Australian National University

Optimising control programs for soil-transmitted helminths

Naomi Elizabeth Clarke

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Declaration and statements of contribution

This thesis is submitted as a Thesis by Compilation in accordance with the Australian National University policy "Higher Degree by Research—Thesis by Compilation".

The research presented in this thesis represents original work that has not been submitted towards any degree or diploma at any other university or institution. This thesis comprises research that I carried out during my candidature at the Australian National University.

This thesis by complication incorporates seven jointly-authored papers. My contributions to each paper are detailed in the following statements.

Paper 1

Title: Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis

Authors: Clarke NE, Doi SAR, Wangdi K, Chen Y, Clements ACA, Nery SV

Publication outlet: Clinical Infectious Diseases

Current status of paper: Published

Contribution to paper: I took part in designing the review protocol. I performed the database searches, study selection, data extraction, and quality assessment. I undertook the statistical analysis and produced the tables and figures. I drafted the manuscript, coordinated co-author input, and undertook revisions as requested by reviewers.

Paper 2

Title: Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis

Authors: Clarke NE, Clements ACA, Doi SA, Wang D, Campbell SJ, Gray D, Nery SV

Publication outlet: The Lancet

Current status of paper: Published

Contribution to paper: I took part in designing the review protocol. I performed the database searches, study selection, data extraction, and quality assessment. I undertook the statistical analysis and produced the tables and figures. I drafted the manuscript, coordinated co-author input, and undertook revisions as requested by reviewers.

Paper 3

Title: Investigating the differential impact of school and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for Worms pilot study protocol

Authors: Clarke NE, Clements ACA, Bryan S, McGown J, Gray D, Nery SV

Publication outlet: Pilot & Feasibility Studies

Current status of paper: Published

Contribution to paper: I drafted the manuscript, coordinated co-author input, and undertook revisions as requested by reviewers.

Paper 4

Title: (S)WASH-D for Worms: a pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths

Authors: Clarke NE, Clements ACA, Amaral S, Richardson A, McCarthy JS, McGown J, Bryan S, Gray DJ, Nery SV

Publication outlet: PLoS Neglected Tropical Diseases

Current status of paper: Published

Contribution to paper: I was the trial coordinator, responsible for overseeing participant enrolment, data collection and data entry, managing field staff, and liaising with partner non-government organisations and government departments. I contributed to data entry and undertook data cleaning. I reviewed the literature, conducted the statistical analysis, and produced the tables and figures. I drafted the manuscript, coordinated co-author input and undertook revisions as requested by reviewers.

Paper 5

Title: WASH for WORMS: a cluster-randomized controlled trial of the impact of a community integrated water, sanitation, and hygiene and deworming intervention on soil-transmitted helminth infections

Authors: Nery SV, Traub RJ, McCarthy JS, Clarke NE, Amaral S, Llewellyn S, Weking E, Richardson A, Campbell SJ, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Clements ACA

Publication outlet: The American Journal of Tropical Medicine and Hygiene

Current status of paper: Published

Contribution to paper: I contributed to designing the data analysis plan. I performed the statistical analysis and produced the tables and figures. I made a substantial contribution to revising the drafted manuscript, preparing it for submission, and responding to reviewer comments.

Paper 6

Title: Risk factors for infection with soil-transmitted helminths during an integrated community-level WASH and deworming intervention in Timor-Leste

Authors: Nery SV*, Clarke NE*, Richardson A, Traub RJ, McCarthy JS, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Campbell SJ, Clements ACA (* co-first authors)

Publication outlet: International Journal for Parasitology

Current status of paper: Published

Contribution to paper: I took part in designing the statistical models. I reviewed the literature, performed the statistical analysis, and produced the tables and figures. I drafted the majority of the manuscript with contributions from SVN, coordinated co-author input, and undertook revisions as requested by reviewers.

Paper 7

Title: Quantitative polymerase chain reaction for diagnosis of soil-transmitted helminth infections: a comparison with a flotation-based technique and an investigation of variability in DNA detection

Authors: Clarke NE, Llewellyn S, Traub RJ, McCarthy J, Richardson A, Nery SV

Publication outlet: The American Journal of Tropical Medicine and Hygiene

Current status of paper: Published

Contribution to paper: I coordinated and oversaw the collection of faecal specimens and conducted the laboratory analysis using sodium nitrate flotation. I reviewed the literature and took part in designing the statistical analysis. I performed the statistical analysis and produced the tables and figures. I drafted the manuscript, coordinated co-author input, and undertook revisions as requested by reviewers.

This thesis is approximately 46,000 words in length, excluding the front matter, abstract, tables, figures, references, and appendices.

Naomi Elizabeth Clarke Date

Endorsement by senior authors

I agree that Naomi Clarke made the contributions described above to the papers on which I am the senior author.

8/1/19

Associate Professor Susana Vaz Nery — Date

Senior author on Papers 1–4 and Paper 7

7 JANUARY 2019

Professor Archie Clements — Date

Senior author on Papers 5 and 6

Endorsement by primary supervisor and delegated authority

8/1/19

Associate Professor Susana Vaz Nery — Date

Primary Supervisor

7/1/2019

Professor Cathy Banwell — Date

Delegated Authority

Additional published works

A number of thesis appendices contain additional published works that are relevant to this thesis but do not form part of it.

Appendix 8 contains an authors' reply to a letter to the editor regarding Paper 2. I drafted this reply and co-ordinated co-author input.

Clarke NE, Doi SA, Clements AC, Gray D, Campbell S, Wang D, Nery SV. The expansion of soil-transmitted helminth control strategies – Authors' reply. *Lancet* 2017, 389(10085): 2191. http://doi.org/10.1016/S0140-6736(17)31343-0

Appendix 9 contains an opinion piece, published on the science news website *The Conversation.* I drafted this piece and incorporated co-author and editor input.

Clarke NE, Nery SV. A new approach for controlling intestinal worm infections could help millions of the world's most vulnerable people. 2016. Available at: http://theconversation.com/a-new-approach-for-controlling-intestinal-worm-infections-could-help-millions-of-the-worlds-most-vulnerable-people-70418.

Appendices 10 and 11 contain peer-reviewed publications. For these papers, I contributed to conducting the statistical analyses, revising the drafted manuscripts, and responding to reviewer comments.

Nery SV, Qi J, Llewellyn S, **Clarke NE**, Traub R, Gray DJ, Vallely AJ, Williams GM, Andrews RM, McCarthy JS, Clements ACA. Use of quantitative PCR to assess the efficacy of albendazole against *Necator americanus* and *Ascaris* spp. in Manufahi District, Timor-Leste. *Parasit Vectors* 2018; 11(1): 373. http://doi.org/10.1186/s13071-018-2838-0

Nery SV, Bennett I, **Clarke NE**, Lin A, Rahman Z, Rahman M, Clements ACA. Characterisation of environmental enteropathy biomarkers and associated risk factors in children in the context of a WASH trial in Timor-Leste. *Int J Hyg Environ Health* 2018, 221(6): 901–906. http://doi.org/10.1016/j.ijheh.2018.05.012

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Abstract

Soil-transmitted helminths (STH) are parasitic intestinal worms that are widely prevalent in impoverished populations, causing a significant burden of morbidity worldwide. Recent years have seen a remarkable global commitment to controlling STH infections. Current World Health Organization (WHO) guidelines for STH control focus on large-scale deworming programs, in which anthelminthic medications—albendazole or mebendazole—are delivered regularly to at-risk population groups in endemic countries. The WHO target for 2020 is regular deworming of 75% of at-risk children; as such, existing control programs have a strong emphasis on treating children.

The overarching aim of this thesis is to provide empirical evidence to guide the optimisation of STH control programs. To achieve this aim, I address four key evidence gaps in the understanding of STH control, by evaluating the potential impact of additional or alternative control strategies, compared to existing strategies and guidelines.

First, I examine the efficacy of alternative anthelminthic drugs. I present the most comprehensive comparison of anthelminthic medications, conducted using network meta-analysis. Findings of this analysis show that several anthelminthic drug combinations, including albendazole–ivermectin and albendazole–oxantel pamoate, are more efficacious than the current standard treatment against *Trichuris trichiura*, the most challenging STH to control.

Second, I investigate the potential impact of expanding STH control programs community-wide. I report the first experimental evidence—obtained using both meta-analysis and a field-based pilot study—comparing community-wide and child-targeted approaches to STH control. Results of both analyses suggest a greater impact of community-wide STH control programs on STH prevalence in children, compared to child-targeted approaches.

Third, I explore the role of water, sanitation, and hygiene (WASH) in STH control. I present results of the first randomised controlled trial comparing a community-wide WASH and deworming program to deworming alone. These results demonstrate no additional impact of the community WASH intervention on STH infections above that achieved by deworming over a two-year period. I also report the findings of an observational risk factor analysis. These findings demonstrate few associations between WASH and STH, with regular deworming and sociodemographic variables representing the main predictors of infection.

Finally, I examine the potential utility of quantitative PCR (qPCR) for monitoring STH control programs. I present the first quantitative comparison of qPCR and sodium nitrate flotation, a microscopy-based diagnostic technique. Findings confirm the higher diagnostic sensitivity of qPCR, particularly for light-

intensity infections, and demonstrate correlations between infection intensity measurements obtained using the two techniques.

The findings presented in this thesis provide robust evidence that will be instrumental to policymakers at a time when the future of STH control efforts is being vigorously discussed. Specific priorities and recommendations for STH control guidelines arising from this research are: including drug combinations as recommended anthelminthics, expanding deworming programs community-wide, and adding qPCR as a diagnostic option in low-transmission settings. Complementing deworming with WASH interventions should also be encouraged, although additional impact on STH may not be apparent for some time. This thesis argues that we must build on the global momentum towards controlling neglected tropical diseases, and re-evaluate global STH control guidelines to ensure that they reflect the available scientific evidence and maximise benefits to afflicted populations.

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Chapter 1
Introduction and background

1.1 Context

Soil-transmitted helminths (STH) are a group of parasitic nematode worms that affect humans. The major STH that cause disease in humans are *Ascaris lumbricoides* (roundworm), *Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum* (hookworms), and *Trichuris trichiura* (whipworm) [1, 2]. There are also other, less common STH, most notably *Strongyloides stercoralis* (threadworm) [3].

Soil-transmitted helminths are responsible for a tremendous burden of disease worldwide: together, they infect over one billion individuals [4], representing the most common parasitic disease of humans. They are a disease of poverty, predominating in Africa, Southeast Asia, and parts of Latin America [5]. Although rarely fatal, STH infections have chronic, insidious sequelae, particularly among children. Along with other neglected tropical diseases (NTDs), they represent a potent factor in perpetuating the poverty cycle [6].

The last decade has seen a substantial global commitment to controlling STH and other NTDs; this has generated significant policy momentum [7]. Large-scale public health programs aimed at controlling STH infections have been scaled up worldwide, with the major goal of reducing the prevalence and intensity of STH infections among children [8–10]. This PhD research has been conducted in the context of this concerted global effort towards STH control, and aims to raise evidence to optimise existing control strategies and maximise their benefits for afflicted populations. This thesis addresses the major STH of humans—*A. lumbricoides*, hookworms, and *T. trichiura*—which are the focus of current global control efforts.

This introductory chapter presents a brief overview of STH, including their transmission, epidemiology, and clinical features. It then describes STH control strategies, with a focus on current guidelines and global progress. Key evidence gaps and the corresponding research aims of the thesis are then presented, followed by an overview of the thesis structure.

1.2 Clinical and epidemiological features of STH infections

1.2.1 STH transmission

Humans are the only definitive host for *A. lumbricoides*, *N. americanus*, *A. duodenale*, and *T. trichiura* [1], while *A. ceylanicum* infects both dogs and humans [11]. As depicted in Figure 1, STH are transmitted through contact with soil that is contaminated with their infective stages (eggs and larvae). Infection with *A. lumbricoides* and *T. trichiura* occurs through the faecal-oral route via ingestion of helminth eggs [1]. Hookworm infection generally occurs following skin penetration by larvae, but can also occur following oral ingestion of *A. duodenale* larvae [2].

Once ingested, *A. lumbricoides* and *T. trichiura* eggs hatch, releasing larvae in the small intestine [3]. *T. trichiura* larvae travel directly to the colon, whereas *A. lumbricoides* larvae migrate through the liver and lungs, before being swallowed and returning to the small intestine [12]. Adult worms of both *T. trichiura* and *A. lumbricoides* develop 2–3 months after initial egg ingestion [1]. After skin penetration, hookworm larvae travel through the pulmonary circulation and lungs prior to entering the small intestine [13], where they develop into adult worms 5–9 weeks after initial infection [1]. Adult worms vary in size among STH species: hookworms measure 7–13mm in length, *T. trichiura* are between 30 and 50mm long, and *A. lumbricoides* represents the largest STH, measuring 150–400mm in length [1]. The number of worms harboured depends on the extent of an individual's exposure; STH do not multiply within the human host [12]. The average lifespan of *A. lumbricoides* is approximately one year, while *T. trichiura* worms live on average 1.5–2 years, and hookworms can live up to 10 years [1, 13].

After mating of adult worms within the human intestine, female *A. lumbricoides* worms each lay up to 200,000 eggs per day, while *T. trichiura* and hookworms lay up to 20,000 [12]. The eggs are excreted in the faeces of infected individuals, and in areas where water, sanitation, and hygiene are lacking, soil becomes contaminated with faeces containing helminth eggs. Infective stages of STH remain viable in warm, moist soil. Under favourable conditions, hookworm eggs hatch in the soil within 5–10 days, releasing larvae that can survive for up to several months [14]. *A. lumbricoides* and *T. trichiura* eggs do not hatch before entering the host. *T. trichiura* eggs can survive in soil for several months, and *A. lumbricoides* eggs for years [5, 15].

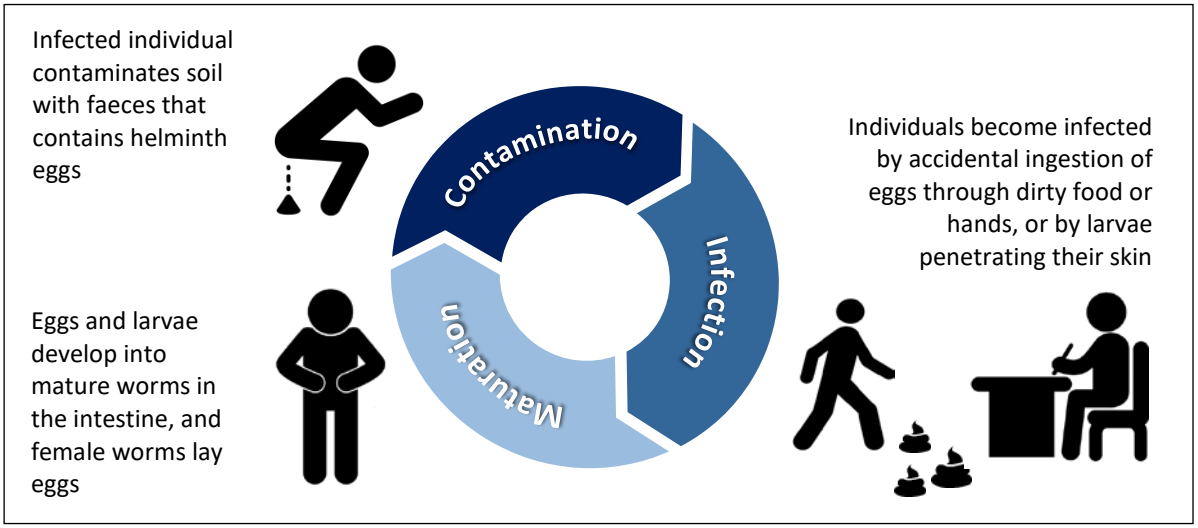

Figure 1. Soil-transmitted helminth lifecycle (adapted from World Health Organization, 2011) [8]

1.2.2 Epidemiology and disease burden

STH represent one of the world's neglected tropical diseases (NTDs), a diverse group of infectious diseases that predominantly affect those living in poverty [6]. Indeed, STH occur almost exclusively in low-income countries, with most infections occurring in Africa, Southeast Asia, China, India, and parts of Latin America [4, 16]. Environmental conditions play an important role in STH transmission. STH predominate in countries with tropical and subtropical climates [5, 16], and infection distribution is affected by temperature, rainfall, soil type, and vegetation [17–19]. Additionally, STH and poverty are inextricably linked. Inadequate access to protected water and improved sanitation is pervasive among poor communities in low-income countries, particularly in rural areas [20]. Such conditions facilitate environmental contamination with faeces, enabling STH transmission [1, 5, 21]. Furthermore, STH infections perpetuate poverty, through mechanisms including impaired child development, lower educational attainment, adverse pregnancy outcomes, and reduced productivity [6, 22].

The most recent estimates of the global prevalence of STH found that in 2010, approximately 439 million people were infected with *A. lumbricoides*, 819 million with hookworm, and 464 million with *T. trichiura* [4]. More recent analyses, as part of the Global Burden of Disease study, estimated the burden attributable to STH at 3.3 million disability-adjusted life years (DALYs) in 2016, down from 4.1 million DALYs in 2006 [23]. Others have suggested that due to underestimation of disability weights for STH and other NTDs, the burden may in fact be higher than this [24–26].

Age-related distribution of infection differs between STH species. For *A. lumbricoides* and *T. trichiura*, infection prevalence and intensity tend to peak in school-aged children and then decline in older age groups [27–30]. On the other hand, hookworm infections tend to increase in prevalence and intensity and plateau in adulthood [13, 27, 31, 32]. In general, males have higher rates of hookworm infection,

while no sex differences are seen for *A. lumbricoides* or *T. trichiura* [13]. The reasons for these age- and sex-related infection patterns are not completely understood, although exposure rates due to behavioural and environmental factors are likely to play an important role [13, 30, 33–35]. It has also been postulated that the decreasing prevalence and intensity of *A. lumbricoides* and *T. trichiura* with age may reflect gradually acquired protective immunity to these parasites [33, 36, 37]; however, the relative contributions of environmental exposure and acquired immunity remain unclear [1].

STH infections are known to be "overdispersed" within affected communities; that is, a small number of individuals harbour the majority of helminths, while most individuals have light infections or are uninfected [29, 38–41]. Additionally, it has been shown that certain individuals are "predisposed" to STH infections, meaning that they have a tendency to become reinfected with a similar worm burden following treatment [42–45]. A variety of factors have been identified as contributing to overdispersion and individual-level predisposition; these include genetic and immune-related factors [46, 47], nutritional status [48], and environmental and behavioural characteristics [34, 46, 49, 50].

1.2.3 Clinical features and morbidity

STH infections are very seldom fatal, with an estimated 4900 deaths worldwide caused by STH in 2016 [51]. Severe acute complications of heavy-intensity *A. lumbricoides* infections can occur; these include hepatobiliary and pancreatic complications such as acute hepatic abscess and pancreatitis, and bowel obstruction that can lead to perforation, peritonitis, and death if untreated [52, 53]. Infections with *T. trichiura* can also cause severe illness, including *Trichuris* dysentery syndrome, associated with chronic diarrhoea and rectal prolapse [54]. The prevalence of these acute complications is poorly documented; however, it has been estimated that approximately 15 cases of severe illness occur per 100,000 infections with *A. lumbricoides* [55], and that approximately 5% of children with heavy-intensity *T. trichiura* infections develop acute complications [56].

These rare complications notwithstanding, the vast majority of the burden of disease attributable to STH infection is due to chronic, insidious sequelae that result from heavy-intensity infections. These sequelae are related primarily to intestinal blood loss (in the case of hookworm) and the effects of STH infections on childhood growth and development [1, 13]. Hookworm infections, particularly those with *A. duodenale*, cause intestinal blood loss; this occurs when hookworms using their "cutting organs" to attach to the intestinal wall, rupturing small blood vessels [13]. The association between hookworm infection and iron-deficiency anaemia is well-documented [57–61], and particularly affects children and women of reproductive age [13, 60]. All STH species impair absorption and utilisation of nutrients and micronutrients [62, 63]; this can lead to impaired childhood growth, manifesting as stunting and/or

wasting [64–68], as well as impaired cognitive ability [69–73]. STH infections may also result in increased school absenteeism [74], as well as reduced work productivity among adults [75].

However, the overall body of evidence examining associations between STH infections and morbidity shows mixed results for many morbidity outcomes, with heterogeneous results seen across different transmission settings, populations, and study designs [26]. Recently, the benefits of mass treatment for STH, in terms of nutritional and educational outcomes, were called into question by large-scale systematic reviews performed by the Cochrane and Campbell collaborations [76, 77]. These reviews were criticised by NTD researchers and policymakers for numerous reasons; in particular, it was argued that pooling infected and uninfected children for outcome assessment would likely obscure potential health benefits among those infected [78, 79]. Additionally, short study follow-up periods and heterogeneity among included studies in terms of transmission levels and predominant STH species could lead to further dilution of potential benefits in pooled analyses [26, 78, 79].

Despite the aforementioned limitations in the evidence, it is understood that STH infections of heavy intensity cause a significant burden of disease, the bulk of which is borne by children and women of child-bearing age living in poverty [80]. Because of this, STH and other NTDs that afflict these vulnerable populations have assumed a dominant place on the global health agenda in the past decade, with intensifying coordinated efforts towards their control [81]. STH control strategies, efforts, and progress will be discussed in the ensuing section.

1.3 Control of STH infections

1.3.1 Preventive chemotherapy

STH infections are treatable with anthelminthic (deworming) medications. These medications kill adult worms, usually by inducing neuromuscular paralysis in the parasites or impairing their ability to absorb glucose [82, 83]. However, humans do not develop lasting immunity to STH, and in the context of ongoing environmental contamination with helminth eggs and larvae, reinfection occurs rapidly following treatment. A large systematic review and meta-analysis found that within six months of treatment, prevalence of all STH species rebounded to at least 50% of pre-treatment levels [43].

The term “preventive chemotherapy” for NTD control was introduced by the World Health Organization (WHO) to describe a public health intervention involving the periodic large-scale distribution of medications to at-risk populations in endemic areas [84]. This strategy, which was designed to enable coordinated treatment against multiple NTDs simultaneously, is the mainstay of current STH control efforts. The primary goal of preventive chemotherapy against STH is to reduce morbidity associated with chronic, heavy-intensity infections by reducing worm burdens in at-risk population groups [9].

The anthelminthic drugs recommended by the WHO for preventive chemotherapy against STH control are the benzimidazoles albendazole (400mg) and mebendazole (500mg) [85]. These drugs have excellent safety profiles when given in single doses [84, 86], and have therefore been deemed safe for widespread distribution without medical supervision [87].

Anthelminthic drugs and their efficacy

The efficacy of single-dose albendazole and mebendazole varies between STH species. Anthelminthic efficacy is measured in terms of cure rate (the proportion of treated individuals who become helminth-egg negative) and egg reduction rate (ERR; the relative decrease in excreted eggs per gram of faeces). Albendazole is highly efficacious against *A. lumbricoides*, with a recent meta-analysis reporting a pooled cure rate of 95.7% and ERR of 98.5% [88]. Mebendazole is similarly efficacious against this parasite, with a pooled cure rate of 96.2% and ERR of 98.0% [88]. For hookworm, albendazole has relatively high efficacy (cure rate 79.5% and ERR 89.6%), while mebendazole displays much lower efficacy (cure rate 32.5% and ERR 61.0%) [88]. Both drugs have low efficacy against *T. trichiura*, with pooled cure rates of 30.7% and 42.1%, and ERR of 49.9% and 66.0%, for albendazole and mebendazole, respectively [88]. This means that preventive chemotherapy programs will have a lesser impact in areas where *T. trichiura* is prevalent, and represents an important limitation of the current standard STH treatments.

A further concern regarding the widespread use of benzimidazoles in preventive chemotherapy programs is the potential for drug resistance to develop. Although there is currently no conclusive evidence of this occurring in humans [89, 90], benzimidazole-resistant helminths are widespread among livestock populations that have been subject to repeated treatment [91]. Resistance to albendazole and mebendazole would be disastrous for STH control efforts; for this reason, as well as the limited efficacy of currently-used drugs against *T. trichiura*, researchers have emphasised an urgent need for alternative drug strategies to treat STH [92, 93]. Identifying alternative anthelminthic medicines (or combinations of existing ones) has additionally been highlighted as a research priority by the WHO [80].

Other drugs that have long been on the WHO Essential Medicines List for STH include pyrantel pamoate and levamisole [94]. Similar to mebendazole, meta-analysis shows that both of these drugs have high efficacy against *A. lumbricoides* (cure rates of 92.6% and 97.3% for pyrantel pamoate and levamisole, respectively) and lower efficacy against hookworm (respective cure rates of 49.8% and 10.3%) and *T. trichiura* (respective cure rates of 20.2% and 29.5%). Both of these drugs were previously used to control STH [95–97]; however, due to their weight-dependent dosing that complicates logistics, they are not routinely recommended for use in large-scale preventive chemotherapy programs [80].

A number of potential alternative drugs have been investigated. Oxantel pamoate, an anthelminthic widely used in veterinary parasitology, was originally investigated for efficacy against STH in the 1980's,

and has also been examined in clinical trials more recently, both on its own and in combination with other anthelminthics [98–102]. Newer agents include nitazoxanide, a broad-spectrum antiparasitic and anti-viral agent that has been licensed for use in humans since 2004 [103, 104], and tribendimidine, an anthelminthic that was developed and licensed for human use in China in 2004 and is being developed for regulatory approval [105, 106], as well as combinations involving these newer agents [107, 108].

Drugs that are used to control other neglected tropical diseases, such as ivermectin (used for lymphatic filariasis (LF) and onchocerciasis control), and diethylcarbamazine (also used for LF control), have been investigated for efficacy against STH [99, 109–111]. The combination of albendazole and ivermectin has shown promise in treating *T. trichiura*, with reported ERR consistently above 90% [112]. In 2017, ivermectin was added to the WHO Essential Medicines List for STH [113].

Few other potential drug candidates exist. Moxidectin has recently been licensed for the treatment of onchocerciasis in humans, and has shown moderate efficacy against hookworm in one study, with a cure rate of 56.7% and ERR of 74.6% [114]. Newer veterinary compounds, such as emodepside, monepantel, and oxfendazole, have also been proposed as potentially useful in treating STH in humans [115, 116]; however, their efficacy profiles are yet to be elucidated.

Preventive chemotherapy for STH control: guidelines and global progress

Large-scale treatment for STH control was mentioned in documents released by the WHO dating back to 1987 [117]. However, it was not until 2001 that a specific target for preventive chemotherapy against STH was defined, in World Health Assembly resolution WHA 54.19. This resolution stipulated that by 2010, regular chemotherapy against STH should be administered to at least 75% of school-aged children living in areas endemic for STH [118]. The focus on school-aged children (generally defined as those aged 5–12 years) was due to their high risk of STH-associated morbidity and the cost-effectiveness of using school-based infrastructure for drug delivery [118–120]. Soon after, the WHO released strategic and operational guidelines for the implementation of STH control programs in endemic countries [121, 122]. In line with the 2010 target, these guidelines focused heavily on school-aged children, but also recommended systematic treatment of other groups at high risk of morbidity, namely preschool-aged children (aged 2–4 years) and women of reproductive age [121].

After the 2010 preventive chemotherapy target was missed, with 31.1% coverage of school-aged children achieved [120], the WHO reaffirmed and strengthened their commitment to NTD control in 2012. Its landmark roadmap for NTD control included specific targets for 17 neglected tropical diseases, to be achieved by the year 2020. For STH, the 2020 target is regular delivery of preventive chemotherapy to 75% of at-risk school- and preschool-aged children [120]. An additional goal, defined in the WHO's 2011–2020 strategic plan for STH control, is to reduce the prevalence of moderate- and heavy-intensity

STH infections in school-aged children to less than 1%, at which point STH would no longer be considered a public health problem in children [9].

The WHO 2020 targets were endorsed in 2012 by a group of public and private stakeholders in the London Declaration on NTDs [123]. In this declaration, non-government organisations, pharmaceutical companies, and donors affirmed their commitment to eradicate, eliminate or control ten NTDs by 2020, inspired by the WHO roadmap targets, and called on the international community and endemic countries to join them in this commitment [123]. The declaration, originally endorsed by 22 partners, has since been joined by an additional 80 organisations [124]. A major component of the London Declaration on NTDs was the commitment of large multinational pharmaceutical companies to donate drugs for use in preventive chemotherapy programs [81]. For STH control, 400 million doses of albendazole and 200 million doses of mebendazole are donated annually by GlaxoSmithKline and Johnson & Johnson, respectively [81]. This is sufficient to cover the estimated 596 million school-aged children (as of 2017) requiring preventive chemotherapy for STH [125]; however, there remains a gap in drug donations for the 272 million at-risk preschool-aged children. The WHO recently reported that this gap was expected to be filled in late 2018, though it is not clear by whom [126].

The progress since 2012 in worldwide coverage of preventive chemotherapy has been remarkable. Between 2010 and 2015, preventive chemotherapy against STH averted an estimated 549,000 DALYs among children [127], reflecting the concerted and coordinated efforts of the WHO, endemic countries, and a large number of partners and stakeholders. In 2017, 68.8% of the 596 million at-risk school-aged children and 69% of the 272 million at-risk preschool-aged children were treated with albendazole or mebendazole through preventive chemotherapy programs, in 77 countries worldwide [125]. Of those treated, 16% of school-aged children and 7% of preschool-aged children received treatment through the Global Programme for the Elimination of Lymphatic Filariasis (GPELF), through which community-wide deworming is currently conducted in 47 countries [125, 126].

WHO guidelines for preventive chemotherapy against STH continue to focus on delivering treatment to groups at high risk of STH-associated morbidity. The most recent guideline was published in 2017 [80], and in keeping with previously published versions [84, 121], recommends regular deworming for preschool-aged children (aged 24–59 months), school-aged children (aged 5–12 years), and women of reproductive age (aged 15–49 years), including lactating women and pregnant women after the first trimester [80]. The most recent guideline additionally includes young children (aged 12–23 months) and adolescent girls (aged 10–19 years) [80], and no longer includes the previous recommendation of treating adults in high-risk occupations [84]. The current WHO guidelines for preventive chemotherapy are summarised in Figure 2.

Conduct baseline assessment of STH prevalence in at-risk populations to determine need for and frequency of preventive chemotherapy programs

↓

Implement PC programs according to baseline prevalence in at-risk groups as follows

	Baseline STH prevalence[a] ≥20% and <50%	**Baseline STH prevalence[a] ≥50%[a]**
Young children (12–23 months)	Albendazole 200mg Once a year	Albendazole 200mg Twice a year
Preschool-aged children (24–59 months) School-aged children (5–12 years)[b] Adolescent girls (10–19 years)[c] Women of reproductive age (15–49 years)[c]	Albendazole 400mg or mebendazole 500mg Once a year	Albendazole 400mg or mebendazole 500mg Twice a year
	Baseline prevalence[a] of hookworm and/or *T. trichiura* ≥20%, AND anaemia prevalence in pregnant women ≥40%	
Pregnant women (in second or third trimester only)[d]	Albendazole 400mg or mebendazole 500mg No interval specified	

↓

Monitor prevalence and intensity of STH infections every 3–4 years

↓

***For school-based deworming programs only*:[e] after 5–6 years with ≥75% treatment coverage, reassess STH prevalence in school-aged children and determine ongoing frequency as follows**

Prevalence <1%	**Prevalence ≥1% and <10%**	**Prevalence ≥10% and <20%**	**Prevalence ≥20% and <50%**	**Prevalence ≥50%**
Cease preventive chemotherapy	Preventive chemotherapy 2-yearly for 4 years	Preventive chemotherapy yearly for 4 years	Maintain previous level of preventive chemotherapy	Intensify frequency, extend to other at-risk groups

↓

Monitor prevalence and intensity of STH infections annually (no further specific guidance)

Figure 2. Current WHO recommendations for preventive chemotherapy for STH control.

Adapted from: Preventive chemotherapy to control soil-transmitted helminth infections in at-risk groups, WHO, 2017 [80] and Helminth control in school-age children: a guide for managers of control programmes, WHO, 2011 [8]. [a] Baseline STH prevalence refers to prevalence in the relevant at-risk population group; for young children, preschool-aged children, and school-aged children, it refers to the overall prevalence among children. [b] In some settings the upper age limit may be 14 years. [c] These groups refer to non-pregnant women. [d] This is a conditional recommendation. Member States advised to consider after debate and involvement from stakeholders. [e] The 2017 guideline provides no specific criteria for determining whether to continue or cease preventive chemotherapy; this information was provided in the 2011 guideline for school-based deworming programs.

Despite the WHO recommendation to treat all at-risk groups, their 2020 targets emphasise school- and preschool-aged children, and this has led to control efforts focusing heavily on these age groups. In addition, drug donations are provided to cover only school-aged children [128], and the specific operational guideline for managers of STH control programs describes only school-based preventive chemotherapy programs targeted to school-aged children [8]. Many endemic countries are therefore implementing school-based deworming programs [8, 85, 129], while treatment of preschool-aged children is generally offered during national child health days and campaigns for immunisation and vitamin A supplementation [125, 130]. Such programs are not easily adapted to reaching women of reproductive age. Approximately 20% of the estimated 688 million women of reproductive age at risk of STH receive albendazole through the GPELF, with very few other reports of preventive chemotherapy programs reaching this group [125, 131]. The development and validation of operational guidelines specifically focusing on STH control for adolescent girls and women of reproductive age has been highlighted as a priority by the relevant WHO Advisory Group [132].

