Does targeting children with hygiene promotion messages work?

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

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

Abstract

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

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

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

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

Introduction

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

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

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

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

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

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

Methods

Search Strategy

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

Screening and Inclusion Criteria

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

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

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

Intervention

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

Outcomes

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

Study Selection, Data Extraction and Analysis

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

Data were extracted into a pre-specified data extraction table, recording the following information: (i) study authors and publication date, (ii) intervention content, (iii) intervention methods, (iv) control group, (v) setting (vi) study design, (vii) intervention length/intensity (intervention intensity was graded as 'low' if intervention activities were implemented at one point in time and 'high' if intervention activities were implemented at multiple points in time over the length of the 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/.

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

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

Quality Assessment

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

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

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

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

Results

Search Results

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

Characteristics of Included Studies

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

Settings and Participants

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

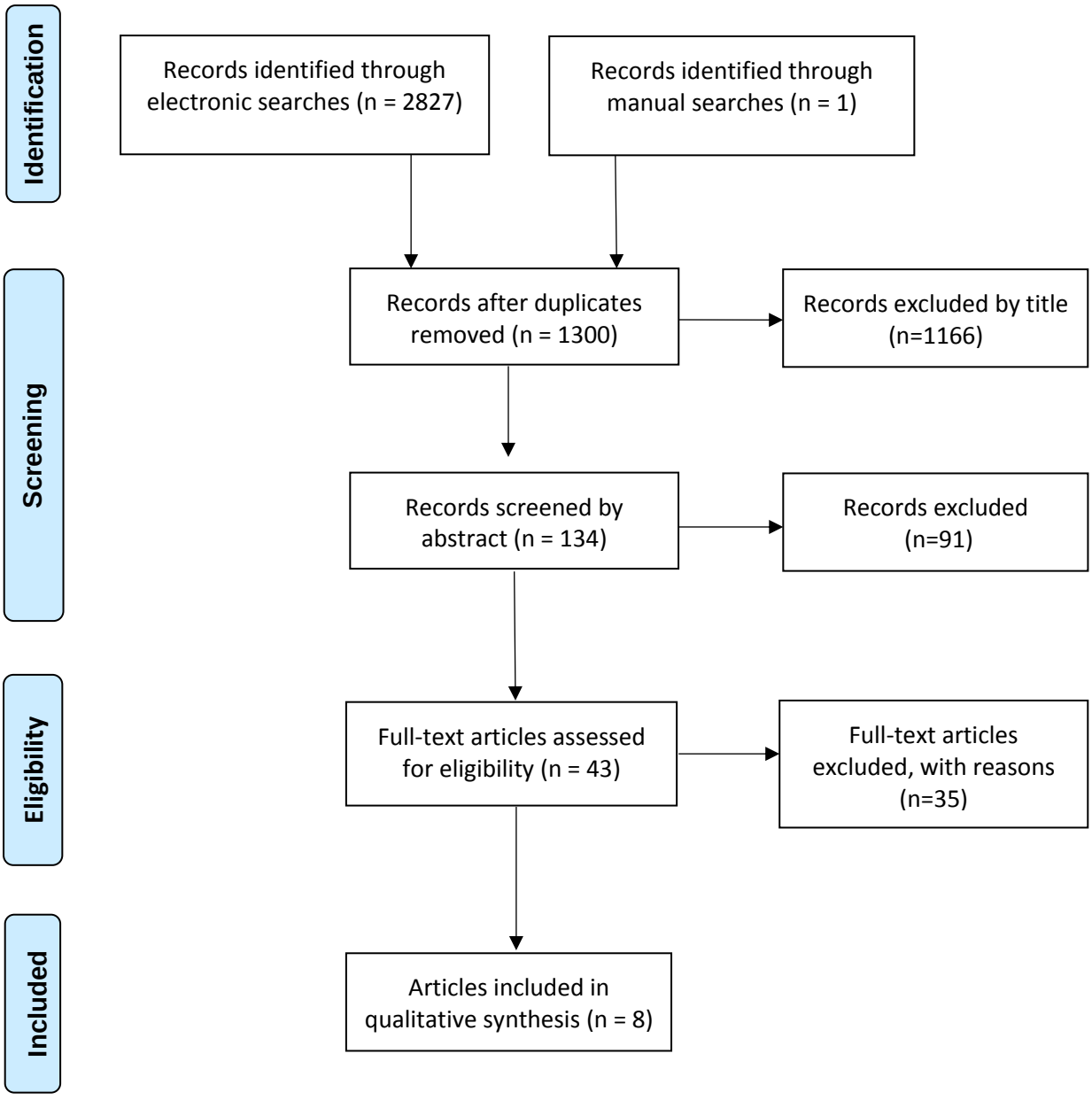
Study Design and Length

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

Intervention

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

Figure 1: PRISMA Flow Diagram



246 **Table 2: Intervention Activities**

Study	Intervention Activities	Intervention Intensity	Soap Provision
Al-delaimy (2014) (24)	Fun activities (comics books, drawing, puppet shows, nursey 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

Outcomes

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

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	*A. lumbricoides* re-infection *A. lumbricoides* infection intensity	× ✓
	Bieri (2013)(25)	Laboratory analysis	STH Incidence STH infection intensity	✓ ×
	Gyorkos (2013)(28)	Laboratory analysis	*A. lumbricoides prevalence* *A. lumbricoides* 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	✓

Handwashing Behaviour Change

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

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

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

Soil-Transmitted Helminth Infection

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

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

Diarrhoea

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

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

Knowledge

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

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

Quality Assessment

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

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

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

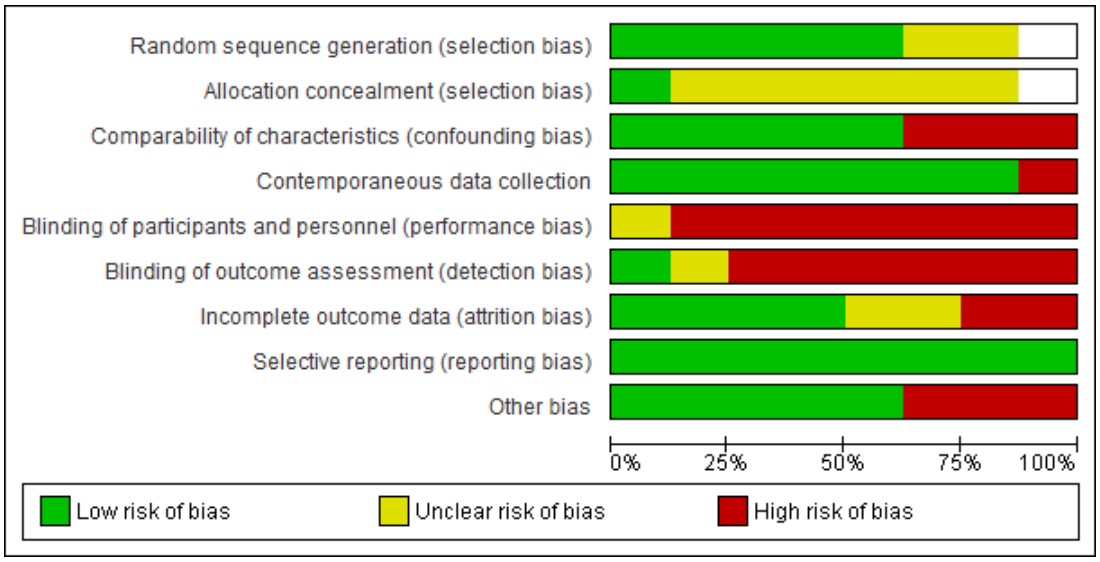
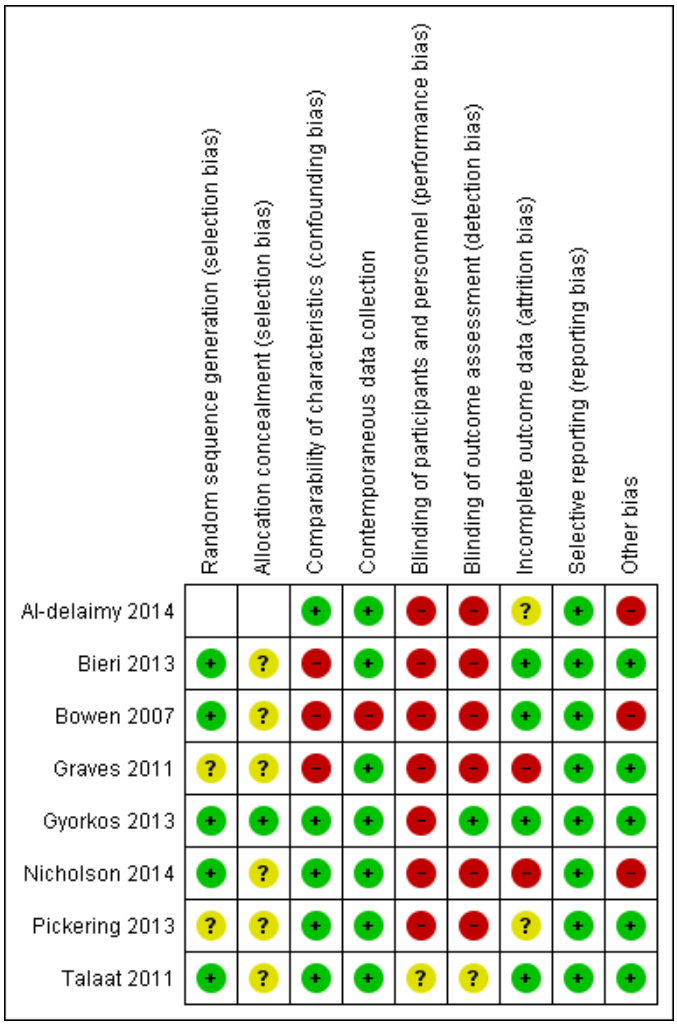


