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1 **Does targeting children with hygiene promotion messages work?**

2 **The effect of handwashing promotion targeted at children, on diarrhoea, soil-**
3 **transmitted helminth infections and behaviour change, in low- and middle-income**
4 **countries: a systematic literature review**

5 Julie A. Watson^{1*}, Jeroen H. J. Ensink¹, Monica Ramos², Prisca Benelli², Elizabeth Holdsworth³, Robert
6 Dreifelbis¹ & Oliver Cumming¹

7 ¹Department for Disease Control, Faculty of Infectious Tropical Diseases, London School of Hygiene
8 and Tropical Medicine, Keppel Street, London, WC1E 7HT, United Kingdom.

9 ²Save the Children UK, 1 St John's Lane, London, EC1M 4AR, United Kingdom

10 ³Department of Health Services Research and Policy, Faculty of Public Health and Policy, London
11 School of Hygiene and Tropical Medicine, Keppel Street, London, WC1E 7HT, United Kingdom.

12 Corresponding author: Julie Watson

13 Email: julie.watson@lshtm.ac.uk

14

15 * This article is dedicated to the late Dr. Jeroen Ensink. As a researcher, and as a teacher, Jeroen
16 made a huge contribution to the field of environmental health. His wisdom, patience and good
17 humour are much missed by his many collaborators and friends around the world.

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30 **Abstract**

31 **Objectives:** To synthesise evidence on the effect of handwashing promotion interventions targeting
32 children, on diarrhoea, soil-transmitted helminth infection and handwashing behaviour, in low and
33 middle income country settings

34 **Methods:** A systematic review of the literature was performed by searching 8 databases and
35 reference lists were hand searched for additional articles. Studies were reviewed for inclusion
36 according to pre-defined inclusion criteria and the quality of all studies was assessed.

37 **Results:** Eight studies were included in this review: seven cluster-randomised controlled trials and
38 one cluster non-randomised controlled trial. All eight studies targeted children aged 5-12 attending
39 primary school but were heterogeneous for both the type of intervention and the reported
40 outcomes so results were synthesised qualitatively. None of the studies were of high quality and the
41 large majority were at high risk of bias. The reported effect of child-targeted handwashing
42 interventions on our outcomes of interest varied between studies. Of the different interventions
43 reported, no one approach to promoting handwashing among children appeared most effective.

44 **Conclusion:** Our review found very few studies that evaluated handwashing interventions targeting
45 children and all had various methodological limitations. It is plausible that interventions which
46 succeed in changing children's handwashing practices will lead to significant health impacts given
47 that much of the attributable disease burden is concentrated in that age group. The current paucity
48 of evidence in this area however does not permit any recommendations to be made as to the most
49 effective route to increasing handwashing with soap practice among children in LMIC.

50

51 **Introduction**

52 The global burden of disease associated with poor water, sanitation, and hygiene (WASH) is
53 concentrated among children and thus promoting the practice of handwashing with soap (HWWS)
54 among children presents an important public health measure (1).

55 Pneumonia and diarrhoea are two of the leading causes of child mortality globally and account for
56 over 900,000, and 500,000 deaths per year in children under-five, respectively (2), many of which
57 may be preventable with improved hygiene (3-5). Systematic reviews have consistently shown that
58 HWWS is effective at reducing diarrhoeal disease, and can reduce the risk of diarrhoea by up to 48%,
59 (1, 6-8), with the current best estimate believed to be around a 23% risk reduction (9). In fact, it has
60 been argued that HWWS is one of the single most cost effective of all public health interventions
61 (10). HWWS acts as an important barrier in the transmission of diarrhoea-causing aetiological agents
62 via the faecal-oral pathway by preventing faeces from entering, and being transmitted in the
63 domestic environment (11).

64 In 2015, the sustainable development goals (SDGs) were launched and the target set for SDG 3.2 was
65 to end, by 2030, the preventable deaths of newborns and children under five years old (12). With
66 pneumonia and diarrhoea among the leading causes of deaths in these age groups, WASH
67 interventions represent one of the most cost-effective methods to help achieve this goal (10).
68 HWWS is a key part of the integrated Global Action Plan for the Prevention and Control of
69 Pneumonia and Diarrhoea (GAPPD) framework, which proposes a cohesive approach to ending
70 preventable pneumonia and diarrhoea deaths (13).

71 Children also represent the population most vulnerable to soil-transmitted helminth (STH) infection,
72 with prevalence and intensity peaking between the ages of 5-14 (14). STHs are parasitic intestinal
73 nematodes passed to humans through contact with soil contaminated with infected faeces and are
74 one of the most common human infections worldwide, with a disproportionate burden in the
75 poorest and most deprived populations (15). STH infection is recognised as one of the most
76 important causes of stunting in children and can also lead to long term effects on cognitive
77 development and educational achievement which may hinder future economic development (14).

78 Whilst, historically, there has been less research assessing the relationship between HWWS and STH
79 than between HWWS and diarrhoea, a recent systematic review has also found handwashing
80 interventions to be an effective measure to prevent the transmission and reduce the infection
81 intensity of *Ascariasis lumbricoides*, a common STH, and can reduce the risk of *A.lumbricoides*
82 infection by up to 62% (16).

83 To our knowledge, there have been no previous systematic reviews that have assessed the
84 effectiveness of targeting handwashing promotion at children in LMICs. A recent Cochrane review of
85 handwashing promotion to prevent diarrhoea did assess the effect of handwashing promotion on
86 preventing diarrhoea, however, results were stratified by setting before being stratified by age, and,
87 within these settings, the author did not analyse the effect of targeting handwashing promotion at
88 children but only the effect of *any* handwashing promotion on diarrhoeal episodes in children (1).
89 The purpose of this systematic review is to assess if handwashing promotion, targeted at children in
90 LMICs, is effective at increasing handwashing behaviour and consequently reducing diarrhoea and
91 STH infection among children and their families. Handwashing behaviour is a primary outcome of
92 interest in this review as this is the proposed mechanism to achieve reductions in communicable
93 disease. Diarrhoeal disease is also a primary outcome of interest as this outcome is commonly used
94 to measure the effectiveness of hygiene interventions and the link between diarrhoea and WASH is
95 well known (8, 9). Including STH infection as a primary outcome offers a measure which potentially
96 has a lower risk of bias because diarrhoea is often measured by self-report, whilst STH can be
97 measured objectively through standard diagnostic tests, such as the commonly used Kato-Katz
98 method and the more sensitive FLOTAC method (17). Although there is only evidence that
99 handwashing reduces *A. lumbricoides* infection, this helminth is commonly grouped together with
100 the helminths *Trichuris trichuria* and hookworm, and referenced as 'STH'.

101 **Methods**

102 **Search Strategy**

103 Searches were carried out in July 2016, using eight bibliographic databases: Medline, Embase, Global
104 Health, CINHAL Plus, Scopus, IBSS, Africa-Wide Information, and Web of Science. The search strategy
105 incorporated terms related to: (i) children; AND (ii) handwashing promotion; AND (ii) (diarrhoea OR
106 soil-transmitted helminths, OR behaviour). The search strategy was originally developed for Medline
107 (MESH terms were identified), before being adapted for use in bibliographic databases using
108 database-specific controlled vocabulary terms and search filters. Reference lists of included studies
109 were hand searched for additional relevant citations. A full description of the search strategy and
110 search terms for the Medline database can be found in Appendix S1.

111 **Screening and Inclusion Criteria**

112 Studies were eligible for inclusion if they were published in a peer-reviewed journal, on any date up
113 until 7th July 2016, and available in English. Qualitative studies and studies that were published as
114 conference abstracts or posters were excluded. Eligible study designs included: randomised
115 controlled trials (RCTs), non-randomised controlled trials (NRCTs), and controlled before-after (CBA)

116 studies (with a concurrently enrolled control group). These study designs were selected to limit the
117 risk of bias.

118 Following screening, articles needed to meet five criteria to be included: (i) the study evaluated a
119 clearly described hygiene promotion intervention including, or exclusively focussed on messages
120 around handwashing; (ii) the evaluated intervention targeted children between the ages of five and
121 eighteen; (iv) the study was conducted within a low- or middle-income country, as defined by the
122 World Bank (18); (v) the study reported an effect on one or more of the outcomes of interest
123 (detailed below). We excluded studies in which water, sanitation, or other health interventions (with
124 the exception of soap provision) were implemented concurrently, unless the study was able to
125 report the effect of the hygiene promotion component targeting children separately. Similarly,
126 studies in which children were not the only main targets of the intervention were excluded unless
127 the effects of a distinct intervention component targeting only children could be clearly stratified.

128 ***Intervention***

129 We included interventions that promoted handwashing (with or without soap) at any specified key
130 moment, for example: after toilet use (defecation or urination), before preparing or handling food,
131 before eating, after sneezing and coughing, upon arriving at school, after playing with soil, and
132 during bathing. Intervention activities could include, for example: hygiene education, posters, group
133 discussions, theatre, peer-monitoring, teacher monitoring, handwashing pledges, videos, comic
134 books, songs, poems, games, drawing, puppet shows, mascots, rewards, competitions and
135 environmental cues.