Recently, concerns about the limited impact of school-based (child-targeted) deworming programs on community-level STH transmission [133], along with concerns regarding the coverage of non-school enrolled children and other high risk groups [134, 135], and the long-term cost-effectiveness of school-based deworming [136], have led to increasing interest in expanding STH control programs community-wide. As many countries achieve their LF elimination targets and begin to scale down their LF control programs, determining platforms for future STH control programs is a vital issue for NTD policymakers. It has previously been suggested that school-based deworming can lead to reduced STH infections among adults, as a result of reduced overall transmission [95, 137]. However, more recently, mathematical modelling studies have indicated that in many settings, school-based deworming alone will have limited impact on community-wide transmission [133, 138].

Results of mathematical modelling studies suggest that expanding preventive chemotherapy programs community-wide (i.e., to all age groups, including adults) will lead to additional reductions in STH infections in children as well as in the community as a whole [139]. Further, modelling shows that broadening coverage beyond school-aged children is generally required to achieve morbidity control according to the WHO target [140, 141]. When considering the prospect of interrupting STH transmission (i.e., reducing STH prevalence and intensity levels to a point at which ongoing transmission cannot be sustained), modelling studies indicate that in most transmission settings, a community-wide approach will be needed to achieve transmission interruption, particularly for hookworm [133, 138, 142, 143]. Cost-effectiveness modelling has also shown that community-wide approaches to STH control are significantly more cost-effective than approaches targeted to children, in terms of controlling both STH transmission and morbidity [144, 145].

1.3.2 Water, sanitation, and hygiene

In addition to preventive chemotherapy, the WHO has repeatedly highlighted water, sanitation, and hygiene (WASH) interventions an important component of STH control since 1987 [9, 80, 117–121, 146]. WASH interventions may encompass provision of any or all of the following components: a protected water supply, improved sanitation facilities designed to safely dispose of human excreta, and hygiene education, particularly relating to handwashing at critical times [147, 148]. WASH is known to have a major impact on health, in particular in terms of reducing child mortality, diarrhoeal illness, and other diseases such as trachoma [147, 149–152]. Access to clean water and sanitation is recognised by the United Nations (UN) as a basic human right [153], and achieving universal and equitable access to water, sanitation, and hygiene is the focus of the sixth UN Sustainable Development Goal for 2030 [154].

As described previously, STH transmission depends on both faecal contamination of soil and subsequent human exposure to this contaminated soil. Therefore, interventions that successfully separate humans from their excreta could theoretically interrupt STH transmission, by preventing reinfections from occurring after deworming in endemic areas. Specifically, provision of improved sanitation facilities should theoretically prevent ongoing environmental contamination with STH, while handwashing with soap—facilitated by provision of water and hygiene education—should prevent individuals from ingesting contaminated soil and becoming infected [155].

Empirical evidence regarding WASH and STH

Despite the clear theoretical link, the empirical evidence for the impact of WASH on STH is mixed. A large number of observational studies have examined associations between WASH and STH infections. Meta-analyses of observational studies have demonstrated that a number of WASH characteristics are associated with reduced odds of infection with one or more STH, including use of treated water, piped water access, availability and use of latrines, handwashing before eating and after defecating, soap use and availability, and footwear use [156–159]. All of these meta-analyses noted that included studies mainly had cross-sectional designs and were generally of low quality.

Evidence from intervention studies is more limited. A number of intervention studies have examined the impact of hygiene or health education interventions on STH reinfection and intensity; these have predominantly been conducted through schools, and have shown mixed results. A randomised controlled trial (RCT) conducted in China found that a school-based health education intervention led to a 50% reduction in odds of STH reinfection following deworming [160]. On the other hand, a school-based health education program implemented in an RCT in Peru reduced *A. lumbricoides* infection intensity, but had no impact on hookworm or *T. trichiura* intensity or on prevalence of any species [161]. A smaller, non-randomised trial in Malaysia demonstrated significant impact of a school-based health

education program on intensity of all three STH species and on hookworm prevalence, but not on *A. lumbricoides* or *T. trichiura* prevalence [162], while a small trial in Indonesia found no impact of school-based health education on *A. lumbricoides* prevalence [163].

In terms of household and community-level hygiene or health education interventions, a study of household-level handwashing and nail clipping interventions in Ethiopia found that both of these strategies led to lower odds of reinfection with intestinal parasites, including STH [164]. On the other hand, a Ugandan study of a community-based hygiene and sanitation education program in the context of regular deworming found no additional impact on STH infections among young children [165].

Fewer intervention studies have looked at the impact of sanitation on STH infections. Three large RCTs have examined the impact of sanitation on health outcomes, including STH. Two were conducted in the context of India's Total Sanitation Campaign, which encouraged construction of household latrines by implementing education and social mobilisation activities, supporting the availability of affordable materials, and providing small government subsidies. Both of these studies demonstrated no impact of the sanitation intervention on STH infections, likely due to suboptimal intervention uptake [166, 167]. A third RCT was conducted in Indonesia, in the context of the rural Total Sanitation and Sanitation Marketing program, that consisted mainly of community mobilisation activities and improving the availability of sanitation materials. This study similarly found relatively low intervention uptake and detected no impact on STH infections [168].

Two smaller studies have investigated the impact of combined sanitation and hygiene interventions on STH. A study in China showed that compared to chemotherapy alone, a combined chemotherapy, sanitation, and hygiene intervention led to greater prevalence reduction for *A. lumbricoides* and *T. trichiura*, but not hookworm [169]. A pilot study of an integrated sanitation, hygiene, and deworming program in Côte d'Ivoire reported greater reductions in hookworm intensity compared to deworming alone, but no impact on other STH species; this study was limited by imbalanced baseline prevalence and intensity between study arms [170].

Finally, two RCTs have examined the impact of integrated water, sanitation, and hygiene interventions on STH infections. One was conducted in Kenya and compared a school-based WASH and deworming program to deworming alone. The school WASH intervention resulted in lower odds of infection with *A. lumbricoides*, but not hookworm or *T. trichiura* [171]. The other RCT, WASH Benefits, compared the effects of household- and compound-level water, sanitation, handwashing, and nutrition interventions on a range of child health outcomes in Kenya and Bangladesh [172]. Results—to date available only from the Kenyan study—showed that the prevalence and intensity of *A. lumbricoides* (but not hookworm or *T. trichiura*) infection was reduced by the water-only intervention and the combined

water, sanitation, and hygiene interventions, while no impact was seen from the sanitation-only or handwashing-only interventions [173].

WASH and STH control in policy and practice

Although available evidence for the impact of WASH interventions on STH is limited and findings are inconsistent, it is undisputable that poor sanitation and hygiene play a crucial role in STH transmission. Improvements to WASH represent an essential component of broader control strategies for STH and other NTDs; this is exemplified by the WHO listing provision of safe water, sanitation, and hygiene as one of the five key interventions for NTD control [120]. However, WASH is generally mentioned in WHO guidelines and strategic plans for STH control as "a long-term strategy" [9], without specific recommendations for implementation or evaluation, and no clear targets or goals [174]. With WHO NTD targets focused on deworming coverage, a lack of guidance regarding WASH for NTD control program managers, and limited resources allocated to NTD control, STH control efforts have concentrated on scaling up preventive chemotherapy programs. On the other hand, in recent years, researchers have repeatedly highlighted the need for intersectoral collaboration between the WASH and NTD sectors to achieve sustained control or elimination of NTDs, including STH [135, 175–178].

Roundtable discussions regarding WASH and NTDs took place in 2012 and 2014, hosted by the Bill & Melinda Gates Foundation and Sanitation and Hygiene Applied Research for Equity (SHARE), respectively [177, 179]. These discussions included researchers, practitioners, donors, and NGO representatives, and aimed to identify opportunities to foster cross-sectoral collaboration at global, national, and district levels. While overall progress has been made in terms of WASH and NTD sector collaboration, progress specifically towards integrating WASH into STH control programs has been limited [180]. However, one notable example includes a recent report that Cambodia and the Lao People's Democratic Republic have improved WASH efforts targeting communities affected by STH and schistosomiasis, as a result of consultations between the NTD and WASH sectors [10].

The WHO recently acknowledged that WASH has been neglected relative to its importance in NTD control [10] and, in an important step towards improving cross-sectoral collaboration, released the first global strategy document focused on WASH and NTD control [181]. This document calls on actors in the WASH and NTD sectors to work together towards NTD targets, encouraging WASH implementers to target endemic areas with programs that maximise impact on NTDs, and advocating for countries to prioritise integration. Key actions to be undertaken by WASH and NTD actors in endemic countries are set out, and although no disease-specific recommendations are provided, the development of operational guidelines for integrated program implementation is highlighted as a key WHO priority [181].

1.3.3 The importance of diagnostics

Diagnostic methods for detecting and quantifying STH represent a crucial component of STH control efforts. Knowledge of infection prevalence and intensity in target populations and communities is critical for planning STH control programs, monitoring and evaluating their impact, and detecting any emerging drug resistance [182]. Currently, the prospect of STH transmission interruption (also known as transmission elimination) is receiving increasing attention among NTD researchers as control programs are scaled up worldwide [183, 184]. In this context, accurate determination of infection levels in populations becomes even more important, because the ability to confirm interruption (or re-emergence) of transmission, and thus make decisions regarding ongoing control efforts, depends on the ability to detect light-intensity infections that may remain in low-transmission settings [185].

There is currently no true gold standard test for diagnosing and quantifying STH infections [186]. The test currently recommended by the WHO is the Kato-Katz technique [8], a simple, microscopy-based technique conducted using a kit that is mostly reusable [187]. Using a template provided in the kit, a specific amount of faecal material is prepared on a microscope slide (41.7mg for standard templates, though other sizes are also available [188]). Eggs of each STH are manually enumerated by trained microscopists [189], and infection intensity is then calculated in eggs per gram of faeces. Infection intensity is classified as light, moderate or heavy based on WHO-specified cut-offs [121]. The major benefits of the Kato-Katz technique are its low cost, minimal equipment, and ability to be performed in the field. However, this technique also has a number of important drawbacks, including the requirement for analysis within one hour of slide preparation to avoid hookworm egg degeneration [190] and, more importantly, its low sensitivity in low-transmission settings [186]. The Kato-Katz technique has a high false-negative rate when light-intensity infections predominate [182], even when duplicate slides are prepared and read for each individual, as recommended to increase sensitivity [121, 186]. This represents a significant limitation in the utility of Kato-Katz within large-scale STH control programs.

A range of alternative microscopy-based diagnostic tests for STH exist; these can be generally classified into concentration-based [191] and flotation-based techniques [192–195]. A number of microscopy-based techniques, including FLOTAC and sodium nitrate flotation, have demonstrated higher sensitivity than the Kato-Katz technique [186, 195], while others, including mini-FLOTAC and McMaster, appear to have equivalent or lower sensitivity [186]. On the other hand, polymerase chain reaction (PCR)-based techniques have consistently demonstrated higher sensitivity compared to the Kato-Katz technique [195–199]. PCR is a molecular approach that does not rely on visualisation of helminth eggs, but rather uses DNA primers and probes to amplify and detect DNA sequences specific to each STH. Therefore, this technique provides the additional benefit of differentiating between hookworm species, which is

not possible using microscopy-based techniques [200]. To increase efficiency, PCR can be adapted to detect multiple different STH simultaneously [196, 198].

PCR-based assays can also provide a quantitative measure of infection, using a real-time procedure known as quantitative PCR (qPCR), that measures the number of DNA amplification cycles required to detect a signal exceeding background levels [197]. DNA concentration results obtained using qPCR have been shown to correlate with infection intensity results (eggs per gram of faeces) obtained using the Kato-Katz technique [196, 197, 201, 202]. The high sensitivity of qPCR, along with its potential ability to measure infection intensity, has generated significant interest among STH researchers in its applicability for monitoring the impact of large-scale STH control programs [185].

1.4 Evidence gaps and research objectives

The overarching aim of the research presented in this thesis is to address evidence gaps in the current understanding of STH control, in order to support the optimisation of current control strategies. Specifically, this research aims to determine the potential impact of additional or alternative strategies, as compared to the current standard control programs based on WHO guidelines and the 2020 targets. Four key evidence gaps relating to STH control are addressed in this thesis.

The first evidence gap relates to the **efficacy of anthelminthic drugs**. As discussed above, identifying alternative drug strategies for STH control is a key priority, given low efficacy of existing drugs against *T. trichiura*, and concerns regarding the potential for benzimidazole resistance to emerge. A variety of alternative options have been investigated in clinical trials, including older anthelminthics, multiple-dose approaches, drug combinations, and newer anti-parasitic agents. However, at the time this PhD was undertaken, there was no comprehensive comparison of the efficacy of available anthelminthic drugs against STH. Existing meta-analyses were limited to four standard anthelminthic drugs [88, 203], or to individual drug combinations [112]. An evaluation with a wider scope could provide essential insight to inform evidence-based decisions regarding anthelminthic selection for mass drug administration programs.

The second key evidence gap relates to **community-wide deworming strategies**. As described previously, although school- and preschool-aged children represent the current focus of STH control efforts, there has recently been increasing interest in expanding control programs to include all community members [135]. When this PhD was commenced, the empirical evidence for the impact of community-wide drug administration on STH was limited, with most evidence coming from mathematical modelling analyses [138, 142, 145]. Apart from one very small trial using levamisole in the 1980's [95], there were no trials comparing child-targeted and community-wide approaches to drug

delivery. Furthermore, there was no synthesis of the large body of evidence from existing studies of deworming programs. Empirical evidence showing the potential impact of community-wide deworming on STH infections in children is needed to lend support to calls for expanding STH control programs community-wide.

The third evidence gap relates to the **role of WASH in STH control**. As previously elucidated, there is limited experimental evidence of the impact of WASH on STH control, with most evidence obtained from low-quality observational studies [156, 158]. The potential importance of WASH as a complementary intervention to deworming for STH control has been repeatedly highlighted by researchers [176, 177]. However, at the commencement of this PhD, there were no published trials comparing the impact of deworming alone to deworming combined with a community-level water, sanitation, and hygiene intervention. Well-designed RCTs investigating the impact of WASH interventions on STH infections are essential to encourage the specific inclusion of WASH in STH control guidelines. Furthermore, detailed risk factor analyses, including longitudinal data, may be instrumental in identifying key focus areas for WASH interventions specifically designed to improve STH control.

The final evidence gap relates to the **validation of qPCR** for determining STH infection intensity. Given the limitations of the currently-recommended Kato-Katz technique, research into alternative diagnostic techniques is an important priority [182]. qPCR has shown significant promise as a highly sensitive diagnostic technique [196, 200]. However, evidence gaps remain in terms of validating quantitative results obtained using qPCR for STH diagnosis. At the starting point of this PhD, there were very few comparisons between infection intensity measurements obtained using qPCR and microscopy-based techniques other than the Kato-Katz technique. Additionally, there were no studies examining variability in DNA detection from STH-positive stool samples over time, an important consideration given that stool samples must be preserved and transported to a reference laboratory for analysis. Such evidence gaps must be addressed before qPCR can be recommended for routine use in STH control programs.

In order to address these four key evidence gaps, this thesis has four research objectives:

1. To compare the efficacy of a broad range of anthelminthic medications, in order to determine if there are more efficacious drugs or drug combinations compared to the current standard treatment (Chapter 2);
2. To determine if community-wide approaches to STH control have a greater impact on STH infections in children, compared to approaches targeted only to children (Chapters 3 and 4);
3. To investigate the impact of community-level WASH interventions and individual- and household-level WASH characteristics on STH infections (Chapter 5); and

4. To further investigate the role of qPCR in monitoring STH control programs, by comparing qPCR with sodium nitrate flotation and investigating variability in DNA detection (Chapter 6).

1.5 Thesis structure

This thesis includes seven chapters, as shown in Figure 3. **Chapter 1** (this chapter) has described the context in which this research was conducted, and provided background on STH infections, their global disease burden, and strategies and guidelines for STH control. It has described the evidence gaps and research aims that will be addressed throughout the thesis.

The subsequent five chapters (**Chapters 2–6**) are a compilation of seven published manuscripts that address the research objectives described above. Each chapter begins with a context statement, positioning the chapter within the overall thesis narrative.

Chapter 2 contains a systematic review and meta-analysis comparing the efficacy of 21 different anthelminthic drug regimens (Paper 1). In this paper, I synthesized a large body of evidence examining anthelminthic efficacy, and used a novel network meta-analytic technique to compare anthelminthic drugs and drug combinations to the current standard treatment, single-dose albendazole.

Chapter 3 also presents a systematic review and meta-analysis. This paper examines the differential impact of community-wide and child-targeted distribution of deworming medications (Paper 2). For this analysis, I synthesized evidence examining either of these two approaches to STH control and compared their effectiveness using a generalised linear model to account for several confounding variables.

Chapter 4 contains the study protocol (Paper 3) and results (Paper 4) from the (S)WASH-D for Worms pilot study. This was a field-based trial conducted in Timor-Leste, comparing the impact of school-based and community-wide approaches to STH control. This study was a key component of my PhD. I was the study coordinator and was present for all fieldwork activities, trained and supervised a team of eight people, liaised with the relevant WASH agencies and government departments, and conducted the analysis of trial data.

Chapter 5 includes two papers, both of which present results from the WASH for WORMS study. WASH for WORMS was a cluster-randomised controlled trial that was conducted in Timor-Leste, investigating the impact of a community-based deworming and WASH intervention on STH infections, compared to deworming alone. This trial finished shortly after I commenced my PhD, and while I had no operational role in the trial, I conducted the statistical analysis of the main trial outcomes (Paper 5) and also undertook a risk factor analysis (Paper 6) to further explore the role of WASH in STH transmission.

Chapter 6 contains a paper comparing the diagnostic performance of qPCR to sodium nitrate flotation, and investigating variability in DNA detection using qPCR (Paper 7). For this project, I oversaw the collection of stool samples (as part of the (S)WASH-D for Worms pilot study), conducted STH diagnosis using sodium nitrate flotation, and led the data analysis.

Finally, **Chapter 7** presents a summary of the key research findings and an integrated discussion of the policy implications of these findings. Future research priorities are identified and strengths and limitations of the thesis are explored, prior to brief concluding remarks.

Chapter 1: Introduction and background

Research objective 1

To determine if there are more efficacious drugs or drug combinations, compared to the current standard treatment against STH

Chapter 2: Anthelminthic efficacy

Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis (Paper 1)

Research objective 2

To determine if community-wide approaches to STH control have a greater impact on STH infections in children, compared to approaches that are targeted only to children

Chapter 3: Delivery of deworming programs

Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis (Paper 2)

Chapter 4: Delivery of integrated STH control programs

Investigating the differential impact of school and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for Worms pilot study protocol (Paper 3)

(S)WASH-D for Worms: a pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths (Paper 4)

Research objective 3

To investigate the impact of community-level WASH interventions and individual- and household-level WASH characteristics on STH infections

Chapter 5: The role of WASH in STH control

WASH for WORMS: a cluster-randomized controlled trial of the impact of a community-integrated water, sanitation, and hygiene and deworming intervention on soil-transmitted helminth infections (Paper 5)

Risk factors for infection with soil-transmitted helminths during an integrated community-level WASH and deworming intervention in Timor-Leste (Paper 6)

Research objective 4

To further investigate the role of qPCR in monitoring STH control programs

Chapter 6: qPCR for STH diagnosis

Quantitative polymerase chain reaction for diagnosis of soil-transmitted helminth infections: a comparison with a flotation-based technique and an investigation of variability in DNA detection (Paper 7)

Chapter 7: Discussion and conclusion

Figure 3. Thesis structure, including research objectives and paper titles

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Chapter 2
Anthelminthic efficacy

2.1 Chapter context

This chapter addresses the first research objective, namely to compare the efficacy of a broad range of anthelminthic medications, in order to determine if there are more efficacious drugs or drug combinations compared to the current standard treatment. This chapter is presented as a peer-reviewed journal article, published in *Clinical Infectious Diseases* (Paper 1).

As described in Chapter 1, preventive chemotherapy is the cornerstone of STH control efforts, and the anthelminthic drugs recommended by the WHO are delivered to hundreds of millions of people every year. Therefore, one of the most critical decisions for NTD policymakers is which drugs to endorse for distribution in large-scale preventive chemotherapy programs. Albendazole and mebendazole are currently recommended due to their excellent safety profile, single fixed dosage, and relatively high efficacy against most STH species. However, their efficacy against *T. trichiura* is poor, and there is increasing concern that benzimidazole resistance may develop in humans. Therefore, alternative chemotherapeutic options for STH control must be identified as a matter of priority, and there is a burgeoning evidence base examining safety, tolerability, and efficacy of various anthelminthic drugs, and their combinations, against STH.

However, existing syntheses of anthelminthic efficacy have been limited to a small number of standard drugs, providing limited information for policymakers. The work presented in this chapter was undertaken to provide an updated and comprehensive synthesis of available anthelminthic drugs and drug combinations for STH infections, including a rigorous comparison of the efficacy of these available

treatments. To ensure the relevance of this analysis to policymakers, all treatments were compared to the current standard treatment, single-dose albendazole.

A network meta-analysis was conducted to enable inclusion of the broadest possible range of studies. Network meta-analysis is a powerful statistical technique that enables comparison of three or more treatments, pooling direct comparisons and indirect comparisons based on a common comparator. This allowed inclusion of not only placebo-controlled trials, but also trials comparing any two (or more) anthelminthic agents. Using this technique, 21 different drug treatments were compared, representing the most comprehensive available comparison of anthelminthic drug efficacy against each STH species. The findings presented in this chapter have important implications for both researchers and policymakers in the NTD sector, highlighting priority chemotherapeutic agents for further research and integration into STH control guidelines.

2.2 Paper 1

Clarke NE, Doi SAR, Wangdi K, Chen Y, Clements ACA, Nery SV. Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis. *Clin Infect Dis* 2019; 68(1): 96–105. http://doi.org/10.1093/cid/ciy423

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This chapter includes a pre-copyedited, author-produced version of an article accepted for publication in *Clinical Infectious Diseases* following peer review. The published version is available online at: https://academic.oup.com/cid/article-abstract/68/1/96/4996920.

Title: Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis

Authors: Naomi E. Clarke[1], Suhail A. R. Doi, PhD[1,2], Kinley Wangdi[1], Yingxi Chen[1], Archie C. A. Clements[1,3], Susana V. Nery[1,4]

[1] Research School of Population Health, Australian National University, Canberra, Australia

[2] Department of Population Medicine, College of Medicine, Qatar University, Doha, Qatar

[3] Faculty of Health Sciences, Curtin University, Perth, Australia

[4] Kirby Institute, University of New South Wales, Sydney, Australia

Corresponding author:

Naomi E Clarke

Ph: +61 2 6125 0538

Email: naomi.clarke@anu.edu.au

Keywords: Soil-transmitted helminths, drug efficacy, benzimidazoles

Running title: Anthelminthic drug efficacy meta-analysis

Summary: This network meta-analysis compares efficacy of 21 treatment regimens against soil-transmitted helminths. Albendazole-ivermectin, albendazole-oxantel pamoate and tribendimidine-oxantel pamoate show higher efficacy against *Trichuris trichiura* than standard treatment. Efforts towards including drug combinations in soil-transmitted helminth control guidelines should be prioritized.

Abstract

Background: Periodic mass distribution of benzimidazole anthelminthic drugs is the key strategy to control soil-transmitted helminths (STH) globally. However, benzimidazoles have low efficacy against *Trichuris trichiura*, and there are concerns about benzimidazole resistance potentially emerging in humans. Therefore, identifying alternative drug regimens is a pressing priority. We present a systematic review and network meta-analysis, comparing the efficacy of 21 different anthelminthic drug regimens, including standard, novel, and combination treatments.

Methods: We searched PubMed, MEDLINE, Embase, Web of Science, and Cochrane databases and identified studies comparing anthelminthic treatments to each other or placebo. The outcomes calculated were relative risk (RR) of cure and difference in egg reduction rates (dERR). We used an automated generalized pair-wise modelling framework to generate mixed treatment effects against a common comparator, the current standard treatment (single-dose albendazole). This study is registered with PROSPERO (CRD42016050739).

Findings: Our search identified 4876 studies, of which 114 were included in meta-analysis. Results identified several drug combinations with higher efficacy than single-dose albendazole for *T. trichiura*, including albendazole-ivermectin (RR of cure 3.22, 95%CI 1.84-5.63; dERR 0.97, 95%CI 0.21-1.74), albendazole-oxantel pamoate (RR 5.07, 95%CI 1.65-15.59; dERR 0.51, 95%CI 0.450-0.52), mebendazole-ivermectin (RR 3.37, 95%CI 2.20-5.16), and tribendimidine-oxantel pamoate (RR 4.06, 95%CI 1.30-12.64).

Interpretation: There are several promising drug combinations that may enhance the impact of STH control programs on *T. trichiura*, without compromising efficacy against *A. lumbricoides* and hookworm. We suggest further, large-scale trials of these drug combinations and consideration of their use in STH control programs where *T. trichiura* is present.

Introduction

Soil-transmitted helminths (STH) are the most prevalent of the neglected tropical diseases (NTDs), a diverse group of chronic infections that afflict the world's most vulnerable populations [1]. STH – roundworms (*Ascaris lumbricoides*), hookworms (*Necator americanus* and *Ancylostoma duodenale*) and whipworms (*Trichuris trichiura*) – infect an estimated 1.45 billion people worldwide [2], and have been associated with chronic sequelae including impaired growth and cognitive development [3].

Significant global progress has been made towards control of NTDs since the landmark London Declaration on NTDs in 2012, with ambitious targets focusing on ten preventable NTDs, including STH [4]. Controlling STH depends on regular delivery of anthelminthic drugs through mass drug administration programs, typically focused on school- and preschool-aged children due to their high risk of STH-associated morbidity [5].

The most commonly-used anthelminthic drugs for STH control are the benzimidazoles mebendazole and albendazole. These drugs, along with pyrantel pamoate and levamisole, are recommended by the World Health Organization (WHO) for STH control [6], and have well-established safety profiles [7, 8]. Large pharmaceutical companies donate mebendazole and albendazole for use in STH control programs, which treated over 630 million children worldwide in 2016 [9]. There have recently been calls to extend STH control programs community-wide [10, 11], with the prospect of interrupting parasite transmission receiving increasing recognition [12, 13].

A recent meta-analysis shows that single-dose albendazole and mebendazole are highly efficacious against *A. lumbricoides*, with pooled cure rates of 95.7% and 96.2% respectively and egg reduction rates of 98.5% and 98% respectively [14]. Albendazole is also efficacious against hookworm, with a pooled cure rate of 79.5% and egg reduction rate of 89.6% [14]. However, both albendazole and mebendazole have poor efficacy against *T. trichiura*, with cure rates of 30.7% and 42.1% and egg reduction rates of 49.9% and 66.1% respectively [14]. Controlling this parasite remains a significant challenge. Furthermore, there are significant concerns about the potential for benzimidazole resistance – already well-established in livestock populations – to emerge in humans as STH control programs continue to be scaled up worldwide [15, 16].

For these reasons, researchers have repeatedly declared the need for new drug regimens for controlling STH [15, 17, 18]. Recent years have seen an increase in both preclinical and clinical studies of novel drugs [19-21], as well as older drugs that had previously shown promise in controlling STH in humans [22, 23]. Furthermore, with few new drugs currently in development, the use of drug combinations has been increasingly investigated, combining existing standard anthelminthics with each other or with more novel anthelminthic agents [24-26].

In the context of a significant commitment by the WHO, endemic countries, and other key stakeholders to STH control, determining whether current guidelines reflect the most appropriate drug regimen is

crucial. A quantitative synthesis of the evidence base represents a powerful tool to guide future clinical trials, and to inform guidelines for control programs. Existing meta-analyses of anthelminthic drug efficacy have been limited to a small number of standard drugs [14, 27] or to individual drug combinations [28]. This study presents a comparison of the efficacy of a broad range of both standard and novel anthelminthic drugs and drug combinations, using network meta-analysis to compare these to the current standard treatment (single-dose albendazole).

Methods

This systematic review and meta-analysis was performed according to PRISMA guidelines [29]. The review protocol is available in PROSPERO, registration number CRD42016050739.

Search strategy

We searched PubMed, MEDLINE, Embase, Web of Science, and the Cochrane Central Register of Controlled Trials on March 10th, 2018, with no limitations on year or language. Search terms related to STH, anthelminthic drugs, and outcome measurements. The full search strategy is shown in the supplementary material. We sought additional papers from reference lists of relevant review papers [7, 17, 27, 30, 31] and included studies.

NEC, KW, SARD and SVN screened titles and abstracts, and NEC, KW, YC and SVN examined full-text papers for eligibility. Disagreements were resolved through consensus.

Selection criteria

Eligible studies compared the efficacy of two or more of the following drugs: albendazole, mebendazole, levamisole, pyrantel pamoate, ivermectin, diethylcarbamazine, oxantel pamoate, nitazoxanide, tribendimidine, or placebo, or combinations of the above drugs. We restricted studies to those that examined efficacy between 10 days and six weeks after treatment. Studies focusing exclusively on HIV-positive patients were excluded. Both randomized controlled trials (RCTs) and quasi-experimental studies with control groups were included.

If a study reported efficacy at multiple time points after treatment, the measurement closest to 14 days was selected, in accordance with WHO guidelines [32, 33]. Because we used a pair-wise modelling approach (described below), if studies compared an odd number of eligible treatments, we selected a pair (or multiple pairs) of treatments for inclusion, prioritizing those currently in widespread use for STH control, and novel treatments.

Data extraction and quality assessment

Data extraction was performed by NEC, KW and YC. We extracted the study year, country and design; study population; drug regimens; sample size; number of participants cured; arithmetic and/or geometric

mean egg counts before treatment; egg reduction rate and/or mean egg counts after treatment; diagnostic method; and time between treatment and efficacy assessment.

We assessed quality of included studies using a modification of a previously published checklist, based on GRADE guidelines and the Cochrane Collaboration's tool for assessing risk of bias [34-36]. There were 9 questions with a maximum score of 12 (see supplementary material). The quality effects model was used to assess whether study quality deficiencies significantly impacted results [37].

Statistical analysis

We examined both cure rate (proportion of treated participants that became egg-negative) and egg reduction rate (relative decrease in faecal egg count). The outcomes calculated for each study were the relative risk (RR) of cure and the absolute difference in egg reduction rates (dERR). The dERR was calculated from mean egg counts before and after treatment, and the standard error computed using a Markov Chain Monte Carlo (MCMC) procedure, detailed in the supplementary material. When only the geometric mean was available this was used in lieu of the arithmetic mean.

We used an automated generalized pair-wise modelling (GPM) framework [38] to generate mixed treatment effects against single-dose albendazole. This involved: (1) pooling effect sizes for direct comparisons between each combination of two treatments; (2) performing indirect comparisons by automated generation of all possible closed loops of three treatments; and (3) pooling direct and indirect effects to give a final effect size comparing each treatment to the common comparator [38]. For the dERR analysis, in order to reduce sparseness of the network, we excluded drug regimens that were reported in one study only. To pool estimates, we used the inverse variance heterogeneity model, which uses a quasi-likelihood based variance structure without distributional assumptions [39]. For comparison, all analyses were re-run using the random effects model within a multivariate frequentist framework [40].

One cluster RCT was included in the analysis after using the design effect to account for clustering [41]. The intra-cluster correlation coefficients for each STH were obtained from a study examining STH reinfection after deworming [42].

We assessed statistical heterogeneity across pooled direct effects using Cochran's Q and the H index, and assessed transitivity across the network by examining inconsistency using the weighted pooled H index ($\bar{H}$), with values < 3 considered to indicate minimal inconsistency [38]. Publication bias was assessed using 'comparison-adjusted' funnel plots [43].

Sensitivity analyses were performed based on restricting treatment networks to studies that utilized the Kato-Katz diagnostic method, and to studies that examined efficacy within 14–21 days, both as recommended by the WHO [44, 45]. We performed an additional sensitivity analysis for dERR, in which all studies reporting the geometric mean were excluded.

All analyses involved in the GPM framework were conducted using MetaXL version 5.3 (EpiGear International; Brisbane, Australia). MCMC analyses were conducted using the Ersatz software implementation (Epigear International, Noosa, Australia). Funnel and network plots were created in Stata version 14.1 (College Station, TX, USA).

Results

We identified 114 studies meeting inclusion criteria for the systematic review and network meta-analysis. Figure 1 depicts a PRISMA flow diagram of the study selection process. Details of included studies are shown in Supplementary Table 1.

Characteristics of included studies

The majority of studies were RCTs (82 studies; 71.9%), with a smaller number of quasi-experimental studies with control groups (26 studies; 22.8%). The study design was not described in six instances.

More than half the studies diagnosed STH infections using the Kato-Katz technique (69 studies; 60.5%), while the remaining studies used other microscopy-based methods (Supplementary Table 1). Efficacy was assessed at the following times: between 10 days and two weeks inclusive (27 studies; 23.9%), more than two and up to three weeks (38 studies; 33.3%), more than three and up to four weeks (18 studies; 15.8%), at one month (10 studies; 8.8%), and between a month and six weeks (5 studies; 4.4%). The remaining 16 studies assessed efficacy across several of the above time points. Cure rate was reported in 108 studies (94.7%). Useable data for egg reduction rate was provided in 76 studies (66.7%), of which 30 (39.5%) reported the geometric mean only.

