealth. Given the important

685   and potentially non-linear relationship between age and pathogen prevalence (Supplemental

686   Figure 4), we also considered inclusion of a higher order age term (age squared) in our models

687   (Supplemental Table 10). Additional covariates (Supplemental Table 9) were considered for

688   inclusion in multivariable models if they were imbalanced between arms at baseline (>0.1

689   standardized difference in prevalence or mean) and resulted in a meaningful change in the DID

690   effect estimate (±10% change in 12-month DID prevalence ratio). We assessed the potential

691   impact of seasonality on our results in three ways: (1) inclusion of binary indicator of wet

692   (November – April) and dry (May – October) season in multivariable models, (2) inclusion of a

693   variable representing cumulative rainfall (mm) 30 days prior to sample or survey collection in

694   multivariable models, and (3) inclusion of sine and cosine functions of sample and survey dates

695   in multivariable models (Supplemental Tables 9 and 11). We used the same statistical approach

696   for sub-group analyses. All analyses were performed on complete case data, and a missing data

697   table is presented in the supplemental information (Supplemental Table 16). We performed all

698   statistical analyses with Stata version 16 (StataCorp, College Station, USA).

699   *Registration*

700   The trial was pre-registered at ClinicalTrials.gov (NCT02362932).

706 early field work. We would like to thank staff at the Instituto Nacional de Saúde, specifically

707 Josina Mate, Acacio Sabonete, and Jeronimo Langa, for their support throughout the project. The

708 study would not have been possible without our implementing partners at Water and Sanitation

709 for the Urban Poor (WSUP), in particular Guy Norman, Carla Costa, Vasco Parente, and

710 Jonathan Stokes. At the Georgia Institute of Technology, we gratefully acknowledge the project

711 and laboratory support provided by Aaron Bivins, Kevin Zhu, David Berendes, Fred Goddard,

712 Olivia Ginn, Sarah Lowry, Olivia Stehr, Haley Lewis, Jonathan Pennie, Derek Whaler, Mio

713 Unno, Catherine Reynolds, Joel Seibel, Diana Chumak, Felicitas Schneider, Katherine Brand,

714 Jiaxin Li, and Meredith Lockwood. We would also like to thank Ben Arnold, Jack Colford, Radu

715 Ban, Jay Graham, Tony Kolb, Eddy Perez, Jan Willem Rosenboom, Tom Slaymaker, Larry

716 Moulton, and Darren Saywell for technical and study design input. We would like to recognize

717 the early contributions of the late Dr. Jeroen Ensink to the MapSan trial protocol. As a colleague,

718 and as a friend, he is sorely missed. Finally, we would like to acknowledge and thank all of the

719 participants and their families who graciously welcomed us into their homes and were so

720 generous with their time.

721 *Role of the Funding Source*

34

727 *Contributors*

728 JB as principal investigator and OC as co-principal investigator designed the trial and drafted the

729 study protocol with input from WPS, JK, DH, PK, JS, VZ, and RN. JK was the study manager,

730 co-led laboratory work and data analysis with TS, and drafted the manuscript. TS curated the

731 data, designed data collection tools and activities with JK, and produced figures. ZA led field

732 data collection. CA, FB, DC, VC, EM, JMB, CR, WZ helped with sample organization and

733 laboratory analysis. WPS designed the analytical approach and JK, DH, and AM helped refine it.

734 JB and OC secured funding for the trial. All authors contributed critically to the final version of

735 the manuscript.

736 *Data sharing*

737 De-identified participant data which underlie the results reported in this manuscript is publicly

738 available on the MapSan trial Open Science Forum website (https://osf.io/p5shk). The published

739 trial protocol can be accessed at: https://bmjopen.bmj.com/content/5/6/e008215 .

740 *Competing interests*

741 All authors have completed the ICMJE uniform disclosure form

742 at *www.icmje.org/coi_disclosure.pdf* and declare: no support from any organization for the

743 submitted work; no financial relationships with any organizations that might have an interest in

744 the submitted work in the previous three years; no other relationships or activities that could

745 appear to have influenced the submitted work.

746 *Source data for Figures and Tables*

747 Figure 1 source data 1 and source code 1

35

748   Table 1 source data 1 and source code 1

749   Table 2 source data 1 and source code 1

750   Table 3 source data 1 and source code 1

751

References

Arnold, B. F., Galiani, S., Ram, P. K., Hubbard, A. E., Briceño, B., Gertler, P. J., & Colford, J. M. (2013). Optimal Recall Period for Caregiver-reported Illness in Risk Factor and Intervention Studies: A Multicountry Study. *American Journal of Epidemiology*, *177*(4), 361–370. https://doi.org/10.1093/aje/kws281

Baqui, A. H., Black, R. E., Yunus, M., Hoque, A. R. A., Chowdhury, H. R., & Sack, R. B. (1991). Methodological issues in diarrhoeal diseases epidemiology: Definition of diarrhoeal episodes. *International Journal of Epidemiology*, *20*(4), 1057–1063. https://doi.org/10.1093/ije/20.4.1057

Barreto, M. L., Genser, B., Strina, A., Teixeira, M. G., Assis, A. M. O., Rego, R. F., … Cairncross, S. (2010). Impact of a citywide sanitation program in Northeast Brazil on intestinal parasites infection in young children. *Environmental Health Perspectives*, *118*(11), 1637–1642. https://doi.org/10.1289/ehp.1002058

Barreto, M. L., Genser, B., Strina, A., Teixeira, M. G., Marlucia, A., Assis, O., … Cairncross, S. (2007). Effect of city-wide sanitation programme on reduction in rate of childhood diarrhoea in northeast Brazil: assessment by two cohort studies. In *www.thelancet.com* (Vol. 370). Retrieved from www.thelancet.com

Benjamin-Chung, J., Pilotte, N., Ercumen, A., Grant, J. R., Maasch, J. R. M. A., Gonzalez, A. M., … Colford, J. M. (2020). Comparison of multi-parallel qPCR and double-slide kato-katz for detection of soil-transmitted helminth infection among children in rural Bangladesh. *PLoS Neglected Tropical Diseases*, *14*(4), 1–23.

773   https://doi.org/10.1371/journal.pntd.0008087

774   Berendes, D. M., Sumner, T. A., & Brown, J. M. (2017). Safely Managed Sanitation for All

775   Means Fecal Sludge Management for at Least 1.8 Billion People in Low and Middle

776   Income Countries. *Environmental Science and Technology*, *51*(5), 3074–3083.

777   https://doi.org/10.1021/acs.est.6b06019

778   Bick, S., Buxton, H., Chase, R. P., Ross, I., Adriano, Z., Capone, D., … Dreibelbis, R. (2021).

779   Using path analysis to test Theory of Change: a quantitative process evaluation of the

780   MapSan trial. *BMC Public Health (Under Review)*. https://doi.org/10.21203/rs.3.rs-

781   234718/v1

782   Bottomley, C., Kirby, M. J., Lindsay, S. W., & Alexander, N. (2016). Can the buck always be

783   passed to the highest level of clustering? *BMC Medical Research Methodology*, *16*(1).

784   https://doi.org/10.1186/s12874-016-0127-1

785   Briscoe, J. (1984). Intervention studies and the definition of dominant transmission routes.

786   *American Journal of Epidemiology*, *120*(3), 449–456.

787   https://doi.org/10.1093/oxfordjournals.aje.a113909

788   Brown, J., & Cumming, O. (2019). Stool-Based Pathogen Detection Offers Advantages as an

789   Outcome Measure for Water, Sanitation, and Hygiene Trials. *Am. J. Trop. Med. Hyg*, *0*(0),

790   1–2. https://doi.org/10.4269/ajtmh.19-0639

791   Brown, J., Cumming, O., Bartram, J., Cairncross, S., Ensink, J., Holcomb, D., … Schmidt, W.-P.

792   (2015). A controlled, before-and-after trial of an urban sanitation intervention to reduce

793 enteric infections in children: research protocol for the Maputo Sanitation (MapSan) study,

794 Mozambique. *Bmj Open*, *5*(6), e008215. https://doi.org/10.1136/bmjopen-2015-008215

795 Caruso, B. A., Cooper, H. L. F., Haardörfer, R., Yount, K. M., Routray, P., Torondel, B., &

796 Clasen, T. (2018). The association between women's sanitation experiences and mental

797 health: A cross-sectional study in Rural, Odisha India. *SSM - Population Health*, *5*, 257–

798 266. https://doi.org/10.1016/j.ssmph.2018.06.005

799 Chhabra, P., Gregoricus, N., Weinberg, G. A., Halasa, N., Chappell, J., Hassan, F., … Vinjé, J.

800 (2017). Comparison of three multiplex gastrointestinal platforms for the detection of

801 gastroenteritis viruses. *Journal of Clinical Virology*, *95*, 66–71.

802 https://doi.org/10.1016/j.jcv.2017.08.012

803 Chisenga, C. C., Bosomprah, S., Makabilo Laban, N., Mwila- Kazimbaya, K., Mwaba, J.,

804 Simuyandi, M., & Chilengi, R. (2018). Aetiology of Diarrhoea in Children Under Five in

805 Zambia Detected Using Luminex xTAG Gastrointestinal Pathogen Panel. *Pediatric*

806 *Infectious Diseases: Open Access*, *03*(02), 1–6. https://doi.org/10.21767/2573-0282.100064

807 Claas, E. C., Burnham, C. A. D., Mazzulli, T., Templeton, K., & Topin, F. (2013). Performance

808 of the xTAG® gastrointestinal pathogen panel, a multiplex molecular assay for

809 simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis.

810 *Journal of Microbiology and Biotechnology*, *23*(7), 1041–1045.

811 https://doi.org/10.4014/jmb.1212.12042

812 Clasen, T., Boisson, S., Routray, P., Torondel, B., Bell, M., Cumming, O., … Schmidt, W.-P.

813 (2014). Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted

814   helminth infection, and child malnutrition in Odisha, India: a cluster-randomised trial.

815   *Lancet Global Health*, *2*(11), E645–E653. https://doi.org/10.1016/s2214-109x(14)70307-9

816   Cools, P., Vlaminck, J., Albonico, M., Ame, S., Ayana, M., José Antonio, B. P., … Levecke, B.

817   (2019). Diagnostic performance of a single and duplicate Kato-Katz, Mini-FLOTAC,

818   FECPAKG2 and qPCR for the detection and quantification of soil-transmitted helminths in

819   three endemic countries. *PLOS Neglected Tropical Diseases*, *13*(8), e0007446.