136 ***Outcomes***

137 The primary outcomes of interest were: (1) handwashing behaviours (cleansing hands with water,
138 with soap and water, or with hand sanitizer, at any key moment as listed above); (2) diarrhoea
139 morbidity [prevalence or incidence] or mortality [regardless of aetiology and case confirmation]; and
140 (3) one or more Soil-Transmitted Helminth¹ infection [including prevalence and/or intensity]. Any
141 reported change in knowledge with regard to handwashing with soap was a secondary outcome of
142 interest. For all outcomes of interest, we included measurements taken at an individual or cluster
143 level, and for either the target children or their families since evidence suggests children can be
144 effective agents of change (20). For the handwashing behaviours outcome, we included studies using
145 either direct measures of handwashing behaviours or soap consumption as a proxy measure.

146 ***Study Selection, Data Extraction and Analysis***

147 All results retrieved from database searches were exported into Endnote X7.1 (Thomson Reuters,
148 New York, USA) and duplicates removed. Results were screened, by title and abstract, by a single
149 reviewer (JW) and non-eligible studies excluded. The full text for eligible studies were then
150 independently reviewed by two reviewers (JW and OC) and a final decision on the inclusion of
151 studies was reached by consensus.

152 Data were extracted into a pre-specified data extraction table, recording the following information:
153 (i) study authors and publication date, (ii) intervention content, (iii) intervention methods, (iv)
154 control group, (v) setting (vi) study design, (vii) intervention length/intensity (intervention intensity
155 was graded as 'low' if intervention activities were implemented at one point in time and 'high' if
156 intervention activities were implemented at multiple points in time over the length of the
157 intervention), (viii) outcomes, (ix) participants, (x) soap provision, (xi) results. A quantitative meta-

¹ The main species that infect humans are roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichuria*), and hookworm (*Necator americanus* and *Ancylostoma duodenale*). 19. The World Health Organisation. Soil-transmitted helminth infections: fact sheet 2016 [Available from: <http://www.who.int/mediacentre/factsheets/fs366/en/>].

158 analysis was not conducted due to the limited number of studies, and the heterogeneity in study
 159 interventions and outcomes, and instead a narrative synthesis of results was undertaken. Studies
 160 were grouped by outcome measure (behaviour change, diarrhoea, and STH infection) and by
 161 secondary outcome (knowledge) to allow for qualitative comparison.

162 The review was reported according to the Preferred Reporting Items for Systematic Reviews and
 163 Meta-Analyses (PRISMA guidelines) (21). A PRISMA checklist can be found in Appendix S2.

164 **Quality Assessment**

165 Two reviewers (JW and OC) independently assessed the risk of bias in studies selected for inclusion
 166 in the review using the Cochrane 'Risk of Bias' Assessment Tool (22). This tool is designed to assess if
 167 adequate steps have been taken to reduce bias across five domains by assessing sources of bias in
 168 each domain. 'Risk of bias' judgements were categorised as 'high risk', 'low risk' or 'unclear risk'.
 169 Table 1 outlines the assessment undertaken for each domain.

170 **Table 1: Tool for assessing risk of bias**

DOMAIN	SOURCE OF BIAS	ASSESSMENT
Selection bias	Random sequence generation	Studies were categorised as 'low risk' if method used to generate allocation was sufficient to produce comparable groups
	Allocation concealment	Studies were categorised as 'low risk' if concealment of allocation before assignment was sufficient to ensure intervention allocations could not have been foreseen before or during enrolment
Performance bias	Blinding of participants and personnel	Studies were categorised as 'low risk' if trial participants and researchers were blinded from knowledge of which intervention a participant received and if intended blinding was effective
Detection bias	Blinding of outcome assessment	Studies were categorised as 'low risk' if outcome assessment was blind from knowledge of which intervention a participant received and if intended blinding was effective
Attrition bias	Incomplete outcome data	Studies were categorised as 'low risk' if outcome data was complete for each main outcome, including attrition and exclusions from the analysis. The reviewers assessed if attrition and exclusions were reported, the numbers in each intervention group (compared with total randomised participants), if reasons for attrition or exclusions were reported, and any re-inclusions in analyses for the review
Reporting bias	Selective reporting	Studies were categorised as 'low risk' if publication of outcomes measured, or of analyses performed, was complete

171

172 To assess the quality of NRCTs and CBAs, two additional criteria were included, as used in a recent
 173 relevant Cochrane Review (23):

- 174 (i) comparability of baseline characteristics - studies were categorised as 'low risk' if
175 baseline characteristics were similar between the intervention and control groups.
176 (ii) contemporaneous data collection - studies were categorised as 'low risk' if data were
177 collected at similar points in time in the intervention and control groups.

178 **Results**

179 **Search Results**

180 A total of 2,827 studies were identified from Medline (349), Embase (494), Global Health (390),
181 Cinhal (183), Africa-Wide Information (125), Scopus (865), IBSS (19) and Web of Science (402). One
182 further study was identified from reference-list scanning and was also included in the final analysis.
183 After de-duplication, 1,300 studies were screened by title and abstract and 43 studies selected for
184 full-text screening. Applying the pre-defined inclusion criteria, 8 studies were selected for inclusion
185 in the final analysis (24-31). The flow diagram in Figure 1 outlines the results of the database
186 searches and the screening process, according to PRIMSA guidelines (21). Appendix S3 lists the
187 reasons for excluding the 35 studies on full-text screening.

188 **Characteristics of Included Studies**

189 Full details of the characteristics of included studies can be found in Appendix S4.

190 **Settings and Participants**

191 Studies were conducted across six different countries; Malaysia (1), Peru (1), India (1), Egypt (1),
192 China (2), and Kenya (2). All studies targeted children of primary-school age, between the ages of
193 five and twelve. Seven of the studies selected for inclusion were implemented in primary schools
194 (24-28, 30, 31) and the one remaining study (Nicholson, 2014) (29) was implemented in
195 communities, but targeted five-year-old children attending the first grade of a primary school.

196 **Study Design and Length**

197 Of the eight included studies, seven were cluster-RCTs (25-31) and one was a cluster-NRCT (24). No
198 eligible CBAs were identified. Six of the cluster-RCTs used schools as the unit of randomisation (25-
199 28, 30, 31) and the other used low-income communities (29). The NRCT used schools as the unit of
200 allocation (24). The intervention length of the included studies ranged from eight to forty-one weeks
201 and intervention intensity was graded as 'high' in the six of the studies (24-26, 28, 29, 31).

202 **Intervention**

203 Of the eight included studies, four employed interventions focussed exclusively on handwashing
204 promotion (26, 27, 29-31) and three studies employed interventions that promoted general hygiene
205 messages around STH transmission and prevention, including handwashing (24, 25, 28). One study
206 (Pickering, 2013), a three-arm cluster RCT, compared two independent interventions of combined
207 soap provision and handwashing promotion versus a waterless hand sanitizer and hand cleaning
208 promotion (30). For this study we considered the results of both the soap and hand sanitizer
209 interventions. The interventions in five of the studies included soap or hand sanitizer provision (24,
210 26, 29-31), whereas, soap was not provided as part of the intervention in the other three studies (25,
211 27, 28). Table 1 outlines the intervention activities, intervention intensity, and soap provision in each
212 of the studies. More detailed characteristics of included studies can be found in appendix S4.

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Figure 1: PRISMA Flow Diagram