Included drugs and combinations

Twenty-one different drug regimens were included in the network meta-analysis. These treatments are summarized in Table 1, with full details given in Supplementary Table 2. Network plots showing the comparison groups for each STH are shown in Figure 2 and Figure S1.

Quantitative synthesis

For *A. lumbricoides*, RR of cure was examined in 76 studies and dERR in 50 studies. Results of the network meta-analyses are shown in Figures 3 and 4. Single-dose oxantel pamoate was significantly less efficacious than single-dose albendazole in terms of both RR of cure and dERR, and single-dose tribendimidine was less efficacious in terms of dERR only. No treatments were more efficacious than single-dose albendazole in terms of RR of cure. For dERR, multiple-dose mebendazole was marginally more efficacious (dERR 0.08, 95% CI 0.00-0.15). All direct, indirect and final effects are shown in Supplementary Tables 3 and 4.

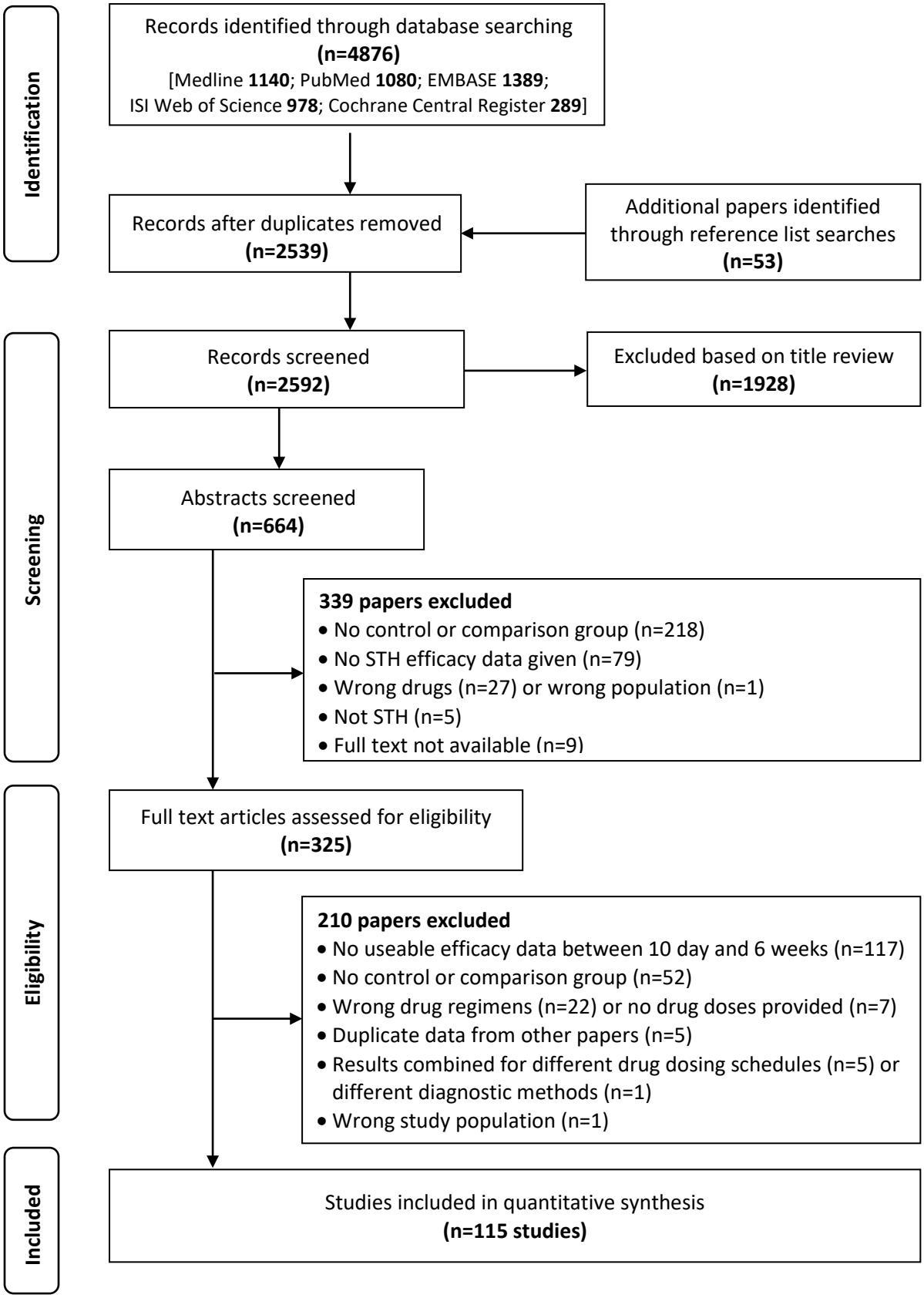

Figure 1. PRISMA flow diagram: process of selection of studies for inclusion in the systematic review and meta-analysis

Table 1. Summary of included studies according to drug treatment, stratified by STH

	Number of studies[a]		
	A. lumbricoides	**Hookworm**	***T. trichiura***
Single-dose albendazole (400mg or greater)	46 studies	45 studies	46 studies
Multiple-dose albendazole	11 studies	8 studies	12 studies
Single-dose mebendazole (400mg or greater)	26 studies	29 studies	30 studies
Multiple-dose mebendazole	21 studies	19 studies	22 studies
Single-dose pyrantel pamoate	18 studies	16 studies	10 studies
Single-dose ivermectin	5 studies	4 studies	5 studies
Single-dose levamisole	9 studies	6 studies	4 studies
Single-dose oxantel pamoate	3 studies	2 studies	4 studies
Nitazoxanide (any dose)	2 studies	1 study	2 studies
Single-dose tribendimidine	5 studies	7 studies	4 studies
Single-dose albendazole + ivermectin	4 studies	4 studies	6 studies
Single-dose mebendazole + ivermectin	1 study	1 study	1 study
Single-dose albendazole + mebendazole	1 study	1 study	2 studies
Single-dose albendazole + diethylcarbamazine	2 studies	1 study	3 studies
Single-dose albendazole + nitazoxanide	1 study	1 study	1 study
Single-dose albendazole + oxantel pamoate	3 studies	3 studies	3 studies
Single-dose oxantel pamoate + pyrantel pamoate	9 studies	6 studies	12 studies
Multiple-dose oxantel pamoate + pyrantel pamoate	2 studies	3 studies	5 studies
Single-dose tribendimidine + oxantel pamoate	1 study	1 study	1 study
Single-dose tribendimidine + ivermectin	1 study	1 study	1 study
Placebo / no treatment	23 studies	29 studies	26 studies
Total	**83 studies**	**80 studies**	**85 studies**

[a] Full references for included studies are presented in Supplementary Table 2

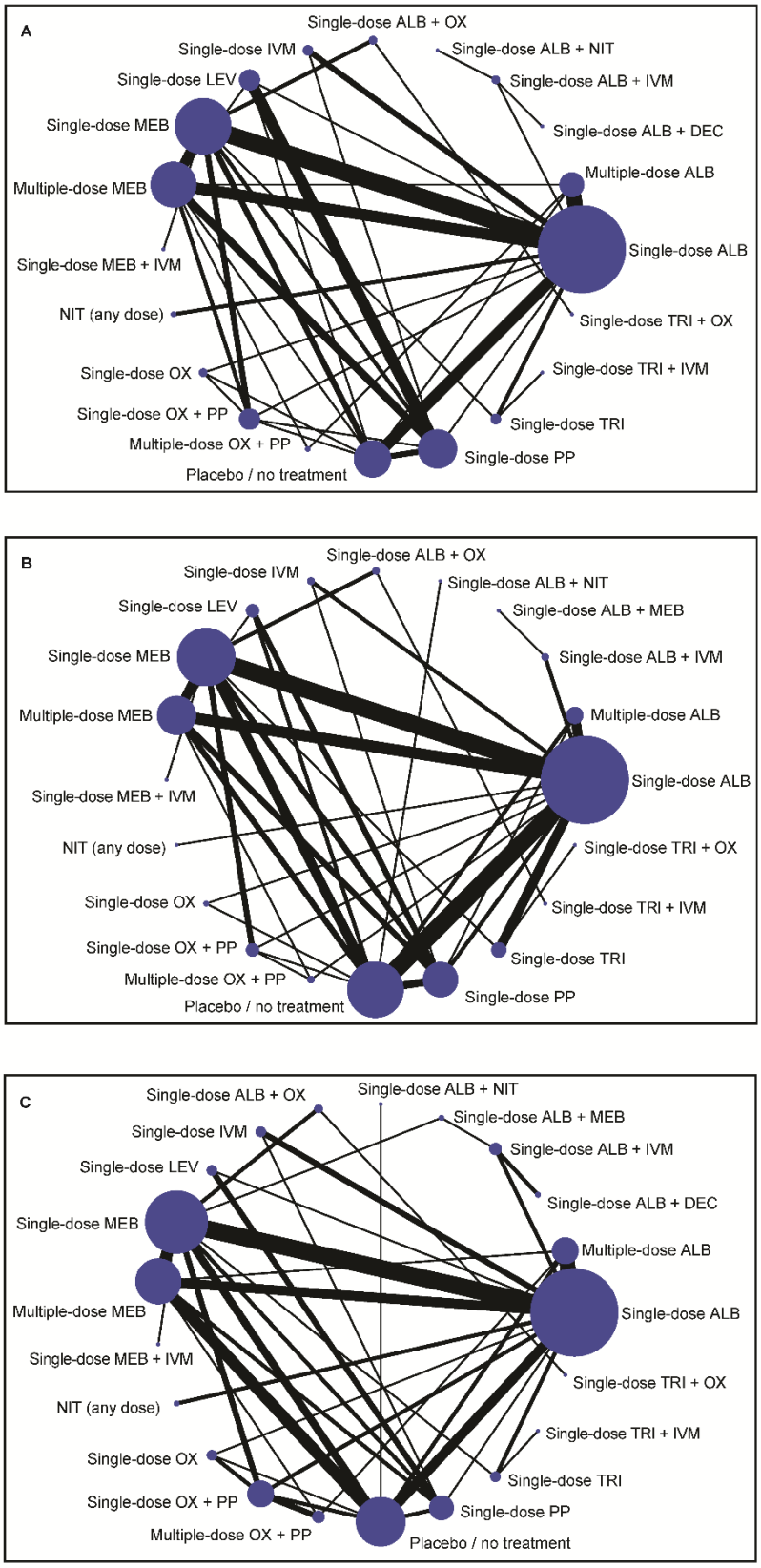

Figure 2. Network plots showing the comparison groups for relative risk of cure for *A. lumbricoides* (A), hookworm (B) and *T. trichiura* (C). Circle size is proportional to number of study arms; line width is proportional to number of pairs. ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine

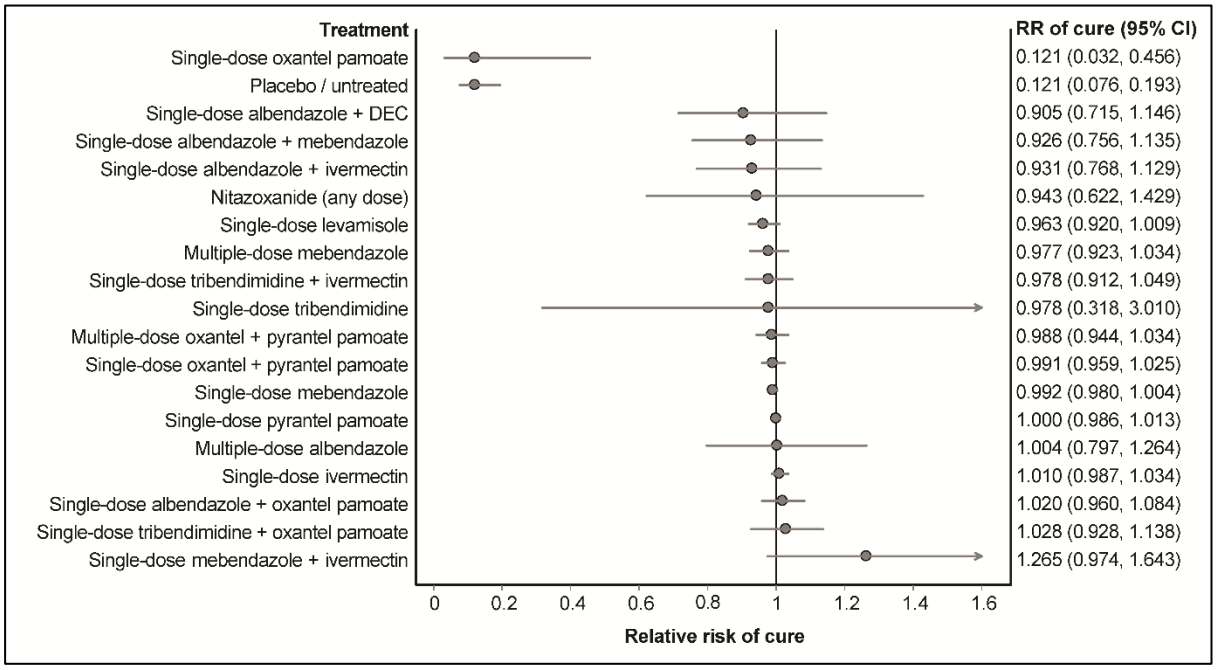

Figure 3. Results of network meta-analysis for *A. lumbricoides*, showing relative risk of cure for each treatment, compared to single dose albendazole. CI = confidence interval; DEC = diethylcarbamazine

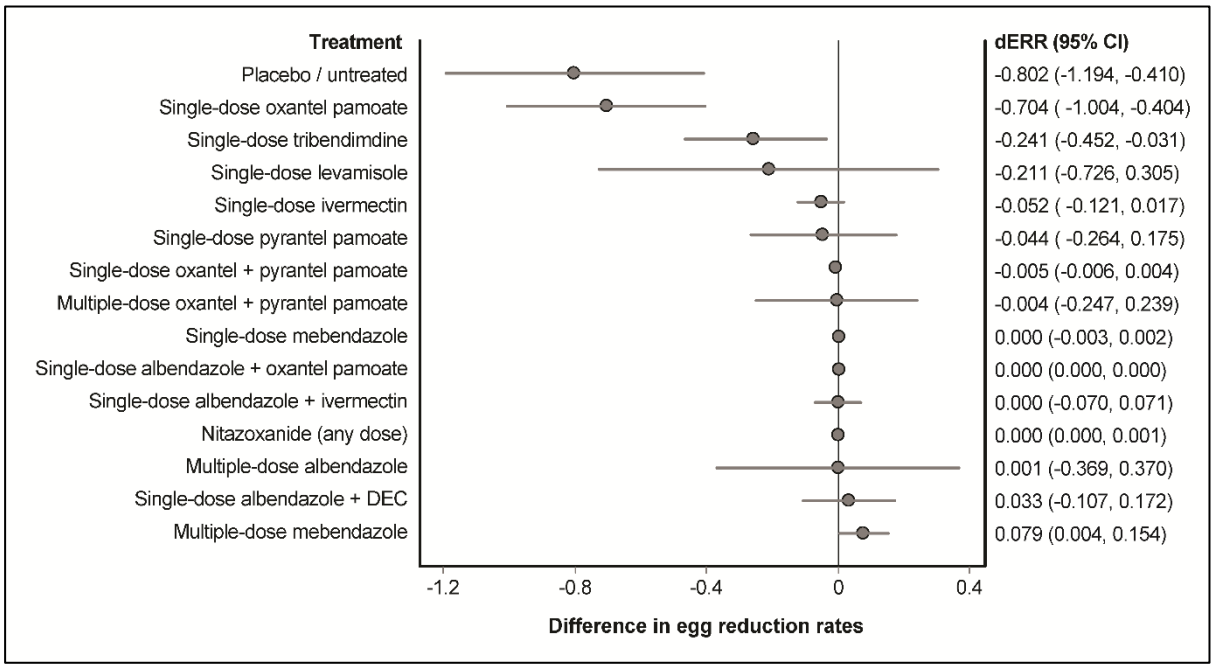

Figure 4. Results of network meta-analysis for *A. lumbricoides*, showing difference in egg reduction rates for each treatment, compared to single dose albendazole. CI = confidence interval; dERR = difference in egg reduction rates

For hookworm, 77 studies were included for RR of cure and 46 for dERR. As shown in Figures 5 and 6, single-dose oxantel pamoate, ivermectin, and mebendazole were significantly less efficacious than single-dose albendazole on both RR of cure and dERR, and single-dose mebendazole-ivermectin and oxantel-pyrantel pamoate were less efficacious in terms of RR of cure only. Multiple-dose albendazole was found to be marginally more efficacious than single-dose albendazole in terms of RR of cure (RR 1.14, 95%CI 1.05-1.24). Full results of all comparisons are shown in Supplementary Tables 5 and 6.

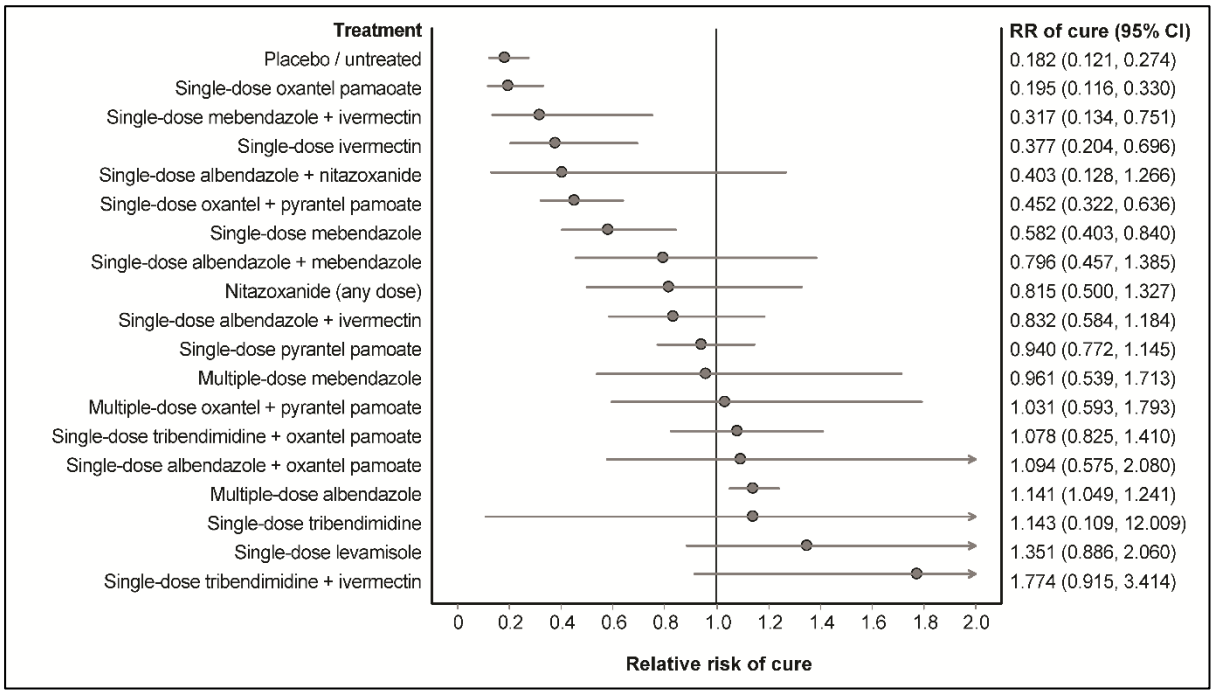

Figure 5. Results of network meta-analysis for hookworm, showing relative risk of cure for each treatment, compared to single dose albendazole. CI = confidence interval

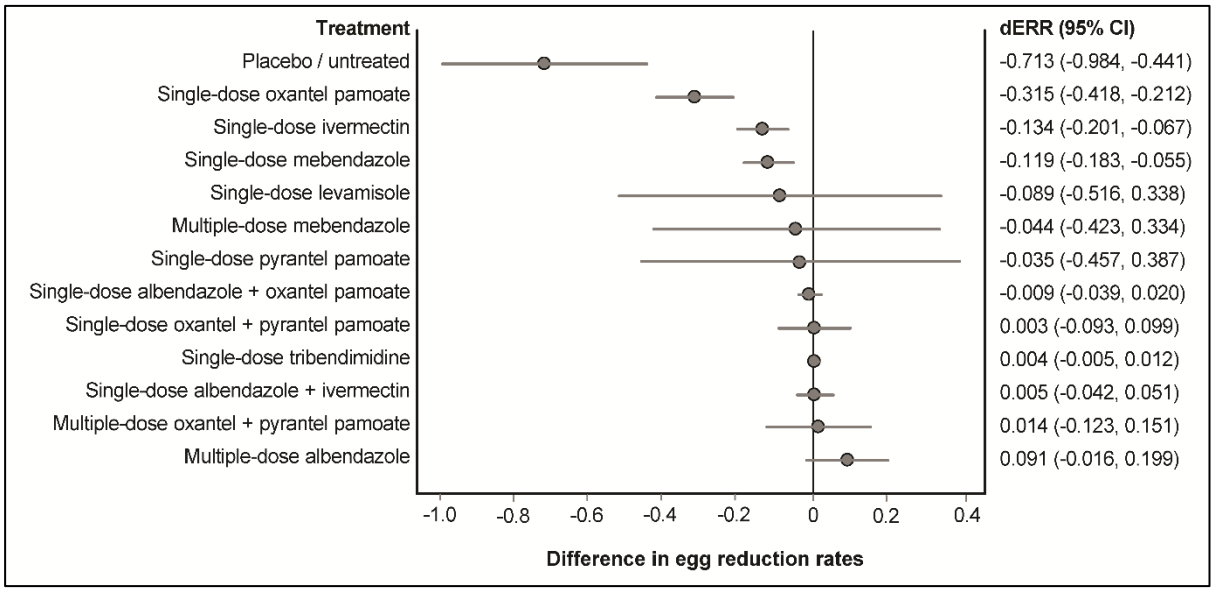

Figure 6. Results of network meta-analysis for hookworm, showing difference in egg reduction rates compared to single dose albendazole. CI = confidence interval; dERR = difference in egg reduction rates

For *T. trichiura*, 80 studies examined RR of cure and 54 examined dERR. Network meta-analysis results are shown in Figures 7 and 8. Single doses of pyrantel pamoate and levamisole were significantly less efficacious than single-dose albendazole in terms of RR of cure. Single doses of albendazole-ivermectin (RR 3.22, 95%CI 1.84-5.63; dERR 0.97, 95%CI 0.21-1.74), albendazole-oxantel pamoate (RR 5.07, 95%CI 1.65-15.59; dERR 0.51, 95%CI 0.450-0.52), and multiple-dose mebendazole (RR 1.72, 95%CI 1.07-2.77; dERR 0.307, 95% CI 0.238-0.377) were more efficacious than single-dose albendazole in terms of both RR of cure and dERR. In addition, mebendazole-ivermectin (RR 3.37, 95%CI 2.20-5.16), tribendimidine-oxantel pamoate (RR 4.06, 95%CI 1.30-12.64), and multiple-dose oxantel-pyrantel pamoate (RR 2.20, 95%CI 1.74-2.79) were more efficacious in terms of RR of cure only. Full results are shown in Supplementary Tables 7 and 8.

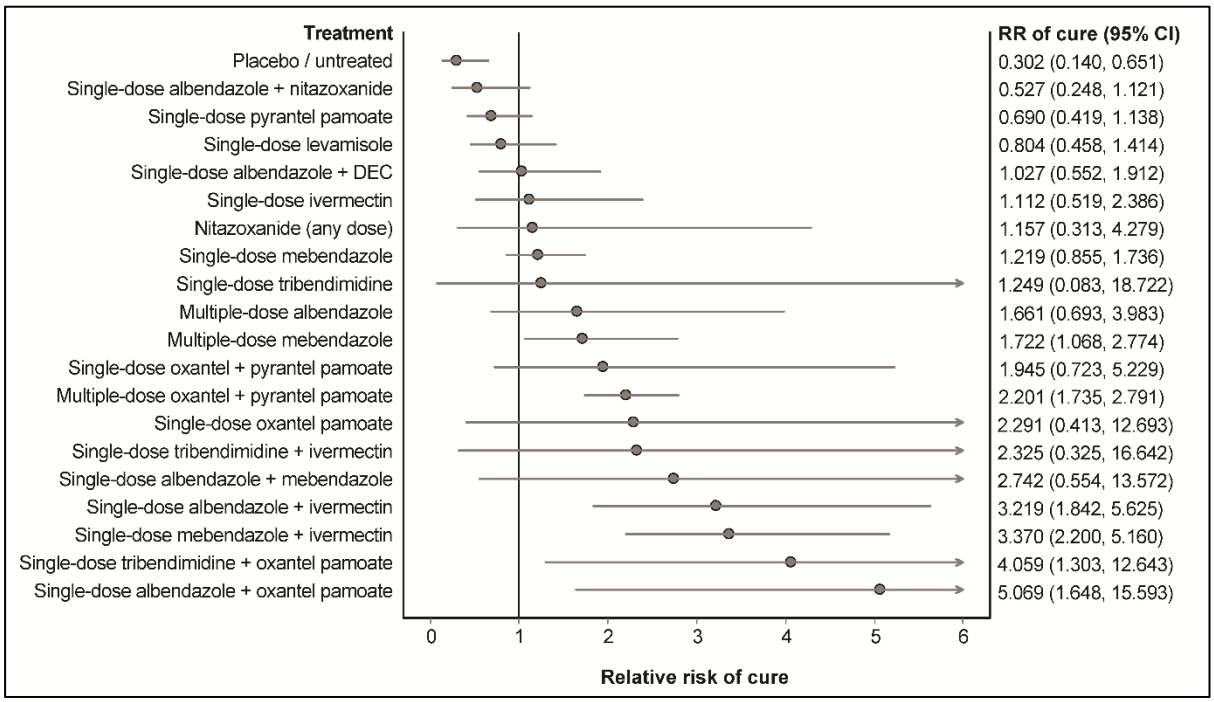

Figure 7. Results of network meta-analysis for *T. trichiura*, showing relative risk of cure for each treatment, compared to single dose albendazole. CI = confidence interval; DEC = diethylcarbamazine

Results comparing the GPM and multivariate frequentist frameworks are depicted in Supplementary Tables 9 and 10. Pooled estimates were similar, but differed in terms of error estimation (confidence intervals). GPM estimates are more reliable because this framework makes no assumptions, while distributional assumptions underpin the multivariate frequentist framework, which requires augmented datasets (using fictional study arms of high variance) when studies lack the reference treatment [40].

Sensitivity analysis and assessment of bias

Sensitivity analyses restricting the network to studies that utilized the Kato-Katz diagnostic method, and to studies that assessed efficacy at 14–21 days after treatment, showed that the results remain robust to these selection criteria. Removing geometric means reduced the number of included drug regimens, but

did not significantly change results for those that remained. Full results are shown in Supplementary Tables 11 and 12.

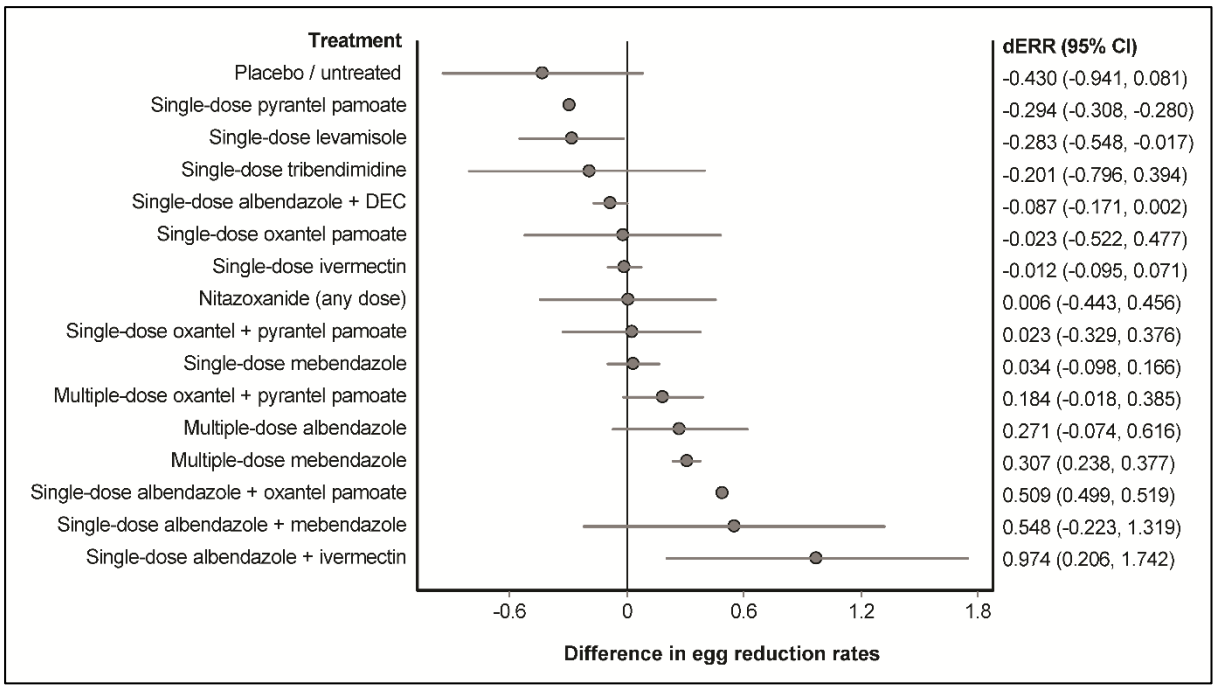

Figure 8. Results of network meta-analysis for *T. trichiura*, showing difference in egg reduction rates for each treatment, compared to single dose albendazole. CI = confidence interval; DEC = diethylcarbamazine; dERR = difference in egg reduction rates

Quality assessment results for studies in the network meta-analysis are shown in Supplementary Table 13. Study quality was highly variable, with scores ranging between 3 and 12. Results after application of the quality effects model did not differ from the main results (data not shown).

There was minimal inconsistency across treatment networks for RR of cure, with $\overline{H}$ = 1.18 for *A. lumbricoides*, $\overline{H}$ = 1.15 for hookworm, and $\overline{H}$ = 1.74 for *T. trichiura*, and minimal inconsistency across direct and indirect effects for each treatment comparison pair (Supplementary Table 11). Inconsistency was higher for dERR, with $\overline{H}$ = 3.45 for *A. lumbricoides*, $\overline{H}$ = 6.87 for hookworm, and $\overline{H}$ = 4.70 for *T. trichiura*, and some inconsistency across direct and indirect effects (see Supplementary Table 12). Comparison-adjusted funnel plots demonstrated little evidence of asymmetry for RR of cure (Supplementary Figure 2). The funnel plot for dERR (Supplementary Figure 3) was not interpretable; however, this was expected given that the effect size has a similarity to the proportion [46].

Discussion

In view of poor efficacy of standard drugs against *T. trichiura*, and concerns about benzimidazole resistance emerging, efforts are being made to identify alternative drug regimens for STH control. Older anthelminthics [22], new drugs [47, 48], and anthelminthic combinations [26, 49] have all been

investigated in recent years. In view of this burgeoning evidence base, and the global commitment to STH control, ensuring that control programs use the best available treatments is imperative.

We compared the efficacy of a wide range of anthelminthic drug regimens using network meta-analysis. Our results show that for *A. lumbricoides* and hookworm, single-dose albendazole is equally or more efficacious when compared to other drug regimens, with the exception of multiple-dose mebendazole for *A. lumbricoides* and multiple-dose albendazole for hookworm. These results confirm that there are currently no superior treatments for these STH within the existing framework of control operations, given the operational and financial challenges involved in multiple dosing.

For *T. trichiura*, the most challenging STH to control, our results identify several single-dose drug combinations that are superior to single-dose albendazole. These include albendazole-ivermectin, albendazole-oxantel pamoate, mebendazole-ivermectin, and tribendimidine-oxantel pamoate. Of these, mebendazole-ivermectin is less efficacious than single-dose albendazole against hookworm. However, albendazole-ivermectin, albendazole-oxantel pamoate, and tribendimidine-oxantel pamoate are equally efficacious as single-dose albendazole against *A. lumbricoides* and hookworm, and may therefore enhance *T. trichiura* control without compromising efficacy against other STH. In existing studies, albendazole-ivermectin consistently reduced *T. trichiura* egg counts by over 90% [24, 26, 50, 51] and albendazole-oxantel pamoate by over 95% [26, 49, 52], while the only study of tribendimidine-oxantel pamoate reported an ERR above 99% [49]. In the only direct comparison of albendazole-ivermectin and albendazole-oxantel pamoate, albendazole-oxantel pamoate was significantly more efficacious than albendazole-ivermectin in terms of both cure rate and ERR [26]. Albendazole-oxantel pamoate was significantly more efficacious than tribendimine-oxantel pamoate in terms of cure rate, but not ERR, in the only direct comparison [49]. Albendazole-ivermectin and tribendimidine-oxantel pamoate have not been directly compared.