820   https://doi.org/10.1371/journal.pntd.0007446

821   Delahoy, M. J., Wodnik, B., McAliley, L., Penakalapati, G., Swarthout, J., Freeman, M. C., &

822   Levy, K. (2018). Pathogens transmitted in animal feces in low- and middle-income

823   countries. *International Journal of Hygiene and Environmental Health*, *221*(4), 661–676.

824   https://doi.org/10.1016/j.ijheh.2018.03.005

825   Deng, J., Luo, X., Wang, R., Jiang, L., Ding, X., Hao, W., … Che, X. (2015). A comparison of

826   Luminex xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) and routine tests for the

827   detection of enteropathogens circulating in Southern China. *Diagnostic Microbiology and*

828   *Infectious Disease*, *83*(3), 325–330. https://doi.org/10.1016/j.diagmicrobio.2015.07.024

829   Devamani, C., Norman, G., & Schmidt, W.-P. (2014). A Simple Microbiological Tool to

830   Evaluate the Effect of Environmental Health Interventions on Hand Contamination.

831   *International Journal of Environmental Research and Public Health*, *11*(11), 11846.

832   Retrieved from http://www.mdpi.com/1660-4601/11/11/11846

833   Duong, V. T., Phat, V. V., Tuyen, H. T., Dung, T. T. N., Trung, P. D., Minh, P. Van, … Baker,

834   S. (2016). Evaluation of luminex xTAG gastrointestinal pathogen panel assay for detection

835   of multiple diarrheal pathogens in fecal samples in Vietnam. *Journal of Clinical*

836   *Microbiology*, *54*(4), 1094–1100. https://doi.org/10.1128/JCM.03321-15

837   Else, K. J., Keiser, J., Holland, C. V., Grencis, R. K., Sattelle, D. B., Fujiwara, R. T., … Cooper,

838   P. J. (2020, December 1). Whipworm and roundworm infections. *Nature Reviews Disease*

839   *Primers*, Vol. 6, pp. 1–23. Nature Research. https://doi.org/10.1038/s41572-020-0171-3

840   Ercumen, A., Benjamin-Chung, J., Arnold, B. F., Lin, A., Hubbard, A. E., Stewart, C., … Luby,

841   S. P. (2019). Effects of water, sanitation, handwashing and nutritional interventions on soil-

842   transmitted helminth infections in young children: A cluster-randomized controlled trial in

843   rural Bangladesh. *PLOS Neglected Tropical Diseases*, *13*(5), e0007323.

844   https://doi.org/10.1371/journal.pntd.0007323

845   Ercumen, A., Mertens, A., Arnold, B. F., Benjamin-Chung, J., Hubbard, A. E., Ahmed, M. A.,

846   … Colford, J. M. (2018). Effects of single and combined water, sanitation and handwashing

847   interventions on fecal contamination in the domestic environment: a cluster-randomized

848   controlled trial in rural Bangladesh. *Environmental Science & Technology*, *22*,

849   acs.est.8b05153. https://doi.org/10.1021/acs.est.8b05153

850   Ercumen, A., Pickering, A. J., Kwong, L. H., Mertens, A., Arnold, B. F., Benjamin-Chung, J., …

851   Colford, J. M. (2018). Do Sanitation Improvements Reduce Fecal Contamination of Water,

852   Hands, Food, Soil, and Flies? Evidence from a Cluster-Randomized Controlled Trial in

853   Rural Bangladesh. *Environmental Science and Technology*, *52*(21), 12089–12097.

854   https://doi.org/10.1021/acs.est.8b02988

855   Evans, B., Hueso, A., Johnston, R., Norman, G., Pérez, E., Slaymaker, T., & Trémolet, S. (2017).

856   Limited services? The role of shared sanitation in the 2030 agenda for sustainable

857   development. *Journal of Water Sanitation and Hygiene for Development*, *7*(3), 349–351.

858   https://doi.org/10.2166/washdev.2017.023

859   Ezeh, A., Oyebode, O., Satterthwaite, D., Chen, Y. F., Ndugwa, R., Sartori, J., … Lilford, R. J.

860   (2017). The history, geography, and sociology of slums and the health problems of people

861   who live in slums. *The Lancet*, *389*(10068), 547–558. https://doi.org/10.1016/S0140-

862   6736(16)31650-6

863   Fink, G., Günther, I., & Hill, K. (2014). Slum Residence and Child Health in Developing

864   Countries. *Demography*, *51*, 1175–1197. https://doi.org/10.1

865   Freeman, K., Tsertsvadze, A., Taylor-Phillips, S., McCarthy, N., Mistry, H., Manuel, R., &

866   Mason, J. (2017). Agreement between gastrointestinal panel testing and standard

867   microbiology methods for detecting pathogens in suspected infectious gastroenteritis: Test

868   evaluation and meta-analysis in the absence of a reference standard. *PLOS ONE*, *12*(3),

869   e0173196. https://doi.org/10.1371/journal.pone.0173196

870   Freeman, M. C., Garn, J. V., Sclar, G. D., Boisson, S., Medlicott, K., Alexander, K. T., …

871   Clasen, T. F. (2017). The impact of sanitation on infectious disease and nutritional status: A

872   systematic review and meta-analysis. *International Journal of Hygiene and Environmental

873   Health*, *220*(6), 928–949. https://doi.org/10.1016/j.ijheh.2017.05.007

874   Fuhrmeister, E. R., Ercumen, A., Pickering, A. J., Jeanis, K. M., Crider, Y., Ahmed, M., …

875   Nelson, K. L. (2020). Effect of Sanitation Improvements on Pathogens and Microbial

876   Source Tracking Markers in the Rural Bangladeshi Household Environment. *Environmental*

877   *Science and Technology*, *54*(7), 4316–4326. https://doi.org/10.1021/acs.est.9b04835

878   Fuller, J. A., & Eisenberg, J. N. S. (2016). Herd Protection from Drinking Water, Sanitation, and

879   Hygiene Interventions. *The American Journal of Tropical Medicine and Hygiene*, *95*(5),

880   1201–1210. https://doi.org/10.4269/ajtmh.15-0677

881   Fuller, J. A., Villamor, E., Cevallos, W., Trostle, J., & Eisenberg, J. N. S. (2016). I get height

882   with a little help from my friends: herd protection from sanitation on child growth in rural

883   Ecuador. *International Journal of Epidemiology*. https://doi.org/10.1093/ije/dyv368

884   Grembi, J. A., Lin, A., Karim, M. A., Islam, M. O., Miah, R., Arnold, B. F., … Luby, S. P.

885   (2020). Effect of Water, Sanitation, Handwashing, and Nutrition Interventions on

886   Enteropathogens in Children 14 Months Old: A Cluster-Randomized Controlled Trial in

887   Rural Bangladesh. *The Journal of Infectious Diseases*. https://doi.org/10.1093/infdis/jiaa549

888   Halpenny, C. M., Koski, K. G., Valdés, V. E., & Scott, M. E. (2012). Prediction of Child Health

889   by Household Density and Asset-Based Indices in Impoverished Indigenous Villages in

890   Rural Panamá. *The American Journal of Tropical Medicine and Hygiene*, *86*(2), 280–291.

891   https://doi.org/10.4269/ajtmh.2012.11-0289

892   Harris, M., Alzua, M. L., Osbert, N., & Pickering, A. (2017). Community-Level Sanitation

893   Coverage More Strongly Associated with Child Growth and Household Drinking Water

894   Quality than Access to a Private Toilet in Rural Mali. *Environmental Science &*

895   *Technology*, *51*(12), 7219–7227. https://doi.org/10.1021/acs.est.7b00178

896   Heijnen, M., Cumming, O., Peletz, R., Chan, G. K.-S., Brown, J., Baker, K., & Clasen, T.

897   (2014). Shared Sanitation versus Individual Household Latrines: A Systematic Review of

898   Health Outcomes. *PLoS ONE*, *9*(4), e93300. https://doi.org/10.1371/journal.pone.0093300

899   Holcomb, D. A., Knee, J., Capone, D., Sumner, T., Adriano, Z., Nalá, R., … Stewart, J. R.

900   (2021). Bayesian analysis of source tracking markers to estimate the effects of an urban

901   sanitation intervention on human fecal contamination in Mozambique. *BioRxiv*,

902   2021.02.19.432000. https://doi.org/10.1101/2021.02.19.432000

903   Holcomb, D. A., Knee, J., Sumner, T., Adriano, Z., de Bruijn, E., Nalá, R., … Stewart, J. R.

904   (2020). Human fecal contamination of water, soil, and surfaces in households sharing poor-

905   quality sanitation facilities in Maputo, Mozambique. *International Journal of Hygiene and*

906   *Environmental Health*, *226*(February), 113496. https://doi.org/10.1016/j.ijheh.2020.113496

907   Huang, R. S. P., Johnson, C. L., Pritchard, L., Hepler, R., Ton, T. T., & Dunn, J. J. (2016).

908   Performance of the Verigene® enteric pathogens test, Biofire FilmArray^{TM} gastrointestinal

909   panel and Luminex xTAG® gastrointestinal pathogen panel for detection of common

910   enteric pathogens. *Diagnostic Microbiology and Infectious Disease*, *86*(4), 336–339.

911   https://doi.org/10.1016/j.diagmicrobio.2016.09.013

912   Humphrey, J. H., Mbuya, M. N. N., Ntozini, R., Moulton, L. H., Stoltzfus, R. J., Tavengwa, N.

913   V, … Makoni, T. (2019). Independent and combined effects of improved water, sanitation,

914   and hygiene, and improved complementary feeding, on child stunting and anaemia in rural

915   Zimbabwe: a cluster-randomised trial. *The Lancet. Global Health*, *7*(1), e132–e147.

916   https://doi.org/10.1016/S2214-109X(18)30374-7

917   Julian, T. R. (2016). Environmental transmission of diarrheal pathogens in low and middle

44

918   income countries. *Environmental Science: Processes and Impacts*, *18*(8), 944–955.