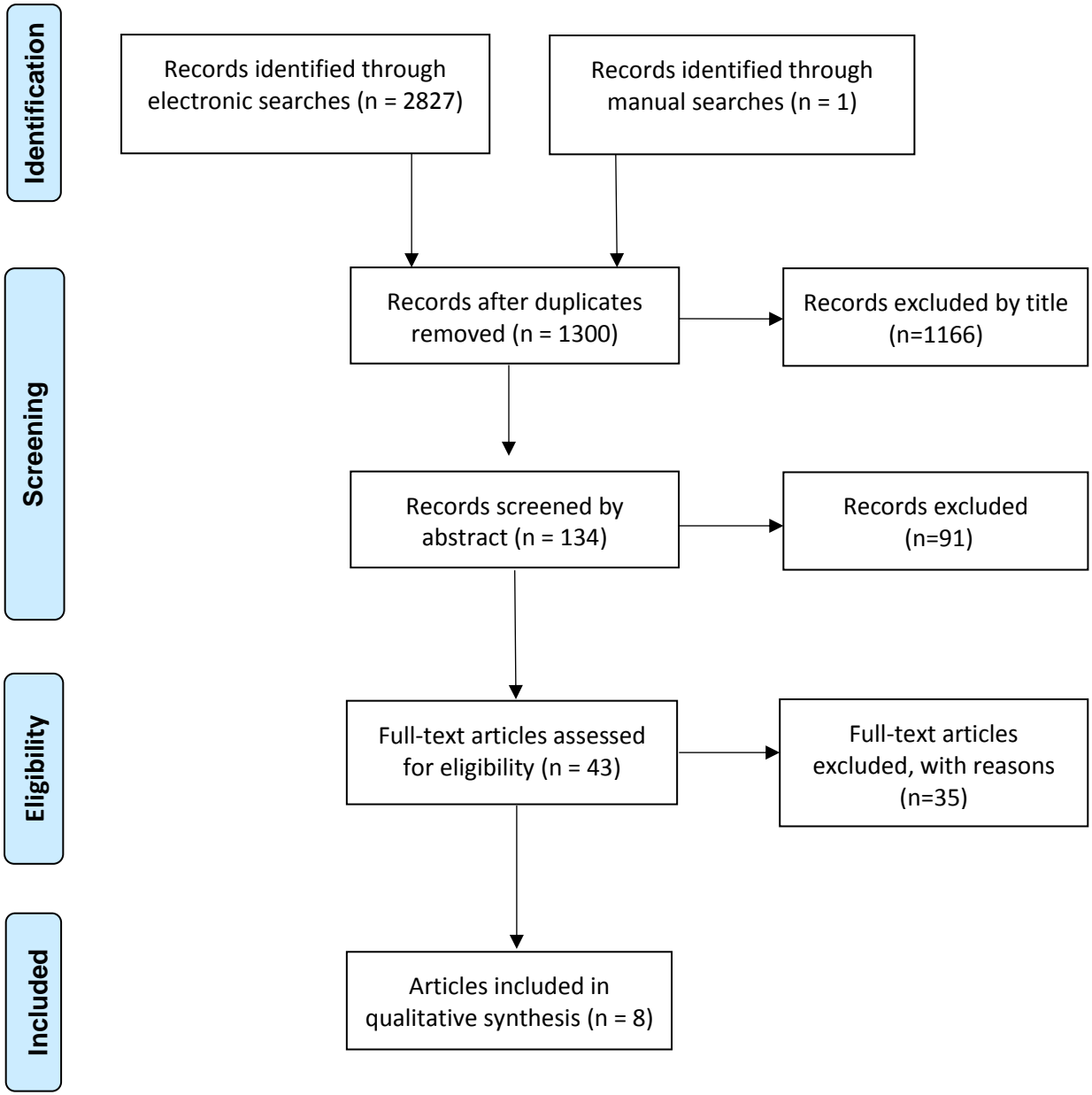


Table 2: Intervention Activities

Study	Intervention Activities	Intervention Intensity	Soap Provision
Al-delaimy (2014) (24)	Fun activities (comics books, drawing, puppet shows, nurse song videos, mascot)	High - activities repeated regularly throughout length of intervention (up to twice a week]	Soap provided
Bieri (2013) (25)	'Magic Glasses' cartoon, group discussions, drawing and essay competitions	High - activities throughout length of intervention	No soap provided
Bowen (2007) (26)	Standard intervention: 40-minute classroom session (animated videotape, hygiene competition, posters) Expanded intervention: standard intervention plus peer handwashing monitors	Standard: Low – 1 session only Expanded: High - 1 session plus regular input from peer monitors	Standard: one soap bar (hygiene pack) Expanded: continuous supply
Graves (2011) (27)	Poster design competition	Low – 1 session only	No soap provided
Gyorkos (2013) (28)	60 minute class on STH transmission and prevention and poster display	High - initial 1 hour session followed by 30 minute refresher activities every 2 weeks throughout length of intervention	No soap provided
Nicholson (2014) (29)	Fun activities (songs, poems and stories), environmental cues (wall hanger etc.), HWWS rewards (stickers, toys, animals etc.), children encouraged to advocate HWWS at home), HWWS pledges for children and mothers, 'Best Mums' club.	High - activities throughout length of intervention	Soap provided
Pickering (2013) (30)	Distribution of hygiene promotion kits for teacher-use (posters, stickers, classroom activities, DVD, promotional songs)	Unclear	Soap schools: liquid soap provided Sanitizer schools: liquid hand sanitizer provided
Talaat (2011) (31)	Fun activities (e.g. games), poster displayed near sinks, songs. Supervised HWWS twice daily.	High - activities repeated throughout length of intervention (at least one activity per week)	Soap provided

247 **Outcomes**

248 Table 3 shows a summary of the outcomes measured in each study and if a positive effect was
 249 observed. To facilitate comparison, the studies were categorised according to their outcomes.
 250 Studies were marked as having a ‘positive effect’ if there was an increase in handwashing behaviour,
 251 a reduction in diarrhoea, a reduction in STH infection, and/or an increase in knowledge related to
 252 handwashing, in the intervention group compared to control group, and the effect was statistically
 253 significant at P<0.05. Due to heterogeneity of the studies in terms of interventions and outcome
 254 measures, a meta-analysis was not considered appropriate and a narrative summary of the results is
 255 presented below. The magnitude of the positive effect is also presented in the narrative summary.

256 **Table 3: Study Outcomes and Effects**

Outcome	Study	Outcome Measurement	Outcomes measured	Positive Effect
BEHAVIOUR	Al-delaimy (2014) (24)	KAP survey	Washing hands before eating	✓
			Washing hands after defecation	✓
			Washing hand with soap	✓
	Bieri (2013) (25)	Observations	Washing hands after toilet	✓
	Graves (2011) (27)	Observations	Handwashing	✗
	Gyorkos (2013) (28)	KAP survey	Washing hands after toilet	✗
			Using soap when washing hands after toilet	✗
			Washing hands before eating	✗
			Using soap when washing hands before eating	✗
	Nicholson (2014) (29)	Soap wrapper collection	Soap consumption	✓
Pickering (2013) (30)	Observations	Soap Intervention		
		Hand cleaning after toilet use	✗	
		Soap intervention – hand cleaning before eating	✗	
		Hand Sanitizer Intervention		
Hand cleaning after toilet use	✓			
Before eating	✗			
DIARRHOEA	Bowen (2007) (26)	Teacher records	Standard Intervention	
			Diarrhoea Incidence	✗
	Expanded Intervention			
	Diarrhoea Incidence	✗		
	Nicholson (2014) (29)	Caregiver interviews	<u>Predictive relative risk reduction (Intention to treat analysis)</u>	
			Target children	✗
			Children aged ≤ 5 (non-target)	✓
Children 6-15 (non-target)			✓	
Whole families	✓			
Pickering (2013) (30)	Student interviews	Soap Intervention		
		Diarrhoea prevalence	✗	
Sanitizer Intervention				
Diarrhoea prevalence	✗			

Outcome	Study	Outcome Measurement	Outcomes measured	Positive Effect
	Talaat (2011) (31)	Teacher records	School absence due to diarrhoea	✓
STH	Al-delaimy (2014)(24)	Laboratory analysis	<i>A. lumbricoides</i> re-infection <i>A. lumbricoides</i> infection intensity	✗ ✓
	Bieri (2013)(25)	Laboratory analysis	STH Incidence STH infection intensity	✓ ✗
	Gyorkos (2013)(28)	Laboratory analysis	<i>A. lumbricoides</i> prevalence <i>A. lumbricoides</i> infection intensity	✗ ✓
KNOWLEDGE	Al-delaimy (2014)(24)	KAP survey	Knowledge of handwashing as a STH infection preventative measure	✓
	Bieri (2013)(25)	KAP survey	Knowledge of handwashing as a STH infection preventative measure	✓
	Gyorkos (2013)(28)	KAP survey	Knowledge of handwashing as a STH infection preventative measure	✓

257

258 **Handwashing Behaviour Change**

259 Six studies measured the effect of handwashing promotion on handwashing behaviour change (24,
260 25, 27-30).

261 Across the studies, three methods were used to measure handwashing behaviour change. Al-
262 delaimy (2014) (24) and Gyorkos (2013) (28) used self-reported measures. Bieri (2013) (25), Graves
263 (2011) (27) and Pickering (2013) (30) used structured observations and Nicholson (2014) (29)
264 indirectly assessed handwashing behaviour using soap consumption as a proxy measure (soap
265 wrapper collection).