Albendazole-ivermectin is already in widespread use for lymphatic filariasis (LF) control programs in areas where onchocerciasis is co-endemic [53]. It has a well-established safety profile [28, 54] and was last year added to the WHO Essential Medicines List for STH treatment [55]. On the other hand, albendazole-oxantel pamoate is not yet listed on the WHO Essential Medicines List, and has been investigated in only a small number of studies. To date, safety trials have described mainly mild adverse events [26, 52]. Tribendimidine was approved for human use in China in 2004 [56], but has not yet been approved by the US Food and Drug Administration [49]. Its combination with oxantel pamoate has only been examined in one study, with mainly mild adverse events seen [49]. Larger scale clinical trials are urgently required to further investigate efficacy and safety of these drug combinations, and to identify a fixed dose for oxantel pamoate; 500mg has been suggested in one dose-ranging study [23].

Given the above, albendazole-ivermectin currently represents the best candidate for inclusion in STH control guidelines to improve treatment of *T. trichiura*. Along with field trials [26, 52] and a recent

synthesis [28], mathematical modelling supports the use of albendazole-ivermectin to enhance impact against *T. trichiura* [57]. However, ivermectin cannot be used in settings where *Loa loa* is endemic, due to the risk of severe neurological complications [58]. This further highlights the need for researchers and policy makers to prioritise efforts towards including alternative highly efficacious options, in particular albendazole-oxantel pamoate, in STH control guidelines.

Operational and financial barriers to including drug combinations in large-scale deworming programs must be considered, especially the requirement for drug donors. Over 270 million doses of ivermectin are donated annually for use in LF and onchocerciasis programs [59], but significantly more would be required to incorporate ivermectin into STH control programs. In the long term, however, co-administration of ivermectin could lead to cost savings, given that it greatly increases the feasibility of breaking *T. trichiura* transmission [57]. An additional benefit of ivermectin is its efficacy against other NTDs, including not only LF and onchocerciasis but also *Strongyloides stercoralis* and scabies [60].

A limitation of this analysis was the use of geometric means to calculate ERR when arithmetic means were not available. Although sensitivity analysis did not reveal a significant impact on study results, this may have resulted in less precise estimates and explain the higher inconsistency in this analysis.

Additionally, combining studies that utilized different diagnostic techniques, at varying times following drug administration, may have decreased the precision of effect estimates. Both diagnostic sensitivity and timing since treatment influence measurements of drug efficacy [33, 61] although sensitivity analyses again did not suggest a significant impact on results. Other factors that may impact drug efficacy include baseline infection intensity and host factors [62].

Finally, the sparseness of the network in some treatment arms represents a limitation of this meta-analysis. Some drug combinations were reported in only a small number of studies, limiting the strength of conclusions that can be drawn from meta-analysis. This highlights the need for large-scale trials to strengthen the evidence base for these treatment combinations.

In conclusion, this network meta-analysis identified several drug combinations that could improve current STH control efforts by enhancing efficacy against *T. trichiura*. Research into novel treatments must remain a priority; however, with few drugs in development [30, 63], the immediate focus should be on selecting the best treatment from those currently available. We suggest that albendazole-ivermectin should be added to global guidelines for use in mass drug administration programs where *T. trichiura* is present (excluding areas where *L. loa* is present), and that further investigations of albendazole-oxantel pamoate and tribendimidine-oxantel should be prioritized.

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Declaration

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Chapter 3
Delivery of deworming programs

3.1 Chapter context

This chapter addresses the second research objective of the thesis: to determine if community-wide approaches to STH control have a greater impact on STH infections in children, compared to approaches targeted only to children. This chapter is presented as a peer-reviewed journal article, published in *The Lancet* (Paper 2).

The previous chapter focused on available anthelminthic drugs that could be distributed in large-scale preventive chemotherapy programs for STH control. The work presented in this chapter continues to focus on preventive chemotherapy, examining to whom regular treatment should be delivered. As detailed in Chapter 1, the 2020 WHO target for STH control focuses on treating preschool- and school-aged children, and drug donations for STH programs cover only these age groups. Existing operational guidelines and STH control programs therefore focus heavily on children. On the other hand, preventive chemotherapy for other NTDs, including lymphatic filariasis and onchocerciasis, are delivered community-wide (i.e., to all age groups), and in recent years there have been calls to expand preventive chemotherapy programs for STH control community-wide. This has mainly been prompted by evidence from mathematical modelling studies, showing that in most settings, regular deworming of children alone cannot achieve interruption of STH transmission. These studies suggest that community-wide treatment may be required to drive infection prevalence and intensity to levels at which STH transmission cannot be sustained and regular deworming can be ceased.

Considering this projected greater impact of community-wide deworming on STH transmission, it follows that this approach is likely to result in fewer STH reinfections among children. This is particularly

relevant for policymakers, given that children are emphasised in STH control targets due to their high burden of associated morbidity. However, when this PhD was commenced, there was no existing empirical evidence comparing community-wide and child-targeted deworming using benzimidazoles. The work presented in this chapter takes a step towards addressing this key evidence gap.

There is a large body of literature reporting the impact of deworming programs on STH prevalence, including the impact of community-wide albendazole treatment administered through the Global Program to Eliminate Lymphatic Filariasis (GPELF). This chapter presents a systematic review of studies reporting the impact of community-wide or child-targeted deworming on STH prevalence in school-aged children. Using meta-analysis and generalised linear models, the impact of these two deworming approaches was directly compared, adjusting for key confounding variables. The findings presented in this chapter are likely to be instrumental in the ongoing discussion regarding the future of STH control, a key issue for NTD policymakers currently, with community-based deworming programs through the GPELF being scaled down in many countries, and program targets beyond 2020 being deliberated.

3.2 Paper 2

Clarke NE, Clements ACA, Doi SA, Wang D, Campbell SJ, Gray DJ, Nery SV. Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis. *Lancet* 2017; 389(10066): 287–297. http://doi.org/10.1016/S0140-6736(16)32123-7

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Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis

Naomi E Clarke, Archie C A Clements, Suhail A Doi, Dongxu Wang, Suzy J Campbell, Darren Gray, Susana V Nery

Summary

Background Soil-transmitted helminth infections are a major global health issue, causing substantial morbidity in the world's poorest populations. Regular delivery of anthelmintic drugs is the mainstay for global soil-transmitted helminth control. Deworming campaigns are often targeted to school-aged children, who are at high risk of soil-transmitted-helminth-associated morbidity. However, findings from modelling studies suggest that deworming campaigns should be expanded community-wide for effective control of soil-transmitted helminth transmission. We aimed to do a systematic review and meta-analysis to compare the effect of mass (community-wide) and targeted (children only) anthelmintic delivery strategies on soil-transmitted helminth prevalence in school-aged children.

Methods In this systematic review and meta-analysis, we searched MEDLINE, Embase, and Web of Science for articles published on or before Nov 5, 2015, reporting soil-transmitted helminth prevalence before and after distribution of albendazole or mebendazole, either targeted to children or delivered to the whole community. We excluded studies in which drug delivery was restricted to infected individuals or to a subset of the community or school, or if follow-up time was less than 3 months or greater than 18 months after drug delivery. We extracted data on study year, country, drug administration strategy, drug dose, number of deworming rounds, treatment coverage, diagnostic method, follow-up interval, and soil-transmitted helminth prevalence before and after treatment. We used inverse variance weighted generalised linear models, with prevalence reduction as the outcome variable, to examine the effect of mass versus targeted drug administration, as well as baseline prevalence, number of drug doses, and follow-up time. This study is registered with PROSPERO, number CRD42016026929.

Findings Of 10 538 studies identified, 56 studies were eligible for the systematic review and 38 of these were included in meta-analysis. Results of the regression models showed that mass deworming led to a significantly greater reduction in prevalence in children than targeted deworming, for both hookworm (odds ratio 4·6, 95% CI 1·8–11·6; p=0·0020) and *Ascaris lumbricoides* (16·4, 2·1–125·8; p=0·0092), with no effect seen for *Trichuris trichiura*. There was significant heterogeneity across studies; for targeted studies I^2 was 97% for *A lumbricoides* and hookworm, and 96% for *T trichiura*, and for mass studies, I^2 was 89% for *A lumbricoides*, 49% for hookworm, and 66% for *T trichiura*.

Interpretation The results of this meta-analysis suggest that expanding deworming programmes community-wide is likely to reduce the prevalence of soil-transmitted helminths in the high-risk group of school-aged children, which could lead to improved morbidity outcomes. These findings are in support of recent calls for re-evaluation of global soil-transmitted helminth control guidelines.

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Research School of Population Health, Australian National University, Canberra, ACT, Australia (N E Clarke MBBS, Prof A C A Clements PhD, Prof S A Doi PhD, D Wang PhD, S J Campbell MPH, D Gray PhD, S V Nery PhD); **College of Medicine, Qatar University, Doha, Qatar** (Prof S A Doi); **and School of Agricultural, Computational and Environmental Sciences, University of Southern Queensland, Toowoomba, QLD, Australia** (Prof S A Doi)

Correspondence to: Dr Naomi E Clarke, Research School of Population Health, Australian National University, Canberra, ACT 0200, Australia **naomi.clarke@anu.edu.au**

Introduction

Infection with the soil-transmitted helminths, roundworms (*Ascaris lumbricoides*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and whipworms (*Trichuris trichiura*), is the most common parasitic human disease worldwide, with an estimated 1·45 billion individuals infected.[1] Chronic infection with soil-transmitted helminths can lead to impaired physical and cognitive development, which is of particular concern in school-aged children, who have the highest burden of *A lumbricoides* and *T trichiura* infections and are at high risk of hookworm-associated morbidity.[2,3] Overall, soil-transmitted helminth infection is estimated to cause more than 3 million disability-adjusted life-years worldwide.[4]

The benzimidazole anthelmintics albendazole and mebendazole are the mainstay of treatment for the reduction of disease prevalence and burden.[2] These drugs have excellent safety records;[5] both drugs have high efficacy against *A lumbricoides*, albendazole is efficacious against hookworm, and both drugs are less efficacious against *T trichiura*.[6] Regular repeated treatment is necessary because reinfection can occur rapidly after treatment.[7] As such, soil-transmitted helminth control programmes consist of annual or biannual distribution of anthelmintic drugs to at-risk populations, in accordance with WHO guidelines.[5,8]

Given the high burden of soil-transmitted-helminth-associated morbidity in children, large-scale anthelmintic

Research in context

Evidence before this study

Regular distribution of deworming medications albendazole or mebendazole is the mainstay of control for soil-transmitted helminth infections. Deworming campaigns for soil-transmitted helminth control are typically targeted to school-aged children, who have the highest burden of morbidity. However, mathematical modelling and cost-effectiveness studies have advocated for the expansion of large-scale deworming programmes to all community members. We searched MEDLINE, Embase, and Web of Science to identify articles published in any language before November, 2015, and included papers reporting soil-transmitted helminth prevalence before and after distribution of albendazole or mebendazole, either targeted to children or deliverved to the whole community. Many studies were identified, but none have been synthesised in a systematic review and meta-analysis.

Added value of this study

To our knowledge, this systematic review and meta-analysis is the first to synthesise existing literature reporting the effect of either targeted or mass distribution of deworming medications on the prevalence of soil-transmitted helminth infections in children. Our findings suggest that for both *Ascaris lumbricoides* and hookworm, mass treatment programmes have a greater effect on prevalence reduction than targeted treatment programmes.

Implications of all the available evidence

The results of this meta-analysis contribute to the evidence base surrounding the benefits of expanding drug therapy programmes for control of soil-transmitted helminths to all members of the community. Our findings support those of modelling and cost-effectiveness studies. We suggest that soil-transmitted helminth control guidelines should be re-evaluated with consideration of expansion to community-wide drug administration in endemic areas.

distribution programmes typically focus on targeted delivery to school-aged children (aged 5–14 years).[5] Current WHO guidelines also suggest delivery of anthelmintics to preschool-aged children (aged 2–4 years), women of childbearing age, and people in high-risk occupations (eg, tea pickers).[5,9] In 2012, the London Declaration on Neglected Tropical Diseases announced a cross-sectoral commitment to help eliminate or control preventable neglected tropical diseases by 2020, inspired by WHO roadmap targets.[10] This commitment included a goal of treating 75% of children at risk of soil-transmitted helminth infection in all endemic countries. To this end, 600 million doses of albendazole and mebendazole are donated annually by pharmaceutical companies, enough to treat nearly 70% of the 876 million at-risk children worldwide.[11]

Since this resolution, demand for government-led, school-based deworming programmes has increased worldwide.[12,13] Using school-based infrastructure for anthelmintic delivery is considered a practical and cost-effective method of reaching a large proportion of the population at high risk of soil-transmitted-helminth-associated morbidity,[14] and some evidence has suggested collateral benefits to other age groups in the community, owing to reduced transmission within the population.[15,16]

Interest in the optimal design of soil-transmitted helminth control programmes has increased over the past 5 years. Mathematical modelling has been used to explore the effect of anthelmintic drug therapy on transmission and worm burden in the host population. Results suggest that, in many settings, child-targeted programmes might have limited effect on overall transmission in the community, and that deworming campaigns should be expanded to all age groups.[17–23] Furthermore, findings from cost-effectiveness modelling studies show that community-wide approaches are highly cost-effective;[21] particularly for hookworm,[23] for which adults can act as substantial reservoirs of infection.

Many published studies have investigated the effectiveness of anthelmintic delivery programmes.[7] However, to our knowledge, no comparison of studies has examined mass and targeted delivery strategies (panel). To fill this gap in the literature, this systematic review and meta-analysis aimed to describe existing literature reporting the effects of mass or targeted administration of albendazole or mebendazole on soil-transmitted helminth prevalence in school-aged children, and to examine the differential effects of mass and targeted drug delivery on soil-transmitted helminth prevalence in school-aged children.

Methods

Search strategy and selection criteria

This systematic review and meta-analysis was done according to PRISMA guidelines.[24] Eligible papers were published studies that reported soil-transmitted helminth prevalence before and after mass or targeted delivery of albendazole or mebendazole. Studies that examined other control strategies in addition to anthelmintic drug therapy, including water, sanitation, and hygiene (WASH) improvements, and medications for other neglected tropical diseases (eg, schistosomiasis and lymphatic filariasis) were included. Randomised trials were included if randomisation occurred at the community level or school level, rather than at the household or individual level.

Studies were excluded if anthelmintic delivery was restricted to infected individuals, a random selection of the population, or a specific group of students in a school; if positive cases were re-treated shortly after

initial drug administration; if soil-transmitted helminth prevalence before and after drug administration was not available; if follow-up time was less than 3 months or greater than 18 months; or if albendazole or mebendazole were not used.

The following additional exclusion criteria were applied for the purposes of meta-analysis: number of doses or follow-up time was not reported; different parasitological diagnostic methods were used at baseline and follow-up; data were combined for mass and targeted distribution strategies, several different dosing schedules, or several different follow-up periods; initial prevalence was less than 5%; or time between baseline assessment and first anthelmintic distribution was more than 12 months.

We searched MEDLINE, Embase, and Web of Science on Nov 5, 2015, with no limitations on year or language of publication. We used the following search terms that related to soil-transmitted helminth infection: "helminth" or "soil-transmitted helminth" or "STH" or "nematode" or "geohelminth" or "hookworm" or "roundworm" or "whipworm" or "Trichuris" or "Ascaris" or "Ancylostoma" or "Necator"; and to intervention: "chemotherapy" or "albendazole" or "mebendazole" or "anthelminthic" or "anthelmintic" or "benzimidazoles" or "deworming" or "mass drug administration". The complete search strategy is provided in the appendix (p 2). We sought further studies by hand-searching reference lists of relevant review papers,[7,25,26] WHO guidelines,[8,9] and included papers.

Potentially relevant studies were imported into EndNote (version X7). Study titles and abstracts were screened by NEC and DW, and full-text papers were retrieved for all candidate studies. Studies published in English were examined by two independent researchers (NEC and SJC), discrepancies were discussed with a third reviewer (SVN), and a consensus reached. Studies published in languages other than English (Chinese, French, Spanish, and Portuguese) were reviewed by researchers fluent in those languages (SVN and DW). All studies were assessed for eligibility against the review protocol. The review protocol is available in PROSPERO, registration number CRD42016026929.

Data extraction and quality assessment

Data were extracted by NEC and DW. Data extracted from eligible papers included study year and country; study population; sample size; drug-delivery strategy (mass or targeted); drug dose, frequency, and number of rounds; treatment coverage; additional interventions; and prevalence of each soil-transmitted helminth before and after drug delivery.

If more than one drug regimen was reported in the same study, data were extracted for each regimen separately. Similarly, if multiple populations were examined in the same study (eg, rural and urban), data were extracted for each population separately. In trials with a control group who had drug treatment only, and an intervention group who received an additional intervention (eg, sanitation improvements), only data from the control groups were extracted.

***Panel*: Mass and targeted drug delivery**

WHO defines different modalities of drug therapy, including:

- Mass drug administration: the entire population of an area (eg, state, region, province, district, subdistrict, or village) is given anthelmintic drugs at regular intervals, irrespective of individual infection status
- Targeted drug therapy: specific risk groups in the population, defined by age, sex, or other social characteristic such as occupation (eg, school-aged children, or fishermen) are given anthelmintic drugs at regular intervals, irrespective of individual infection status[8]

In this Article, we use the term mass drug delivery to describe programmes that give anthelmintic drug therapy to all community members, and targeted drug delivery to describe programmes that provide anthelmintic drug therapy only to children.

We contacted 33 authors to request additional information, including age-stratified soil-transmitted helminth prevalence, sample size, drug dose, follow-up time, and treated population. Five authors provided numerical data, which were previously only published in figure format, four authors clarified the treated population, three authors provided sample sizes, two authors clarified drug doses or follow-up time, and two authors provided age-segregated data.

See Online for appendix

We assessed study quality using a scale modified from the validated scale described by Hoy and colleagues,[27] which was designed to assess risk of bias in prevalence studies. Modifications were made to account for most studies being quasi-experimental studies without a control group, consisting of pre-post prevalence surveys. We used the National Heart, Lung, and Blood Institute quality assessment tools for observational cohort and cross-sectional studies,[28] and pre-post design studies,[29] to make these modifications, which included addition of items relating to consistent participant selection and sampling across timepoints, and coverage of the intervention. We assessed studies against nine safeguards, each of which provided additional assurance that there was no bias in the measurement of soil-transmitted helminth prevalence. Both internal and external validity items were included, as suggested for prevalence studies.[27] Quality assessment was done by NEC and cross-checked by SVN, with disagreements resolved through consensus.

For the **study protocol** see http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016026929

Statistical analysis

All analyses were done separately for each soil-transmitted helminth, because of the differences in age distribution, cure and reinfection rates after treatment, and environmental resilience.[2,6,7]

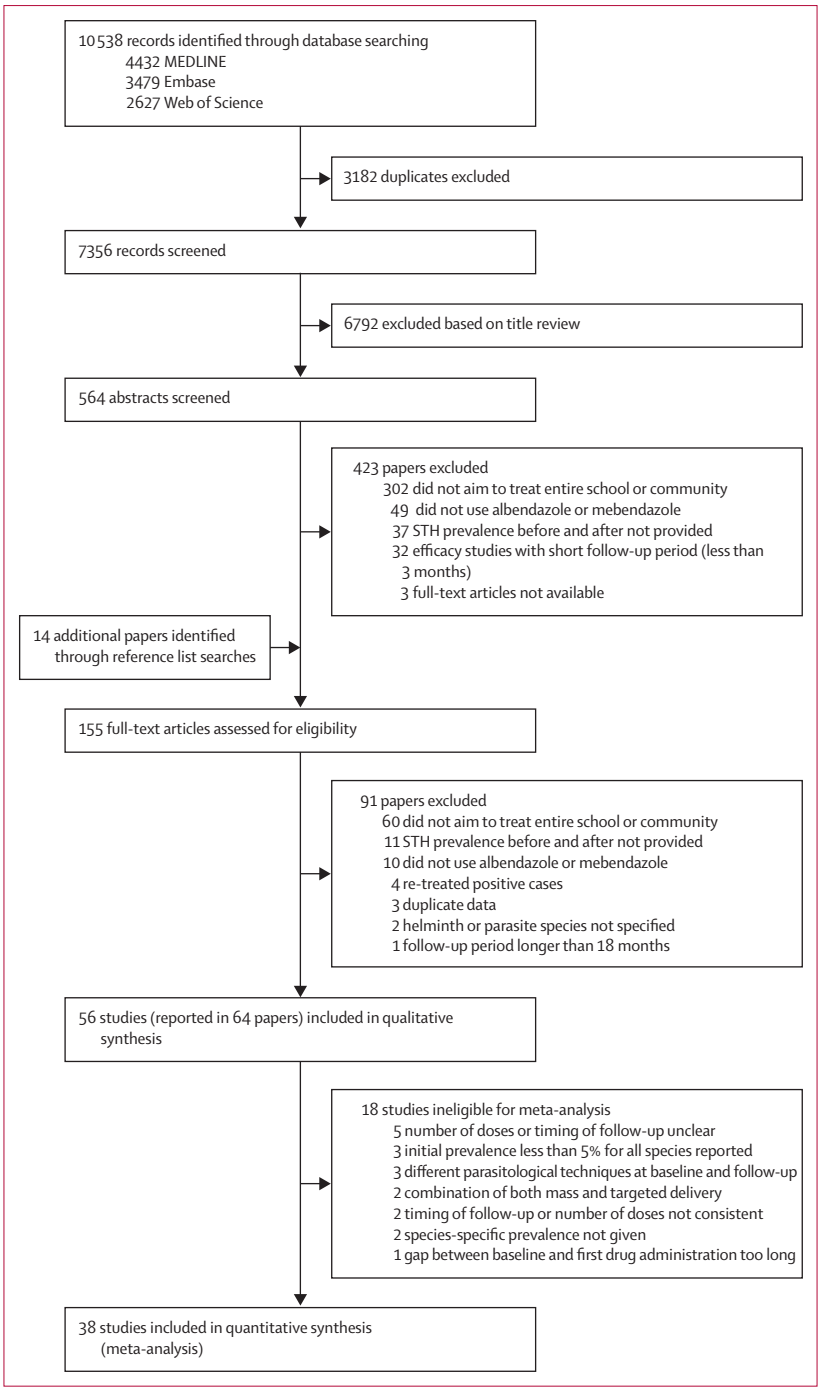

***Figure 1*: Study selection**
STH=soil-transmitted helminth.

Where age-stratified soil-transmitted helminth prevalence was not available (ten studies), we estimated prevalence in school-aged children from community prevalence with scaled age weights[30] and estimates of community age distribution obtained from UN datasets for the relevant country and 5 year period.[31]

The first timepoint at which data were available was considered the baseline. We considered this approach acceptable because soil-transmitted helminth infections rapidly recur after treatment,[7] and many populations in the studies included probably had some previous exposure to anthelmintics.

Given the heterogeneity between studies in terms of number of drug doses, dosing interval, and follow-up period, we used an inverse variance weighted generalised linear model with robust error variances to quantify the effect of these covariates. This regression used the inverse of the variance of each study as weights, so that observations with the least variance provided the most information to the model.

The outcome variable in the model was the prevalence reduction (PReduc). This was defined as $(p_1 - p_2) / p_1 = 1 -$ prevalence ratio, where p_1 is the pre-intervention prevalence proportion and p_2 is the post-intervention prevalence proportion, and p_2/p_1 is the prevalence ratio (PRatio). Only one follow-up prevalence, p_2, was entered per study. In an attempt to achieve consistency, follow-up prevalence was selected as follows: if prevalence was reported after multiple different doses, the assessment closest to the fourth dose was selected; and if prevalence was reported at multiple timepoints after the chosen dose, the assessment closest to 6 months was selected.

PReduc was truncated at its lower boundary so that any prevalence increase was reset to zero; thus, the truncated distribution mirrored that of a proportion. This truncated response variable could then be modelled using a logit link function to linearise it with predicted values.[32] This approach made sense because any increase would be unrelated to the intervention, implying no effect. Coefficients were exponentiated to generate weighted odds ratios based on the study-level predictors.[32] Link specifications were tested using the linktest command in Stata, to assess variance explained by the squared linear predictor.

Due to disproportionately high weights in some studies with very small variances, for the purposes of the weighted regression model, any weights that were more than five times greater than the upper quartile were truncated and replaced with the weight at the threshold. This action stabilised the variance of the regression coefficients and the point estimates.

The following covariates were entered into the model: (1) mass versus targeted distribution; (2) baseline prevalence; (3) number of doses between baseline and follow-up assessments; and (4) follow-up time (months) between most recent dose and prevalence assessment. Cumulative time between first dose and follow-up assessment was co-linear with number of doses, and thus not used. Regression outliers were examined using a leverage against residual squared plot and removed from the analysis.

We did a secondary analysis to synthesise PReduc (non-truncated) for each soil-transmitted helminth. To

	Ascaris lumbricoides	Hookworm	*Trichuris trichiura*	Overall STHs only	Total studies with references
Targeted delivery	20 studies, 23 papers	19 studies, 21 papers	20 studies, 22 papers	2 studies	25 studies, 28 papers
Mass delivery	18 studies, 22 papers	21 studies, 25 papers	18 studies, 22 papers	1 study	24 studies, 28 papers
Both targeted and mass delivery	7 studies, 8 papers	7 studies, 8 papers	7 studies, 8 papers	0 studies	7 studies, 8 papers

Full references for included studies are presented in the appendix. STH=soil-transmitted helminth.

Table 1: **Numbers of included studies according to method of drug delivery, stratified by type of STH**

do this we pooled PRatio, but reported results as 1−PRatio=PReduc. Results from each study were pooled using the inverse variance heterogeneity model,[33] which uses a quasi-likelihood-based variance structure without distributional assumptions and has been shown to perform better than the random effects method.[34] Heterogeneity was assessed using Cochran's Q test and Higgins' I^2, with I^2 greater than 50% considered to indicate significant heterogeneity. Publication bias and evidence of small-study effects were assessed using visual inspection of funnel plots,[35] and Egger's regression test (two-tailed p<0·1 considered indicative of asymmetry).[36]

Sensitivity analyses were done based on the following criteria: exclusion of influential studies (defined as studies with weight ≥30%); restriction to studies published in Africa; restriction to studies published in Asia; restriction to studies that used the Kato-Katz diagnostic method, recommended by WHO;[8] exclusion of studies that implemented WASH improvements; and prevalence reduction truncated as in the generalised linear model.

All meta-analyses, sensitivity analyses and the generalised linear model were re-run using random effects model weights for comparison. Meta-analyses were done with MetaXL (version 5.1). The generalised linear model was run in Stata (version 14.1).

Role of the funding source

There was no funding source for this study. The corresponding author (NEC) and senior author (SVN) had full access to all the data and had final responsibility for the decision to submit for publication.

Results

After title and abstract screening, 155 full-text articles were considered for inclusion, including 14 which were identified from manual searching of reference lists. 64 papers representing 56 individual studies met the inclusion criteria for the systematic review. 38 of these studies were suitable for meta-analysis (figure 1). Details of studies that were included and excluded are in the appendix (pp 3–7).

25 (45%) of 56 included studies reported on targeted drug administration and 24 (43%) studies reported on mass drug administration. Seven (13%) studies used both strategies (table 1). Most studies of targeted delivery used school-based deworming (23 [92%] of 25 studies) and treated only primary-school-aged children, generally aged 5–14 years (20 [80%] of 25; appendix p 8). Only four studies of school-based deworming included an attempt to include non-enrolled children.[37–40] In studies of mass delivery, the most common exclusion criteria for treatment were pregnancy (11 studies), and children younger than 2 years (11 studies) or 3 years (four studies; appendix p 8).

	Odds ratio (95% CI)	p value	R^2
Ascaris lumbricoides			
Mass *vs* targeted treatment	16·4 (2·1–125·8)	0·0092	0·724
Baseline prevalence*	2·7 (0·03–239·7)	0·6555	
Number of drug doses	1·8 (0·51–6·1)	0·3507	
Follow-up time	0·37 (0·27–0·51)	<0·0001	
Hookworm			
Mass *vs* targeted treatment	4·6 (1·8–11·6)	0·0020	0·336
Baseline prevalence*	0·07 (0·01–0·77)	0·0304	
Number of drug doses	0·82 (0·39–1·7)	0·5906	
Follow-up time	0·92 (0·81–1·0)	0·1797	
Trichuris trichiura			
Mass *vs* targeted treatment	2·1 (0·30–14·8)	0·4281	0·362
Baseline prevalence*	0·09 (0·004–2·0)	0·1228	
Number of drug doses	0·76 (0·35–1·6)	0·4568	
Follow-up time	0·55 (0·25–1·2)	0·1186	

STH=soil-transmitted helminth. *Baseline prevalence data were entered into the model on a scale of 0–1.

Table 2: **Odds ratio for selected covariates, stratified by STH (inverse variance weighted logit-linear regression with robust error variance)**

Of the seven studies that used both mass and targeted delivery, four studies alternated between the two strategies over time,[41–44] whereas three studies used different strategies in different regions, depending on the setting (rural *vs* urban),[45] *Schistosoma mansoni* prevalence,[40] or lymphatic filariasis prevalence.[46] There were no head-to-head comparisons of mass and targeted strategies in any study.

The number of anthelmintic drug doses varied from one to 16 doses, with dosing intervals ranging from 3 to 12 months, although interruptions in planned dosing schedules occasionally led to longer intervals.[41,45,47] The most common dosing intervals were 6 and 12 months, reported in 19 studies (6 months) and 20 studies (12 months). Drug administration strategies, as well as drug doses and study populations, are further described in the appendix (p 8).

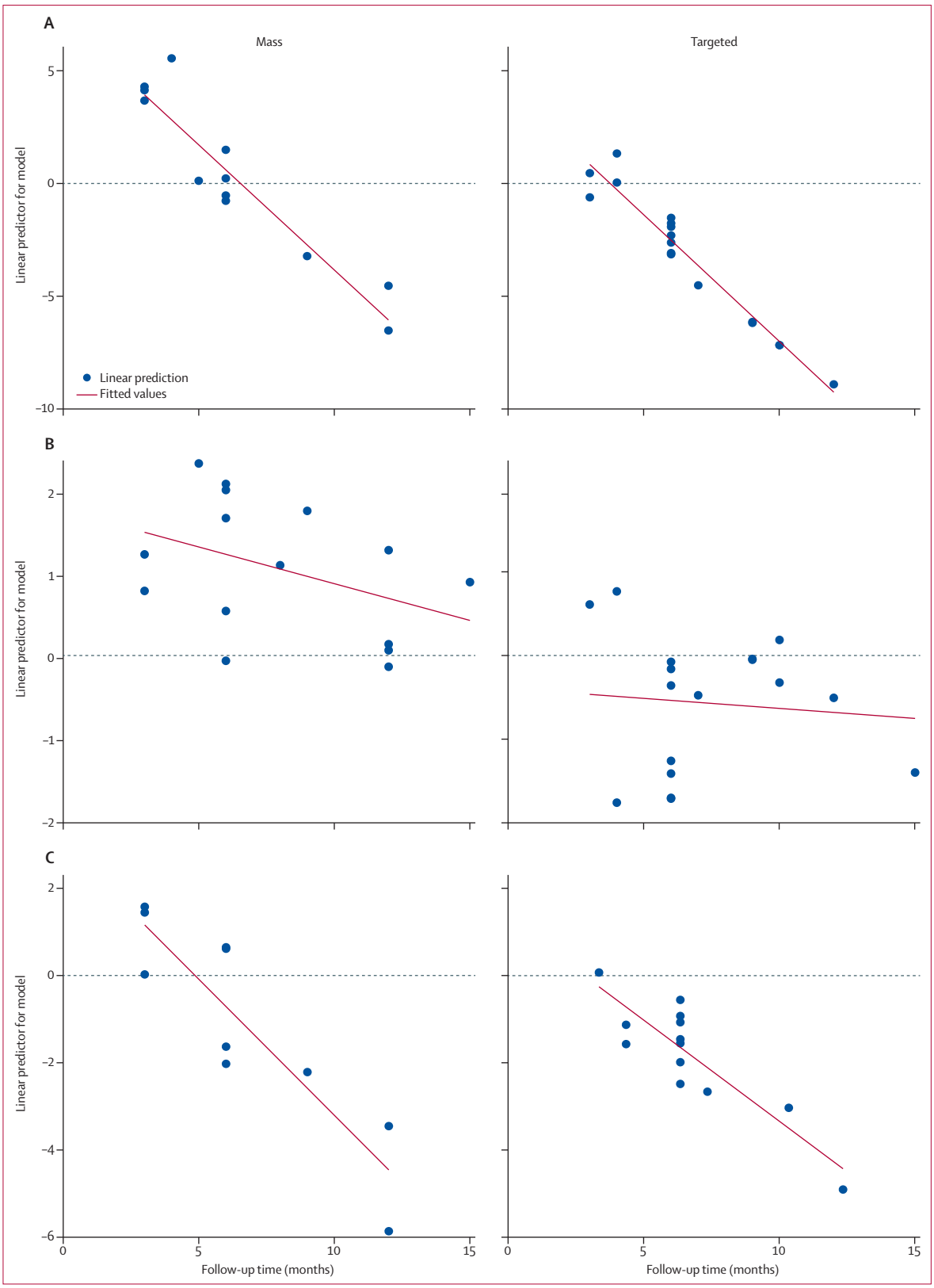

***Figure 2*: Contribution of follow-up time to the model**
(A) *Ascaris lumbricoides*. (B) Hookworm. (C) *Trichuris trichiura*. Relationship between the linear predictor from the model and follow-up time, stratified by method of delivery (mass *vs* targeted). The line depicts an overlaid linear fit to the plot data.