Figure 3: Risk of Bias Summary: review authors' judgements about each risk of bias item for each included study



Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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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 (*trichuriasis, ascariasis*, 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 *trichuriasis*, *ascariasis* and hook worm (respectively) compared to control. The intensity of *Trichuris, Ascaris* 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 (normally displayed in schools)	China (Linxiang City District, Hunan province) / primary schools	Cluster RCT	36 weeks/ High (activities throughout length of intervention)	Outcome 1: STH incidence (*ascaris* and *trichuris*) 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 *ascariasis* 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** = standard intervention + 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: *Ascaris lumbricoides* prevalence aOR = 0.88 (0.57, 1.34) *Trichuris trichiura* 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
										intervention and control group found in: * 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 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).
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: 1026, control: 1026), 2469 other children under 5 years (intervention: 1190, control: 1279), 3519 children 6 to 15 years (intervention: 1784, control: 1735), 3685 adults (intervention: 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)		Predicted RRR = 22.5% (36.5%-5.3%), p=0.013 *Not adjusted for clustering Outcome 2: Median soap consumption in control households = 45g per household per week 235g per household per week in intervention households
Pickering (2013)	Handwashing education. Key messages: 1. Handwashing before eating 2. Handwashing after using the toilet	**Soap Intervention arm** 1. Teacher training sessions on germ theory and hygiene 2. Hygiene promotion kits distributed including: posters, stickers, classroom activities, DVD presentation	No intervention	Kenya (Nairobi) /primary schools	Cluster-RCT	8 weeks/ unclear	Outcome 1; hand cleaning after using the toilet and before eating Methods: both interview and structured observations Outcome 2: diarrhoeal rates	Number: 4 schools (2 intervention, 2 control), 929 students (460 intervention, 469 control). Age 5 - 10	Liquid Soap or hand sanitizer provided to intervention schools (spot check revealed that in control schools soap almost never available	8-week follow-up 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) 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)

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

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 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

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
			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

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
			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

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
						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