266 Al-delaimy (2014) (24) measured the handwashing behaviour of the parents of target children, at 12-
267 weeks follow-up, and reported that the proportion of the parents practising handwashing in the
268 intervention group was three-and-a-half times higher than the proportion of parents practising
269 handwashing in the control group, both before eating (odds ratio [OR] 3.5, 95% confidence interval
270 [CI]: 1.9-6.4), and after using the toilet (OR 3.5, 95% CI: 1.7-7.1). Soap was supplied in this
271 intervention and the odds of HWWS was six and a half times higher in the parents in the
272 intervention group, compared to parents in the control group (95% CI: 3.2-13.1). Gyorkos (2013) (28)
273 found no statistically significant difference (at the 5% significance level) between proportions of
274 children washing their hands before eating or after visiting the toilet at the 16-week follow-up, and
275 no difference in children using soap to wash their hands. Bieri (2013) (25) found a statistically
276 significant increase in the number of children who washed their hands after toilet use in the
277 intervention group compared to the control group (44.6% increase, 95% CI: 10.1%-79.1%, P=0.005)
278 at 36-weeks follow-up. Graves (2011) (27) reported no significant difference in the proportion of
279 children practicing handwashing after toilet use, at 16-weeks follow-up; the mean difference in the
280 proportion of students washing their hands was 0.07 (95% CI: -0.13, 0.27). Pickering (2013) (30)
281 reported no significant differences in handwashing at intervention schools compared to control
282 schools after toilet use in (prevalence ratio = 1.0, 95% CI: 0.3-3.8) and before eating (prevalence ratio
283 = 1.2, 95% CI: 0.7-2.0). Nicholson (2014) (29) reported a median soap consumption of 45g per
284 household in control group, compared to 235g per household in the intervention group.

285 **Soil-Transmitted Helminth Infection**

286 Three studies reported the effect of hygiene promotion interventions, which included messages
287 around handwashing, on STH infections (24, 25, 28).

288 Although Al-delaimy (2014) (24) showed a significant decrease in hookworm infection rates in the
289 intervention group compared to the control group 24-weeks after deworming (75.5% vs 39.6%,
290 $P < 0.05$), the reduction in *A. lumbricoides* infection rates in the intervention group were not
291 significant (82.3% vs 63.3% $P > 0.05$). This study did however show a significant decrease in the
292 intensity of *A. lumbricoides* at the 24-week follow-up, assessed as the mean *A. lumbricoides* egg
293 count per gram of faeces. Bieri (2013) (25) reported significant reductions in incidence of STH
294 infections, 36 weeks after deworming, between the intervention group and control group (OR 0.50,
295 $P < 0.001$), but not in the intensity of infections (OR 1.12, $P = 0.12$), assessed as the geometric mean
296 number of eggs per gram of faeces. Although researchers present results as 'all STHs', 100% of the
297 infections detected were *A. lumbricoides* and thus were amenable to the handwashing promotion
298 intervention (25). Gyorkos (2013) (28) showed no significant difference in *A. lumbricoides* infection
299 between the intervention group and the control group 16-weeks post-deworming (adjusted odds
300 ratio 0.88, 95% CI: 0.57-1.34), however, the intensity of *A. lumbricoides* infection was significantly
301 lower in the intervention group (adjusted incidence rate ratio 0.42, 95% CI: 0.21-0.85).

302 **Diarrhoea**

303 Four studies measured the effect of handwashing on diarrhoea (26, 29-31).

304 Talaat (2011) (31) measured the incidence of school absence due to diarrhoea among children (in
305 the first three grades of primary school) and reported incidence was 33% lower in the intervention
306 school compared to the control school ($P < 0.0001$, no 95% CI given). This intervention included a
307 'Hand Hygiene Team' comprising three teachers who supervised children to ensure handwashing
308 was being practised, a method that may account for the pronounced effect of the intervention.
309 Bowen (2007) (26) also measured diarrhoea incidence using teacher records of school absence due
310 to diarrhoea, as well as diarrhoea reported during school time, however, the incidence of diarrhoea
311 was reported to be zero in control, standard intervention, and expanded intervention groups, and
312 thus no significant difference reported. Pickering (2013) (30) measured prevalence of diarrhoea, as
313 reported in interviews with children, and found no significant effect in either the soap intervention
314 group (risk ratio 0.84, 95% CI: 0.58-1.22, $p = 0.36$) or the waterless hand sanitizer group (risk ratio
315 0.89, 95% CI 0.61-1.30, $p = 0.56$) at 8-weeks follow-up, although the authors highlight that the study
316 was not designed to have adequate power to detect effects on health outcomes. Nicholson (2014)
317 (29) reported the effect of the intervention on diarrhoea incidence in the target children (age 5), and
318 in household members stratified by different age groups (under-5's, ages 6-15, and adults),
319 measured by interviews with caregivers. In the per-protocol analysis, the target children in the
320 intervention group were reported to have a predictive relative risk reduction (PRRR) of 21.3% (95%
321 CI: 36.6%-2.3%), however, in the intention-to-treat (ITT) analysis the PRRR was no longer significant.
322 The PRRRs for the under-5's, 6-15-year olds, and whole families was similar to that of the target
323 children, however, all remained significant in the ITT analysis.

324

325 **Knowledge**

326 The three studies that focused on education around STH also measured changes in knowledge as a
327 secondary outcome, along with STH infection and handwashing behaviour, and all reported
328 statistically significant increases in knowledge (24, 25, 28). Bieri (2013) (25) reported a 32.8
329 percentage point increase (95% CI: 28.9%-36.8%, $p < 0.001$) in the KAP scores (measuring knowledge
330 of STH transmission, symptoms, prevention and treatment) of the intervention group compared to
331 the control group, however, these results may be biased as KAP scores were also higher in the

332 intervention group at baseline. Gyorkos (2013) (28) reported significantly higher KAP scores in the
333 target children in the intervention group compared to the control group (OR 18.4, 95% CI: 12.7-26.6)
334 and Al-delaimy (2014) (24) measured knowledge of handwashing as a STH infection preventative
335 measure in parents of the target children, using KAP surveys, and recorded significantly higher
336 scores from parents in the intervention group compared to parents in the control group (OR 2.5,
337 95% CI: 1.5-4.1).

338 **Quality Assessment**

339 Judgements about the risk of bias are summarised in Figure 2 and Figure 3. The full quality
340 assessment is presented in Appendix S5.

341 The random sequence was judged to be adequately generated in five out of the seven cluster-RCTs
342 and these studies were classed as having a 'low risk' of bias (25, 26, 28, 29, 31). In the other two
343 cluster-RCTs the sequence generation was unclear (27, 30). The method of allocation concealment
344 was classed as 'low risk' in Gyorkos (2013) (28), whilst the risk was 'unclear' in all other cluster-RCTs.
345 Five of the studies were at 'low risk' of confounding bias (24, 28-31) and the other three studies
346 were classed as 'high risk' because of differences in soap availability (Graves [2011]) (27), KAP scores
347 (Bieri [2013]) (25), household water and sanitation, and student age (Bowen [2007]) (26), at
348 baseline. Data were collected contemporaneously, and classed as 'low risk', in all studies except for
349 Bowen (2007) (26), which was classed as 'high risk' due to the replacement of some schools in the
350 study during the second week of data collection. Seven studies were judged to have a 'high risk' of
351 performance bias as neither of the participants or the personnel were blinded (24-30), whilst the
352 blinding status of participants or personnel could not be determined in Talaat (2011) (31). Seven of
353 the studies had a 'high risk' or 'unclear risk' of detection bias as the outcome assessors were not
354 blinded to intervention status or blinding was unclear (24-27, 29-31), whilst Gyorkos (2013) (28) was
355 judged to have a 'low risk' of detection bias as the laboratory technologists testing STH in stool
356 samples were blinded to the intervention. In four of the studies, over 80% of those allocated to the
357 study were included in the analysis and these studies were classed as 'low risk' of attrition bias (25,
358 26, 28, 31). Al-delaimy (2014) (24) and Pickering (2013) (30) did not report loss-to-follow-up and
359 hence, the risk of attrition bias was unclear. Graves (2011) (27) and Nicholson (2014) (29) were
360 classed as 'high risk' of attrition bias, with less than 80% of participants allocated to the study,
361 included in the analysis. Other sources of bias identified in the studies were lack of adjustment for
362 clustering in the analysis (Nicholson [2014] (29) and Al-delaimy [2014] (24)) and misrepresentation
363 of the source population (Bowen [2007] (26) and Al-delaimy [2014] (24)).