Follow-up prevalence assessment ranged from 3 months to 3 years after the final drug dose; 6 months was the most common follow-up time (21 [38%] of 56 studies).

Most studies used the Kato-Katz method for diagnosis of soil-transmitted helminth infection (47 [84%] of 56 studies). Other methods included the formalin-ether sedimentation technique (five studies), direct smear technique (three studies), and the Harada-Mori technique (two studies). The formalin-detergent sedimentation technique,[48] single coproculture,[49] double coverslip method,[50] and real-time PCR for *A lumbricoides* only[51] were used in one study each. Two studies did not report the parasitological technique that was used.[38,52]

The most common additional medications were praziquantel (16 studies), diethylcarbamazine (nine studies), and ivermectin (five studies). Health education (eg, posters, leaflets, and information sessions) was reported in 14 studies. WASH improvements were described in eight studies, two of which had control groups that received drug treatment only. Additional interventions are summarised in the appendix (p 9).

34 studies (61%) reported treatment coverage for at least one round of drug administration. Two of these studies relied on self-reporting to measure coverage, whereas the remainder reported coverage recorded by the team responsible for drug administration. Coverage rates were highly variable, even within studies (at different rounds or in different regions), with the lowest reported coverage 29·3% and the highest 100%.

Nine potential deficiencies were assessed in terms of risk of bias (appendix pp 10–11). Of these deficiencies, the most common were response rate of less than 75% (or not reported) in 27 studies, deworming medications delivered to less than 75% of target population (or not reported) in 24 studies, use of different population sampling methods at baseline and follow-up (or not reported) in 11 studies, and non-representativeness of the general population (or target population not reported) in ten studies. All other deficiencies were less common and observed in a maximum of seven studies.

Results from the weighted regression model are shown in table 2. For *A lumbricoides*, 29 studies were included in the model. Mass drug distribution had a significantly greater effect on prevalence reduction than targeted drug distribution (OR 16·4, 95% CI 2·1–125·8; p=0·0092). Follow-up time was also strongly associated with prevalence reduction; for each 1 month increase, the odds of prevalence reduction decreased by 63% compared with baseline (0·37, 0·27–0·51; p<0·0001). Number of drug doses and baseline prevalence did not significantly contribute to prevalence reduction.

For hookworm, 32 studies were included in the model after exclusion of one study that was an outlier causing unstable estimates.[43] Mass drug distribution had a significantly greater effect on prevalence reduction than targeted distribution (OR 4·6, 95% CI 1·8–11·6; p=0·0020; table 2). Baseline prevalence was also associated with prevalence reduction (0·07, 0·01–0·77, p=0·0304). Follow-up time and number of drug doses did not have a significant effect on prevalence reduction.

Based on 23 studies included in the model, no significant effect was seen for mass versus targeted delivery, follow-up time, number of doses, or baseline prevalence for *T trichiura*.

Link specification tests showed that the models were correctly specified (squared linear predictor was not statistically significant; data not shown).[53] A scatter plot of the linear predictor against the true value of the outcome variable showed a reasonable fit through visual inspection of the data (data not shown).

The contribution of follow-up time to the variance explained by the linear model for each soil-transmitted helminth is shown in figure 2. The graphs are stratified by delivery strategy, depicting the differential effects of mass and targeted strategies as assessed by the model (the outcome variable PReduc is presented on the logit scale).

The results of the secondary analyses synthesising the non-truncated prevalence reduction estimates from individual studies are shown in table 3. Results are presented separately for studies of mass and targeted distribution, stratified by follow-up time. Heterogeneity among included studies was high. In targeted studies, I^2 was 97% for *A lumbricoides* and hookworm, and 96% for *T trichiura*. In mass studies, I^2 was 89% for *A lumbricoides*, 49% for hookworm, and 66% for *T trichiura*.

Sensitivity analyses to examine effect sizes when only studies from geographically similar locations were included, when only studies that used the Kato-Katz method were included, when influential studies were excluded, when studies that implemented WASH improvements were excluded, and when prevalence reduction was truncated as in the generalised linear model, showed that the results remain robust when these selection criteria are applied (appendix p 12).

The results of analyses using the random effects model weights are depicted in the appendix (pp 13–15). Re-analysis with this conventional approach did not substantially alter the results.

Egger's regression showed evidence of funnel plot asymmetry for *A lumbricoides* (intercept –3·68, p=0·0024), hookworm (intercept –4·34, p<0·0001), and *T trichiura* (intercept –2·578, p=0·0095). Funnel plots for each soil-transmitted helminth are shown in the appendix (p 16); to account for heterogeneity, plots were created separately according to delivery strategy and follow-up time. On visual inspection, minor asymmetry was noted for *T trichiura*, with more asymmetry for hookworm and *A lumbricoides*.

Discussion

Although studies examining the control of soil-transmitted helminth infections have been reported in

	Follow-up time	PReduc* (95% CI)	Cochran's Q	p value (Cochran's Q)	Number of study datasets
Ascaris lumbricoides					
Mass	6 months or less	0·52 (–0·10 to 0·79)	86·9	<0·0001	9
	More than 6 months	0·23 (0·02 to 0·40)	0·35	0·8390	3
Targeted	6 months or less	0·38 (0·12 to 0·57)	243·6	<0·0001	11
	More than 6 months	–0·01 (–0·45 to 0·30)	41·7	<0·0001	6
Hookworm					
Mass	6 months or less	0·72 (0·51 to 0·84)	14·1	0·0495	8
	More than 6 months	0·67 (0·47 to 0·79)	12·3	0·0546	7
Targeted	6 months or less	0·11 (–0·20 to 0·33)	336·4	<0·0001	10
	More than 6 months	0·30 (–0·21 to 0·59)	246·4	<0·0001	8
Trichuris trichiura					
Mass	6 months or less	0·14 (–0·22 to 0·40)	15·8	0·0148	7
	More than 6 months	0·23 (–0·49 to 0·60)	10·1	0·0066	3
Targeted	6 months or less	0·12 (–0·11 to 0·30)	294·9	<0·0001	9
	More than 6 months	0·13 (–0·08 to 0·30)	16·5	0·0003	3

Data are shown separately for mass and targeted studies for each STH and stratified by follow-up time. STH=soil-transmitted helminth. *PReduc=1–PRatio.

***Table 3*: Meta-analysis results synthesising non-truncated prevalence reduction estimates from individual studies**

the literature for over 90 years,[54] global interest in controlling these highly prevalent infections has surged in the past two decades. Resources committed to soil-transmitted helminth control have substantially increased; as such, identification of optimal drug delivery strategies is crucial to ensure effective use of these resources. To our knowledge, this systematic review and meta-analysis is the first synthesis of existing empirical evidence of the effect of mass and targeted drug distribution strategies on soil-transmitted helminth prevalence in school-aged children.

The results of this meta-analysis show that prevalence reduction of hookworm in school-aged children is significantly greater after mass deworming than after targeted deworming. This finding fits with existing knowledge that prevalence and intensity of hookworm infections peak in adulthood,[55] and that child-targeted programmes are thus unlikely to significantly reduce community transmission.[19] Because hookworm larvae have a short life expectancy in soil,[56] differential effects of targeted and mass deworming on environmental contamination and reinfection should become apparent soon after deworming. Our findings concur with results from mathematical modelling studies, which suggest that community-wide treatment would have a larger impact on environmental hookworm reservoirs, and therefore on reinfection, than would targeted treatment.[17,19,20]

Notably, results of this meta-analysis also show that mass deworming has a greater effect on prevalence reduction of *A lumbricoides* than does targeted deworming. Unlike hookworm, prevalence and intensity of *A lumbricoides* is highest in school-aged children,[2] and its infective stages can persist for several months in the environment.[56] Although findings from a modelling study[17] suggest that the current child-focused WHO guidelines will have a major impact on *A lumbricoides* levels by 2020, our results suggest that greater gains could be made if treatment was expanded to the community. The strong inverse association seen in our regression model between prevalence reduction and follow-up time for *A lumbricoides* agrees with a systematic review of soil-transmitted helminth reinfection following drug treatment,[7] which lends support to the validity of our findings.

No effect of mass versus targeted drug distribution on prevalence reduction was seen for *T trichiura*. Albendazole and mebendazole are known to have poor efficacy against *T trichiura*.[6,57] Therefore, it is unsurprising that community-wide treatment would not significantly enhance prevalence reduction, because environmental reservoirs of infective stages would remain high, and reinfection would occur rapidly after any successful treatment. This finding highlights the need for new drugs and drug combination strategies in areas with high *T trichiura* prevalence.[17,58]

There was significant heterogeneity in prevalence reduction among included studies, with wide CIs around odds ratios obtained in our regression models. This result is unsurprising, because studies were done in different countries, with variation in environmental conditions, WASH access, and economic contexts. Heterogeneity was particularly high in studies of targeted control programmes, suggesting that the effect of mass treatment programmes could be more consistent across different settings.

Egger's regression and funnel plots showed evidence of asymmetry, which probably reflects heterogeneity among studies. Small studies, which focus on a small number of schools or communities, might have led to greater prevalence reductions than large studies because of higher deworming coverage in smaller target populations. Publication bias is another possible reason, wherein studies showing little effect of deworming could be less likely to be published than studies showing significant impact. Such concerns have previously been raised in systematic reviews of the effect of deworming on morbidity indicators.[59]

This systematic review and meta-analysis adheres to PRISMA guidelines,[24] and a comprehensive search strategy was used. However, several limitations must be acknowledged. Heterogeneity among studies introduces the possibility of confounding by variables that were not included in our regression model. We were unable to control for factors such as environmental conditions, WASH access, and socioeconomic situation, all of which are known to influence the effect of deworming programmes.[60] Additionally, deworming coverage was not taken into account in our analyses. As we aimed to measure the differential effect of mass and targeted drug administration campaigns in real-life settings, we felt it inappropriate to exclude studies with low deworming

coverage, because coverage and compliance issues are important challenges facing these campaigns.[61]

We used soil-transmitted helminth prevalence to measure the effect of deworming programmes. High-intensity infections are known to cause most soil-transmitted-helminth-associated morbidity,[56] and some individuals harbour a disproportionately high worm burden.[62] Thus, prevalence might not accurately reflect associated morbidity in children in a community. However, preva-lence is the most widely-reported outcome measure in studies assessing deworming campaigns. Mean intensity of infection is not thought to be a reliable indicator of soil-transmitted-helminth-associated morbidity or an appropriate measure of the effect of soil-transmitted helminth control programmes.[63] Insufficient numbers of studies have reported on the prevalence of moderate-intensity and high-intensity infections for the analysis of pooled estimates.

The Kato-Katz diagnostic method, used by most studies in this analysis, is known to have reduced sensitivity in low-intensity settings.[64] This represents a potential source of measurement error that would bias results towards the null hypothesis, resulting in an underestimation of the differential effect of mass and targeted treatment.

Finally, we used standardised weights to calculate prevalence in school-aged children when age-stratified data were not available. These weights have been used in large-scale analyses including a global epidemiological disease burden study in 2010.[1] However, distribution of both age and soil-transmitted helminth prevalence might vary between communities, and prevalence reduction in school-aged children might differ from other age groups.

The results of this meta-analysis support the benefits of expanding drug treatment programmes to all community members. Given the potential for bias due to unmeasured confounders, these results also highlight the need for adequately powered cluster-randomised controlled trials examining the differential effect of mass and targeted treatment programmes. We are currently investigating the differential effect of school-based and community-based integrated soil-transmitted helminth control programmes in a pilot study in Timor-Leste.[65] A large cluster-randomised controlled trial assessing the effect of school-based versus community-based deworming on soil-transmitted helminth prevalence is also underway in Kenya.[66]

One concern is that the scaling up of mass drug administration programmes could exert additional drug pressure on soil-transmitted helminths, and potentially select for anthelmintic-resistant parasite genotypes.[5,67] Although no conclusive evidence exists for anthelmintic resistance of soil-transmitted helminths in human beings,[67] benzimidazole resistance is widespread in livestock.[68] Close monitoring of drug effectiveness during mass drug administration campaigns, as well as development of new anthelmintics, are important priorities for researchers, countries in which these campaigns are implemented, and their implementation partners.[2,67,68]

Integration of deworming programmes with WASH improvements should also be emphasised. By reducing environmental contamination with, and human exposure to, helminth infective stages, WASH interventions are a key component of sustainable soil-transmitted helminth control.[69–71] Such interventions are more expensive and complex than deworming campaigns, requiring infra-structure improvements and long-term behavioural change, and should be implemented alongside drug administration programmes designed to reduce soil-transmitted helminth prevalence and infection intensity.[72]

From a programmatic point of view, scaling up from targeted drug administration to mass drug administration has important economic implications for drug donation and soil-transmitted helminth control programmes. Current donations from pharmaceutical companies reach approximately 70% of at-risk children; expanding to mass treatment would require a substantial increase in the amount of drugs required. An increase in resources to support implementation—probably including additional international aid—would also be needed.[23] Although mass treatment campaigns for neglected tropical diseases such as onchocerciasis and lymphatic filariasis show the feasibility of providing community-wide treatment,[73,74] sustaining community-wide deworming long term might be difficult in some areas of sub-Saharan Africa and southeast Asia,[22] because of limited health system resources and capacity. However, in many transmission settings, mass deworming might eventually interrupt soil-transmitted helminth transmission such that drug treatment is no longer needed, whereas this could not be achieved in most settings with targeted deworming.[20,22,70]

Our analysis of existing empirical evidence agrees with mathematical modelling[20,22,23] and cost-effectiveness analyses,[21,23] highlighting the benefits of expanding soil-transmitted helminth control programmes to all age groups in endemic countries. Our findings lend support to calls to re-evaluate global soil-transmitted helminth control guidelines.[75] In view of the substantial global disease burden of soil-transmitted helminth infections and worldwide attention focused on the elimination of neglected tropical diseases, consideration of expansion to community-wide treatment needs to be prioritised.

Contributors

NEC did the database searches, data extraction, quality assessment, and statistical analysis, and drafted the manuscript. SAD and ACAC provided statistical guidance. SAD created and reviewed the statistical models. DW and SJC assisted with database searches, data extraction, and drafting of the manuscript. SVN conceived and designed the review protocol, and assisted with data extraction, quality assessment, and drafting the manuscript. ACAC and DG provided input into the review protocol. All authors edited and revised the manuscript.

Declaration of interests

We declare no competing interests.

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Chapter 4
Delivery of integrated STH control programs

4.1 Chapter context

This chapter builds on the preceding chapter in addressing the second research objective, to determine if community-wide approaches to STH control have a greater impact on STH infections in children, compared to approaches targeted only to children. This chapter is presented as two published peer-reviewed journal articles.

As detailed in the previous chapter, despite recent calls to expand STH control programs community-wide, at the time this PhD was commenced, there was virtually no empirical evidence comparing child-targeted and community-wide approaches to STH control in terms of impact on STH infections among children. This evidence gap was addressed in the previous chapter using a systematic review and meta-analysis; in that paper, the need for field-based studies directly comparing these two approaches to STH control was highlighted as a significant research priority.

In this context, a field-based pilot study was undertaken as a key component of this PhD, comparing community-wide and child-targeted approaches to STH control. The (S)WASH-D for Worms pilot study was conducted in Timor-Leste in 2015–2016. The pilot study compared the impact of integrated STH control programs—consisting of both deworming and WASH improvements—when delivered community-wide versus when delivered only to children. The pilot phase of the study was designed to assess feasibility and acceptability, and to establish proof of principle for the hypothesis that control programs will have a greater impact on STH infections among school-aged children when delivered community-wide.

The first paper in this chapter is the study protocol for the (S)WASH-D for Worms pilot study, and is published in *Pilot & Feasibility Studies* (Paper 3). The second paper presents the results of the pilot study, and is published in *PLoS Neglected Tropical Diseases* (Paper 4). The results of the pilot study complement those presented in Chapter 3, building the evidence base for expanded community-wide deworming for STH control.

4.2 Paper 3

Clarke NE, Clements ACA, Bryan S, McGown J, Gray D, Nery SV. Investigating the differential impact of school and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for Worms pilot study protocol. *Pilot Feasibility Stud* 2016; 2: 69. http://doi.org/10.1186/s40814-016-0109-4

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Pilot and Feasibility Studies

STUDY PROTOCOL Open Access

Investigating the differential impact of school and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for Worms pilot study protocol

Naomi E. Clarke[1*], Archie C. A. Clements[1], Stuart Bryan[2], John McGown[3], Darren Gray[1] and Susana V. Nery[1]

Abstract

Background: Water, sanitation and hygiene (WASH) interventions represent an important component of soil-transmitted helminth (STH) infection control, alongside the administration of anthelmintic drugs, which are generally targeted to school-aged children. Recent modelling studies have suggested that STH control programmes should be broadened to include all age groups across the community. We describe the protocol for a pilot study investigating the impact of school-versus-community-based delivery of integrated WASH and deworming programmes on STH infections in school-aged children in Timor-Leste.

Methods: The (S)WASH-D for Worms pilot is a two-arm, non-randomised cluster intervention study. The aims are to determine feasibility and acceptability of the intervention and study procedures and to establish proof of principle for the hypothesis that STH control programmes directed to the entire community will lead to greater reductions in STH infections in children than programmes directed only to school-aged children. Of the six participating communities, three receive a school-based integrated WASH and deworming programme and three additionally receive a community-based integrated WASH and deworming programme. The primary outcomes are the proportions of eligible children who enrol in the study and participate in the data collection, and outcomes relating to WASH and deworming programme completion, coverage, and use. Secondary outcomes are the cumulative incidence and mean intensity of STH infection in school-aged children at 6-month follow-up, mean haemoglobin concentration and several anthropometric indices. Results will inform the design of a cluster-randomised controlled trial (RCT).

Discussion: This pilot study is being conducted in preparation for a cluster-RCT investigating the differential impact of school- and community-based integrated STH control programmes on STH infections in school-aged children. It aims to establish feasibility and proof of principle, while results of the subsequent RCT could have significant implications for global STH control policy.

Trial registration: Australian New Zealand Clinical Trials Registry, ACTRN12615001012561

Keywords: Soil-transmitted helminths, Water, Sanitation and hygiene, Mass drug administration

* Correspondence: naomi.clarke@anu.edu.au
[1]Research School of Population Health, Australian National University, Canberra ACT 0200, Australia
Full list of author information is available at the end of the article

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Background

Soil-transmitted helminths (STHs) represent a group of parasitic nematode worms which fall into the category of neglected tropical diseases—a group of infections which predominantly affect people living in extreme poverty [1]. The soil-transmitted helminths include hookworms (*Necator americanus* and *Ancylostoma duodenale*), roundworms (*Ascaris lumbricoides*) and whipworms (*Trichuris trichiura*). Together, it is estimated that approximately 1.45 billion people worldwide are infected with at least one of these species of worms [2], with an estimated disease burden in excess of five million disability-adjusted life years (DALYs) [3].

STH infections are transmitted when helminth eggs are excreted in the faeces of infected individuals, contaminating soil in areas without adequate sanitation. Infections are subsequently acquired through direct penetration of the skin by hookworm larvae, or accidental ingestion of *A. lumbricoides* or *T. trichiura* eggs, or hookworm larvae [4]. These infectious stages of STH can remain viable in soil for a period of time ranging from several weeks for hookworm larvae to several years for *A. lumbricoides* eggs [5–7].

Chronic STH infections result in malabsorption of nutrients and micronutrients, and a number of studies show evidence for malnutrition, iron-deficiency anaemia, poor growth and impaired cognitive development in STH-infected individuals [8–14], with children harbouring the largest burden of morbidity [4, 7, 15, 16]. Both *A. lumbricoides* and *T. trichiura* infections have a peak incidence and intensity in children between the ages of 5 and 15, with a decline in both frequency and intensity in adulthood [4]. Hookworm infections, on the other hand, tend to maintain a high prevalence and intensity in adulthood [4, 5]. Despite this, children, along with women of child-bearing age, remain at the highest risk of hookworm-associated anaemia and other related morbidities [5, 17].

Regular treatment with the benzimidazole anthelmintic albendazole leads to rapid and significant decreases in STH prevalence, particularly *A. lumbricoides* and hookworm [18]. Regular anthelmintic delivery—also known as deworming—has been shown in a number of studies to improve STH morbidity indicators, including growth, anaemia, cognitive abilities and school attendance [11, 15, 19, 20], although some of this evidence has recently been called into question [21–23].

Following the administration of anthelmintic drugs, STH infections rapidly recur in the context of ongoing environmental contamination [24]. Therefore, to achieve sustainable control of STH infections, facilitating improvements in water, sanitation and hygiene (WASH) in order to interrupt the cycle of ongoing reinfection is thought to be important [25, 26]. The impact of adequate water and sanitation infrastructure, as well as good hygiene practice, on preventing enteric infections and diarrhoeal illness is widely understood [27–29]. Intervention studies and systematic reviews specifically examining the link between WASH components and STH infections show evidence, albeit not consistent, that access to improved water and sanitation, and exposure to health education or hygiene promotion, are associated with reduced odds of STH infection [30, 31] or reduced risk of reinfection following drug treatment [32–36].

The optimal strategy for delivery of integrated deworming and WASH approaches remains uncertain. Due to the heavy burden of STH morbidity in school-aged children, and the cost-effectiveness of using school-based infrastructure [37], the World Health Organization (WHO) guidelines have focused predominantly on school-aged children as major targets of anthelmintic drug programmes [14, 38], with more recent recommendations including preschool-aged children, women of child-bearing age and adults in high-risk occupations [16]. School-based deworming programmes have been widely advocated and have become a cornerstone of STH control [15, 16]. The London Declaration on Neglected Tropical Diseases (NTDs) in 2012 saw 600 million annual doses of anthelmintic drugs donated towards the control of STH in children [39], a step towards achieving the WHO target of 75% deworming coverage of at-risk preschool- and school-aged children by 2020 [16]. This has resulted in a large global scale-up of chemotherapy programmes targeting school- and preschool-aged children [40].

However, recent modelling studies have raised concerns about the impact of child-targeted control programmes on the transmission of STH in the wider community [41–44]. These studies suggest that targeted programmes may not significantly impact the overall level of transmission [41, 42] and that child-focused strategies may be ineffective in reducing the overall community burden of the disease, particularly in areas where hookworm infections are predominant [43, 44]. Therefore, expanding treatment programmes to the whole community may result in improved STH control [42]. Cost-effectiveness modelling has demonstrated that community-based drug administration programmes for STH control are highly cost-effective when compared with treatment of school-aged children only [44, 45].

Intervention studies examining the impact of one or more components of WASH on STH infections have been conducted, or are currently underway, both in schools [32–36] and in communities [46–49]; however, the relative merits of the two delivery strategies have not been discussed in the literature. Furthermore, to our knowledge, there are no studies which have directly compared school-based and community-based integrated WASH and deworming programmes. The (School) Water, Sanitation,

Hygiene and Deworming for Worms ((S)WASH-D for Worms) study aims to contribute to this evidence gap by comparing an integrated approach focused on school children with an integrated community-based approach. This report describes the protocol of the (S)WASH-D for Worms pilot study, which is being conducted in preparation of a full-scale cluster-randomised controlled trial. This protocol has been developed using the SPIRIT 2013 guidelines (see Additional file 1) [50].

The objectives of this pilot study are as follows:

1. To examine the feasibility and acceptability of conducting a trial that recruits school-aged children and implements distribution of deworming medications along with school- and community-based WASH programmes. Specifically,
   (a) To determine the feasibility and acceptability of study procedures by estimating rates of participant consent, recruitment, participation in data collection and retention
   (b) To determine the feasibility and acceptability of the WASH and deworming programme by observing completion, uptake and usage
   (c) To identify operational issues for consideration when planning the full-scale trial
   (d) To obtain the initial estimates of STH prevalence, infection intensity and nutritional indicators for the purpose of informing sample size calculation
2. To establish "proof of principle" (preliminary evidence) for our hypothesis that a community-based deworming and WASH intervention is more effective in reducing STH infections in children than an exclusively school-based approach, by comparing estimates of the impact of the interventions

Methods

Design

This pilot study is a two-arm, non-randomised cluster intervention study. The six participating clusters, each based around a local primary school, are located in Aileu and Manufahi municipalities of Timor-Leste. Three clusters comprise the "control" arm of the study: in these, a WASH programme is delivered to the primary school, and albendazole is distributed to the schoolchildren. The other three clusters comprise the "intervention" arm, in which a WASH programme is delivered to both the primary school and the community in which the school is located, and albendazole is distributed to all community members. The follow-up period for the pilot study is 6 months following the distribution of albendazole, which will allow sufficient time for STH reinfection to occur [24], and represents the follow-up interval planned for the full-scale trial, which will take place over a 2-year period. Figure 1 depicts a flow diagram for the pilot study. This pilot study is registered with the Australian New Zealand Clinical Trials Registry (registration number ACTRN12615001012561).

Setting

Timor-Leste is placed 133rd out of 187 countries on the Human Development Index [51], with 31.5% of the population living in severe poverty and a further 21.4% living in near poverty [51]. Over 50% of children under 5 years of age suffer from stunting, indicating chronic malnutrition [52]. A national survey in 2012 showed an overall STH prevalence of 29% in school-aged children in Timor-Leste, with 26 and 30% prevalence in the Manufahi and Aileu municipalities, respectively [53].

Open defecation in rural communities poses significant health risks. In 2015, 73% of the rural households in Timor-Leste did not have access to improved sanitation facilities, and 39% did not have access to improved water supplies [54]. Furthermore, a UNICEF survey in 2011 reported that 35% of the primary schools in Timor-Leste did not have latrines, and 62% of schools did not have regular access to a water supply [55].

Improved water and sanitation facilities across rural areas of Timor-Leste has been highlighted as a priority by the government of Timor-Leste [56], and multiple non-governmental organisations (NGOs) are also working in Timor-Leste to provide rural communities with improved access to reliable protected water sources and sanitation facilities and to promote hygiene behaviour [57–61].

Integrated STH control programme

The WASH programmes in the pilot study are implemented by partner NGOs working in Timor-Leste. In order to ensure that the WASH programme would be completed within the planned time frames, two partner NGOs were selected. Plan International Timor-Leste is the implementer of the school- and community-based WASH programmes in the intervention arm of the study. These programmes are being delivered as part of a 4-year EU-funded water and sanitation project (FED/2011/270-630). Cruz Vermelha Timor-Leste (CVTL; a partner of Red Cross Australia) is the implementer of school-based WASH programmes in the control arm of the study, which are being delivered as a special project within the Integrated Community Based Risk Reduction program, funded by the Australian NGO Cooperation Program (ANCP 777-PRG01-PRJ08).

School WASH programme

All study clusters receive a school-based WASH programme, which includes three of the following components:

(A) Providing access to a protected source of water which will be available year-round. This involves the

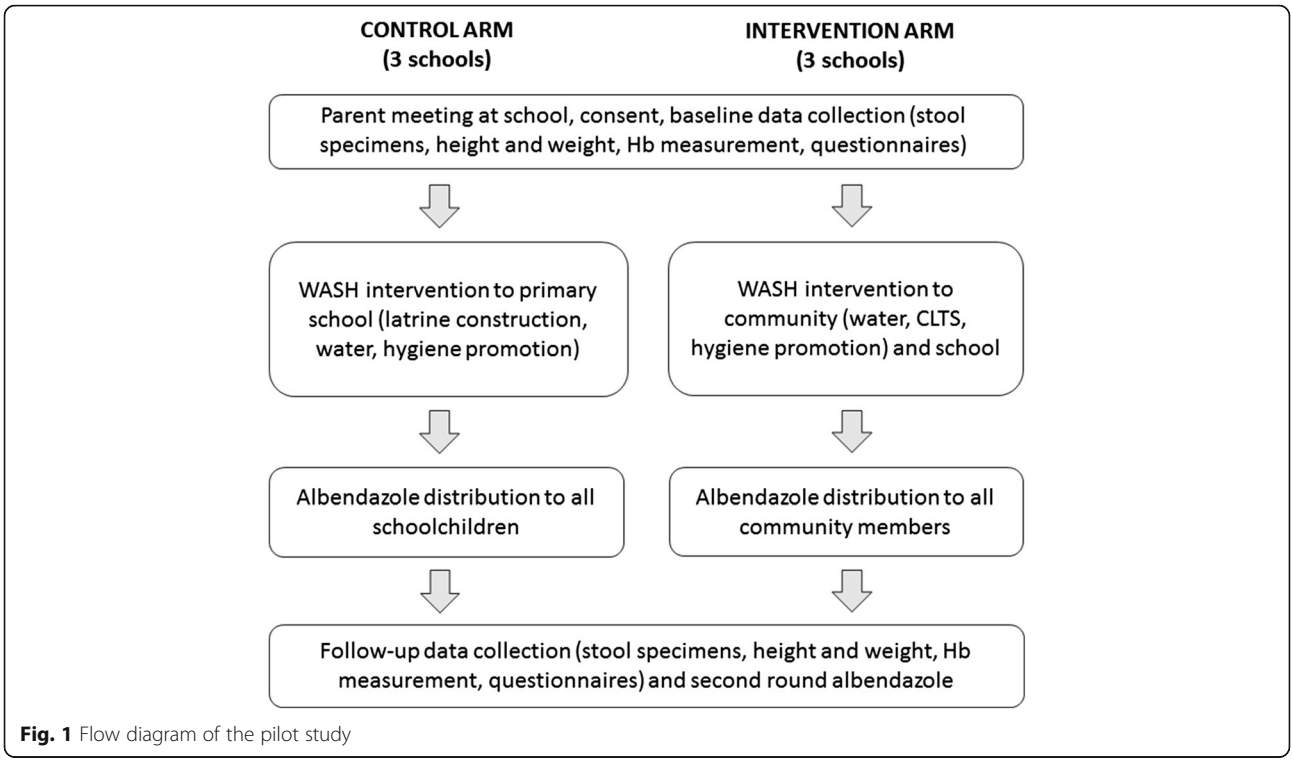

Fig. 1 Flow diagram of the pilot study

construction of a new water system where required or the development or rehabilitation of existing water systems to improve water supply to the schools

(B) Providing access to sanitation, achieved through either the construction of new school latrines or rehabilitation of existing, non-functional school latrines. Latrines are built following the Timor-Leste WASH in schools guidelines, which provide standards for the construction of sufficient, gender-segregated, accessible, private, secure, clean and culturally appropriate toilets for schoolchildren and staff, including facilities for use by menstruating students and staff [62]. Toilets are designed so that they are hygienic to use and easy to clean. At study schools, pour-flush latrines are constructed (or rehabilitated). The latrine pits are lined with concrete rings, and the superstructures are built with concrete blocks for durability. Handwashing stations are also constructed

(C) Improving hygiene behaviour through hygiene promotion sessions conducted at primary schools. These sessions focus on using latrines, handwashing with soap at key times and keeping the environment clean. Strategies to communicate these messages include the use of flip charts, banners and posters, as well as game-oriented activities and participatory demonstrations

Community WASH programme

In addition to the school-based WASH programme, the three intervention clusters also receive a WASH programme delivered at the community level, including the same three components:

(A) Providing access to a protected primary source of water that will be available year-round. This involves the construction of a new water system, or rehabilitation of an existing one, and comprehensive training for community-based water committees about operation and maintenance

(B) Access to improved sanitation, achieved by increasing the number of household latrines. Plan International Timor-Leste and its partners utilise the community-led total sanitation (CLTS) approach, which encourages all households to take responsibility for building and using their own household latrines, thus eliminating open defecation in their communities [63]. CLTS challenges community members to reflect on their defecation practices through a series of sessions collectively called "triggering" that include transect walks, mapping open defecation areas, calculating the amount of faeces produced daily by each household, explanations on faecal-oral disease transmission routes and costs of medical treatment for gastrointestinal illnesses. If triggering is conducted optimally, community members come to the realisation that they are consuming each other's faeces. Finally,

community members make an activity plan and pledge to build or repair their household toilets [63]. CLTS facilitators provide information and lead discussions around the types of latrines which could be built and local materials that could be used in their construction. The two most common types of latrines that are built are simple direct pit latrines and offset pit pour-flush latrines

(C) Hygiene promotion, conducted at the community level by Plan International and its partner NGOs as well as village health volunteers. This involves regular house-to-house visits to promote construction and use of latrines, handwashing with soap at key times and treatment and storage of drinking water

Administration of albendazole

In control clusters, albendazole is distributed to school-aged children only, while in intervention clusters, albendazole is distributed to every eligible member of the community. This includes all community residents, except for children under 1 year of age and pregnant women in the first trimester, in line with the WHO guidelines [64]. All doses are given as a single oral dose of 400 mg albendazole (Albenza, GlaxoSmithKline, Research Triangle Park, NC), taken under direct observation of the field staff.

In control clusters, albendazole distribution occurs at the primary school on a day agreed with the head teacher, with the first round given following the completion of school latrines and water systems. In intervention clusters, albendazole distribution occurs both at the primary school, again on a day agreed with the head teacher, and house-by-house, over a period of 1 to 2 days, with the first round given once 80% of households have latrines, and following the completion of school latrines and water systems. In both arms of the study, a second round of albendazole will be given at follow-up 6 months later, following the collection of follow-up stool specimens from the study participants.