919   https://doi.org/10.1039/c6em00222f

920   Jung, Y. T., Lou, W., & Cheng, Y.-L. (2017). Exposure-response relationship of neighbourhood

921   sanitation and children's diarrhoea. *Tropical Medicine & International Health*, *22*(7), 857–

922   865. https://doi.org/10.1111/tmi.12886

923   Kellner, T., Parsons, B., Chui, L., Berenger, B. M., Xie, J., Burnham, C. A. D., … Freedman, S.

924   B. (2019). Comparative evaluation of enteric bacterial culture and a molecular multiplex

925   syndromic panel in children with acute gastroenteritis. *Journal of Clinical Microbiology*,

926   *57*(6). https://doi.org/10.1128/JCM.00205-19

927   Keusch, G. T., Denno, D. M., Black, R. E., Duggan, C., Guerrant, R. L., Lavery, J. V, …

928   Brewer, T. (2014). Environmental Enteric Dysfunction: Pathogenesis, Diagnosis, and

929   Clinical Consequences. *Clinical Infectious Diseases*, *59*, S207–S212.

930   https://doi.org/10.1093/cid/ciu485

931   Khare, R., Espy, M. J., Cebelinski, E., Boxrud, D., Sloan, L. M., Cunningham, S. A., …

932   Binnicker, M. J. (2014). Comparative evaluation of two commercial multiplex panels for

933   detection of gastrointestinal pathogens by use of clinical stool specimens. *Journal of*

934   *Clinical Microbiology*, *52*(10), 3667–3673. https://doi.org/10.1128/JCM.01637-14

935   Knee, J., Sumner, T., Adriano, Z., Berendes, D., de Bruijn, E., Schmidt, W.-P., … Brown, J.

936   (2018). Risk factors for childhood enteric infection in urban Maputo, Mozambique: A cross-

937   sectional study. *PLOS Neglected Tropical Diseases*, *12*(11), e0006956.

938   https://doi.org/10.1371/journal.pntd.0006956

939   Kosek, M. N., Ahmed, T., Bhutta, Z. Z. A., Caulfield, L., Guerrant, R. L., Houpt, E., … Trigoso,

940   D. R. (2017). Causal Pathways from Enteropathogens to Environmental Enteropathy:

941   Findings from the MAL-ED Birth Cohort Study. *EBioMedicine*, *18*, 109–117.

942   https://doi.org/http://dx.doi.org/10.1016/j.ebiom.2017.02.024

943   Kwong, L. H., Ercumen, A., Pickering, A. J., Arsenault, J. E., Islam, M., Parvez, S. M., … Luby,

944   S. P. (2020). Ingestion of Fecal Bacteria along Multiple Pathways by Young Children in

945   Rural Bangladesh Participating in a Cluster-Randomized Trial of Water, Sanitation, and

946   Hygiene Interventions (WASH Benefits). *Environmental Science & Technology*,

947   acs.est.0c02606. https://doi.org/10.1021/acs.est.0c02606

948   Lin, A., Ali, S., Arnold, B. F., Rahman, M. Z., Alauddin, M., Grembi, J., … Luby, S. P. (2019).

949   Effects of Water, Sanitation, Handwashing, and Nutritional Interventions on Environmental

950   Enteric Dysfunction in Young Children: A Cluster-randomized, Controlled Trial in Rural

951   Bangladesh. *Clinical Infectious Diseases*. https://doi.org/10.1093/cid/ciz291

952   Lin, A., Ercumen, A., Benjamin-Chung, J., Arnold, B. F., Das, S., Haque, R., … Luby, S. P.

953   (2018). Effects of Water, Sanitation, Handwashing, and Nutritional Interventions on Child

954   Enteric Protozoan Infections in Rural Bangladesh: A Cluster-Randomized Controlled Trial.

955   *Clinical Infectious Diseases*, *67*(10), 1515–1522. https://doi.org/10.1093/cid/ciy320

956   Liu, J., Kabir, F., Manneh, J., Lertsethtakarm, P., Begum, S., Gratz, J., … Houpt, E. R. (2014).

957   Development and assessment of molecular diagnostic tests for 15 enteropathogens causing

958   childhood diarrhoea: a multicentre study. *Lancet Infect Dis*, *14*(8), 716–724.

959   https://doi.org/10.1016/s1473-3099(14)70808-4

960   Liu, J., Platts-Mills, J. A., Juma, J., Kabir, F., Nkeze, J., Okoi, C., … Houpt, E. R. (2016). Use of

961   quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a

962   reanalysis of the GEMS case-control study. *The Lancet*, *388*(10051), 1291–1301.

963   https://doi.org/https://doi.org/10.1016/S0140-6736(16)31529-X

964   Luby, S. P., Rahman, M., Arnold, B. F., Unicomb, L., Ashraf, S., Winch, P. J., … Colford, J. M.

965   (2018, January). Effects of water quality, sanitation, handwashing, and nutritional

966   interventions on diarrhoea and child growth in rural Bangladesh: A cluster randomised

967   controlled trial. *The Lancet Global Health*. https://doi.org/10.1016/S2214-109X(17)30490-4

968   Majorin, F., Torondel, B., Chan, G. K. S., & Clasen, T. (2019). Interventions to improve disposal

969   of child faeces for preventing diarrhoea and soil-transmitted helminth infection. *Cochrane*

970   *Database of Systematic Reviews*, *2019*(9).

971   https://doi.org/10.1002/14651858.CD011055.pub2

972   MAL-ED Network Investigators. (2018). Early childhood cognitive development is affected by

973   interactions among illness, diet, enteropathogens and the home environment: findings from

974   the MAL-ED birth cohort study. *BMJ Global Health*, *3*(4), e000752.

975   https://doi.org/10.1136/bmjgh-2018-000752

976   Medgyesi, D., Sewell, D., Senesac, R., Cumming, O., Mumma, J., & Baker, K. K. (2019). The

977   landscape of enteric pathogen exposure of young children in public domains of low-income,

978   urban Kenya: The influence of exposure pathway and spatial range of play on multi-

979   pathogen exposure risks. *PLOS Neglected Tropical Diseases*, *13*(3), e0007292.

980   https://doi.org/10.1371/journal.pntd.0007292

981   Moser, W., Schindler, C., & Keiser, J. (2017). Efficacy of recommended drugs against soil

982   transmitted helminths: systematic review and network meta-analysis. *BMJ*, *358*, 4307.

983   https://doi.org/10.1136/bmj.j4307

984   Navidad, J. F., Griswold, D. J., Gradus, M. S., & Bhattacharyya, S. (2013). Evaluation of

985   Luminex xTAG gastrointestinal pathogen analyte-specific reagents for high-throughput,

986   simultaneous detection of bacteria, viruses, and parasites of clinical and public health

987   importance. *J Clin Microbiol*, *51*(9), 3018–3024. https://doi.org/10.1128/jcm.00896-13

988   Norman, G., Pedley, S., & Takkouche, B. (2010). Effects of sewerage on diarrhoea and enteric

989   infections: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, *10*(8),

990   536–544. https://doi.org/10.1016/S1473-3099(10)70123-7

991   Null, C., Stewart, C. P., Pickering, A. J., Dentz, H. N., Arnold, B. F., Arnold, C. D., … Colford,

992   J. M. (2018). Effects of water quality, sanitation, handwashing, and nutritional interventions

993   on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial. *The*

994   *Lancet Global Health*, *6*(3), e316–e329. https://doi.org/10.1016/S2214-109X(18)30005-6

995   Parker, E. P., Ramani, S., Lopman, B. A., Church, J. A., Iturriza-Gómara, M., Prendergast, A. J.,

996   & Grassly, N. C. (2018). Causes of impaired oral vaccine efficacy in developing countries.

997   *Future Microbiology*, *13*(1), 97–118. https://doi.org/10.2217/fmb-2017-0128

998   Patel, A., Navidad, J., & Bhattacharyya, S. (2014). Site-specific clinical evaluation of the

999   Luminex xTAG gastrointestinal pathogen panel for detection of infectious gastroenteritis in

1000   fecal specimens. *Journal of Clinical Microbiology*, *52*(8), 3068–3071.

1001   https://doi.org/10.1128/JCM.01393-14

1002   Patil, S. R., Arnold, B. F., Salvatore, A. L., Briceno, B., Ganguly, S., Colford Jr., J. M., &

1003   Gertler, P. J. (2014). The Effect of India's Total Sanitation Campaign on Defecation

1004   Behaviors and Child Health in Rural Madhya Pradesh: A Cluster Randomized Controlled

1005   Trial. *Plos Medicine*, *11*(8). https://doi.org/10.1371/journal.pmed.1001709

1006   Penakalapati, G., Swarthout, J., Delahoy, M. J., McAliley, L., Wodnik, B., Levy, K., & Freeman,

1007   M. C. (2017). Exposure to Animal Feces and Human Health: A Systematic Review and

1008   Proposed Research Priorities. *Environmental Science & Technology*, *51*(20), 11537–11552.

1009   https://doi.org/10.1021/acs.est.7b02811

1010   Pickering, A. J., Djebbari, H., Lopez, C., Coulibaly, M., & Alzua, M. L. (2015). Effect of a

1011   community-led sanitation intervention on child diarrhoea and child growth in rural Mali: a

1012   cluster-randomised controlled trial. *The Lancet Global Health*, *3*(11), e701–e711.

1013   https://doi.org/10.1016/S2214-109X(15)00144-8

1014   Pickering, A. J., Ercumen, A., Arnold, B. F., Kwong, L. H., Parvez, S. M., Alam, M., … Luby,

1015   S. P. (2018). Fecal Indicator Bacteria along Multiple Environmental Transmission Pathways

1016   (Water, Hands, Food, Soil, Flies) and Subsequent Child Diarrhea in Rural Bangladesh.

1017   *Environmental Science & Technology*, *52*(14), 7928–7936.

1018   https://doi.org/10.1021/acs.est.8b00928

1019   Pickering, A. J., Njenga, S. M., Steinbaum, L., Swarthout, J., Lin, A., Arnold, B. F., … Null, C.

1020   (2019). Effects of single and integrated water, sanitation, handwashing, and nutrition

1021   interventions on child soil-transmitted helminth and Giardia infections: A cluster-

1022   randomized controlled trial in rural Kenya. *PLOS Medicine*, *16*(6), e1002841.