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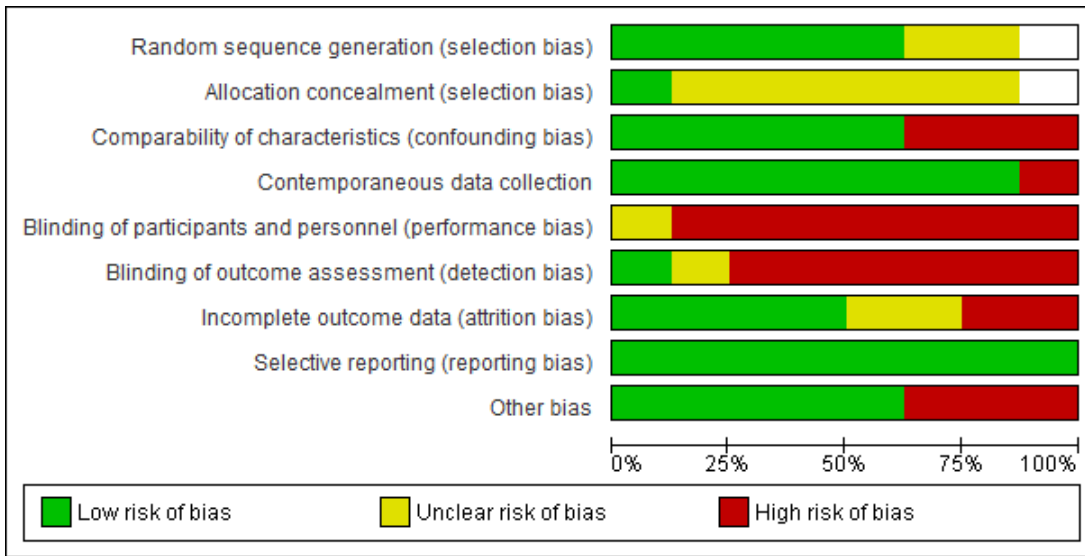
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371 **Figure 2:** Risk of Bias Graph: review authors' judgements about each risk of bias item presented as
 372 percentages across all included studies



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375 **Figure 3:** Risk of Bias Summary: review authors' judgements about each risk of bias item for each
 376 included study

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Comparability of characteristics (confounding bias)	Contemporaneous data collection	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Al-delaimy 2014			+	+	-	-	?	+	-
Bieri 2013	+	?	-	+	-	-	+	+	+
Bowen 2007	+	?	-	-	-	-	+	+	-
Graves 2011	?	?	-	+	-	-	-	+	+
Gyorkos 2013	+	+	+	+	-	+	+	+	+
Nicholson 2014	+	?	+	+	-	-	-	+	-
Pickering 2013	?	?	+	+	-	-	?	+	+
Talaat 2011	+	?	+	+	?	?	+	+	+

377

378 **Discussion**

379 The aim of this systematic review was to synthesise evidence on the effectiveness of handwashing
380 promotion targeted at children on diarrhoea, STH infection and handwashing behaviour, in LMICs.

381 The main finding from the review is that the evidence base for child-focused handwashing
382 promotion in LMICs, is extremely limited; only eight relevant studies were found (24-31) and meta-
383 analysis was not deemed possible due to heterogeneity in the interventions and measurement of
384 outcomes across the studies. This was also evident in a recent review of the effect of handwashing
385 promotion on diarrhoea, in which only three trials were identified that were conducted in schools or
386 day care centres in LMICs (1). Studies also suffered from a number of design limitations which
387 compromised the validity of their findings. The heterogeneity of the results, however, reflect the
388 'real-world' circumstance of handwashing promotion and hence a qualitative approach to
389 synthesising the evidence is necessary.

390 Our review showed mixed evidence on the effectiveness of handwashing promotion, targeted at
391 children, on infection with the STH, *A.lumbricoides*. Only one of the three studies identified showed
392 a statistically significant reduction in *A.lumbricoides* infection in children (25), whilst two of the
393 studies showed a significant reduction in *A.lumbricoides* intensity (24, 28). These studies, however,
394 may have been affected by bias due to a lack of blinding of the assessors. In one study that did blind
395 the laboratory technologists assessing STH infection, and therefore was at a low risk of detection
396 bias, no significant effect on *A.lumbricoides* infection was recorded (28).

397 Handwashing promotion targeted at children was only reported to have a significant effect on
398 diarrhoea in the intervention target children in one study, in which handwashing was obligatory and
399 teacher-supervised, potentially masking the true effects of the other hygiene promotion activities in
400 this study (31). No other significant effects on diarrhoea incidence were reported in the other
401 studies, however, incidence of diarrhoea was measured by self-report or through care-giver reports
402 across all studies. As the responders were not blinded to the intervention, these reports are at high
403 risk of response bias, influenced by perceived social desirability, and thus diarrhoea is likely to be
404 under-reported and may not accurately represent the effectiveness of the interventions (32). A
405 meta-analysis in Ejemot's (2015) review did show handwashing promotion to have a positive effect
406 on the diarrhoea incidence of children within child day-care centres or schools in LMICs (rate ratio
407 0.66, 95% CI: 0.43-0.99), however this meta-analysis only included two trials which were both
408 graded as low quality (1).

409 All three of the studies in this review which used hygiene-related knowledge as a secondary
410 outcome measure of intervention effect recorded a significant increase in knowledge post-
411 intervention (24, 25, 28). However, although knowledge is quick and easy to measure it is not a good
412 proxy indicator of behaviour change as it does not necessarily translate into behaviour change (33),
413 as evident in Gyorkos' (2013) (28) study where children in receipt of the intervention scored
414 significantly higher on a STH-related knowledge survey but no significant change in handwashing
415 behaviour was recorded. This intervention also had no significant effect on *A. lumbricoides* infection.
416 By contrast, Bieri (2013) (25) and Al-delaimy (2014) (24) did both show a significantly higher increase
417 in knowledge as well as behaviour in the intervention group compared to the control group.
418 However, all studies measured behaviour outcomes in different ways - observations of target-
419 children's handwashing in Bieri (2013) (25), self-report of target-children's handwashing in Gyorkos
420 (2013) (28) and self-report of parent's handwashing in Al-delaimy (2014) (24) – and hence,
421 comparisons should be made with caution. Though knowledge is necessary for behaviour change it is

422 not always sufficient and thus studies assessing the effect of handwashing promotion interventions
423 should also include direct measures of behaviour change wherever possible.

424 Only three of the eight studies in our review used direct observations to measure handwashing
425 behaviour change (25, 27, 30), whilst the remaining studies measuring handwashing behaviour used
426 self-report, via KAP surveys (24, 28), or soap consumption as a proxy measure (29). Whilst using self-
427 reported behaviour and soap consumption to measure handwashing may be easier and less
428 expensive than direct observations, as less enumerator time and training is required, the validity of
429 these measures is questionable. Participant awareness of the social desirability of handwashing,
430 coupled with possible courtesy bias, is likely to lead to an overestimation of self-reported
431 handwashing behaviour (32) and proxy measures such as soap consumption do not necessarily
432 correlate with actual practice or prevalence of handwashing (34). Direct observation of behaviours is
433 considered the current 'gold standard' for measuring handwashing (34), though it is still at risk of
434 bias; the presence of an observer has been shown to introduce reactivity and observed individuals
435 may over-perform, leading to overestimates of actual behaviour (35, 36). However, only one of the
436 studies with observed handwashing behaviour (25) saw an overall statistically significant increase in
437 the handwashing practices of children post-intervention compared to pre-intervention, which may
438 suggest the effect of reactivity bias in schools was minimal. Though Nicholson (2014) (29) did record
439 an increase in hand cleaning after using the toilet in the hand sanitizer intervention no such effect
440 was recorded in the soap intervention group.

441 The range of methods used to assess changes in behaviours across the studies made direct
442 comparisons of findings difficult. Meta-analysis would be facilitated if future studies used more
443 consistent measures of behaviour change to enable comparison. Direct observation should be the
444 outcome measure selected where possible to improve the validity of results. Furthermore, a
445 standard unit of measurement, such as the proportion of participants HWWS at a specified moment,
446 such as after defecation, would better enable comparative analysis. The use of covert video cameras
447 in both schools and homes has become increasingly common; however video surveillance has also
448 been shown to introduce reactivity (37) and remains logistically difficult and expensive.

449 All of the handwashing promotion interventions identified in this review were targeted at children
450 attending primary school, between the ages of five and twelve. There is a clear lack of handwashing
451 promotion interventions targeting teenagers, who may represent a potentially very important group
452 in the disruption of the pathogen transmission considering the high adolescent fertility rate in low
453 income settings, which may indicate a large number teenagers in caregiving roles (38). Another
454 overlooked target group, identified by this review, is children who do not attend school, the
455 numbers of which are substantially higher in LMICs than in high income countries (38). The findings
456 of Ejemot's (2015) review also highlights this, with no trials included which were focussed on
457 teenagers or out-of-school children (1).

458 A lack of good quality evidence exists to prioritise specific handwashing promotion interventions
459 targeted at children in LMICs. A variety of intervention methods are being employed to promote
460 handwashing among children and not one accepted method of implementation or outcome measure
461 has yet come to the forefront as the most effective. Due to the limited number of studies and
462 heterogeneity of interventions, we were not able to assess the relationship between intervention
463 effectiveness and the duration or intensity of the intervention. However, a recent systematic review
464 of school-based interventions to modify dietary behaviour found no relationship between
465 intervention intensity and effectiveness (39).

466 There has been some recent innovation in handwashing behaviour change science. The Behaviour
467 Centred Design (BCD) framework offers a new generalized approach to behaviour change which
468 incorporates both a theory of change for behaviour as well as a practical process for designing and
469 evaluating interventions (40). BCD aims to change behaviour through surprise, reevaluation and
470 disruption of performance rather than traditional ‘messaging’ and has been used successfully in the
471 design and evaluation of handwashing interventions, for example the SuperAmma programme in
472 rural India (41). Central to the BCD framework is changing both the environment and the brain
473 (cognitive processes related to a specific behaviour). Pilot research in Bangladesh found large,
474 sustained changes in handwashing behaviour associated with nudges – environmental changes in
475 schools that included brick paths and painted symbols that prompted handwashing behaviours (42).
476 Larger trials examining the effect of environmental modification on handwashing outcomes in
477 schools are underway (43). While more evidence is needed, environmental modification may
478 present a viable approach to changing handwashing behaviours in schools.