Albendazole is widely distributed in large mass drug administration programmes globally; side effects are minor [64]. Parents are advised to seek healthcare at the local community health centre if their child is unwell following drug distribution, and community health centres are notified of study activities during field visits.

Study outcomes

The primary outcomes will be used to examine feasibility and acceptability (objective 1). Primary outcomes relating to study feasibility and acceptability in terms of recruitment and participation (objective 1A) are as follows: the proportion of eligible children whose parents provide informed consent; the proportion of eligible children who provide stool samples, complete questionnaires and undergo measurement of height, weight and haemoglobin; and the retention rate of participants between baseline and follow-up.

Primary outcomes relating to the feasibility and acceptability of the WASH and deworming programmes (objective 1B) are as follows: the proportion of children and eligible community members who receive albendazole; the time taken for the completion of the school WASH programmes in each study cluster; the time taken to achieve 80% household latrine coverage in each intervention cluster; the proportion of schools and households with latrines and access to a reliable source of improved water; the proportion of schools with handwashing stations; and the proportion of children who report using latrines and handwashing stations.

Secondary outcomes, which will be used to inform sample size calculation (objective 1D) and to examine the study hypothesis (objective 2), are as follows: 6-month cumulative incidence of infection with STH (*Ascaris* spp., *N. americanus*, *Ancylostoma* spp. and *T. trichiura*) at follow-up; mean intensity of STH infection (measured as average number of eggs per gramme of faeces) at follow-up; mean haemoglobin concentration; and four anthropometric indices: weight-for-age, height-for-age, weight-for-height and body mass index (BMI)-for-age *Z*-scores (to identify underweight, stunting, wasting and thinness, respectively). Cumulative incidence of STH infection is the planned primary outcome in the full-scale trial. It should be noted that the term "cumulative incidence" is used for simplicity, as cases diagnosed at follow-up will include both incident infections and prevalent infections not cured by albendazole, particularly in the case of hookworm and *T. trichiura* infections [65].

All outcomes (see Table 1) will be compared between control and intervention clusters.

Selection and recruitment of clusters

Because each of the two partner NGOs only had capacity to conduct the WASH programme in one of the study arms within the required study time frame, and operated in neighbouring but different administrative areas, a randomised design could not be used for the pilot project. In the full-scale study, the intention is to randomise communities to the intervention and control arms.

For the pilot study, communities were considered eligible if they contained a primary school which was suitable for a school-based WASH programme (i.e. did not have access to functional improved latrines) and were located in a village with low sanitation coverage (i.e. less than 50% of households with latrines). Communities were selected in consultation with the implementing

Table 1 Study outcomes

Primary outcomes		
	Proportion of eligible children who:	Provide parental informed consent
		Provide stool samples
		Complete questionnaires
		Undergo measurement of height, weight and haemoglobin
	Participant retention rates between baseline and follow-up (defined as the proportion of baseline participants who were retained in the study at follow-up)	
	Proportion of children and eligible community members who take albendazole	
	Time taken to complete school WASH programmes in each study cluster	
	Time taken to achieve 80% household latrine coverage in each intervention cluster	
	Proportion of schools and households with functional and clean latrines	
	Proportion of children who report using household and school latrines	
	Proportion of schools and households with access to a reliable primary source of improved water	
	Proportion of schools with handwashing stations	
	Proportion of children who report using handwashing stations at school	
Secondary outcomes		
	Cumulative incidence of infection with:	*Ascaris* spp.
		T. trichiura
		N. americanus
		Ancylostoma spp.
	Mean intensity of infection (calculated as the average number of eggs per gramme of faeces) of:	*Ascaris* spp.
		T. trichiura
		N. americanus
		Ancylostoma spp.
	Mean haemoglobin concentration	
	Weight-for-age (underweight) *Z*-score	
	Weight-for-height *Z*-score (wasting)	
	Height-for-age *Z*-score (stunting)	
	Body mass index (BMI)-for-age *Z*-score (thinness)	

partner NGOs, based on their upcoming activities which fit into the study time frame.

Members of the (S)WASH-D for Worms research team accompanied NGO staff to community meetings in each cluster. For intervention communities, this occurred at the community "triggering", and for control communities, this was a pre-arranged meeting with community and school leaders to explain plans for the school-based WASH programme. In all clusters, the study was explained to the village leader and head of school, who provided consent for the study activities to take place in their community. During the triggering in intervention communities, trial staff were also given the opportunity to explain the research study to the community. Following these initial meetings, plans were made for the research team to return for baseline data collection at the schools, within 1 to 3 weeks of the first meeting and prior to the commencement of the WASH programme.

Participants

Participants in the data collection in both arms of the study are children attending the local primary school. The study has no specific exclusion criteria; all children enrolled in the local primary school are eligible for participation, provided a parent or guardian is available to provide informed consent.

Prior to the baseline field visit, teachers were asked to organise a parent meeting at the school on the day the research team arrived. At this meeting, the study was explained in detail to the parents by the Timorese project manager. Parents were provided with both written and schematic information sheets and given the opportunity to ask questions about the study prior to providing written informed consent.

At baseline, informed consent was obtained for 522 out of 602 eligible children (i.e. those who were enrolled in the local primary school), representing a recruitment rate of 87%. Of the 80 children who were not recruited to the study, 39 were absent from school during the baseline visit, and 41 were present but their parents were unable to attend the school to provide informed consent. No refusals of consent were recorded among children whose parents attended.

At the 6-month follow-up visit, which will take place in a new academic year, teachers will again be asked to arrange a parent meeting, and consent will be sought from any parents who did not attend at baseline, as well as from parents of children who are new to the school, including all children in the new grade 1 cohort.

Data collection

Questionnaires

Study participant questionnaires are administered as interviews at both baseline and follow-up. They are conducted by trained local fieldworkers and include two components. The first component consists of questions asked directly to the children, relating to diarrhoea history, access to deworming medications, presence and use of a household latrine, defecation practices, handwashing practices and shoe wearing at home, at school and while defecating. The second component consists of questions directed to the caregiver, relating to household water source, household assets, education and occupation. Questionnaires are also administered to school and village leaders and include questions relating to school and community latrines and water sources.

Stool samples

Stool samples are collected at both baseline and 6-month follow-up. On the first day of each field visit, all participating children are given an explanation of the study and requested to provide a stool sample as part of their participation. Each child is given a labelled plastic container and provided with instructions on the collection of a faecal sample, ideally to be done the following morning and returned to the field team at the school.

Upon receipt of the stool specimens by the field team, two aliquots of 2–3 g are taken and preserved in 15-mL centrifuge tubes—one containing 8 mL of 10% formalin, and the other containing 5 mL of 5% potassium dichromate.

The formalin-fixed samples are transported to the University of Melbourne, Victoria, Australia, for diagnostic processing using microscopy. This is achieved using a simple sodium nitrate flotation technique and direct microscopy to quantify the number of STH eggs (*A. lumbricoides*, hookworm spp. and *T. trichiura*) in each faecal sample [66].

The potassium dichromate-fixed samples are sent to the QIMR Berghofer Medical Research Institute, Brisbane, Australia, for diagnostic processing using a polymerase chain reaction (PCR) technique. DNA is extracted using the PowerSoil DNA extraction kit, with modifications [67], and a real-time multiplex PCR is then undertaken to detect and quantify soil-transmitted helminths (*Ascaris* spp., *N. americanus*, *Ancylostoma* spp. and *T. trichiura*) [68].

Measurement of height, weight and haemoglobin

At both baseline and follow-up field visits, all children for whom informed consent has been provided undergo measurement of height (to the nearest 0.1 cm) and weight (to the nearest 0.5 kg), obtained as a single measurement. A fingerprick blood sample is also obtained for measurement of haemoglobin. These measurements are done by the (S)WASH-D for Worms field team, which includes a nurse, utilising a portable height rod (Wedderburn, WSHRP), digital scale (Livingstone, SCLBATHDIG) and a portable haemoglobin analyser (Hb 201+, HemoCue, Angelholm, Sweden).

Height and weight measurements will be used, along with age, to calculate anthropometric values indicative of nutritional status in children: weight-for-age, height-for-age, weight-for-height and BMI-for-height. These will be calculated as *Z*-scores, the number of standard deviations from the mean of the standard population, with malnutrition and severe malnutrition defined as values 2 and 3 standard deviations, respectively, below the mean score of the standard population [69], using the 2006 WHO database for child growth standards [70]. Anaemia is defined as per the WHO classification guidelines, adjusted for altitude in communities more than 1000 m above sea level.

Data management and confidentiality

Questionnaire data, as well as height, weight and haemoglobin measurements and results of parasitological examinations, are entered into a password-protected database. Data are entered twice by two different data clerks, and the database has in-built range checks for appropriate variables. The final study dataset will be accessible only by the study investigators. Original questionnaires are kept in a locked cabinet in the study office in Timor-Leste and will be destroyed after 7 years. Stool samples are labelled using the participant's unique study ID number, with no identifying information. Results of the parasitological examinations are entered into the study database described above.

Analysis

For the primary outcomes, analyses will be mainly descriptive. The proportions of eligible participants who gave informed consent and participated in each aspect of data collection will be calculated (with 95% confidence intervals (CIs)) and compared across the two study arms, at both baseline and follow-up, and will also be examined separately by gender and age group. The proportion of baseline participants retained at follow-up will also be calculated and compared across the study arms. Descriptive statistics will be used to examine the completeness of data collected using the study questionnaires.

The proportion of children and eligible community members who received albendazole, the proportion of schools and households with access to various WASH components and the proportion of children who report using various WASH components will be calculated (with 95% CIs) and compared across the two study arms, at both baseline and follow-up. The time taken to complete the school and community WASH programmes will be examined using descriptive statistics (mean, median, range).

For the secondary outcomes, prevalence at baseline and cumulative incidence at follow-up will be calculated (with 95% CIs) for each STH as will the mean and standard deviation of the infection intensity, expressed as eggs per gramme of faeces. Mean and standard deviation of the haemoglobin concentration and *Z*-scores for the four anthropometric indices will also be calculated. These outcomes will be compared across both arms of the trial using mixed effects multivariable regression models that account for clustering of participants within schools and villages. Cumulative incidence of infection will be modelled using multivariable Bernoulli logistic regression, with age and sex entered as covariates, baseline infection

status as a fixed effect, and school and village as random effects. The study arm will be entered as a binary fixed effect to estimate differences in cumulative incidence between the study arms, using a cumulative incidence ratio (CIR). Intensity of infection will also be modelled with random and fixed effects as described above. Mixed effects linear regression will be used to model anthropometric *Z*-scores and mean haemoglobin concentration. Stata software will be used for all analyses (StataCorp LP, College Station, TX).

Dissemination

The results of this pilot study will be published in peer-reviewed journals and presented at national and international conferences. Results will also be conveyed to, and discussed with, the Timor-Leste Ministry of Health and relevant WASH programme stakeholders.

Discussion

The current WHO guidelines for STH control focuses strongly on school- and preschool-aged children, who experience the highest burden of disease-related morbidity. In the context of significant global interest in the control of neglected tropical diseases, including significant donations from pharmaceutical companies, and recognition of the potential added benefit of WASH interventions for sustainable control, there is increasing interest in the optimal control strategies for STH.

The (S)WASH-D for Worms pilot study primarily represents a feasibility study in preparation for a cluster-randomised controlled trial (RCT) investigating the differential impact of school- and community-based integrated STH control programmes. The integrated control programme implemented in the study includes both deworming medications, distributed by the research team, and a water, sanitation and hygiene intervention, implemented by partner NGOs. The pilot study will provide an indication of the rate of recruitment and participation which could be expected in a full-scale RCT, which will be used to inform sample size calculations for the full-scale trial. The pilot study will also provide an opportunity to test the study procedures and data collection forms and to examine the feasibility and acceptability of the deworming and WASH programmes, in particular, the time frames for completion of the WASH programmes and their ability to achieve improved WASH access and use. Furthermore, it will allow for the identification of operational challenges involved in implementing such a trial in a developing country. In particular, the pilot study will give an estimation of the time frames required for the completion of the school- and community-based WASH interventions.

The pilot study sample size does not allow sufficient power to detect significant differences in secondary outcomes between study arms. Initial estimates of the secondary outcomes obtained in this pilot study will be used to provide preliminary evidence for our study hypothesis that a community-wide intervention is more effective at reducing STH infections in children than a school-based intervention and to inform sample size calculation. Results of hypothesis testing will be interpreted with caution; emphasis will be given to confidence intervals, rather than *p* values, and results will be presented in terms of assessment of "proof of principle", rather than establishment of causation.

Conclusion

Expanding existing school-based STH control programmes to all community members has the potential to result in improved STH control among school-aged children. The (S)WASH-D for Worms pilot study is the precursor to a cluster-RCT which will contribute to the current evidence gap and could have significant implications for global STH policy.

Additional file

Additional file 1: Table S1. SPIRIT 2013 Checklist—recommended items to address in a clinical trial protocol and related documents*. (DOC 123 kb)

Abbreviations

BMI: Body mass index; CI: Confidence interval; CIR: Cumulative incidence ratio; CLTS: Community-led total sanitation; CVTL: Cruz Vermelha Timor-Leste; DALY: Disability-adjusted life years; NGO: Non-governmental organisation; NTDs: Neglected tropical diseases; PCR: Polymerase chain reaction; RCT: Randomised controlled trial; STH: Soil-transmitted helminth; WASH: Water, sanitation and hygiene; WHO: World Health Organization

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Authors' contributions

NC is the trial coordinator and drafted the manuscript. SVN is the principal investigator of the trial and co-drafted the manuscript. AC and DG are study investigators who participated in the development of the study protocol. SB and JM are the co-ordinators of the WASH programmes. All authors contributed to the editing and revising of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethics approval has been obtained from the Human Research Ethics Committees at the Australian National University (HREC 2015/111) and the Timor-Leste Ministry of Health (2015/196). Written consent has been

provided by the village and school leaders in all participating communities, as well as parents of all participating students, with a thumbprint used in place of a signature where necessary.

Author details
[1]Research School of Population Health, Australian National University, Canberra ACT 0200, Australia. [2]Cruz Vermelha Timor-Leste, Dili, Timor-Leste. [3]Plan International Timor-Leste, Dili, Timor-Leste.

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4.3 Paper 4

Clarke NE, Clements ACA, Amaral S, Richardson A, McCarthy JS, McGown J, Bryan S, Gray D, Nery SV. (S)WASH-D for Worms: a pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths. *PLoS Negl Trop Dis* 2018; 12(5): e0006389. http://doi.org/10.1371/journal.pntd.0006389

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PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

(S)WASH-D for Worms: A pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths

Naomi E. Clarke[1]*, Archie C. A. Clements[1], Salvador Amaral[1], Alice Richardson[1], James S. McCarthy[2], John McGown[3], Stuart Bryan[4], Darren J. Gray[1], Susana V. Nery[1]

1 Research School of Population Health, Australian National University, Canberra, Australia, **2** QIMR Berghofer Medical Research Institute, Brisbane, Australia, **3** Plan International Timor-Leste, Dili, Timor-Leste, **4** Cruz Vermelha Timor-Leste, Dili, Timor-Leste

* naomi.clarke@anu.edu.au

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Data Availability Statement: Some restrictions apply to the data for the (S)WASH-D for Worms pilot. All relevant aggregated data are presented within the paper and its supporting information files. Individual data cannot be made public in compliance with the protocol approved by the research ethics board in order to respect participant privacy. The ANU ethics committee can be contacted at human.ethics.officer@anu.edu.au.

Abstract

Background

Soil-transmitted helminths (STH) infect nearly 1.5 billion individuals globally, and contribute to poor physical and cognitive development in children. STH control programs typically consist of regular delivery of anthelminthic drugs, targeting school-aged children. Expanding STH control programs community-wide may improve STH control among school-aged children, and combining deworming with improvements to water, sanitation and hygiene (WASH) may further reduce transmission. The (S)WASH-D for Worms pilot study aims to compare the differential impact of integrated WASH and deworming programs when implemented at primary schools only versus when additionally implemented community-wide.

Methodology/Principal findings

A two-arm, non-randomized cluster intervention study was conducted. Six communities were identified by partner WASH agencies and enrolled in the study. All communities received a school-based WASH and deworming program, while three additionally received a community-based WASH and deworming program. STH infections were measured in school-aged children at baseline and six months after deworming. Over 90% of eligible children were recruited for the study, of whom 92.3% provided stool samples at baseline and 88.9% at follow-up. The school WASH intervention improved school sanitation, while the community WASH intervention reduced open defecation from 50.4% (95% CI 41.8–59.0) to 23.5% (95% CI 16.7–32.0). There was a trend towards reduced odds of *N. americanus* infection among children who received the community-wide intervention (OR 0.42, 95% CI 0.07–2.36, $p = 0.32$).

Conclusions

This pilot study provides proof of principle for testing the hypothesis that community-wide STH control programs have a greater impact on STH infections among children than

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school-based programs, and supports the rationale for conducting a full-scale cluster randomized controlled trial. High recruitment and participation rates and successful implementation of school WASH programs demonstrate study feasibility and acceptability. However, eliminating open defecation remains a challenge; ongoing work is required to develop community sanitation programs that achieve high and sustainable latrine coverage.

Trial registration

Australian New Zealand Clinical Trials Registry (ANZCTR) ACTRN12615001012561

Author summary

Soil-transmitted helminth (STH) infections are widespread globally, and their health impacts include poor growth and impaired cognitive development in children. STH control programs therefore usually focus on delivering deworming medications to school-aged children. However, expanding such programs to the whole community may result in larger benefits for children. Additionally, using water, sanitation and hygiene (WASH) interventions has been encouraged, to augment deworming programs by reducing environmental transmission. We conducted a pilot study to investigate the impact of an integrated WASH and deworming program implemented at primary schools only with a similar program implemented community-wide. Results confirm that the study procedures were feasible and acceptable to participants. Furthermore, results provide preliminary evidence that a community-wide control program will result in greater reductions in STH prevalence among school-aged children, compared to a school-based program.

Introduction

Soil-transmitted helminthiases–caused by roundworm (*Ascaris lumbricoides*), hookworm (*Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum*) and whipworm (*Trichuris trichiura*)–constitute the world's most common parasitic diseases of humans, with an estimated 1.45 billion people affected globally [1]. Soil-transmitted helminth (STH) infections are diseases of poverty, spread through fecal contamination of soil in areas that lack adequate water, sanitation and hygiene (WASH) [2].

Heavy STH infections are associated with iron-deficiency anaemia, poor growth, and impaired cognitive development [2], despite recent controversy over the benefits of treatment in terms of morbidity reversal [3]. Children are believed to suffer the majority of STH-associated morbidity, partly due to a peak in *A*. *lumbricoides* and *T*. *trichiura* prevalence and infection intensity among school-aged children [2]. Although hookworm tends to increase in prevalence towards adulthood, children and pregnant women are most at risk of the adverse effects of iron-deficiency anaemia [4].

Regular distribution of anthelminthic drugs, aiming to reduce morbidity, is the cornerstone of STH control programs, as advocated by the World Health Organization (WHO) [5]. Current guidelines focus on distribution of these medications to school-aged children, through school-based deworming programs, whereby deworming tablets are administered by teachers to all children, regardless of infection status [6]. Preschool-aged children, women of childbearing age, pregnant women after the first trimester, and adults in high-risk occupations have recently been recommended as additional targets, although no clear guidelines have been

implemented to guide distribution mechanisms to reach these groups [5, 7, 8]. The 2020 WHO target for STH control is regular deworming of 75% of at-risk children [9]; with over 550 million children treated in 2015, progress is on track to achieve this goal [10].

In the context of substantial global attention to neglected tropical disease (NTD) control, there is evolving interest in the most effective and sustainable ways to control STH infections. Integration of deworming programs with WASH improvements has been advocated, and included in recent WHO policies [11–13]. By reducing both environmental contamination with helminth eggs and larvae, and human exposure to these infective stages, WASH interventions may be essential for achieving long-term control of STH. Meta-analyses of mainly observational studies of WASH components suggest a reduction in STH infection [14, 15], and a cluster randomized controlled trial (RCT) examining a school-based WASH intervention reported reduced *A. lumbricoides* prevalence [16]. School-based health education programs have also been shown to reduce STH infections and intensity [17, 18]. However, RCTs of community-based WASH interventions have failed to show an impact on STH infection, potentially due to low intervention uptake and use [19, 20].

Expanding the target population of deworming programs to improve STH control has also been advocated [21]. In a recent meta-analysis, it was concluded that expanding deworming programs to whole communities would likely lead to additional benefits for children, indicating a significantly greater reduction in STH prevalence following community-wide deworming compared to child-targeted deworming [22]. With increasing interest in STH transmission interruption, mathematical modelling and cost-effectiveness studies have also highlighted the importance of expanding beyond school-based deworming [23–26]. In particular, it has been shown that the elimination of hookworm, where adults act as a substantial reservoir, will only be possible if mass drug administration campaigns include adults [27].

No prospective studies have directly compared school-based STH control programs with community-wide STH control programs, although a trial is currently underway in Kenya [28]. We conducted a pilot study, in preparation of a fully-powered cluster RCT, to compare the impact of school-based and community-wide integrated control programs, consisting of both WASH and deworming, on STH infections among school aged children.

The specific objectives of this study are as follows:

1. To assess the feasibility and acceptability of conducting a trial studying the impact of school- vs community-based distribution of deworming medications along with WASH programs, by examining study participation and recruitment rates and intervention outputs;
2. To establish "proof of principle" (preliminary evidence) to support our hypothesis that a community-wide STH control program is more effective than an exclusively school-based approach in reducing STH infections and infection intensity in school-aged children, by comparing estimates of intervention impact.

Methods

Study design and participants

This was a two-arm, non-randomized cluster intervention trial (see S1 Checklist) [29]. Six primary schools (clusters) were included in the pilot study. Three received only a school-based WASH and deworming program (control arm), while three additionally received a community-based WASH and deworming program in the community where the school was located (intervention arm).

The study was undertaken in Aileu and Manufahi municipalities of Timor-Leste between April 2015 and June 2016. Schools and their communities were identified in consultation with partner WASH agencies, who were responsible for implementing the WASH interventions. Different partner agencies were used for each of the two study arms, due to logistical and timing constraints that rendered it impossible to identify a partner agency with capacity to implement both study arms in the pilot phase of the study. Cruz Vermelha Timor-Leste (CVTL; Timor-Leste Red Cross Society) implemented the WASH programs in the control clusters, while Plan International implemented the WASH programs in the intervention clusters.

Participants in both arms of the study were school-aged children. All children attending the local primary school were eligible for participation in the study; there were no exclusion criteria.

Ethics statement

The protocol for this pilot study was developed to reflect that planned for a large scale cluster RCT, and has been published previously [30]. The study is registered with the Australian New Zealand Clinical Trials Registry, registration number ACTRN12615001012561 (see S1 Protocol).

This study received ethical approval from the Human Research Ethics Committees at Australian National University (2015/111) and the Timor-Leste Ministry of Health (2015/196). The study was explained to students and their parents or guardians at a meeting at the primary school; written and schematic information sheets were provided. Written informed consent was obtained from parents or guardians via signature or ink thumbprint.

Study interventions

The study interventions are described in detail in the protocol paper [30]. Briefly, following baseline data collection, all schools received a WASH program, consisting of: (a) provision of access to a reliable source of protected water for use by the school, achieved through constructing tapped water tanks gravity-fed from protected local springs; (b) construction of pour-flush concrete-lined pit latrines with concrete superstructures for use by students and teachers, following the Timor-Leste "WASH in Schools" guidelines [31]; and (c) hygiene education sessions conducted at school, emphasizing the importance of using latrines, handwashing with soap at key times, and keeping the environment clean, achieved through the use of flipcharts, posters and participatory demonstrations.

In the intervention arm, a WASH program was also implemented in the community where the school was located. This consisted of: (a) a sanitation intervention, aiming to increase the number of household latrines through a process known as Community-Led Total Sanitation, which challenges community members to reflect on their defecation practices and encourages them to take responsibility for building household latrines [32]; and (b) community-wide hygiene education, emphasizing key health promotion messages as above, conducted by WASH agency staff at a household level. The three intervention communities had previously received a community-level water intervention, which involved construction of tap stands supplied by gravity-fed systems from protected springs, providing access to a reliable source of protected water for use by all community members.

Following completion of the WASH intervention (defined as completed functional school latrines and 80% community latrine coverage, as reported by partner WASH agencies), the research team delivered deworming medication. In both study arms, deworming medication was delivered by study fieldworkers at school to all primary school children. In the intervention clusters, house-by-house delivery of deworming medications was additionally undertaken

by fieldworkers to administer treatment to all community members, excluding children under 12 months of age and pregnant women in the first trimester. A single dose of albendazole (400mg) was used, as per WHO guidelines [5]. All tablets were taken under direct observation of fieldworkers. Study follow-up was conducted six months following albendazole distribution.

Due to the nature of the interventions, participants and intervention implementers (research team and WASH agency staff) could not be blinded to the study arm assignment.

Data collection

At baseline and six month follow-up, students and their parents completed questionnaires, administered as interviews by trained local fieldworkers at the primary schools. Students answered questions relating to their defecation and hygiene practices. Parents answered questions relating to household water source, assets, education and occupation.

Stool samples were collected from participating students at baseline and at follow-up for diagnosis of STH infections. Children were educated on how to provide a stool sample and asked to bring a sample from the following morning to school. Aliquots of 2–3 grams were preserved immediately upon receipt of the samples with 5mL of 5% potassium dichromate, and transported at room temperature to QIMR Berghofer Medical Research Institute in Brisbane, Australia. Samples were analysed using a quantitative polymerase chain reaction (qPCR) technique, which involved DNA extraction, followed by running a real-time multiplex PCR to detect and quantify STH (*Ascaris* spp., *N. americanus*, *Ancylostoma* spp., *T. trichiura*), as described previously [33]. Laboratory staff were not aware of the study arm to which participants belonged.

At both study time points, children underwent anthropometric measurement of height (to the nearest 0.1cm) and weight (to the nearest 0.5kg); a fingerprick blood sample was also collected to measure haemoglobin using a portable analyser (Hb 201+, HemoCue, Angelholm, Sweden).

Outcomes

Primary outcomes related to study feasibility and acceptability, and secondary outcomes related to study intervention impact (see Box 1).

Infection intensity categories were defined as "higher intensity", "lower intensity", and "no infection", and were used to examine relative changes in infection intensity over time. Samples were categorized based on the cycle threshold (Ct) values obtained by qPCR; lower Ct values reflect higher infection intensity. We determined the median Ct value for all positive samples at baseline, and classified individuals with Ct values lower than baseline median as "higher intensity" infections, and those with Ct values higher than baseline median as "lower intensity" infections (see S1 Appendix). These categories were not intended to correspond with WHO thresholds for heavy, moderate and light-intensity infections because PCR-based values have not yet been identified that accurately correspond with these thresholds.

Anaemia was diagnosed from altitude-adjusted haemoglobin measurements using WHO thresholds [34]. The 2006 WHO database for child growth standards were used to calculate *Z*-scores for the following anthropometric indices: weight-for-age (for children aged $\leq$10 years only), height-for-age, and body mass index-for-age. These *Z*-scores were used to determine presence of underweight, stunting, and thinness, respectively, with scores below –2 indicative of malnutrition [35].

Box 1. Study outcomes (measured at baseline and six month follow-up).

Primary outcomes

Proportion of eligible children for whom parental informed consent is gained

Proportion of eligible children who provide stool samples

Proportion of eligible children who complete questionnaires

Proportion of eligible children who undergo measurement of height, weight and haemoglobin

Proportion of eligible children and community members who receive albendazole

Time taken to complete school WASH interventions in all clusters

Time taken to achieve 80% household latrine coverage in intervention clusters

Proportion of schools and households with functional and clean latrines

Proportion of children who report practicing open defecation

Proportion of schools with handwashing stations

Proportion of children who practise handwashing with soap at critical times

Secondary outcomes

Infection with *Ascaris* spp., *N. americanus*, *Ancylostoma* spp., and *T. trichiura*

Infection intensity category of *Ascaris* spp., *N. americanus*, *Ancylostoma* spp., and *T. trichiura*

Anaemia

Malnutrition indicators: stunting, thinness, and underweight

Statistical analysis

For the primary outcomes, descriptive statistics were used to determine the proportion of eligible participants who gave informed consent and participated in study procedures, as well as outcomes relating to completion and coverage of the WASH interventions. WASH outputs were compared between intervention and control arms using a difference in differences (DID) approach, where DID = $(\text{Intervention}_{\text{Follow-up}} - \text{Intervention}_{\text{Baseline}}) - (\text{Control}_{\text{Follow-up}} - \text{Control}_{\text{Baseline}})$.

For unadjusted analysis of secondary outcomes, the proportions of children with each STH infection, higher-intensity infection, anaemia, stunting, thinness and underweight, were compared between study arms using a difference in differences approach. We also calculated the prevalence reduction $([\text{Prevalence}_{\text{Baseline}} - \text{Prevalence}_{\text{Follow-up}}] / \text{Prevalence}_{\text{Baseline}})$ of each STH infection, with confidence intervals calculated using a bootstrap resampling method with 2000 replicates.

Generalized linear mixed models [36] were then used to calculate adjusted odds ratios (OR) for infection presence and intensity group, comparing study arms at follow-up. Bernoulli logistic regression was used for infection presence, and ordinal logistic regression for infection intensity group (no, lower-intensity, and higher-intensity infection). Age, sex and baseline infection status were entered as fixed effects. Due to discordances between study arms in terms of hygiene behaviour and access to improved water at baseline, handwashing after defecation and access to improved water were also included as fixed effects. School was entered as a random effect to account for clustering. Due to very low baseline prevalence of *Ancylostoma* spp. and *T. trichiura*, and a highly imbalanced baseline prevalence of *Ascaris* spp. across study arms, generalized linear mixed models were run only for *N. americanus* infections. All analyses were conducted using Stata version 14.1 (College Station, TX, USA).

Results

Baseline characteristics

At study baseline, informed consent was obtained for 522 students across the six participating schools. Owing to size differences in the schools, there were three times as many participants in the control arm compared to the intervention arm. The CONSORT trial profile [37] is shown in Fig 1.

Baseline characteristics of participating students and schools, including baseline STH infections and morbidity indicators, are shown in Table 1. Age and sex were balanced across study arms. The prevalence of *Ascaris* spp. was significantly imbalanced between the two study arms, with baseline prevalence of 48.7% (95% confidence interval (CI) 43.6–53.8) in the control arm vs 7.6% (3.8–14.4) in the intervention arm ($p = 0.007$). The prevalence of *N. americanus* was

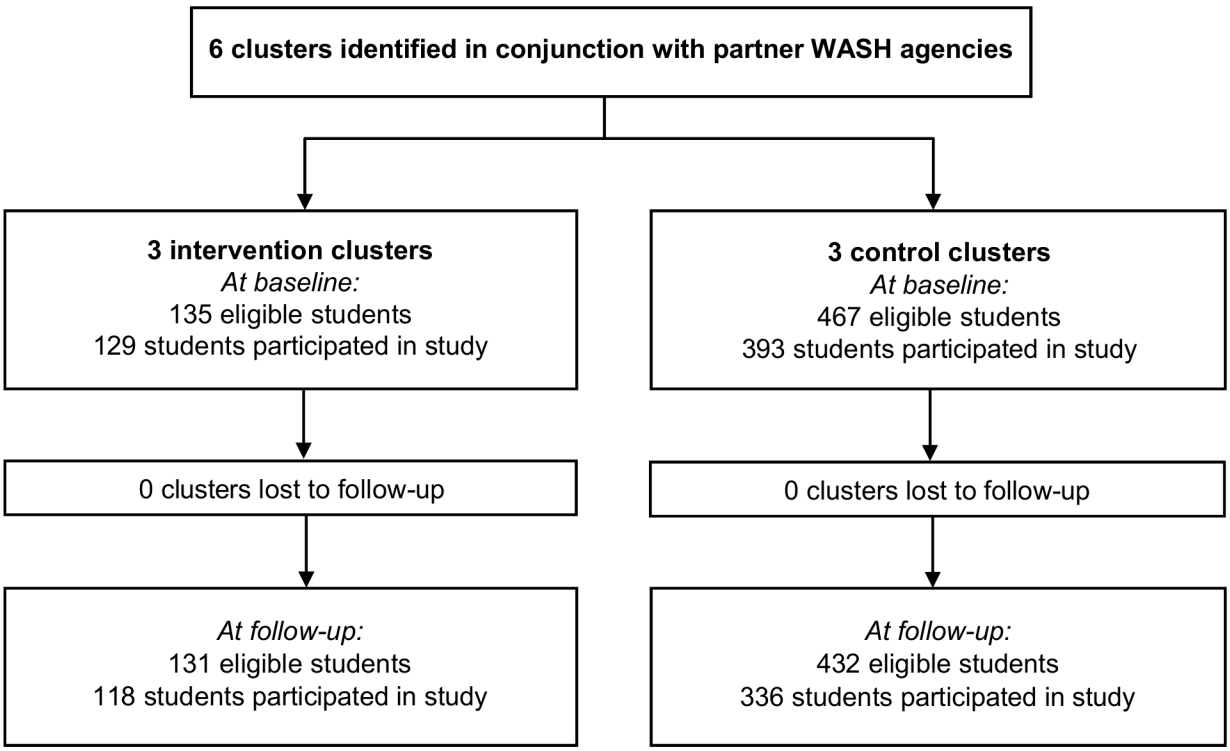

Fig 1. CONSORT flow diagram of the pilot study. Study participation is defined as providing a questionnaire and/or stool sample.