1023 https://doi.org/10.1371/journal.pmed.1002841

1024 Platts-Mills, J. A., Liu, J., & Houpt, E. R. (2013). New concepts in diagnostics for infectious

1025 diarrhea. *Mucosal Immunology*, *6*(5), 876–885. https://doi.org/10.1038/mi.2013.50

1026 Prendergast, A. J., & Kelly, P. (2016). Interactions between intestinal pathogens, enteropathy and

1027 malnutrition in developing countries. *Current Opinion in Infectious Diseases*, *29*(3), 229–

1028 236. https://doi.org/10.1097/QCO.0000000000000261

1029 Rahman, M., Wojtyniak, B., Mujibur Rahaman, M., & Aziz, K. M. S. (1985). Impact of

1030 Environmental Sanitation and Crowding on Infant Mortality in Rural Bangladesh. *The*

1031 *Lancet*, *326*(8445), 28–30. https://doi.org/10.1016/S0140-6736(85)90068-6

1032 Robb, K., Null, C., Teunis, P., Yakubu, H., Armah, G., & Moe, C. L. (2017). Assessment of

1033 Fecal Exposure Pathways in Low-Income Urban Neighborhoods in Accra, Ghana:

1034 Rationale, Design, Methods, and Key Findings of the SaniPath Study. *Am. J. Trop. Med.*

1035 *Hyg*, *97*(4), 1020–1032. https://doi.org/10.4269/ajtmh.16-0508

1036 Rogawski, E. T., Bartelt, L. A., Platts-Mills, J. A., Seidman, J. C., Samie, A., Havt, A., … MAL-

1037 ED Network Investigators, the. (2017). Determinants and Impact of Giardia Infection in the

1038 First 2 Years of Life in the MAL-ED Birth Cohort. *Journal of the Pediatric Infectious*

1039 *Diseases Society Giardia Epidemiology and Impact • JPIDS*, *2017*(6), 153–160.

1040 https://doi.org/10.1093/jpids/piw082

1041 Rogawski, E. T., Liu, J., Platts-Mills, J. A., Kabir, F., Lertsethtakarn, P., Siguas, M., … Nyathi,

1042 E. (2018). Use of quantitative molecular diagnostic methods to investigate the effect of

50

1043   enteropathogen infections on linear growth in children in low-resource settings: longitudinal

1044   analysis of results from the MAL-ED cohort study. *The Lancet Global Health*, *6*(12),

1045   e1319–e1328. https://doi.org/10.1016/S2214-109X(18)30351-6

1046   Rogawski McQuade, E. T., Platts-Mills, J. A., Gratz, J., Zhang, J., Moulton, L. H., Mutasa, K.,

1047   … Houpt, E. R. (2020). Impact of water quality, sanitation, handwashing, and nutritional

1048   interventions on enteric infections in rural zimbabwe: The sanitation hygiene infant

1049   nutrition efficacy (SHINE) trial. *Journal of Infectious Diseases*, *221*(8), 1379–1386.

1050   https://doi.org/10.1093/infdis/jiz179

1051   Rogawski McQuade, E. T., Shaheen, F., Kabir, F., Rizvi, A., Platts-Mills, J. A., Aziz, F., …

1052   Iqbal, N. T. (2020). Epidemiology of Shigella infections and diarrhea in the first two years

1053   of life using culture-independent diagnostics in 8 low-resource settings. *PLOS Neglected*

1054   *Tropical Diseases*, *14*(8), e0008536. https://doi.org/10.1371/journal.pntd.0008536

1055   Satterthwaite, D., Beard, V. A., Mitlin, D., & Du, J. (2019). *Untreated and Unsafe□: Solving the*

1056   *Urban Sanitation Crisis in the Global South Solving the Urban Sanitation Crisis in the*

1057   *Global South Untreated and Unsafe□:* Washington DC. Retrieved from

1058   www.citiesforall.org

1059   Sclar, G.D., Penakalapati, G., Caruso, B. A., Rehfuess, E. A., Garn, J. V., Alexander, K. T., …

1060   Clasen, T. (2018). Exploring the relationship between sanitation and mental and social well-

1061   being: A systematic review and qualitative synthesis. *Social Science & Medicine*, *217*, 121–

1062   134. https://doi.org/10.1016/J.SOCSCIMED.2018.09.016

1063   Sclar, Gloria D., Penakalapati, G., Amato, H. K., Garn, J. V., Alexander, K., Freeman, M. C., …

1064   Clasen, T. (2016). Assessing the impact of sanitation on indicators of fecal exposure along

1065   principal transmission pathways: A systematic review. *International Journal of Hygiene*

1066   *and Environmental Health*, *219*(8), 709–723.

1067   https://doi.org/https://doi.org/10.1016/j.ijheh.2016.09.021

1068   Shiras, T., Cumming, O., Brown, J., Muneme, B., Nala, R., & Dreibelbis, R. (2018). Shared

1069   latrines in Maputo, Mozambique: Exploring emotional well-being and psychosocial stress.

1070   *BMC International Health and Human Rights*, *18*(1). https://doi.org/10.1186/s12914-018-

1071   0169-z

1072   Spears, D., Ghosh, A., & Cumming, O. (2013). Open Defecation and Childhood Stunting in

1073   India: An Ecological Analysis of New Data from 112 Districts. *PLoS ONE*, *8*(9), e73784.

1074   https://doi.org/10.1371/journal.pone.0073784

1075   Steinbaum, L., Mboya, J., Mahoney, R., Njenga, S. M., Null, C., & Pickering, A. J. (2019).

1076   Effect of a sanitation intervention on soil-transmitted helminth prevalence and concentration

1077   in household soil: A cluster-randomized controlled trial and risk factor analysis. *PLOS*

1078   *Neglected Tropical Diseases*, *13*(2), e0007180.

1079   https://doi.org/10.1371/journal.pntd.0007180

1080   Stolwijk, A. M., Straatman, H., & Zielhuis, G. A. (1999). Studying seasonality by using sine and

1081   cosine functions in regression analysis. *Journal of Epidemiology and Community Health*,

1082   *53*(4), 235–238. https://doi.org/10.1136/jech.53.4.235

1083   Tidwell, J. B., Chipungu, J., Ross, I., Antwi-Agyei, P., Alam, M.-U., Tumwebaze, I. K., …

1084   Simiyu, S. (2020). Where Shared Sanitation is the Only Immediate Option: A Research

1085   Agenda for Shared Sanitation in Densely Populated Low-Income Urban Settings. *The*

1086   *American Journal of Tropical Medicine and Hygiene*, *104*(2), 429–432.

1087   https://doi.org/10.4269/ajtmh.20-0985

1088   UN-Habitat. (2016). *World Cities Report: Urbanization and Development Emerging futures*.

1089   Nairobi. Retrieved from http://wcr.unhabitat.org/wp-content/uploads/2017/03/Chapter1-

1090   WCR-2016.pdf

1091   UNICEF/WHO. (2019). *Progress on household drinking water, sanitation and hygiene 2000-*

1092   *2017. Special focus on inequalities.* New York. Retrieved from https://washdata.org

1093   Wolf, J., Hunter, P. R., Freeman, M. C., Cumming, O., Clasen, T., Bartram, J., … Prüss-Ustün,

1094   A. (2018). Impact of drinking water, sanitation and handwashing with soap on childhood

1095   diarrhoeal disease: updated meta-analysis and meta-regression. *Tropical Medicine &*

1096   *International Health*, *23*(5), 508–525. https://doi.org/10.1111/tmi.13051

1097   Zhan, Z., Guo, J., Xiao, Y., He, Z., Xia, X., Huang, Z., … Zhang, J. (2020). Comparison of

1098   BioFire FilmArray gastrointestinal panel versus Luminex xTAG Gastrointestinal Pathogen

1099   Panel (xTAG GPP) for diarrheal pathogen detection in China. *International Journal of*

1100   *Infectious Diseases*, *99*, 414–420. https://doi.org/10.1016/j.ijid.2020.08.020

1101   Zhuo, R., Cho, J., Qiu, Y. Y., Parsons, B. D., Lee, B. E., Chui, L., … Pang, X. (2017). High

1102   genetic variability of norovirus leads to diagnostic test challenges. *Journal of Clinical*

1103   *Virology*, *96*(May), 94–98. https://doi.org/10.1016/j.jcv.2017.10.003

1104

BASELINE

454 children enrolled & 365 stools collected in INTERVENTION sites

- 366 households
- 208 compounds

533 children enrolled & 400 stools collected in CONTROL sites

- 443 households
- 287 compounds

152 children unavailable at 12-month

- 1 died
- 151 traveling or moved

220 children unavailable at 12-month

- 2 died
- 211 traveling or moved
- 7 refused

160 children newly eligible

- 92 born in
- 52 moved in
- 16 traveling at baseline

164 children newly eligible

- 85 born in
- 50 moved in
- 28 traveling at baseline
- 1 refused baseline enrollment

12-MONTH

462 children visited & 408 stools collected during 12-month phase

- 351 households
- 198 compounds

477 children visited & 397 stools collected during 12-month phase

- 369 households
- 240 compounds

132 children unavailable at 24-month:

- 2 died
- 130 traveling or moved

163 children unavailable at 24-month

- 1 refused
- 136 traveling or moved

26 censored from dataset*

143 children newly eligible

- 75 born in
- 29 moved in
- 39 traveling during prior visits

29 enrolled at baseline but absent at 12-month

155 children newly eligible

- 84 born in
- 27 moved in
- 44 traveling during prior visits

30 enrolled at baseline but absent at 12-month

24-MONTH

502 children visited & 462 stools collected during 24-month phase

- 372 households
- 197 compounds

499 children visited & 460 stools collected during 24-month phase

- 352 households
- 211 compounds

1105

1106   Figure 1: Trial profile. †Eligible for enrollment at baseline and/or 12-month but traveling at time of visit. ‡Children
1107   removed from 24-month analysis because their compound received an intervention after completion of the baseline
1108   phase. Source files available in Figure 1 – source data 1 and Figure 1 – source code 1.