479 This review had some limitations. Firstly, because the studies were judged too heterogeneous to
480 conduct a meaningful meta-analysis, no quantitative conclusions could be drawn. Due to the
481 heterogeneity of the studies it was also not feasible to assess publication bias, however, many of the
482 studies did report negative findings indicating that publication bias was not an important bias in this
483 review. One potential method of reducing publication bias would be to include unpublished studies,
484 though unpublished studies may be of lower quality and do not always reduce the publication bias
485 but often alter the effect size (44). Whilst this review only included concurrently controlled trials,
486 there may also be some useful information to gain from those uncontrolled studies excluded from
487 this review, especially as in low-income settings, RCTs and non-randomised controlled trials are
488 often considered ethically or financially challenging. Inclusion of these lower quality studies,
489 however, may have resulted in inclusion of evidence with an unacceptably high risk of bias.
490 Additionally, the exclusion of non-English language studies from this review may limit the
491 generalisability of the findings since we may have excluded valid international work. A final
492 limitation of this review is the exclusion of studies where the effect of the handwashing promotion
493 intervention could not be distinguished from the effect of other WASH improvements. Whilst this
494 was necessary to assess the effectiveness of handwashing promotion interventions, it does not
495 reflect the best approaches to improving health through hygiene where access to water, improved
496 water quality, and sanitation also play an important role. Organizational support is a key factor in the
497 sustainability of health service interventions (45). In the school-based handwashing promotion
498 interventions identified in our review, soap supply, WASH infrastructure and maintenance, along
499 with other organizational aspects of handwashing, over which children have very little agency, will
500 impact the sustainability of these interventions and are important considerations.

501 Whilst regular handwashing with soap is regarded as an effective and cost-effective public health
502 measure, no previous reviews have assessed whether interventions targeting children are effective
503 in changing handwashing behaviours nor health outcomes. Our review found just eight studies that
504 evaluated such interventions and those identified were heterogeneous in nature and had various
505 methodological limitations. As much of the hygiene attributable disease burden is concentrated
506 among children, it is plausible that interventions which succeed in changing children’s handwashing
507 practices will lead to significant health impacts. The current paucity of evidence in this area however
508 does not permit any recommendations to be made as to the most effective route to increasing
509 handwashing with soap practice among children in LMIC.

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- 731

Appendix S1

Detailed Search Strategy and Hits – Medline

Children		Hits
1	(child* or youth* or minor* or adolescent* or teenager* or schoolchild* or "school child*" or "school age" or "school-age" or "school going" or "school-going" or pupil* or "young person*" or "young people" or kid* or junior* or "young adult*").ab,kw,ti,tw.	1814609
Handwashing promotion		
2	(handwash* or "hand-wash*").ab,kw,ti,tw.	3338
3	exp Hand Hygiene/	4871
4	(hand\$1 adj3 (hygien* or clean* or disinfect* or decontaminat* or antisepsis or wash* or sterili* or sanit* or soap*)).ab,kw,ti,tw.	6162
5	2 or 3 or 4	8967
6	(promotion* or education* or intervention* or program* or training* or lesson* or campaign* or project*).ab,kw,ti,tw.	1773319
7	5 and 6	2882
Diarrhoea		
8	diarrh*.ab,kw,ti,tw.	83287
9	exp Diarrhea/	47282
10	"gastroenteri*".ab,kw,ti,tw.	15882
11	exp Gastroenteritis/	174407
12	(enteric adj3 (infection* or disease*)).ab,kw,ti,tw.	3582
13	exp Enterobacteriaceae Infections/ or exp Enterobacteriaceae/	391097
14	(waterborne adj3 (infection* or illness*)).ab,kw,ti,tw.	271
15	(cholera or shigell* or dysenter* or cryptosporid* or giardia* or "Escherichia coli" or "E. coli" or rotavirus* or amoebic or clostridium).ab,kw,ti,tw.	340688
16	8 or 9 or 10 or 11 or 12 or 13 or 14 or 15	748777
Soil-transmitted helminths		
17	("soil-transmitted helminth*" or geohelminth* or "geo-helminth*" or "geo helminth*" or STH" or ascari* or roundworm* or nematode* or trichuri* or whipworm* or ancylostom* or necator* or hookworm*).ab,kw,ti,tw.	40962
18	exp Ascaris/ or exp Ascariasis/ or exp Trichuris/ or exp Trichuriasis/ or exp Ancylostoma or exp ancylostomatoidea/ or exp ancylostomiasis/ or exp necator/ or exp necatoriasis/ or exp hookworm infections/	13910
19	17 or 18	43923
Behaviour		
20	behavio?r.ab,kw,ti,tw	541263
Children & handwashing promotion & (diarrhoea or STH)		
21	16 or 19 or 20	1320439
22	1 and 7 and 21	349

Appendix S2

PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Not applicable
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	2-3
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	2-3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Appendix S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	3
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	4
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Appendix S3
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Not applicable

Appendix S3

Characteristics of Excluded Studies (ordered by study ID)

Study	Reason for exclusion
Ahmed (1994) (46)	Intervention not targeted at children
Ankur (2013) (47)	No concurrent control group
Annesi (2010) (48)	Not published in a peer-reviewed journal
Aslan (2006) (49)	Not published in English
Au (2010) (50)	Conducted in a high-income country
Biran (2009) (51)	Intervention targeted both children and women and results not disaggregated
Birran (2014) (41)	Intervention targeted both children and adults and results not disaggregated
Borzekowski (2015) (52)	Only published as a conference abstract
Boubacar Mainassara (2014) (53)	Multiple water and sanitation interventions implemented concurrently
Dongre (2007) (54)	No concurrent control group
Dreibelbis (2012) (55)	Published only as abstract
Dreibelbis (2014) (56)	Water and sanitation interventions implemented concurrently
Dreibelbis (2016) (42)	No concurrent control group
Fishbein (2011) (57)	Conducted in a high-income country
Freeman (2013) (58)	Water and sanitation interventions implemented concurrently and unclear if handwashing is part of the hygiene promotion
(Freeman 2014a) (59)	Water and sanitation interventions implemented concurrently and unclear if handwashing is part of the hygiene promotion
Galiani (2012) (60)	Not published in a peer-reviewed journal and intervention targeted at both children and communities
Geetharani (2016) (61)	Published only as a conference abstract and no concurrent control group
Gungoren (2007) (62)	Intervention targeting both children and adults and unclear if handwashing is a part of the hygiene promotion
Haggerty (1994) (63)	Intervention not targeted at children
Hosain (2003) (64)	Sanitation intervention implemented concurrently and unclear if handwashing is part of hygiene promotion
Kapadia (2014) (65)	Other health behaviours also promoted regarding nutrition, reproductive health etc.
Kaya (2009) (66)	Not published in English
Lang (2012) (67)	No concurrent control group
Le Thi Thanh (2003) (68)	No concurrent control group
Liao (2014) (69)	Only published as a conference abstract
Luby (2005) (70)	Intervention not targeting children
Luby (2004) (71)	Intervention not targeting children
Onyango-Ouma (2005) (72)	No concurrent control group

O'Reilly (2008) (73)	No concurrent control group and water treatment intervention implemented concurrently
Patel (2012) (74)	Water treatment and access interventions implemented concurrently, unclear if handwashing is part of the hygiene promotion, and intervention also targets community
Pinfold (1999) (75)	Intervention targeted at children as well as the community
Sahin (2008) (76)	Not published in English and no concurrent control group
Shrestha (2015) (77)	No concurrent control group
Trinies (2016) (78)	Multiple water and sanitation interventions implemented concurrently and unclear if handwashing is a part of the hygiene promotion

Appendix S4

Characteristics of Included Studies

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
Al-Delaimy (2014)	Health Education Learning Package (HELP) Key messages on STH prevention: 1. child HWWS before eating, after playing with soil and after toilet use. 2. avoiding open defecation 3. washing fruits and vegetables 4. drinking clean water 5. covering food from flies 6. cutting nails periodically	1. teacher training workshop 2. posters 3. comic book 4. drawing activities 5. puppet show 6. nursery songs videos 7. mascot 8. group discussions 9. distribution of sanitary bags (slippers, hand soap and nail clippers)	No HELP intervention in control school	Malaysia (Lipis, Pahang)/ Orang Asli primary schools	Cluster NRCT	24 weeks/ High (activities repeated regularly over length of intervention [up to twice a week]).	Outcome 1: STH (<i>trichuriasis</i> , <i>ascariasis</i> , hookworm) reinfection rate and reinfection intensity in school children Method: Laboratory testing of faecal samples (intensity measured by egg counts) Outcome 2: handwashing practices of parents Method: KAP survey Outcome 3:	Number: 2 schools, 317 students (Orang Asli) (172 from intervention school, 145 from control school) Age: 6-12 (median age = 9)	Soap supplied	Outcome 1: 24-week follow-up:- intervention group had 3.7% (P>0.05), 19% (P>0.05) and 36.2% (P<0.05) lower reinfection rates of <i>trichuriasis</i> , <i>ascariasis</i> and hook worm (respectively) compared to control. The intensity of <i>Trichuris</i> , <i>Ascaris</i> and hookworm reinfections reduced by 19.4% (P>0.05), 33.2% (P<0.05) and 65.4% (P<0.05) more in the intervention group than in the control group Outcome 2: 12 week follow-up (intervention group vs. control) Washing hands before eating OR = 3.5 (1.9, 6.4), p<0.001 Washing hands after defecation