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Table 1. Baseline characteristics of study participants.

	Control arm	Intervention arm
Demographics	*n = 393*	*n = 129*
Female sex, n (%)	195 (52.7%)	58 (45.0%)
Mean (range) age in years	9.64 (4.2–17.1)	9.05 (5.2–15.8)
STH infections	*n = 372*	*n = 110*
Ascaris spp. infections	48.7% (43.6–53.8)**	7.6% (3.8–14.4)**
Ascaris spp. higher-intensity infections	27.3% (21.1–30.0)	0.9% (0.1–6.5)
N. americanus infections	13.7% (10.6–17.6)	15.1% (9.4–23.3)
N. americanus higher-intensity infections	7.3% (5.0–10.4)	6.6% (3.2–13.3)
Ancylostoma spp. infections	1.1% (0.4–2.8)	0
T. trichiura infections	2.2% (1.1–4.3)	1.9% (0.5–7.3)
Hematological parameters	*n = 381*	*n = 116*
Anaemia	12.6% (9.6–16.3)	4.3% (1.8–10.0)
Growth parameters (all age groups)	*n = 382*	*n = 124*
Stunting	51.7% (46.6–56.8)	62.1% (53.2–70.2)
Thinness	25.5% (21.3–30.1)	42.7% (34.3–51.6)
Growth parameters (age ≤10 years only)	*n = 225*	*n = 86*
Underweight	53.3% (46.7–59.8)	65.1% (54.4–74.5)
School-level variables	*n = 3*	*n = 3*
Mean number of students (SD)	175.3 (97.3)*	52.0 (16.4)*
Mean proportion girls (SD)	50.1 (3.0)	48.5 (10.3)
Mean pupil/teacher ratio (SD)	26.4 (2.3)	18.6 (8.5)

Unless otherwise indicated, results are shown as: proportion (95% confidence interval)

Significant difference between study arms:

* $p<0.05$

** $p<0.01$

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more balanced across study arms (13.7% vs 15.1%; $p = 0.956$), while the prevalence of *Ancylostoma* spp. and *T. trichiura* was very low across both study arms. Stunting and underweight were each present in more than half of the study participants.

Study recruitment and participation

Over 90% of students who were present during the research team visits were recruited (i.e., parental informed consent obtained) at each study time point; there were no refusals of consent. At baseline, among the 522 students for whom informed consent was gained, 92.3% provided stool samples, 100% completed questionnaires, 95.4% provided blood samples, and 96.9% had their height and weight measured. Participation rates remained similarly high at follow-up (see Table 2). There were no differences in participation rates between study arms at either time point. Following the implementation of the WASH programs, albendazole was distributed to 89.4% of eligible schoolchildren, and in the intervention arm of the study, to 395 out of 471 (83.9%) eligible community members.

WASH indicators

Community latrine coverage reached 80% within three months in all intervention communities. The school WASH program was completed within five months in five schools. In the remaining school, it took seven months to complete, and in order to treat participating

Table 2. Recruitment and participation in the pilot study.

	Baseline		Follow-up	
	Control	Intervention	Control	Intervention
Eligible students (n)[a]	467	135	432	131
Students present, n (%)	432 (92.5%)	131 (97.8%)	378 (87.5%)	124 (94.7%)
Consent obtained, n (%)[b]	393 (90.5%)	129 (98.5%)	341 (90.2%)	120 (96.8%)
Provided stool, n (%)[c]	372 (94.7%)	110 (85.3%)	303 (88.9%)	107 (89.2%)
Completed questionnaire, n (%)[c]	393 (100%)	129 (100%)	336 (98.5%)	118 (98.3%)
Provided blood sample, n (%)[c]	382 (97.2%)	116 (89.9%)	324 (95.0%)	112 (93.3%)
Height/weight measured, n (%)[c]	382 (97.2%)	124 (96.1%)	326 (95.6%)	116 (96.7%)
Albendazole taken, n (%)[d]	393/444 (88.5%)	124/134 (92.5%)	359/432 (83.1%)	120/131 (91.6%)

[a] Eligible students defined as all those enrolled to attend the primary school

[b] Proportion calculated out of total students present

[c] Proportion calculated out of total students for whom informed consent was obtained

[d] Proportion calculated out of total students enrolled in primary school at time of albendazole distribution

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children before school holidays commenced, albendazole was administered before school latrine construction was completed. The latrines were completed by the time children returned to school six weeks later.

Outcomes relating to the WASH intervention are depicted in Table 3. The school WASH intervention resulted in all schools having access to handwashing stations and functional latrines, with a mean pupil-to-latrine ratio of 25.4 students in the control arm and 26 students in the intervention arm. All schools had separate toilets for male and female students.

Baseline household latrine coverage was higher than expected in both study arms; approximately two thirds of children reported presence of a household latrine. However, open defecation was reported by 58.5% (53.6–66.3) children in the control arm and 50.4% (41.8–59.0) in the intervention arm at baseline. At follow-up, the proportion of children reporting household latrines increased to 76.4% (71.5–80.8) in the control arm and 84.9% (77.2–90.3) in the intervention arm, with the difference in differences (DID) between intervention and control arms

Table 3. WASH infrastructure and behaviour at baseline and follow-up.

Variable	Baseline		Follow-up	
	Control	Intervention	Control	Intervention
School-level variables	*n = 3*	*n = 3*	*n = 3*	*n = 3*
Schools with functional latrines, n (%)	0	0	3 (100%)	3 (100%)
Mean (SD) number of pupils per latrine	-	-	25.4 (10.6)	26.0 (8.2)
Schools with handwashing stations, n (%)	0	0	3 (100%)	3 (100%)
Individual-level variables[a]	*n = 393*	*n = 129*	*n = 329*	*n = 119*
Students reporting presence of household latrine	67.4% (62.6–71.9)	65.9% (60.7–76.7)	76.4% (71.5–80.8)	84.9% (77.2–90.3)
Students reporting open defecation	58.5% (53.6–66.3)	50.4% (41.8–59.0)	45.9% (40.6–51.3)	23.5% (16.7–32.0)
Students with access to improved water[b]	53.1% (48.5–58.4)	83.7% (76.2–89.2)	69.2% (63.7–74.1)	86.1% (78.4–91.4)
Students reporting use of soap when washing hands	87.3% (83.6–90.2)	72.9% 64.5–79.9)	91.2% (87.6–93.8)	88.2% (81.1–92.9)
Students reporting handwashing after defecation	59.0% (54.1–63.8)	38.0% (30.0–46.7)	70.8% (65.7–75.5)	61.3% (52.3–69.7)
Students reporting handwashing before eating	30.8% (26.4–35.5)	14.7% (9.6–22.0)	44.1% (38.8–49.5)	41.2% (32.6–50.3)

[a] Individual level variables are shown as proportion (95% confidence interval).

[b] Access to improved water defined as main household water source being either piped water, protected spring, or protected dugwell.

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of 6.6% ($p = 0.211$). Children practicing open defecation decreased to 45.9% (40.6–51.3) in the control arm, and 23.5% (16.7–32.0) in the intervention arm, with DID of -14.2% ($p = 0.032$).

At study baseline, more children in the intervention arm had access to an improved water source, compared to the control arm (83.7% vs 53.1%); this difference persisted at study follow-up (86.1% vs 69.2%). On the other hand, children in the control arm displayed better hygiene behaviour than those in the intervention arm at baseline, with a higher proportion of students reporting use of soap (87.3% vs 72.9%), handwashing after defecation (59.0% vs 38.0%), and handwashing before eating (30.8% vs 14.7%). Following the WASH program, hygiene behaviour improved in both study arms. DID between intervention and control arms was 11.5% for use of soap ($p = 0.150$), 11.6% for handwashing after defecation ($p = 0.373$) and 13.2% for handwashing before eating ($p = 0.219$).

Intervention impact

As shown in Fig 2 and S1 Table, six months following the WASH and deworming intervention, the prevalence of *Ascaris* spp. decreased from 48.7% (43.6–53.8) to 23.4% (18.9–28.5) in the control arm and from 7.6% (3.8–14.4) to 0.9% (0.1–6.5) in the intervention arm, representing a prevalence reduction of 52.0% (95% CI 45.2–70.0) in the control arm and 88.2% (95% CI 70.2–100.0) in the intervention arm. The crude DID between intervention and control arms was 18.6% ($p = 0.005$), reflecting the significantly higher baseline prevalence in the control arm. *N. americanus* prevalence decreased from 13.7% (10.6–17.6) to 9.9% (7.0–13.8) in the control arm, and from 15.1% (9.4–23.3) to 5.7% (2.5–12.1) in the intervention arm, representing a prevalence reduction of 27.7% (95% CI 12.7–40.7) in the control arm and 62.3% (95% CI 54.9–67.4) in the intervention arm (see Fig 3 and S1 Table). The crude DID between intervention and control arms was -5.6% ($p = 0.254$). The prevalence of higher-intensity infections also decreased for both STH.

Morbidity indicators remained similar across the six months of the study (S2 Table). There were no significant DID between study arms in terms of nutritional indicators or hematological parameters.

The results of the generalized linear mixed models used to assess impact of the intervention on *N. americanus* infection are shown in Table 4. The odds of *N. americanus* infection at follow-up were 58% lower in the intervention arm compared to the control arm (OR 0.42, 95% CI 0.07–2.36), and the odds of higher-intensity infection were 57% lower (OR 0.43, 95% CI 0.08–2.37), although these did not reach statistical significance. Males had increased odds of infection (OR 2.92, 95% CI 1.07–7.95). Children who had *N. americanus* infection at baseline had significantly increased odds of infection at follow-up (OR 10.20, 95% CI 3.68–28.27). Similarly, those with a higher-intensity infection at baseline had significantly increased odds of higher-intensity infection at follow-up (OR 15.57, 95% CI 5.15–47.06).

Discussion

To our knowledge, this pilot study represents the first direct comparison of the impact of integrated school-based versus community-wide control programs on STH infection in school-aged children. The integrated STH control programs in our pilot study consisted of distribution of anthelminthic medications, regardless of infection status, complemented by WASH improvements, in line with recent WHO recommendations [38].

We achieved high rates of both parental informed consent and participation of school-aged children in all aspects of the study. Participation rates were above 80% for all study procedures (completing questionnaires, providing stool samples, undergoing anthropometric measurement, providing a fingerprick blood sample, and taking albendazole), confirming the

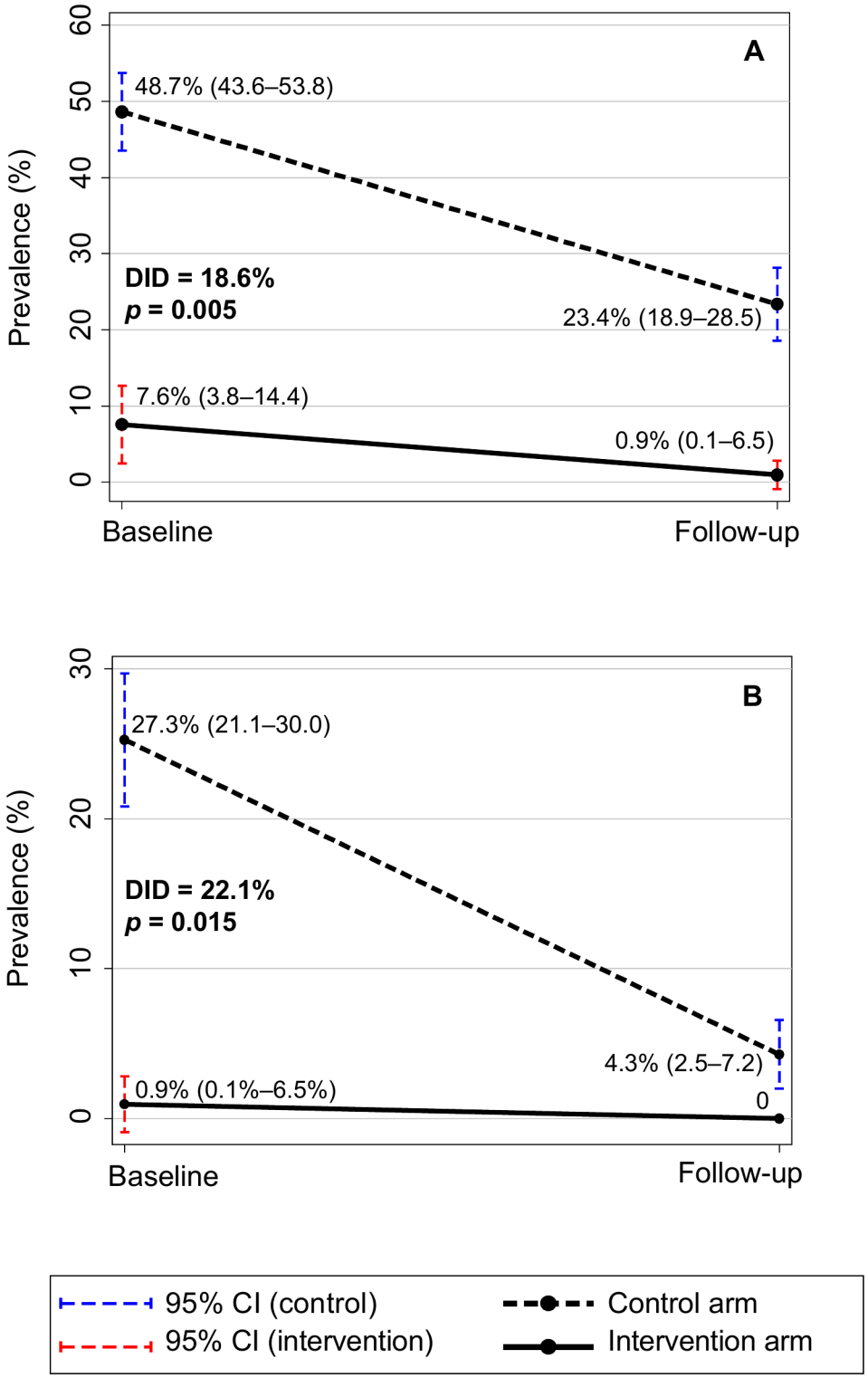

Fig 2. Prevalence of (A) infection and (B) higher-intensity infection with *Ascaris* spp., before and six months following the study intervention. *P* values are based on logistic regression models comparing intervention and control arms, accounting for school-level clustering. CI = confidence interval; DID = difference in differences between intervention and control arms.

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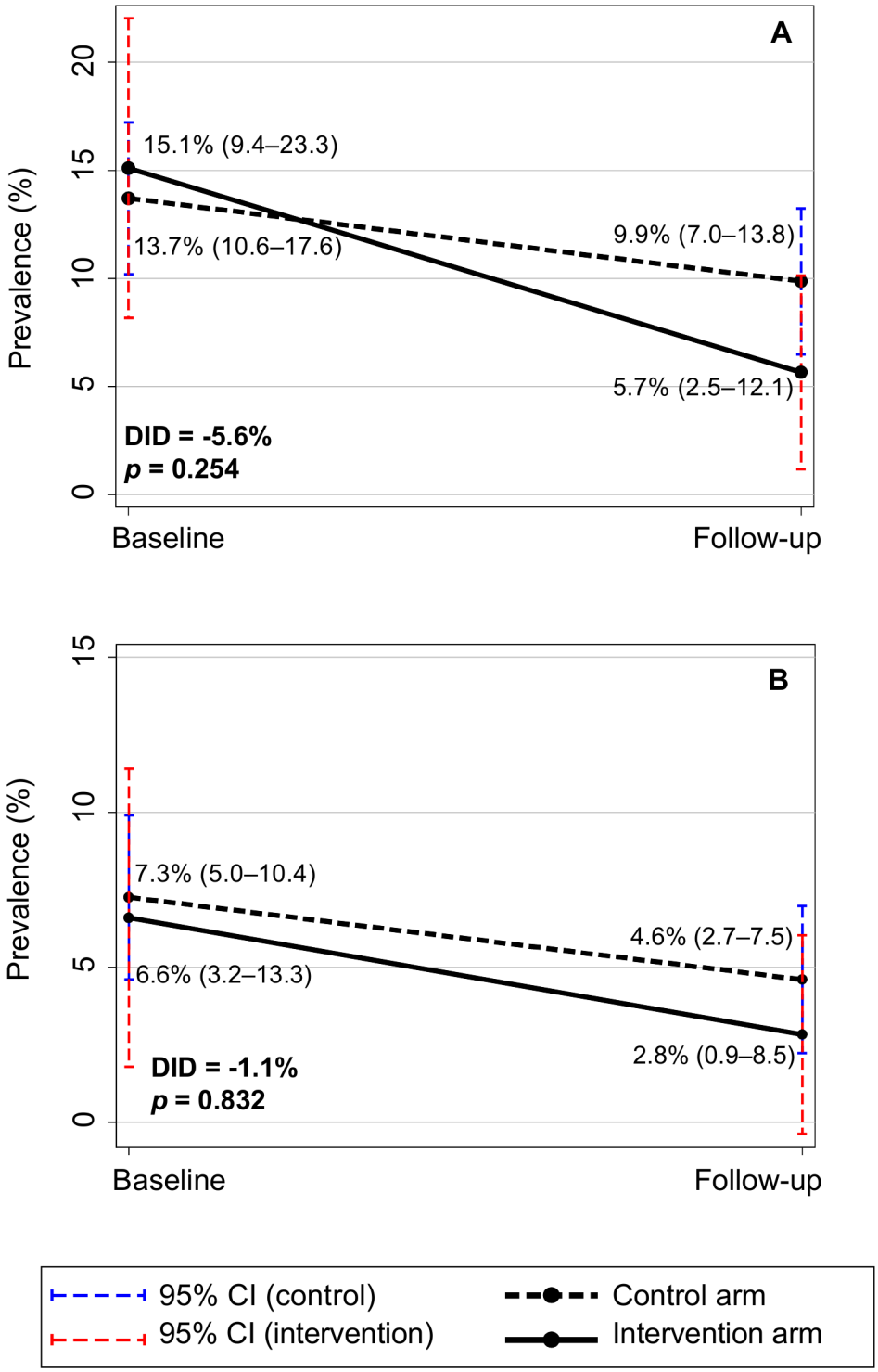

Fig 3. Prevalence of (A) infection and (B) higher-intensity infection with *N. americanus*, before and six months following the study intervention. *P* values are based on logistic regression models comparing intervention and control arms, accounting for school-level clustering. CI = confidence interval; DID = difference in differences between intervention and control arms.

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Table 4. Results of generalized linear mixed models showing effect estimate of the study intervention and other covariates on *N. americanus* prevalence and intensity.

Variable	*N. americanus* infection			*N. americanus* higher-intensity infection		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Study intervention[a]	0.42	0.07–2.36	0.32	0.43	0.08–2.37	0.33
Age in years	1.05	0.85–1.31	0.63	1.13	0.91–1.40	0.26
Male sex[b]	2.92	1.07–7.95	0.04	2.45	0.91–6.58	0.08
Handwashing after defecation[c]	0.55	0.21–1.45	0.23	0.58	0.22–1.55	0.28
Access to improved water[d]	0.51	0.20–1.32	0.17	0.59	0.23–1.50	0.27
N. americanus infection at baseline[e]	10.20	3.68–28.27	<0.01	-	-	-
N. americanus intensity group at baseline[e]						
Lower intensity	-	-	-	5.17	1.35–19.84	0.02
Higher intensity	-	-	-	15.57	5.15–47.06	<0.01
Random effects variance (95% CI)						
School	0.48 (0.06–4.03)			0.48 (0.06–3.89)		

CI = confidence interval; OR = odds ratio. Reference groups are as follows

[a] Control group

[b] Female sex

[c] Does not wash hands after defecation.

[d] No access to improved water. Access to improved water is defined as main household water source being either piped water, protected spring, or protected dugwell.

[e] No infection at baseline.

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acceptability of the study methods and procedures. There were no refusals of parental informed consent, likely because deworming medications are understood to benefit children, and WASH improvements to benefit both children and the wider community. Child refusal to participate in any component of the study was low. In particular, stool samples were collected from a high proportion of participating children (92.3% at baseline and 88.9% at follow-up), suggesting that our results are likely representative of the population STH prevalence in school-aged children.

The school WASH programs were successful in improving school sanitation, handwashing facilities, and hygiene behaviour among school-aged children. The community-wide WASH program reduced the practice of open defecation among school-aged children, such that prevalence of open defecation was significantly lower in the intervention arm compared to the control arm at follow-up. Importantly, the aim of community WASH programs is to eliminate open defecation, and in this respect, the community-wide program implemented in our study did not achieve "open defecation free" status. Nearly 25% of children in the intervention arm still reported that they practised open defecation at study follow-up. These results are consistent with other community-based trials where WASH programs have been implemented and where health improvements have not been detected as a result [19, 20].

The integrated WASH and deworming interventions reduced STH prevalence and intensity in both study arms. Morbidity outcomes, including anaemia, stunting, thinness and underweight, were mainly measured to establish feasibility; significant changes were not anticipated within a six-month time frame. As expected, these indicators remained similar across the six-month study period.

Importantly, results of the pilot study showed a 58% reduction in odds of *N. americanus* infection and 57% reduction in odds of higher-intensity infection in the intervention arm (community-wide control program), compared to the control arm (school-based control program). In the context of a small pilot study, these results did not reach statistical significance.

This was to be expected, because the pilot study was not powered to detect such differences between study arms. However, these results provide preliminary evidence and proof of principle for testing our hypothesis that a community-wide control program will be more effective at reducing STH infections in children than a school-based control program. Our findings agree with a recent meta-analysis, as well as with mathematical modelling studies, highlighting the additional benefits of expanding STH control programs community-wide [22–26].

Study limitations and lessons learned

This pilot study highlighted several important issues for consideration during planning and implementation of a larger-scale trial. Firstly, a significant limitation of this pilot study was the non-randomization of study schools. Randomization was not possible because we were unable to identify a partner agency who was able to implement the WASH interventions in both arms of the study within required timeframes. Therefore, two different partner agencies were used (one for each study arm), who worked in two different municipalities of Timor-Leste. Non-randomization led to a number of discrepancies between study arms at baseline, including school size, *Ascaris* spp. prevalence, and hygiene behaviour. Hygiene behaviour was significantly better among children in the control arm at baseline. Given previous findings that school-based health education programs may significantly reduce STH reinfection [17], this could bias results against showing an intervention impact. Therefore, we adjusted for baseline hygiene behaviour in our final analysis. The highly discordant baseline prevalence of *Ascaris* spp. made interpretation of intervention impact on infection with this parasite impossible, limiting our analysis to *N. americanus*. Such discrepancies would likely be avoided in a fully-powered study that was adequately randomized.

We collected information on household WASH conditions using self-reported data from children and parents in interviews conducted at the school. This approach may result in a response bias; participants may be more likely to report favourable answers if they are reluctant to give negative feedback (courtesy bias), or on the other hand, they may report less favourable answers if they anticipate NGO intervention. In a larger trial, household inspections should be carried out in order to improve accuracy of the measurement of WASH conditions, both before and after intervention implementation.

The school WASH programs were commenced mid-way through the school year, and due to school holiday timing, albendazole was distributed before latrines were completed in one school in the intervention arm. This could have led to higher reinfection rates and biased results against showing an intervention effect. A fully-powered trial should be implemented such that the WASH intervention is conducted close to the beginning of the school year, and it is crucial to ensure that partner WASH agencies have capacity to complete their interventions in a timely and simultaneous manner. Successful study implementation will require co-operation and regular liaison between the research team, WASH agencies, school and community leaders, and the Ministry of Education.

We observed a higher than expected baseline coverage of household latrines; as a result, improvements to sanitation coverage achieved through the community WASH program were modest. Additionally, water improvements were implemented in the intervention communities prior to study baseline; this was adjusted for in our analysis. Both of these factors may have decreased the likelihood of the WASH intervention reducing STH transmission. In a larger trial, a thorough assessment of potential study communities should be conducted to ensure that the communities are appropriate for a WASH intervention, with clearly defined eligibility criteria, such as a cut-off for baseline latrine coverage and water availability.

Finally, although we observed a greater than 50% decrease in open defecation following the community WASH program, the goal of eliminating the practice of open defecation was not

achieved. Our pilot study highlighted the difficulty in achieving high and sustainable latrine coverage that has been seen previously [19, 20], and the need for ongoing studies within the WASH sector to identify and assess innovative strategies for improving and sustaining the coverage and use of sanitation facilities. When designing a larger-scale trial, rigorous discussion and planning with partner agencies should be undertaken in an effort to maximize intervention uptake and fidelity.

Conclusions and future directions

Our results demonstrate proof of principle for testing the hypothesis that an expanded community-wide STH control program will lead to reduced STH reinfection among school-aged children compared to a school-based control program. These results highlight the feasibility and rationale for conducting a full-scale cluster RCT comparing community-wide and school-based STH control approaches to test this hypothesis, and to inform global STH control guidelines.

Supporting information

S1 Checklist. TREND checklist for reporting of non-randomized clinical trials.
(PDF)

S1 Protocol. Full study protocol from the Australian New Zealand clinical trial registry.
(PDF)

S1 Appendix. STH infection intensity categories.
(PDF)

S1 Table. STH infections over time.
(PDF)

S2 Table. Morbidity indicators over time.
(PDF)

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Author Contributions

Conceptualization: Susana V. Nery.

Data curation: Naomi E. Clarke.

Formal analysis: Naomi E. Clarke.

Funding acquisition: Archie C. A. Clements, Darren J. Gray, Susana V. Nery.

Investigation: Naomi E. Clarke, Salvador Amaral, James S. McCarthy, Susana V. Nery.

Methodology: Naomi E. Clarke, Archie C. A. Clements, Alice Richardson, Susana V. Nery.

Project administration: Naomi E. Clarke, Salvador Amaral, John McGown, Stuart Bryan, Susana V. Nery.

Supervision: Naomi E. Clarke, Salvador Amaral, John McGown, Stuart Bryan, Susana V. Nery.

Writing – original draft: Naomi E. Clarke.

Writing – review & editing: Naomi E. Clarke, Archie C. A. Clements, Salvador Amaral, Alice Richardson, James S. McCarthy, John McGown, Stuart Bryan, Darren J. Gray, Susana V. Nery.

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Chapter 5
The role of WASH in STH control

5.1 Chapter context

This chapter addresses the third thesis research objective: to investigate the impact of community-level WASH interventions and individual- and household-level WASH characteristics on STH infections. Two manuscripts are presented in this chapter, both of which are published in peer-reviewed journals.

The preceding chapters have focused mainly on preventive chemotherapy, which is the principal strategy used to address STH as a public health problem. However, it is well established that STH reinfections occur rapidly after treatment in the context of ongoing environmental contamination, and therefore the importance of complementing regular deworming with WASH improvements for sustainable control has been highlighted. However, as discussed in Chapter 1, most existing evidence regarding the impact of WASH on STH infections comes from low-quality cross-sectional studies. Robust evidence from cluster RCTs of WASH interventions is limited, and predominantly relates to school-based interventions or community-based sanitation interventions without water or hygiene components. A sparse and conflicting body of evidence makes it difficult for policymakers to determine what types of WASH interventions will contribute meaningfully to STH control, and therefore how best to utilise limited funds and human resources.

While the pilot study presented in the previous chapter included a WASH component, that study was not designed to determine the relative contributions of WASH and deworming to STH control. The work presented in the current chapter was conducted to explore the specific contribution of WASH to STH control, in the context of regular deworming in an endemic setting. This chapter includes two papers, both of which present results from the WASH for WORMS cluster randomised controlled trial. The

WASH for WORMS trial was conducted in Timor-Leste between 2012 and 2016, and compared the impact of a community-based WASH and deworming intervention to community deworming alone over a two-year period.

The first paper presents the main experimental results of the WASH for WORMS study, namely the impact of a community-based WASH intervention on STH prevalence, intensity, and associated morbidity. It also presents process measures including uptake of the WASH intervention. This paper is published in *The American Journal of Tropical Medicine & Hygiene* (Paper 5). The second paper is a detailed risk factor analysis, examining the impact of individual WASH components on STH infections. This was conducted using longitudinal data collected in the WASH for WORMS trial on WASH conditions and STH infections. Longitudinal data facilitated a more robust risk factor analysis compared to existing cross-sectional analyses, allowing adjustment for a "lag time" in the context of evolving WASH conditions. This paper is published in the *International Journal for Parasitology* (Paper 6).

The work presented in this chapter contributes to the understanding of integrated WASH interventions for STH control, and to disentangling the impact of different WASH components to highlight key focus areas for WASH implementers in the context of STH control. These findings are of particular relevance to policymakers in the NTD and WASH sectors at a time when the WHO and other stakeholders are emphasising the importance of their collaboration in accelerating progress against NTDs.

5.2 Paper 5

Nery SV, Traub RJ, McCarthy JS, **Clarke NE**, Amaral S, Llewellyn S, Weking E, Richardson A, Campbell SJ, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Clements ACA. WASH for WORMS: a cluster-randomized controlled trial of the impact of a community integrated water, sanitation, and hygiene and deworming intervention on soil-transmitted helminth infections. *Am J Trop Med Hyg* 2019; 100(3): 750–761. http://doi.org/10.4269/ajtmh.18-0705

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

Susana Vaz Nery,[1,2]* Rebecca J. Traub,[3] James S. McCarthy,[4] Naomi E. Clarke,[1] Salvador Amaral,[1] Stacey Llewellyn,[4] Edmund Weking,[5] Alice Richardson,[1] Suzy J. Campbell,[1] Darren J. Gray,[1] Andrew J. Vallely,[2] Gail M. Williams,[6] Ross M. Andrews,[1,7] and Archie C. A. Clements[1,8]

[1]*Research School of Population Health, Australian National University, Canberra, Australia;* [2]*The Kirby Institute for Infection and Immunity in Society, University of New South Wales, Sydney, Australia;* [3]*Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Australia;* [4]*Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia;* [5]*WaterAid Australia, Dili, Timor-Leste;* [6]*School of Public Health, University of Queensland, Brisbane, Australia;* [7]*Menzies School of Health Research, Charles Darwin University, Darwin, Australia;* [8]*Faculty of Health Sciences, Curtin University, Perth, Australia*

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

INTRODUCTION

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

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

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

* Address correspondence to Susana Vaz Nery, The Kirby Institute for Infection and Immunity in Society, University of New South Wales, Sydney, NSW 2052, Australia. E-mail: snery@kirby.unsw.edu.au

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

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

MATERIAL AND METHODS

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

RESULTS

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

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

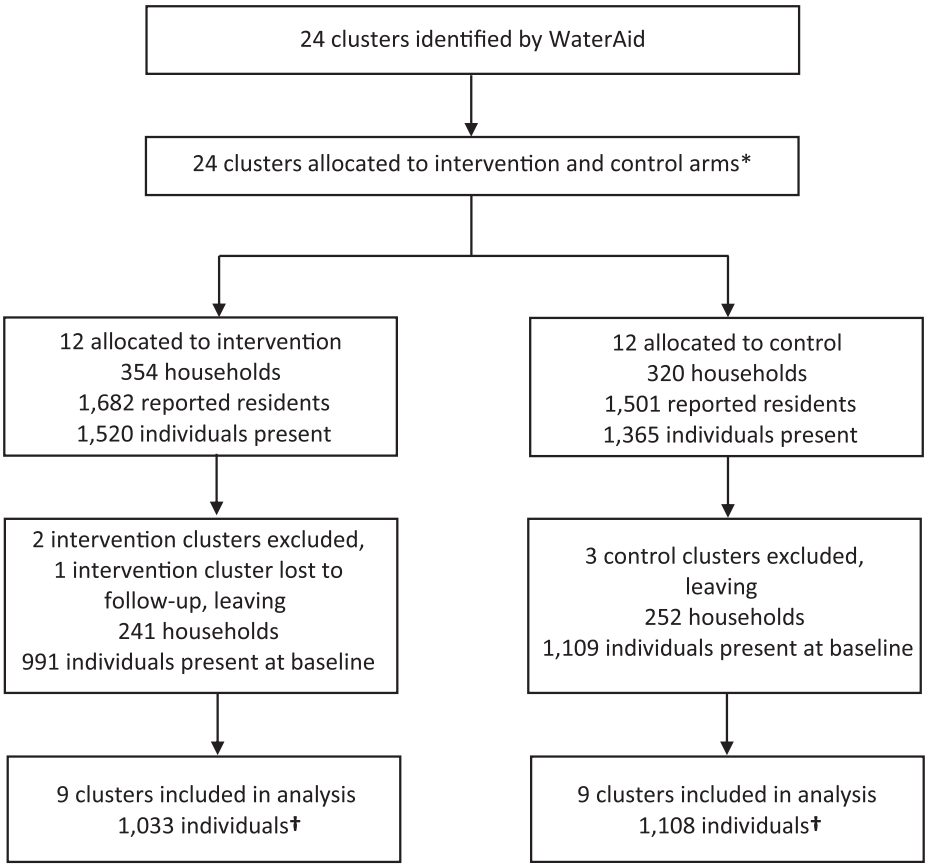

FIGURE 1. Trial profile. *In three blocks. †Individuals who provided a stool sample or questionnaire for at least one follow-up time point.