1109 **Table 1: Baseline characteristics of enrolled children, households, and compounds**

	Control		Intervention	
	N	**n (%) or mean (SD)**	**N**	**n (%) or mean (SD)**
Child level variables				
Age at survey, days[†]	520	700 (405)	441	694 (403)
Sex, female	520	266 (51%)	444	227 (51%)
Child is breastfed with or without complementary feeding	526	169 (32%)	448	143 (32%)
Child is exclusively breastfed	526	49 (9.3%)	448	37 (8.3%)
Child feces reported to be disposed of in a latrine	526	148 (28%)	448	141 (31%)
Child wears diapers	526	342 (65%)	447	294 (66%)
Caregiver completed primary school	528	287 (54%)	451	239 (53%)
Child's mother is alive	513	503 (98%)	435	426 (98%)
Respondent is child's mother	519	368 (71%)	443	284 (64%)
Household level variables				
Household population	441	5.4 (2.4)	365	6.1 (3.0)
Household wealth score, 0 (poorer) - 1 (wealthier)[*]	440	0.45 (0.10)	365	0.44 (0.10)
Household crowding, >3 persons/room	440	54 (12%)	365	60 (17%)
Household floor is covered[‡]	440	426 (97%)	365	333 (91%)
Household wall made of bricks, concrete, or similar[‡]	440	304 (69%)	365	215 (59%)
Household drinking water source inside compound	435	324 (74%)	360	294 (82%)
Latrine used by household has a ceramic or masonry pedestal[‡]	432	153 (35%)	359	142 (40%)
Latrine used by household has a drop-hole cover[‡]	434	232 (53%)	359	224 (62%)
Compound level variables				
Number of compound members	287	14 (6.2)	208	19 (12)
Number of households	287	3.8 (2.1)	208	4.4 (3.7)
Number of water taps in compound	283	0.98 (0.95)	207	1.4 (1.6)
Number of latrines in compound	287	1.0 (0.20)	207	1.1 (0.57)

Number of people sharing a latrine	285	14 (6.2)	197	17 (8.9)
Number of households sharing a latrine	285	3.7 (1.8)	197	4.0 (2.8)
Latrine walls made of brick, concrete or similar[‡]	282	72 (26%)	204	67 (33%)
Compound population density, persons/square meter	281	0.071 (0.04)	205	0.087 (0.05)
Compound has electricity that normally functions	287	251 (87%)	208	189 (91%)
Compound is prone to flooding	287	184 (64%)	208	120 (58%)
Any animals observed in compound[‡]	287	170 (59%)	208	132 (63%)
Dog(s) observed[‡]	287	14 (4.9%)	208	14 (6.7%)
Chicken(s) or duck(s) observed[‡]	287	40 (14%)	208	30 (14%)
Cat(s) observed[‡]	287	149 (52%)	208	116 (56%)

1110   Data are n (%) or mean (standard deviation) and collected by questionnaire unless otherwise noted. [†] Age range 32-1819 days, IQR 339-1021 days. Age

1111   distributions available in Supplemental Figure 3. [*]Assessed using Simple Poverty Scorecard for Mozambique

1112   (http://www.simplepovertyscorecard.com/MOZ_2008_ENG.pdf), [‡]Data collected by direct observation. Calculated as # of people living in the compound

1113   divided by the area of the compound in square meters. Source files available in Table 1 – source data 1 and Table 1 – source code 1.

Table 2: Effect of the intervention on bacterial, protozoan, and STH infection and diarrhea at 12 and 24 months post-intervention.

	Prevalence			12 month Prevalence ratio (95% CI), p-value *		24 month Prevalence ratio (95% CI), p-value □	
	Baseline	**12-month**	**24-month**	**unadjusted**	**adjusted†**	**unadjusted**	**adjusted†**
Any bacterial or protozoan infection‡							
Control	313/392 (80%)	334/395 (85%)	403/459 (88%)	..	..	..	..
Intervention	278/361 (77%)	347/408 (85%)	392/462 (85%)	1.04 (0.94 - 1.15), p=0.41	1.04 (0.94 - 1.15), p=0.41	1.00 (0.91 - 1.10), p=1.0	0.99 (0.91 - 1.09), p=0.89
Any STH infection‡							
Control	170/360 (47%)	143/283 (51%)	142/253 (56%)	..	..	..	..
Intervention	138/329 (42%)	150/305 (49%)	136/292 (47%)	1.12 (0.89 - 1.40), p=0.33	1.11 (0.89 - 1.38), p=0.35	0.94 (0.75 - 1.17), p=0.59	0.95 (0.77 - 1.17), p=0.62
Diarrhea‡							
Control	67/526 (13%)	40/430 (9.3%)	53/390 (14%)	..	..	..	..
Intervention	59/448 (13%)	59/436 (14%)	53/410 (13%)	1.41 (0.80 - 2.48), p=0.24	1.69 (0.89 - 3.21), p=0.11	0.92 (0.55 - 1.54), p=0.76	0.84 (0.47 - 1.51), p=0.56
Any Bacteria							
Control	271/392 (69%)	285/395 (72%)	345/459 (75%)	..	..	..	..
Intervention	227/361 (63%)	292/408 (72%)	324/462 (70%)	1.09 (0.95 - 1.25), p=0.25	1.09 (0.95 - 1.26), p=0.20	1.03 (0.90 - 1.18), p=0.69	1.00 (0.87 - 1.15), p=0.96
Shigella							
Control	179/392 (46%)	204/395 (52%)	269/459 (59%)	..	..	..	..
Intervention	152/361 (42%)	218/408 (53%)	245/462 (53%)	1.13 (0.91 - 1.39), p=0.28	1.12 (0.92 - 1.38), p=0.27	0.98 (0.80 - 1.20), p=0.86	0.95 (0.79 - 1.16), p=0.64
ETEC							
Control	116/392 (30%)	142/395 (36%)	127/459 (28%)	..	..	..	..
Intervention	110/361 (30%)	143/408 (35%)	126/462 (27%)	0.93 (0.68 - 1.28), p=0.66	0.96 (0.69 - 1.33), p=0.81	0.95 (0.67 - 1.35), p=0.77	0.83 (0.57 - 1.19), p=0.31
Campylobacter							
Control	39/392 (9.9%)	32/395 (8.1%)	48/459 (10%)	..	..	..	..
Intervention	21/361 (5.8%)	35/408 (8.6%)	34/462 (7.4%)	1.78 (0.89 - 3.56), p=0.10	1.68 (0.82 - 3.45), p=0.16	1.20 (0.60 - 2.39), p=0.60	1.28 (0.62 - 2.62), 0.50
C. difficile							
Control	22/392 (5.6%)	13/395 (3.3%)	13/459 (2.8%)	..	..	..	..

Intervention	13/361 (3.6%)	17/408 (4.2%)	11/462 (2.4%)	1.95 (0.71 - 5.35), p=0.20	2.09 (0.77 - 5.64), p=0.15	1.32 (0.47 - 3.73), p=0.60	1.41 (0.46 - 4.30), p=0.54
E. coli O157							
Control	13/392 (3.3%)	19/395 (4.8%)	25/459 (5.5%)	..	..	..	..
Intervention	18/361 (5.0%)	14/408 (3.4%)	16/462 (3.5%)	0.48 (0.18 - 1.27), p=0.14	0.46 (0.18 - 1.21), p=0.12	0.43 (0.15 - 1.29), p=0.13	0.52 (0.17 - 1.59), p=0.25
STEC							
Control	3/392 (0.77%)	9/395 (2.3%)	17/459 (3.7%)	..	..	..	..
Intervention	10/361 (2.8%)	5/408 (1.2%)	15/462 (3.3%)	0.14 (0.03 - 0.67), p=0.014	0.15 (0.03 - 0.70), p=0.016	0.23 (0.05 - 1.03), p=0.055	0.24 (0.05 - 1.01), p=0.052
Any Protozoa							
Control	205/392 (52%)	236/395 (60%)	303/459 (66%)	..	..	..	..
Intervention	195/361 (54%)	259/408 (63%)	296/462 (64%)	1.04 (0.87 - 1.24), p=0.69	1.03 (0.86 - 1.22), p=0.76	0.93 (0.78 - 1.11), p=0.40	0.91 (0.76 - 1.09), p=0.29
Giardia							
Control	201/392 (51%)	230/395 (58%)	294/459 (64%)	..	..	..	..
Intervention	186/361 (52%)	251/408 (62%)	289/462 (63%)	1.06 (0.88 - 1.27), p=0.55	1.05 (0.88 - 1.25), p=0.58	0.96 (0.80 - 1.14), p=0.61	0.93 (0.78 - 1.11), p=0.44
Cryptosporidium							
Control	8/392 (2%)	8/395 (2%)	14/459 (3.0%)	..	..	..	..
Intervention	16/361 (4.4%)	15/408 (3.7%)	15/462 (3.3%)	0.89 (0.23 - 3.43), p=0.87	0.89 (0.24 - 3.31), p=0.86	0.46 (0.11 - 1.93), p=0.29	0.53 (0.13 - 2.14), p=0.37
Any virus							
Control	53/392 (14%)	52/395 (13%)	59/459 (13%)	..	..	..	..
Intervention	52/361 (14%)	45/408 (11%)	62/462 (13%)	0.77 (0.45 - 1.32), p=0.35	0.75 (0.44 - 1.27), p=0.29	0.96 (0.55 - 1.68), p=0.88	1.03 (0.57 - 1.86), p=0.92
Norovirus GI/GII							
Control	38/392 (9.7%)	44/395 (11%)	47/459 (10%)	..	..	..	..
Intervention	39/361 (11%)	37/408 (9.1%)	55/462 (12%)	0.71 (0.38 - 1.33), p=0.28	0.68 (0.36 - 1.27), p=0.23	1.00 (0.52 - 1.93), p=0.99	1.10 (0.55 - 2.18), p=0.79
Adenovirus 40/41							
Control	13/392 (3.3%)	9/395 (2.3%)	7/459 (1.5%)	..	..	..	..
Intervention	9/361 (2.5%)	9/408 (2.2%)	6/462 (1.3%)	1.34 (0.34 - 5.23), p=0.68	1.24 (0.32 - 4.83), p=0.76	1.18 (0.23 - 5.98), p=0.84	0.97 (0.18 - 5.19), p=0.97
Coinfection, ≥2 GPP							

59

pathogens							
Control	206/392 (53%)	237/395 (60%)	302/459 (66%)	..	..	..	..
Intervention	185/361 (51%)	257/408 (63%)	282/462 (61%)	1.08 (0.90 - 1.29), p=0.39	1.08 (0.91 - 1.29), p=0.37	0.95 (0.80 - 1.12), p=0.54	0.93 (0.79 - 1.10), p=0.41
Trichuris							
Control	139/360 (39%)	116/283 (41%)	124/253 (49%)	..	..	..	..
Intervention	117/329 (36%)	120/305 (39%)	117/292 (40%)	1.05 (0.82 - 1.35), p=0.68	1.01 (0.79 - 1.28), p=0.96	0.89 (0.69 - 1.16), p=0.40	0.86 (0.67 - 1.10), p=0.22
Ascaris							
Control	95/360 (26%)	82/283 (29%)	78/253 (31%)	..	..	..	..
Intervention	68/329 (21%)	87/305 (29%)	56/292 (19%)	1.26 (0.87 - 1.82), p=0.22	1.33 (0.92 - 1.93), p=0.13	0.80 (0.52 - 1.21), p=29	0.83 (0.54 - 1.27), p=0.39
Coinfection, ≥2 STH							
Control	64/360 (18%)	55/283 (19%)	60/253 (24%)	..	..	..	..
Intervention	47/329 (14%)	57/305 (19%)	37/292 (13%)	1.16 (0.76 - 1.77), p=0.50	1.17 (0.76 - 1.79), p=0.49	0.67 (0.40 - 1.13), p=0.13	0.63 (0.37 - 1.07), p=0.084