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
							Handwashing knowledge of parents Method: KAP survey			OR = 3.5 (1.7, 7.1), p<0.001 Washing hand with soap OR = 6.5 (3.2, 13.1), p<0.001 Outcome 3: 12-week follow-up Knowledge of handwashing as a STH infection preventative measure OR = 2.5 (1.5, 4.1), p<0.001
Bieri (2013)	Health Education Package Key messages on STH transmission and prevention: 1. Handwashing before eating and after toilet use 2. Avoiding open defecation 3. Shoe wearing 4. Covering food 5. Washing fruit and vegetables 6. Seeking treatment for worm infections	1.teacher training workshop 2. 'Magic Glasses' cartoon video on the topic of STH transmission and prevention. 3. Classroom discussions following cartoon. 4. Pamphlet with STH messages distributed.	Health education poster only (normal display in schools)	China (Linxiang City, Hunan province) / primary schools	Cluster RCT	36 weeks/ High (activities throughout length of intervention)	Outcome 1: STH incidence (<i>ascaris</i> and <i>trichuris</i>) in participants Method: laboratory testing of faecal samples. Outcome 2: Handwashing practice after toilet use at school Method: observations	Number: 38 rural primary schools (19 intervention and 19 control schools), 1718 students (825 from intervention school, 893 from control school). Age: 9 to 10	Soap not supplied	36-week follow-up Outcome 1: Incidence of STH OR = 0.50 (95% CI 0.35-0.70), P<0.001 Intensity of infection OR 1.12 (95% CI 0.97-1.29), P=0.12 *Adjusted for sex and school grade *Adjusted for clustering NB. All infection was <i>ascariasis</i> Outcome 2: 44.6% (10.1%-79.1%), P=0.005, more children washed hands after using toilet. *Adjusted for clustering

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
		5. Drawing and essay-writing competitions on STH.					by research staff Outcome 3: Knowledge on STH infection (transmission, treatment, prevention – including handwashing) Method: KAP survey with students			* Not adjusted for age and school grade Outcome 3: KAP score was significantly higher (32.8 percentage points, 95% CI 28.9-36.8, P<0.001) in the intervention group. NB. Also significantly higher at baseline. *adjusted for clustering, sex and school grade
Bowen (2007)	Hand hygiene education Key messages: 1. Handwashing before meals and after using the toilet 2. Proper handwashing technique (5 handwashing steps)	Standard Intervention Teacher training session and teacher-delivered 40 minute classroom session involving;	Standard government hygiene education (received by all arms of intervention) consisting of an annual	China (Fijian Province) / public primary schools	Cluster RCT	20 weeks/ Standard intervention – Low (1 session), Expanded intervention x High (1 session plus regular input from peer monitors)	Outcome 1: diarrhoea incidence in students (as cause of school absence), and in-class diarrhoea incidence Method: Teacher records	Number: 87 schools (28 standard intervention schools, 29 expanded intervention school and 30 control schools), 3962 students	Continuous supply of soap for schools receiving 'expanded intervention' 1 bar soap provided	20-week follow-up Outcome 1: Control intervention 0 episodes of diarrhoea per 100 student weeks Standard intervention 0 episodes of diarrhoea per 100 student weeks Expanded Intervention 0 episodes of diarrhoea per 100 student weeks

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
		1. Animated videotape 2. Classroom hygiene competitions 3. Posters 4. Student take-home pack (hygiene board game, parents' booklet about handwashing, soap) Expanded Intervention n = standard intervention n + 1. Continuous supply of soap at sinks	statement about washing hands					Age = 7 median (first grade students)	in take-home packs	

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
		2. Peer handwashing trainers and peer-monitoring								
Graves (2011)	Handwashing intervention Key messages: 1. HWWS	1. Poster-design competition promoting HWWS. Winning posters displayed in schools NB. Poster intervention was embedded within an existing program (NICHE) that provided handwashing infrastructure	No intervention	Kenya (Nyanza Province) /primary schools	Cluster-RCT	16 weeks/ Low (one session)	Outcome 1: proportion of children handwashing after defecating or urinating (using latrine or outside latrine) at school Methods: observations by researchers	Number: 21 schools (10 intervention, 11 control) Age: Not specified, primary school-age	Soap not supplied	16-week follow-up; Outcome 1: Mean difference in proportion of students washing hands (intervention - control) = 0.07 (-0.13, 0.27) (NS). Mean difference in change in proportion of students washing their hands (intervention - control) = 0.06 (-0.27, 0.38) (NS)

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
Gyorkos (2013)	Health hygiene education Key messages on STH transmission and prevention: 1. HWWS 2. Peeling and washing fruits 3. Wearing shoes 4. Avoiding open defecation 5. Other general hygienic behaviours	1. Half-day workshop for teachers and principals 2. 1-hour class on STH (transmission and prevention) led by research team 3. 30-minute refresher activities every 2 weeks over 4 months 4. Booklet distributed 5. Posters displayed	No intervention received	Peru (Belén, Peruvian Amazon) / primary schools	Cluster RCT	16 weeks/ High (initial one hour session followed by 30 minute refresher activities every two weeks through length of intervention)	Outcome 1: STH infection and intensity Methods: laboratory testing of faecal samples (intensity measured by eggs per gram) Outcome 2: Hygiene behaviours Methods: Interviewer-administered KAP Outcome 3: STH knowledge Methods: KAP survey	Number: 18 schools (9 intervention, 9 control) 1,089 students (518 from intervention schools, 571 from control schools). Age: mean age = 10 (grade 5)	Soap not supplied	Outcome 1: (16-weeks follow-up) No statistically significant differences in prevalence of STH infections found: <i>Ascaris lumbricoides</i> prevalence aOR = 0.88 (0.57, 1.34) <i>Trichuris trichiura</i> prevalence aOR = 0.88 (0.62, 1.25) Hookworm prevalence aOR = 1.13 (0.51, 2.50) Any STH prevalence aOR = 1.00 (0.58, 1.72) Intensity (Incidence rate ratios (IRR)) A. Lumbricoides aIRR = 0.42 (0.21,0.85)* T. trichiura aIRR = 1.14 (0.78,1.67) Hookworm aIRR = 0.11 (0.01,1.49) *aOR and aIRR adjusted for confounding factors Outcome 2: (16-week follow-up, univariate analysis) No statistically significant differences between

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
										<p>intervention and control group found in:</p> <ul style="list-style-type: none"> * Washing hands after going to bathroom * Using soap when washing hands after going to the bathroom * Washing hand before eating * Using soap when washing hands before eating <p>Outcome 3: (16-week follow-up) STH knowledge score – aOR = 18.4 (12.7, 26.6) On average, the odds of having a one point increase in score was 18 times higher in the intervention schools compared with the control schools (adjusted for potential confounders).</p>
Nicholson (2014)	Hand hygiene education Key messages: 1. HWWS after defecating	4. Out-of-school lessons on hand hygiene (including songs	Continued normal handwashing	India, Mumbai (South and West)/low income	Cluster RCT	41 weeks/ High (activities throughout length of intervention)	Outcome 1: Diarrhoea incidence in target children and among	Number: 70 low-income communities (35 intervention, 35 matched control),	Soap provided	41-week follow-up Outcome 1: Diarrhoea incidence (episodes per 100 person weeks) Per-protocol analysis (control vs. intervention)

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
	2. HWWS before eating 3. HWWS during bathing	poems and stories) 5. Environmental cues (wall hangers, danglers etc.) 6. Rewards for handwashing (stickers, coins, toy animals etc.) 7. Children encouraged to advocate HWWS within families 8. Children and mother asked to pledge HWWS in front of peers 9. 'Best Mums' club held every 6 weeks		communities			family of target children Methods: interviews with caregivers. Outcome 2: Handwashing behaviour. Methods: indirectly assessed using soap consumption (soap wrapper collection)	2052 target children who were attending first grade on a municipal school (intervention: n: 1026, control: 1026), 2469 other children under 5 years (intervention: n: 1190, control: 1279), 3519 children 6 to 15 years (intervention: n: 1784, control: 1735), 3685 adults (intervention: n: 1892, control: 1793)		Target children Predicted RRR= 21.3% (95% CI 36.6% - 2.3%), P=0.030 Children aged ≤ 5 (non-target) Predicted RRR = 24.7% (95%CI 41.1%-3.8%), P=0.023 Children 6-15 (non-target) Predicted RRR = 24.3% (95% CI 38.7%-6.6%), P=0.010 Whole families Predicted RRR = 23.1% (37.5%-5.5%), p=0.013 Intention-to-treat analysis (control vs. intervention) Target children Predicted RRR= 21.3% (95% CI 36.6% - 2.3%), P=0.102 Children aged ≤ 5 (non-target) Predicted RRR = 23.6% (95%CI 40.2%-2.5%), P=0.03 Children 6-15 (non-target) Predicted RRR = 21.1% (95% CI 35.3%-3.8%), P=0.019 Whole families