1115 Prevalence results are presented as (n/N (%)). All effect estimates are presented as prevalence ratios (ratio of ratios) and estimated using
1116 generalized estimating equations to fit Poisson regression models with robust standard errors. *Analysis includes all children measured at baseline
1117 and 12-month visits. □Analysis includes all children measured at baseline and 24-month visits. ‡Outcome was pre-specified in trial registration.
1118 All other outcomes are exploratory. †Pathogen outcomes adjusted for child age and sex, caregiver's education, and household wealth index.
1119 Reported diarrhea was also adjusted for baseline presence of a drop-hole cover and reported use of a tap on compound grounds as primary
1120 drinking water source. Sample sizes for adjusted analyses are slightly smaller than numbers presented in prevalence estimates due to missing
1121 covariate data. *Y. enterocolitica, V. cholerae, E. histolytica*, and rotavirus were detected in <2% of samples in each arm at each phase. Descriptive
1122 data for these pathogens are available in the Supplemental Table 2. Source files available in Table 2 – source data 1 and Table 2 – source code 1.

Table 3: Effect of intervention on bacterial, protozoan, and STH infection and reported diarrhea in children born into study sites post-intervention (post-baseline) but by 24-month visit compared with children of a similar age at baseline (<2 years old).

	Prevalence (<2 years old)		Prevalence ratio (95% CI), p-value	
	Baseline	24-month, Born-in	unadjusted	adjusted†
Any bacterial or protozoan infection‡				
Control	158/228 (69%)	79/106 (75%)	..	..
Intervention	129/201 (64%)	71/107 (66%)	0.96 (0.77 - 1.21), p=0.74	0.99 (0.80 - 1.22), p=0.92
Any STH infection‡				
Control	67/205 (33%)	25/68 (37%)	..	..
Intervention	52/183 (28%)	13/75 (17%)	0.52 (0.26 - 1.05), p=0.069	0.51 (0.27 - 0.95), p=0.035
Diarrhea‡				
Control	46/283 (16%)	18/105 (17%)	..	..
Intervention	43/238 (18%)	22/100 (22%)	1.20 (0.57 -2.5), p=0.64	1.37 (0.47 - 4.03), p=0.57
Any Bacteria				
Control	142/228 (62%)	70/106 (66%)	..	..
Intervention	102/201 (51%)	51/107 (48%)	0.89 (0.66 - 1.20), p=0.44	0.90 (0.67 - 1.19), p=0.45
Shigella				
Control	67/228 (29%)	36/106 (34%)	..	..
Intervention	49/201 (24%)	15/107 (14%)	0.48 (0.28 - 0.83), p=0.009	0.49 (0.28 - 0.85), p=0.011
ETEC				
Control	70/228 (31%)	30/106 (28%)	..	..
Intervention	58/201 (29%)	24/107 (22%)	0.84 (0.46 - 1.52), p=0.56	0.85 (0.48 - 1.51), p=0.58
Campylobacter				
Control	27/228 (12%)	14/106 (13%)	..	..
Intervention	14/201 (7%)	13/107 (12%)	1.75 (0.63 - 4.87), p=0.29	1.75 (0.61 - 4.98), p=0.30
C. difficile				
Control	20/228 (8.8%)	7/106 (6.6%)	..	..
Intervention	13/201 (6.5%)	7/107 (6.5%)	1.33 (0.36 - 4.86), p=0.67	1.49 (0.41 - 5.44), p=0.55

E. coli O157				
Control	7/228 (3.1%)	3/106 (2.8%)	..	..
Intervention	9/201 (4.5%)	2/107 (1.9%)	0.45 (0.06 - 3.66), p=0.46	0.53 (0.07 - 4.24), p=0.55
STEC				
Control	1/228 (0.44%)	2/106 (1.9%)	..	..
Intervention	9/201 (4.5%)	1/107 (0.93%)	0.05 (0.00 - 1.13), p=0.059	0.05 (0.00 - 1.26), p=0.070
Any Protozoa				
Control	82/228 (36%)	47/106 (44%)	..	..
Intervention	74/201 (37%)	43/107 (40%)	0.84 (0.55 - 1.28), p=0.42	0.90 (0.62 - 1.30), p=0.58
Giardia				
Control	79/228 (35%)	44/106 (42%)	..	..
Intervention	68/201 (34%)	41/107 (38%)	0.90 (0.58 - 1.39), p=0.63	0.93 (0.64 - 1.36), p=0.70
Cryptosporidium				
Control	7/228 (3.1%)	5/106 (4.7%)	..	..
Intervention	12/201 (6%)	5/107 (4.7%)	0.45 (0.08 - 2.57), p=0.37	0.64 (0.12 - 3.51), p=0.61
Any virus				
Control	34/228 (15%)	18/106 (17%)	..	..
Intervention	36/201 (18%)	18/107 (17%)	0.83 (0.37 - 1.83), p=0.64	0.83 (0.37 - 1.87), p=0.66
Norovirus GI/GII				
Control	26/228 (11%)	12/106 (11%)	..	..
Intervention	26/201 (13%)	17/107 (16%)	1.24 (0.48 - 3.17), p=0.66	1.29 (0.49 - 3.41), p=0.61
Adenovirus 40/41				
Control	7/228 (3.1%)	4/106 (3.8%)	..	..
Intervention	7/201 (3.5%)	0/107 (0.0%)	..▯	..▯
Coinfection, ≥2 GPP pathogens				
Control	92/228 (40%)	52/106 (49%)	..	..
Intervention	74/201 (37%)	39/107 (36%)	0.82 (0.56 - 1.21), p=0.33	0.86 (0.59 - 1.24), p=0.41
Trichuris				

Control	48/205 (23%)	18/68 (26%)	..	..
Intervention	41/183 (22%)	5/75 (6.7%)	0.25 (0.09 - 0.68), p=0.006	0.24 (0.10 - 0.60), p=0.002
Ascaris				
Control	45/205 (22%)	16/68 (24%)	..	..
Intervention	29/183 (16%)	9/75 (12%)	0.70 (0.30 - 1.64), p=0.42	0.68 (0.30 - 1.54), p=0.36
Coinfection, ≥2 STH				
Control	26/205 (13%)	9/68 (13%)	..	..
Intervention	18/183 (9.8%)	1/75 (1.3%)	0.13 (0.02 - 1.08), p=0.059	0.12 (0.01 - 1.02), p=0.052

Analysis includes children <2 years old at baseline and children born into the study after baseline and <2 years old at the time of the 24-month visit. Prevalence results are presented as (n/N (%)). All effect estimates are presented as prevalence ratios (ratio of ratios) and estimated using generalized estimating equations to fit Poisson regression models with robust standard errors ‡Outcome was pre-specified in trial registration. All other outcomes are exploratory. †Pathogen outcomes adjusted for child age and sex, caregiver's education, and household wealth index. Reported diarrhea was also adjusted for baseline presence of a drop-hole cover and reported use of a tap on compound grounds as primary drinking water source. Sample sizes for adjusted analyses are slightly smaller than numbers presented in prevalence estimates due to missing covariate data. ⸗Models would not converge due to sparse data. *Y. enterocolitica, V. cholerae, E. histolytica*, and rotavirus were detected in <2% of samples in each arm at each phase and excluded. Descriptive data for these pathogens are available in the Supplemental Table 2. Source files available in Table 3 – source data 1 and Table 3 – source code 1.

Supplemental Files

Supplemental Figure 1: Proportion of each type of sample collected during the baseline, 12-month, and 24-month phases.

Supplemental Table 1: Number and proportion of sample types collected in each arm at each phase.

Supplemental Figure 2: Enrollment and stool sample collection profile.

Supplemental Figure 3: Distribution of age (years) of enrolled children at each phase.

Supplemental Table 2: Age stratified baseline prevalence of health outcomes.

Supplemental Figure 4: Prevalence of pathogens by age at baseline, 12-month, and 24-month phases.

Supplemental Table 3: Baseline enrollment characteristics of children with and without repeated measures at the 12-month phase

Supplemental Table 4: Baseline enrollment characteristics of children with and without repeated measures at the 24-month phase

Supplemental Table 5: Balance of characteristics measured at 12-month visits between children with repeat observations at baseline and 12-month and children with observations at the 12-month phase only.

Supplemental Table 6: Balance of characteristics measured at 24-month visits between children with repeat observations at baseline and 24-month and children with observations at the 24-month phase only.

Supplemental Table 7: Sensitivity analysis assessing the impact of reported deworming on STH effect estimates 12 and 24 months after the intervention.

Supplemental Table 8: Sensitivity analysis assessing impact of independent upgrading of control sanitation facilities on effect estimates.

Supplemental Table 9: Confounding assessment for primary outcome and both secondary outcomes (any STH, diarrhea) at 12-month.

Supplemental Table 10: Effect estimates (prevalence ratios) for main analyses and all sub-group analyses adjusted for *a priori* covariates and age-squared

Supplemental Table 11: Comparison of effect estimates (prevalence ratios) at 12- and 24 month adjusted for a priori covariates only and for *a priori* covariates and seasonality.

BASELINE

454 children enrolled & 365 stools collected in **INTERVENTION** sites
- 366 households
- 208 compounds

533 children enrolled & 400 stools collected in **CONTROL** sites
- 443 households
- 287 compounds

152 children unavailable at 12-month
- 1 died
- 151 traveling or moved

220 children unavailable at 12-month
- 2 died
- 211 traveling or moved
- 7 refused

160 children newly eligible
- 92 born in
- 52 moved in
- 16 traveling at baseline

164 children newly eligible
- 85 born in
- 50 moved in
- 28 traveling at baseline
- 1 refused baseline enrollment

12-MONTH

462 children visited & 408 stools collected during 12-month phase
- 351 households
- 198 compounds

477 children visited & 397 stools collected during 12-month phase
- 369 households
- 240 compounds

132 children unavailable at 24-month:
- 2 died
- 130 traveling or moved

163 children unavailable at 24-month
- 1 refused
- 136 traveling or moved

26 censored from dataset*

143 children newly eligible
- 75 born in
- 29 moved in
- 39 traveling during prior visits

29 enrolled at baseline but absent at 12-month

155 children newly eligible
- 84 born in
- 27 moved in
- 44 traveling during prior visits

30 enrolled at baseline but absent at 12-month

24-MONTH

502 children visited & 462 stools collected during 24-month phase
- 372 households
- 197 compounds

499 children visited & 460 stools collected during 24-month phase
- 352 households
- 211 compounds