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
								Age: 5 year old children (Target); under-fives, children 6 to 15 years and adults (non-targets)		<p>Predicted RRR = 22.5% (36.5%-5.3%), p=0.013</p> <p>*Not adjusted for clustering</p> <p>Outcome 2: Median soap consumption in control households = 45g per household per week 235g per household per week in intervention households</p>
Pickering (2013)	<p>Handwashing education.</p> <p>Key messages: 1. Handwashing before eating 2. Handwashing after using the toilet</p>	<p>Soap Intervention arm</p> <p>1. Teacher training sessions on germ theory and hygiene 2. Hygiene promotion kits distributed including: posters, stickers, classroom activities, DVD presentation</p>	No intervention	Kenya (Nairobi) /primary schools	Cluster-RCT	8 weeks/unclear	<p>Outcome 1; hand cleaning after using the toilet and before eating Methods: both interview and structured observations</p> <p>Outcome 2: diarrhoeal rates</p>	<p>Number: 4 schools (2 intervention, 2 control), 929 students (460 intervention, 469 control). Age 5 - 10</p> <p>Liquid Soap or hand sanitizer provided to intervention schools (spot check revealed that in control schools soap almost never available</p>	<p>8-week follow-up</p> <p>Outcome 1: Soap intervention Hand cleaning after toilet use (intervention vs. control) Prevalence ratio PR = 1.0 (0.3, 3.8) Before eating PR = 1.2 (0.7-2.0)</p> <p>Use of product (soap) when cleaning hands (intervention vs. control) After using toilet PR = 17.2 (4.4, 67.5) Before eating PR = 143.0 (38.9,525.6)</p>	

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
		<p>on handwashing and promotional song</p> <p>3. Installation of soap dispensers</p> <p>4. Provision of water tank</p> <p>Waterless hand sanitizer arm</p> <p>Hygiene promotion as above, plus installation of hand sanitizer dispensers</p>					<p>Methods: student interviews</p>		<p>at latrines (2%) and eating areas (0%) vs. 90% at both areas in intervention schools)</p>	<p>Hand sanitizer intervention</p> <p>Hand cleaning after toilet use (intervention vs. control)</p> <p>Prevalence ratio PR = 2.2 (1.2, 4.3)</p> <p>Before eating PR = 1.3 (0.8-2.2)</p> <p>Use of product (sanitizer) when cleaning hands (intervention vs. control)</p> <p>After using toilet PR = 38.5 (18.1-81.5)</p> <p>Before eating PR = 126.8 (31.9,503.8)</p> <p>Outcome 2: Diarrhoea prevalence</p> <p>Soap Intervention (vs. control) Risk ratio (RR) 0.84 (0.58-1.22), p=0.36</p> <p>Sanitizer intervention (vs. control) RR 0.89 (0.61-1.30), p=0.56</p> <p>*adjusted for week of follow-up, age, sex and clustering</p>

Study	Intervention Content	Intervention Methods	Control Group	Setting	Study design	Intervention length/intensity	Outcomes	Participants	Soap provision	Results
Talaat (2011)	Hand hygiene education Key messages: 1. HWWS upon arriving at school, before and after meals, after using the bathroom, and after coughing or sneezing.	1. Obligatory HWWS twice daily for children under supervision (during school) 2. Posters near sinks, games and fun activities delivered by teachers. 3. Handwashing songs played 4. Informational leaflets distributed to parents.	No intervention	Egypt (Cairo)/ Primary schools	Cluster RCT	12 weeks/ High (activities repeated throughout length of intervention [at least one activity per week])	Outcome 1: Absence incidence due to diarrhoea Methods: teacher records	Number: 60 schools (30 intervention, 30 control), 44,451 students (20,882 intervention, 23,569 control). Age: median = 8 (elementary school)	Soap provided	4-week follow-up Outcome 1: no significant differences in absence incidence due to diarrhoea 5-8 and 9-12-week follow-up: Absence incidence due to diarrhoea significantly lower in intervention compared to control group 1,316 episodes of absence due to diarrhoea in control school vs. 639 in the intervention school = 33% reduction, p<0.0001

Appendix S5

Risk of Bias

Study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Comparability of characteristics (confounding bias)	Contemporaneous data collection	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Al-delaimy (2014)	N/A to study design	N/A to study design	Low risk Reason: Baseline characteristics did not differ significantly between groups	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded	Unclear risk: (all outcomes) Reason: Loss to follow-up not reported	Low risk Reason: none observed	High risk Reason: i) Only two schools in study means the study population may not be representative of source population ii) Analysis not adjusted for clustering
Bieri (2013)	Low risk Reason: spatial sampling frame	Unclear risk Reason: Not described	High risk: Baseline scores on the KAP questionnaire were significantly higher among students in intervention schools than among	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded	Low risk: (all outcomes) Reason: Of 1934 students enrolled, 216 were lost to follow up because of relocation to another school. 1718 participants were included in the analysis. The 210 new students that registered during the	Low risk Reason: none observed	Low risk: None observed

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			students in the control schools.				study period were excluded from the analysis.		
Bowen (2007)	Low risk Reason: random number generator	Unclear risk Reason: Not described	High risk Reason: Some baseline characteristics differed significantly between groups (grade one student age, household piped water and sanitation)	High risk Reason: replacement schools enrolled during second week of data collection	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded	Low risk (all outcomes) Reason: of the 4256 first graders attending the enrolled schools, 3962 (93%) agreed to participate and were included in the analysis	Low risk Reason: none observed	High risk: Some regions may have been over or under sampled when since investigators had to recruit more control schools as the original control schools were accidentally sent intervention packs and were subsequently excluded.
Graves (2011)	Unclear risk Reason: Not described	Unclear risk Reason: Not described	High risk Reason: significantly higher soap availability reported in intervention	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded:	High risk (all outcomes) Reason: Baseline handwashing behaviour was observed in 10 intervention and 11	Low risk Reason: none observed	Low risk Reason: none observed

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			schools (100%) compared to control schools (67%) at baseline (p=0.04).				comparison schools (One intervention school lacked water at baseline). Follow-up observations were not conducted at 3 intervention, and 3 comparison schools, due to lack of water or transportation challenges for observers. Analyses did not include the one school without baseline and six schools without follow-up observations.		
Gyorkos (2013)	Low risk Reason: random number generator	Low risk Reason: The randomization was executed by an independent statistician blinded to school identity	Low risk Reason: Baseline characteristics similar at baseline	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	Low risk (outcome 1) Reason: laboratory technologists (primary assessors) blinded to intervention High risk (outcome 2)	Low risk (all outcomes) Reason: Of the 1,486 officially enrolled children, informed consent was obtained from 1,339 parents (90.1%) and child assent was obtained from 1,286 students (86.5%). Complete data were obtained	Low risk Reason: none reported	Low risk; None found

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						Reason: not blinded	for 1,089 children, or 84.7% of those who assented and only these children were included in the analysis		
Nicholson (2014)	Low risk Reason: random coin tossing	Unclear risk Reason: not described	Low risk Reason; baseline characteristics reported to be well matched apart from small differences in sanitation	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: loss to follow-up > 20% in both arms. Average attrition in both groups = 18%	Low risk Reason; none observed	High risk Reason: i) analysis not adjusted for clustering ii) no direct measure of behaviour (used a proxy)
Pickering (2013)	Unclear risk Reason: randomisation method not described	Unclear risk Reason: not described	Low risk Reason: Baseline data reported to be similar	Low risk Reason: Data collected at similar points in time	High risk (all outcomes) Reason: not blinded	High risk (all outcomes) Reason: not blinded	Unclear risk (all outcomes) Reason: only reported total observations	Low risk Reason: none observed	Low risk Reason: none observed
Talaat (2011)	Risk: Low risk Reason: computer generated random number table	Risk: Unclear risk Reason: not described	Low risk Reason: Baseline characteristics did not differ significantly between groups	Low risk Reason: Data collected at similar points in time	Risk: Unclear risk (all outcomes) Reason: not described	Risk: Unclear risk (all outcomes) Reason: not described	Low risk (all outcomes) Reason: analysis accounts for all enrolled in the trial	Low risk Reason: none observed	Low risk: Reason: none observed