Types of Collected Samples

(a) Baseline

(b) 12m Follow up

(c) 24m Follow up

(d) All Phases

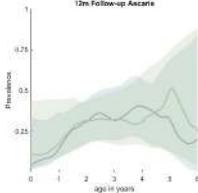| Baseline Any Virus | 12m Follow-up Any Virus | 24m Follow-up Any Virus |
Baseline Shigella	12m Follow-up Shigella	24m Follow-up Shigella
Baseline ETEC	12m Follow-up ETEC	24m Follow-up ETEC
Baseline Salmonella	12m Follow-up Salmonella	24m Follow-up Salmonella
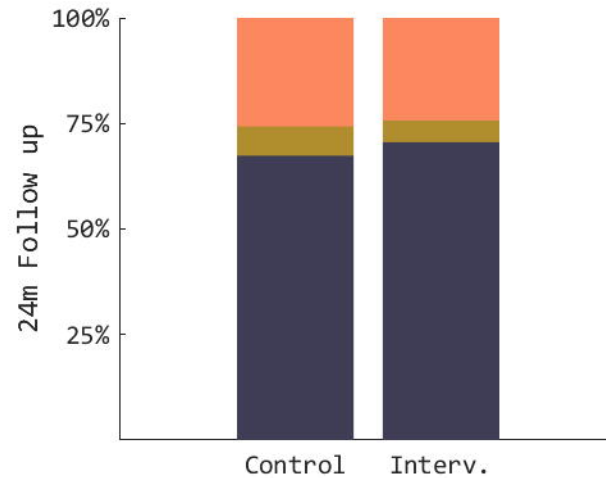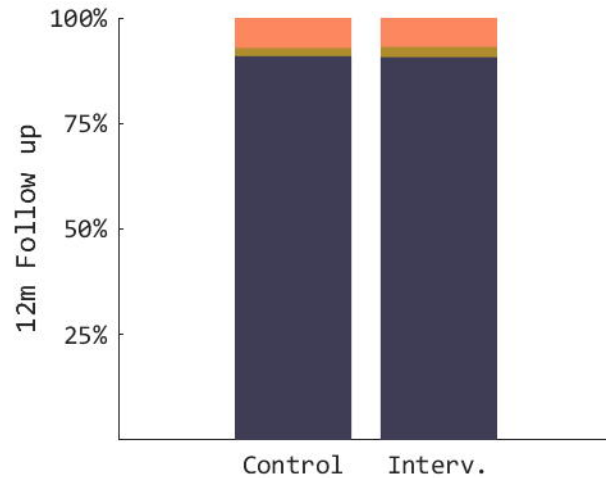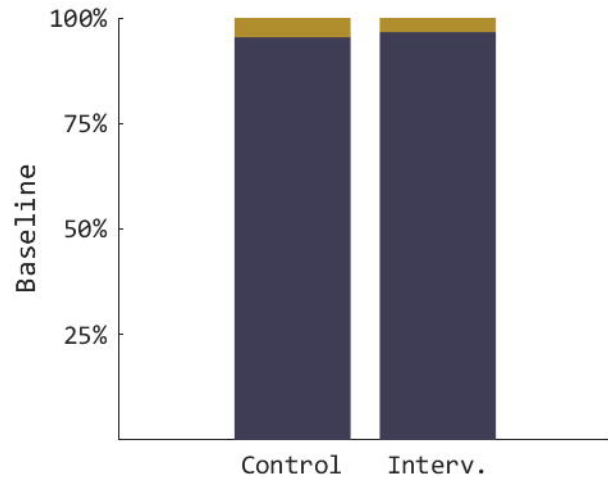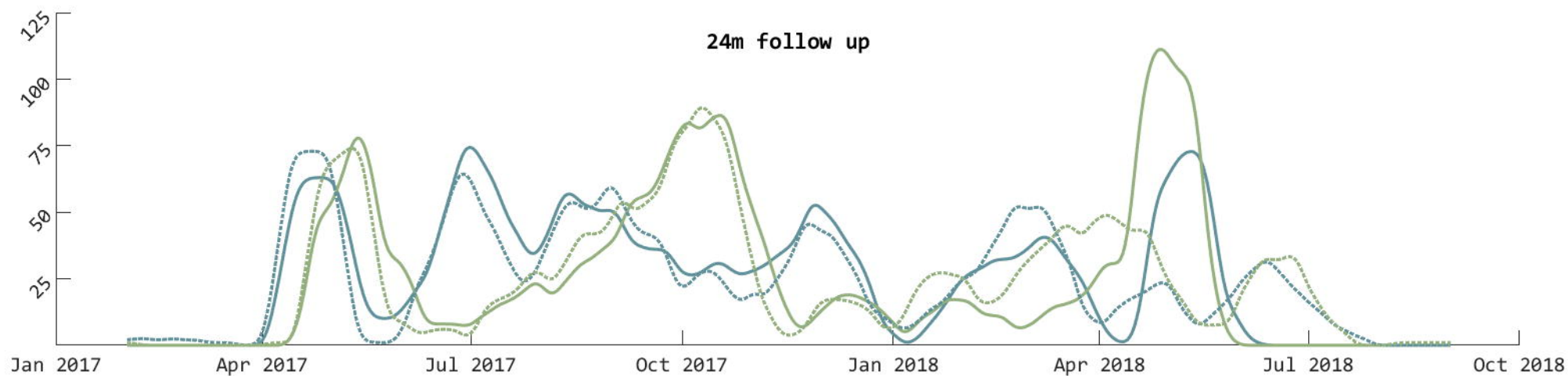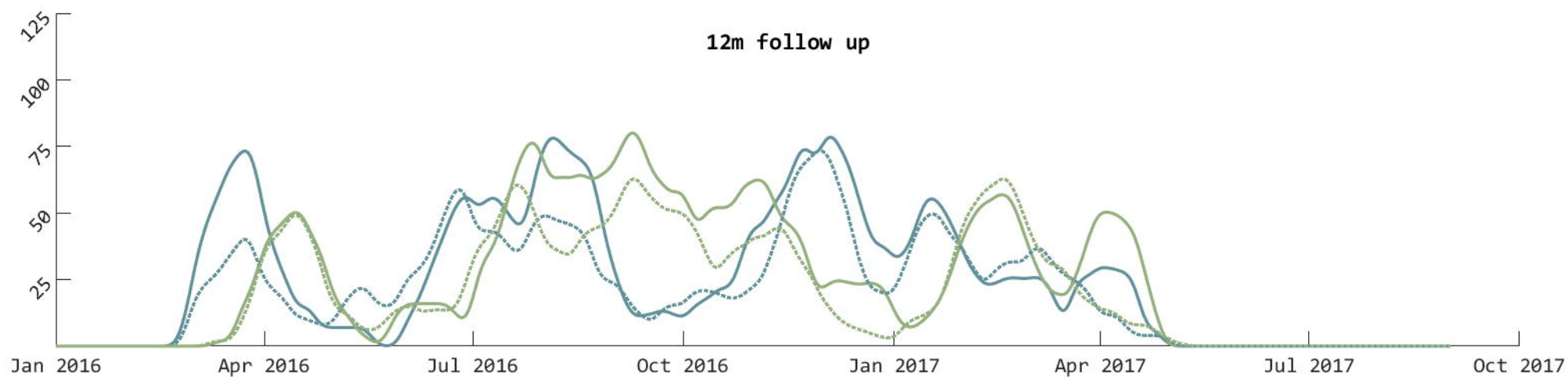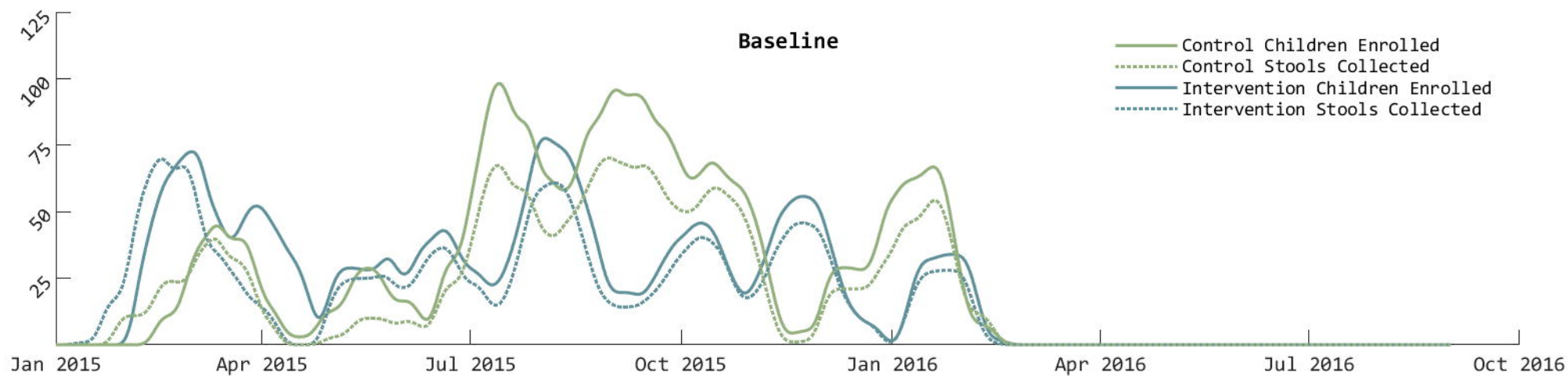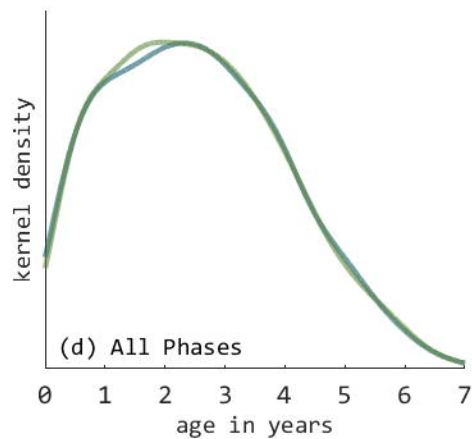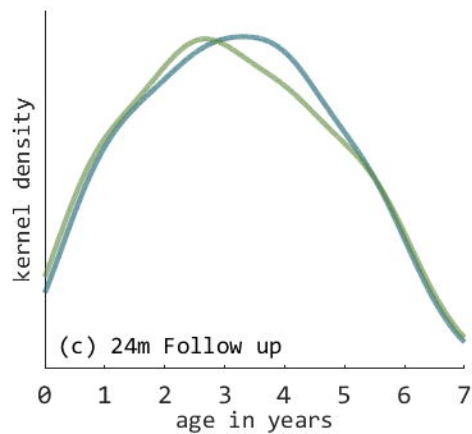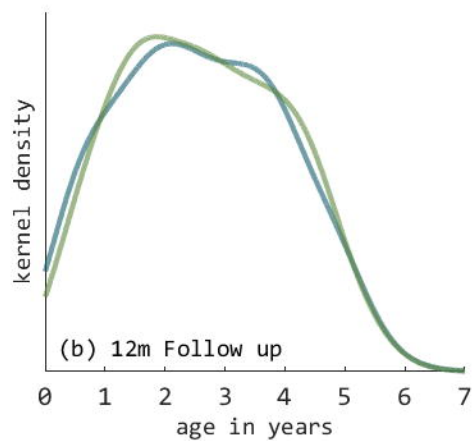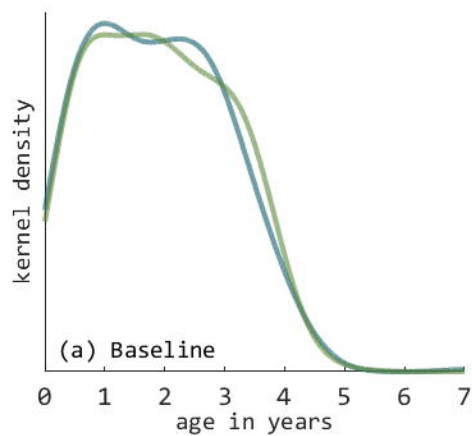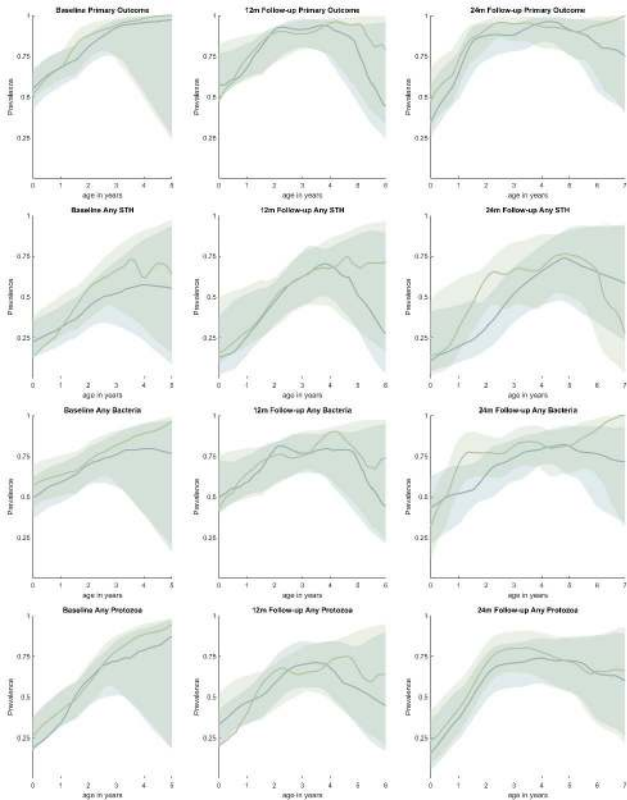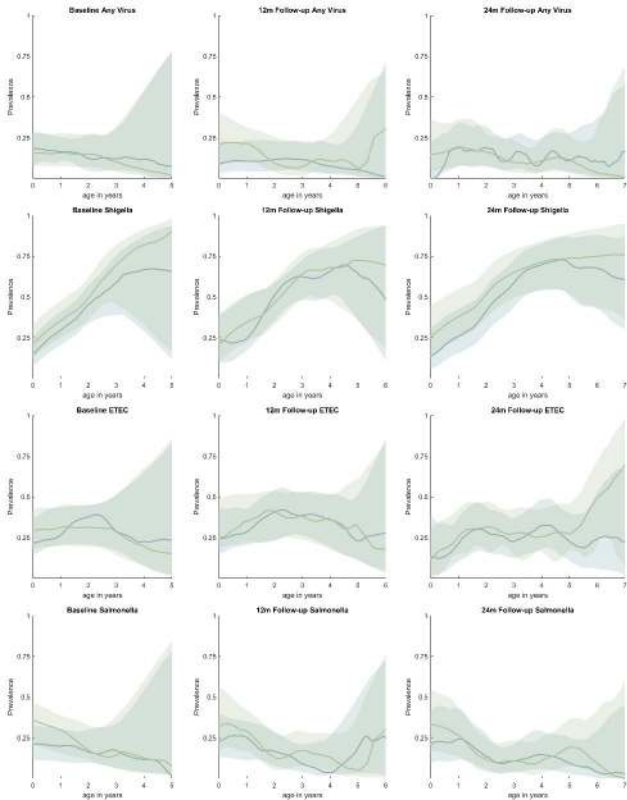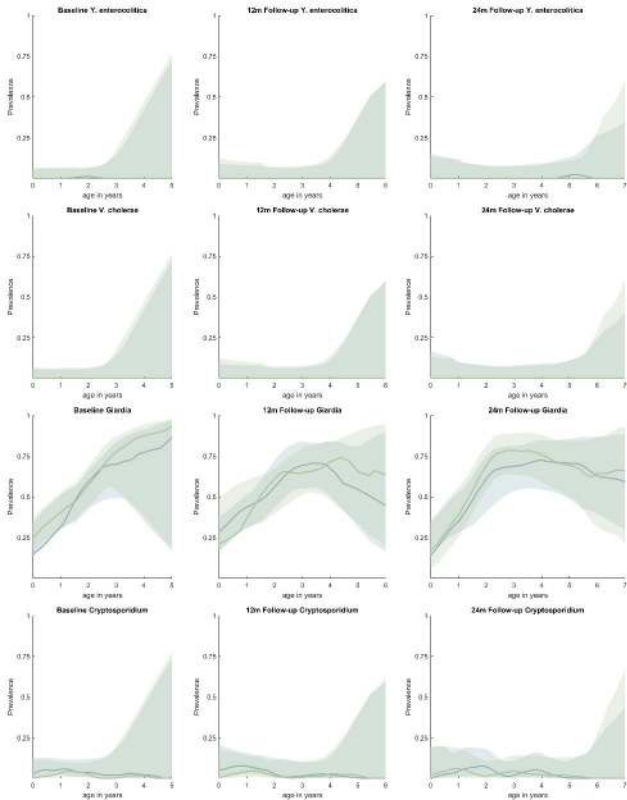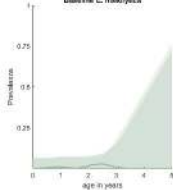
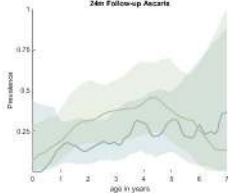| Baseline E. histolytica | 12m Follow-up E. histolytica | 24m Follow-up E. histolytica |
Baseline Norovirus GI/GII	12m Follow-up Norovirus GI/GII	24m Follow-up Norovirus GI/GII
Baseline Adenovirus	12m Follow-up Adenovirus	24m Follow-up Adenovirus
Baseline Rotavirus	12m Follow-up Rotavirus	24m Follow-up Rotavirus
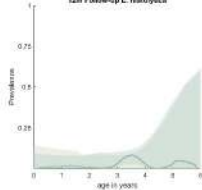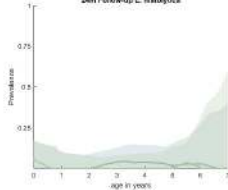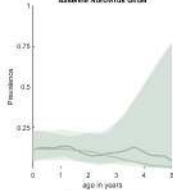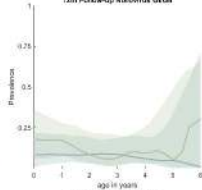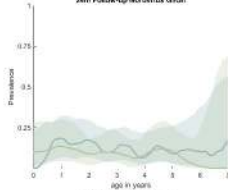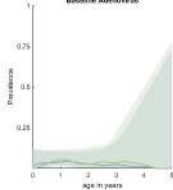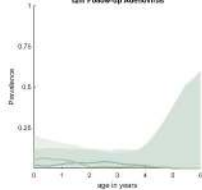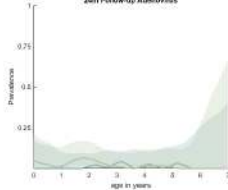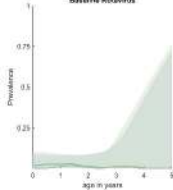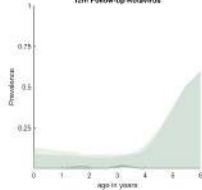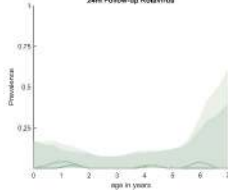
| Baseline Trichuris | 12m Follow-up Trichuris | 24m Follow-up Trichuris |
| Baseline Ascaris | 12m Follow-up Ascaris | 24m Follow-up Ascaris |
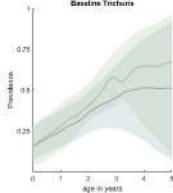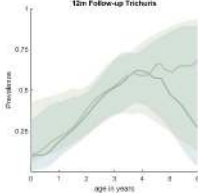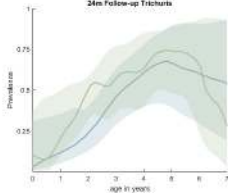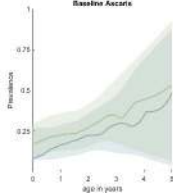
Elevated water storage tank (500L)

Door (entry)

Septic tank

Laundry basin

Pour-flush toilet (pedestal design)

Study Sites

■ Intervention Sites

◆ Control Sites

250 0 250 500 750 1000 m