TABLE 1
Baseline characteristics of study participants* and households

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

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

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

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

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

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

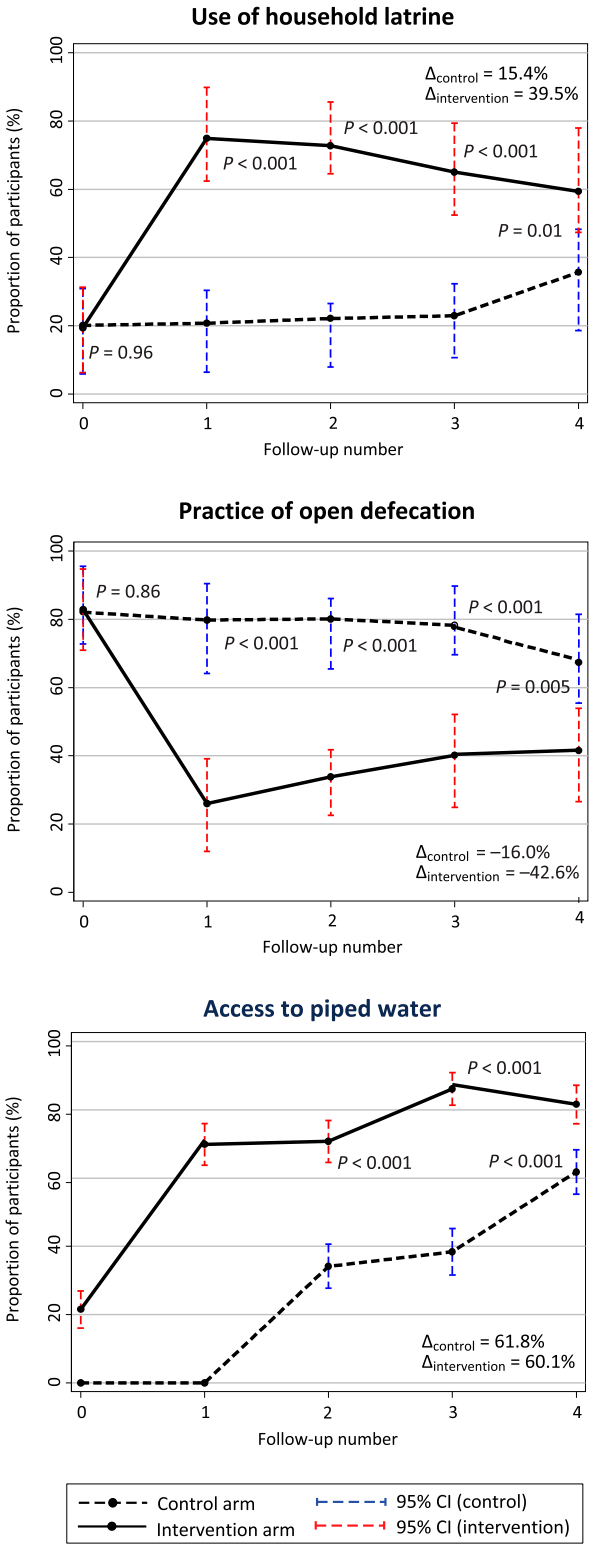

FIGURE 2. Use of household latrines, practice of open defecation, and piped water as main water source in the two study arms over time. *P*-values and 95% CI calculated using logistic regression models accounting for community-level clustering. Δ = absolute change in proportion = Proportion [Follow-up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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

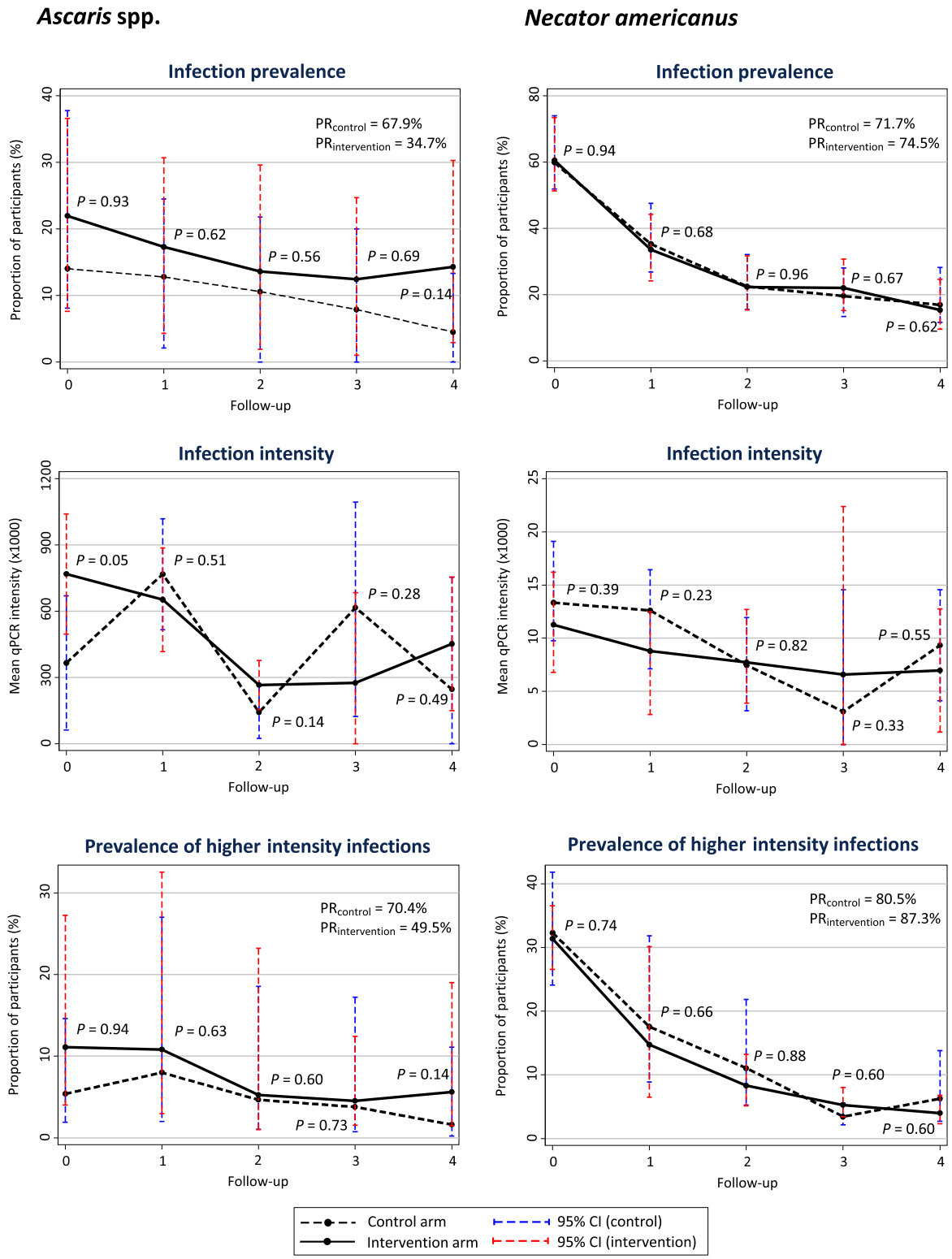

FIGURE 3. Infection prevalence, intensity, and prevalence of high-intensity infections in the two study arms over time. *P*-values and 95% CI calculated using logistic regression models accounting for community-level clustering. PR = prevalence reduction, calculated as: (Prevalence [Baseline]–Prevalence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

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

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

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

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

BMI = body mass index; CI = confidence interval; RR = relative risk. **Bold** text indicates statistically significant *P* value (< 0.05).
* Adjusted RR obtained from generalized linear mixed models, adjusted for age and gender (fixed effects) and clustering at the community, household, and individual levels (random effects). Models included the following numbers of participants in 18 communities: for anemia, 1,598 participants in 428 households; for stunting, 789 participants in 304 households; for thinness, 781 participants in 301 households; for wasting, 231 participants in 157 households; and for underweight 511 participants in 249 households.
† Anthropometric indices is defined as < −2 standard deviation below the mean of a standard population for the following indicators: stunting = weight-for-age; thinness = BMI-for-age, where BMI is calculated as weight (kg)/height^2 (cm); wasting = weight-for-height; and underweight = weight-for-age.

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

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

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

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

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

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

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

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

CONCLUSION

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

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Note: Supplemental tables appear atwww.ajtmh.org.

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Authors' addresses: Susana Vaz Nery and Andrew J. Vallely, Kirby Institute for Infection and Immunity in Society, University of New South Wales, Kensington, Australia, E-mails: snery@kirby.unsw.edu.au and avallely@kirby.unsw.edu.au. Rebecca J. Traub, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Australia, E-mail: rebecca.traub@unimelb.edu.au. James S. McCarthy and Stacey Llewellyn, Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia, E-mails: j.mccarthy@uq.edu.au and stacey.llewellyn@qimrberghofer.edu.au. Naomi E. Clarke, Salvador Amaral, Alice Richardson, and Darren J. Gray, Research School of Population Health, Australian National University, Canberra, Australia, E-mails: naomi.clarke@anu.edu.au, salvadorcoro@yahoo.com, alice.richardson@anu.edu.au, and darren.gray@anu.edu.au. Edmund Weking, WaterAid Australia, Dili, Timor-Leste, E-mail: edmund.weking@wateraid.org.au. Suzy J. Campbell, Evidence Action, Washington, DC, E-mail: suzy.campbell@evidenceaction.org. Gail M. Williams, School of Public Health, University of Queensland, Brisbane, Australia, E-mail: gail.williams@sph.uq.edu.au. Ross M. Andrews, Menzies School of Health Research, Charles Darwin University, Darwin, Australia, E-mail: ross.andrews@menzies.edu.au. Archie C. A. Clements, Faculty of Health Sciences, Curtin University, Bentley, Australia, E-mail: archie.clements@curtin.edu.au.

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5.3 Paper 6

Nery SV*, **Clarke NE***, Richardson A, Traub RJ, McCarthy JS, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Campbell SJ, Clements ACA. Risk factors for infection with soil-transmitted helminths during an integrated community-level WASH and deworming intervention in Timor-Leste. *Int J Parasitol* 2019; 49(5): 389–396. https://doi.org/10.1016/j.ijpara.2018.12.006 (* co-first authors)

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Risk factors for infection with soil-transmitted helminths during an integrated community level water, sanitation, and hygiene and deworming intervention in Timor-Leste

Susana Vaz Nery [a,1,4,*], Naomi E. Clarke [a,1,4], Alice Richardson [a], Rebecca Traub [b], James S. McCarthy [c,d], Darren J. Gray [a], Andrew J. Vallely [e], Gail M. Williams [f], Ross M. Andrews [a,g], Suzy J. Campbell [a,2], Archie C.A. Clements [a,3]

[a] Research School of Population Health, Australian National University, Canberra ACT 0200, Australia
[b] Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, VIC 3052, Australia
[c] Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD 4006, Australia
[d] School of Medicine, University of Queensland, Brisbane, QLD 4006, Australia
[e] Kirby Institute, University of New South Wales, Sydney, NSW 2052, Australia
[f] School of Public Health, University of Queensland, Brisbane, QLD 4006, Australia
[g] Menzies School of Health Research, Charles Darwin University, Darwin, NT 0811, Australia

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ABSTRACT

Water, sanitation and hygiene interventions have been advocated as important complements to deworming programs to improve soil-transmitted helminth control. Evidence for the impact of water, sanitation and hygiene on soil-transmitted helminth infections is mixed, and based mainly on cross-sectional studies. In this study, we assessed associations between individual- and household-level water, sanitation and hygiene variables and soil-transmitted helminth infections, using data collected during the 2 year follow-up study period of the WASH for WORMS randomised controlled trial in Timor-Leste. Data were collected across four surveys, conducted at 6 monthly intervals in 23 communities. We analysed water, sanitation and hygiene and sociodemographic variables as risk factors for infection with *Necator americanus*, *Ascaris* spp., and undifferentiated soil-transmitted helminth infection, using generalised linear mixed models to account for clustering at community, household and participant levels. Water, sanitation and hygiene risk factors were examined both concurrently and with a 6 month lag period that coincided with the most recent deworming. The analysis included 2333 participants. Factors associated with *N. americanus* infection included age group, male sex (adjusted odds ratio (aOR) 3.1, 95% confidence interval (CI) 2.4–4.2), working as a farmer (aOR 1.7, 95% CI 1.2–2.4), and completing secondary school or higher (aOR 0.29, 95% CI 0.16–0.53). Risk factors for *Ascaris* spp. infection included age group, living in a dwelling with more than six people (aOR 1.6, 95% CI 1.1–2.3), having a tube well or borehole as the household water source (aOR 3.7, 95% CI 1.3–10.8), and using a latrine shared between households 6 months previously (aOR 2.3, 95% CI 1.2–4.3). Handwashing before eating was protective against infection with any soil-transmitted helminth (aOR 0.79, 95% CI 0.65–0.95). In the context of regular deworming, few water, sanitation and hygiene-related factors were associated with soil-transmitted helminth infections. Future research examining the role of water, sanitation and hygiene in soil-transmitted helminth transmission is required, particularly in low transmission settings after cessation of deworming. Identifying improved indicators for measuring water, sanitation and hygiene behaviours is also a key priority.

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* Corresponding author at: Kirby Institute, University of New South Wales, Sydney NSW 2052, Australia.
E-mail address: snery@kirby.unsw.edu.au (S. Vaz Nery).
[1] These authors contributed equally to this work.
[2] Present address: Evidence Action: Deworm the World Initiative, Washington, DC 20005, USA.
[3] Present address: Faculty of Health Sciences, Curtin University, Bentley, WA 6102, Australia.
[4] Present address: Kirby Institute, University of New South Wales, Sydney, NSW 2052, Australia.

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1. Introduction

Ascaris lumbricoides, hookworm (*Necator americanus, Ancylostoma duodenale* and *Anyclostoma ceylanicum*) and *Trichuris trichiura* are the most common soil transmitted helminths (STH), and together constitute the most prevalent neglected tropical disease (NTD) worldwide (Hotez et al., 2014). STH transmission occurs through the faecal-oral route after a period of development in soil, or through direct penetration of the skin by hookworm larvae (Bethony et al., 2006). STH are therefore common in poor communities that lack access to safe water, improved sanitation and appropriate hygiene behaviour (Brooker et al., 2006).

The mainstay of STH control is mass treatment with deworming drugs (World Health Organization, 2017); these kill adult worms but must be given on a regular basis due to reinfection after treatment (Jia et al., 2012). In order to interrupt STH transmission and achieve lasting control, interventions that successfully separate humans from excreta may be required, particularly in high transmission areas (Anderson et al., 2015; Coffeng et al., 2015). Interventions aimed at improving water, sanitation and hygiene (WASH) may therefore represent an important component of integrated control efforts against STH (Freeman et al., 2013b; Campbell et al., 2014).

Interventional studies examining the impact of WASH on STH epidemiology have revealed mixed results. School-based health education programs have been shown to reduce STH incidence and/or intensity in children (Bieri et al., 2013; Gyorkos et al., 2013), and a household level handwashing and nail clipping intervention decreased intestinal parasitic infections, including STH (Mahmud et al., 2015). On the other hand, several studies where community level sanitation interventions were implemented failed to demonstrate an impact on STH infections, likely due to poor intervention uptake (Clasen et al., 2014; Patil et al., 2014).

A small number of studies have examined multi-component WASH interventions. In a pilot trial, a combined sanitation, hygiene and deworming intervention had no impact on STH reinfection rates compared with deworming alone, but higher hookworm egg reduction rates were observed in intervention communities (Hürlimann et al., 2018). A school-based integrated WASH and deworming program reduced reinfection with *A. lumbricoides*, but not other STH, compared with deworming alone (Freeman et al., 2013a). The WASH Benefits study in Kenya found that a combined WASH intervention significantly reduced infection with *A. lumbricoides* (but not other STH) compared with the control group (Pickering et al., Integrating water, sanitation, handwashing, and nutrition interventions to reduce child soil-transmitted helminth and *Giardia* infections: a cluster-randomized controlled trial in rural Kenya. BioRxiv 464917 preprint, https://doi.org/10.1101/464917). In that study, a water only intervention achieved similar reductions in *A. lumbricoides* prevalence, while no impact on prevalence was observed following sanitation only or hygiene only interventions.

In our WASH for WORMS randomised controlled trial (RCT), no additional impact on STH infections was identified as a result of an integrated community level deworming and WASH intervention, compared with the significant reductions achieved by regular deworming of the entire community (Nery et al., 2019). Failure to sustain the sanitation intervention may have been a factor, with 40% of participants in the intervention arm practising open defecation at the end of the trial. Additionally, gradual improvements in WASH conditions were observed in the control arm of the study (Nery et al., 2019).

RCTs provide essential high quality evidence regarding the impact of interventions; however, in practice, implementing WASH improvements is challenging, particularly within a trial context (Campbell et al., 2018). Examining the overall impact of an integrated WASH intervention on STH infections is important, but offers limited insights into the relative contributions of discrete WASH components and practices, which may vary considerably among study participants. Investigating relationships between STH infections and specific WASH-related variables may generate important findings for policymakers seeking to understand what types of tailored interventions might have an impact on STH, and may similarly inform the development of future intervention trials.

Meta-analyses of observational studies have shown reduced odds of STH infection associated with a number of WASH-related variables, including access to and use of sanitation facilities (Ziegelbauer et al., 2012; Strunz et al., 2014), access to improved water (Esrey et al., 1991; Strunz et al., 2014), and handwashing behaviours (Strunz et al., 2014), although these results are not consistent across all studies. A cross-sectional analysis of WASH-related risk factors for STH infection conducted at the WASH for WORMS study baseline detected few WASH variables associated with STH infection (Campbell et al., 2016). It was argued that universally poor WASH conditions may have made it difficult to detect any associations (Campbell et al., 2016).

Although most existing analyses of WASH risk factors for STH infections are cross-sectional in nature, WASH conditions at the time of most recent deworming are likely to be important predictors of infection, in addition to concurrent WASH conditions. This is because reinfection can occur soon after deworming (Jia et al., 2012), and it is known that infective stages of hookworm and *T. trichiura* can persist in soil for up to several months (Udonsi and Atata, 1987; Brooker et al., 2006) and *A. lumbricoides* for several years (Muller, 2002). Furthermore, the prepatent interval (time between initial infection occurring and development of egg-laying adult worms) is up to 12 weeks for *A. lumbricoides* and *T. trichiura* and 8 weeks for hookworm (Bethony et al., 2006).

In the present study, we analyse data from the WASH for WORMS study, using an observational design, to investigate associations between specific WASH-related factors and STH infections. The specific objectives are to investigate the associations between STH infections and: (a) concurrent WASH variables (cross-sectional analysis), and (b) WASH variables 6 months previously, at the time of most recent deworming (longitudinal analysis).

2. Materials and methods

2.1. Study setting and design

This study took place in remote communities in Manufahi municipality, Timor-Leste. Data were collected from residents of the 23 communities initially enrolled in the WASH for WORMS RCT (of which five were sequentially, rather than randomly, allocated to a study arm and were therefore not analysed in the main trial) (Nery et al., 2015).

The WASH for WORMS study methods are described in the published protocol (Nery et al., 2015). Briefly, this was a two-armed cluster RCT in which all communities received deworming treatment with albendazole (administered to all residents over 1 year of age, excluding pregnant women in the first trimester), every 6 months for 2 years. Additionally, in the intervention clusters, WaterAid Australia and their local partners implemented a WASH program, which consisted of: providing access to an improved water source; using a Community Led Total Sanitation-based approach to improve household sanitation; and promoting hand-washing with soap at critical times. Characteristics of the

participating communities at the beginning of the study have been published elsewhere (Nery et al., 2015; Campbell et al., 2016).

2.2. Data collection

All community residents aged over 1 year were invited to participate in data collection surveys. These surveys were conducted at baseline and then at four follow-up data collection rounds that occurred at 6 monthly intervals for two years, immediately prior to each deworming round.

Faecal samples were provided by study participants and preserved in 5% (w/v) potassium dichromate. Samples were stored at room temperature and transported for further processing and molecular diagnosis at QIMR Berghofer Medical Research Institute (Brisbane, Australia). We used a multiplex real-time quantitative PCR (qPCR) technique to identify infections with *Ascaris* spp., *N. americanus, Ancylostoma* spp., and *Trichuris* spp. (Llewellyn et al., 2016).

Trained local fieldworkers interviewed participants and heads of households using a structured questionnaire. Information collected included demographic and socio-economic characteristics (age, sex, education, employment, income, and assets), clinical features (history of diarrhoea and deworming), and information related to WASH ownership and behaviour (presence and use of household latrines, defecation practices, availability of water, and hand-washing behaviours). As part of a thorough community census, age and sex were documented for all community members, including those who did not participate in the study. Most information about WASH access and behaviours was collected via self-report, or parental report for young children. Household water sources and the presence, features, and cleanliness of household latrines were directly observed by fieldworkers.

2.3. Statistical analysis

Participants for whom both parasitological and questionnaire data were available at one or more follow-up time points were included in the analysis. A household level wealth index was constructed using principal component analysis as described previously (Campbell et al., 2016), with minor modifications. Scores obtained on the wealth index were divided into quintiles to classify households according to relative poverty.

Due to low prevalence of *Ancylostoma* spp. and *Trichuris* spp., analyses were only performed for *Ascaris* spp. and *N. americanus*, as well as undifferentiated STH infection. We constructed generalised linear mixed models to examine associations between risk factors and infection. Models included random effects for community, household, and participant, to account for clustering at community and household levels, and measurements on the same individuals over time.

Data from all four follow-up time points were included in this analysis. We examined a wide range of WASH and socioeconomic variables as potential predictors; a full list is provided in the Supplementary Data S1. All WASH variables were examined both concurrently (i.e., at the same time point as STH diagnosis) and 6 months previously (i.e., at the previous study time point, when participants were most recently dewormed). Demographic and socioeconomic variables displayed no to minimal variability over the study period, and were examined concurrently only. The study time point was included as a covariate in all analyses to account for the number of deworming rounds that had been delivered. The study intervention was not included as a predictor, because this analysis focused on individual WASH access and behaviours, regardless of study assignment. The experimental findings of the WASH for WORMS RCT have been presented elsewhere (Nery et al., 2019).

To construct the generalised linear mixed models, we investigated multicollinearity between WASH variables using the "collin" command in Stata. Univariable regression models were run for each individual risk factor; these were run separately for concurrent and previous time point predictors. Variables were retained for further analysis if $P < 0.2$. Generalised linear mixed models were then constructed in a two step approach. Firstly, "within domain" multivariable models were constructed for each of 8 risk factor domains: demographic, individual hygiene, individual sanitation, school sanitation, individual socioeconomic, household sanitation, household water, and household socioeconomic variables. These "within domain" models included age, sex, study time point, and all variables retained from univariable analysis for that domain, including both concurrent and previous time point variables. Secondly, variables with $P < 0.1$ from the "within domain" models were retained in a full model and then removed iteratively until only age, sex, study time point, and variables significant at $P < 0.05$ remained in the final model. Analyses were conducted using Stata version 14.1 (College Station, TX, USA).

2.4. Ethics statement

Ethical approval was given by the Human Research Ethics Committees at The University of Queensland (2011000734), The Australian National University (2014/311), and the Timor-Leste Ministry of Health (2011/51). Written consent was provided by participants aged 18 years and older, and parents/guardians of those under 18 years of age. Written assent was provided by participants aged 12–17 years.

3. Results

3.1. Study participants

In total, 2333 individuals were included in this risk factor analysis. A summary of participation over time is shown in Supplementary Table S1. There was a higher proportion of females among participants (51.4%) compared with non-participants (42.6%, $P < 0.001$), mainly due to the fact that men were less likely to provide stool samples than women ($P < 0.001$). Participants ranged in age from 1 year to 94 years, and were slightly younger than non-participants (mean age 26.3 years versus 27.9 years; $P < 0.001$); see Table 1.

WASH characteristics in the study population over time are shown in Table 2. These data were collected following the implementation of the study WASH intervention in 12 of the 23 communities; as a result, overall WASH coverage among the study population was higher than that reported at baseline (Campbell et al., 2016).

At the first study follow-up, the prevalence of *N. americanus* was 33.6% (95% confidence interval (CI) 31.3–36.0), while *Ascaris* spp. prevalence was 17.9% (95% CI 16.1–19.9). As expected given regular deworming in both study arms, prevalence decreased by the end of the study, with final *N. americanus* prevalence of 14.6% (95% CI 12.8–16.5) and *Ascaris* spp. of 10.5% (95% CI 9.0–12.2); see Fig. 1 and Supplementary Table S2.

3.2. Factors associated with STH infections

Univariable analyses revealed a range of WASH, demographic and socioeconomic variables with $P < 0.2$ that were retained for initial (within domain) multivariable models. Supplementary Tables S3–S5 depict the results of univariable analyses of all risk factors examined.

Table 1
Characteristics of study participants.

Characteristic	*n* (%)
Individual characteristics (*n* = 2333)	
Age group	
1–5 years	459 (19.7)
6–11 years	488 (20.9)
12–17 years	238 (10.2)
18–64 years	954 (40.9)
65 + years	194 (8.3)
Male sex	1135 (48.7)
For children aged 6–17 (*n* = 726)	
Attends school	619 (85.3)
For adults aged 18+ (*n* = 1148)	
Education level	
Never went to school	535 (46.6)
Not finished primary school	197 (17.2)
Finished primary but not secondary	283 (24.7)
Finished secondary or higher	126 (11.0)
Employment	
Not employed outside the home	428 (37.3)
Employed as farmer	629 (55.8)
Employed, other occupation	84 (7.3)
Household characteristics (*n* = 565)	
At least one child under 5 years of age	257 (45.5)
More than 6 people sharing a dwelling	222 (39.3)

Participation was defined as providing both a questionnaire and stool sample for at least one follow-up time point. Data in this table are from the first time point at which participation was recorded.

3.2.1. Factors associated with N. americanus infection

Results of the final adjusted multivariable model for *N. americanus* are shown in Table 3. The odds of infection decreased with each follow-up, with lowest odds at the final follow-up (adjusted odds ratio (aOR) 0.15, 95% CI 0.11–0.20). All age groups were found to have significantly higher odds of infection compared with children aged 1–5 years, with highest odds among those aged 18–64 years (aOR 7.8, 95% CI 4.7–12.8) and 12–17 years (aOR 6.0, 95% CI 3.7–9.8). Males had significantly higher odds of infection than females (aOR 3.1, 95% CI 2.4–4.2). Adults who obtained at least primary school education had lower odds of infection compared with those who never went to school, with lowest odds among those who completed secondary school or higher (aOR 0.29, 95% CI 0.16–0.53). Farmers had higher odds of infection compared with those who did not work outside the home (aOR 1.7, 95% CI 1.2–2.4). The only WASH variable that was associated with *N. americanus* was having water available for personal cleaning after defecation, which was associated with marginally increased odds of infection (aOR 1.4, 95% CI 1.0–2.0).

3.2.2. Factors associated with Ascaris spp. infection

Results of the final adjusted multivariable model for *Ascaris* spp. are shown in Table 4. Similar to results for *N. americanus*, odds of infection decreased with each follow-up, with odds at the final follow-up nearly two-thirds lower than the first follow-up (aOR 0.37, 95% CI 0.24–0.55). Adults aged 18–64 (aOR 0.37, 95% CI 0.23–0.61) and 65+ years (aOR 0.37, 95% CI 0.19–0.71) had significantly lower odds of infection compared with children aged 1–5 years. Those living in a household with more than six people had higher odds of infection (aOR 1.7, 95% CI 1.2–2.4). Those who finished primary school but not secondary school were found to have higher odds of infection compared with those who never went to school (aOR 2.1, 95% CI 1.2–3.6). In terms of concurrent

Table 2
Water, sanitation and hygiene characteristics over time.

	Follow-up 1, *n* (%)	Follow-up 2, *n* (%)	Follow-up 3, *n* (%)	Follow-up 4, *n* (%)
Individual sanitation variables	*n = 1598*	*n = 1459*	*n = 1465*	*n = 1412*
Main place of defecation is toilet	768 (48.1)	723 (50.5)	724 (49.4)	813 (57.6)
Practises open defecation	852 (53.4)	802 (55.0)	806 (55.0)	675 (47.8)
Uses water to clean self after defecation	659 (41.2)	635 (43.5)	649 (44.3)	815 (57.7)
Household sanitation variables	*n = 482*	*n = 467*	*n = 442*	*n = 428*
Household has toilet	226 (46.9)	232 (49.7)	224 (50.7)	244 (57.0)
If yes, toilet has slab	102 (45.1)	141 (60.8)	119 (53.1)	125 (51.2)
If yes, toilet is pour-flush latrine	137 (60.6)	171 (73.7)	157 (70.1)	181 (74.2)
If yes, toilet is clean	67 (29.7)	66 (28.5)	42 (18.8)	66 (27.1)
Toilet shared with another dwelling	26 (5.4)	27 (5.8)	28 (6.3)	18 (4.2)
Household garbage disposed of in bush	295 (61.2)	292 (62.5)	296 (67.0)	283 (66.1)
Household garbage buried	54 (11.2)	47 (10.1)	19 (4.3)	21 (4.9)
Household garbage burnt	273 (56.6)	196 (42.0)	174 (39.4)	128 (29.9)
For households with children < 5 years old	*n = 175*	*n = 157*	*n = 161*	*n = 127*
Child waste disposed of hygienically[a]	38 (21.7)	14 (8.9)	24 (14.9)	7 (5.1)
Household water variables	*n = 482*	*n = 467*	*n = 442*	*n = 428*
Main water source				
Piped water	167 (34.7)	240 (51.4)	249 (56.3)	293 (68.5)
Protected spring	13 (2.7)	20 (4.3)	9 (2.0)	8 (1.9)
Tube well/borehole	27 (5.6)	61 (13.1)	30 (6.8)	41 (9.6)
Unprotected spring/dug well	208 (43.2)	59 (12.6)	80 (18.1)	50 (11.7)
Surface water	62 (12.9)	79 (16.9)	70 (15.8)	32 (7.5)
Distance to household water source more than 15 min	124 (25.7)	94 (20.1)	114 (25.8)	95 (22.2)
Water always available from main water source	373 (77.4)	405 (86.7)	270 (83.7)	368 (86.0)
Water stored in covered containers only	431 (89.4)	434 (92.9)	419 (94.8)	393 (91.8)
Household water treated	288 (59.8)	305 (65.3)	259 (58.6)	246 (57.5)
Individual hygiene variables	*n = 1598*	*n = 1459*	*n = 1465*	*n = 1412*
Washes hands using soap or ash	1060 (66.3)	1233 (84.5)	1306 (89.2)	1379 (97.7)
Washes hands before contact with food	745 (46.7)	580 (39.8)	612 (41.8)	709 (50.2)
Washes hands after contact with faeces	956 (59.9)	1127 (77.2)	1192 (81.4)	1243 (88.0)
Washes hands after contact with dirt	939 (58.8)	934 (64.0)	1093 (74.6)	1124 (79.6)
Always wears shoes indoors	493 (30.9)	516 (35.4)	590 (40.3)	694 (49.2)
Always wears shoes outdoors and while toileting	891 (55.8)	802 (55.0)	869 (59.3)	1001 (70.9)

[a] Hygienic disposal defined as disposing of a child's faeces in the household toilet or with household waste.

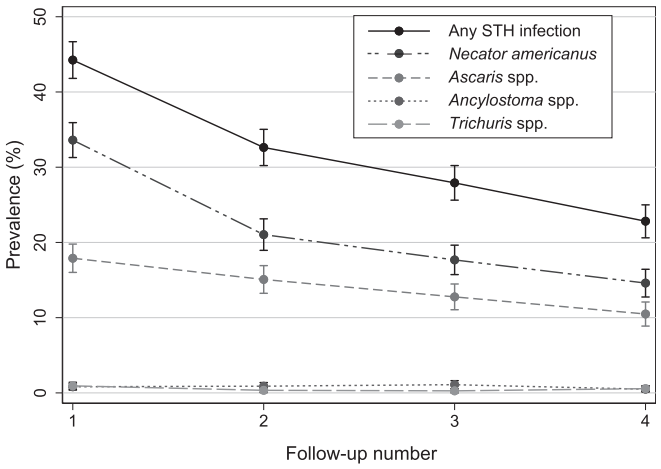

Fig. 1. Prevalence of soil-transmitted helminth infections in the study population over time.

Table 3
Results of final multivariable mixed effects logistic regression examining risk factors for *Necator americanus* infection (n = 2333).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.34**	**0.27–0.44**	**<0.001**
Follow-up 3	**0.24**	**0.18–0.31**	**<0.001**
Follow-up 4	**0.15**	**0.11–0.20**	**<0.001**
Age group[a]			
6–11 years	**3.87**	**2.59–5.76**	**<0.001**
12–17 years	**6.03**	**3.71–9.78**	**<0.001**
18–64 years	**7.76**	**4.69–12.82**	**<0.001**
65 + years	**4.05**	**2.21–7.42**	**<0.001**
Male sex	**3.14**	**2.37–4.16**	**<0.001**
Individual socioeconomic variables			
Education level (adults age 18 + years only)[b]			
Didn't finish primary school	0.79	0.53–1.19	0.259
Finished primary but not secondary school	0.76	0.51–1.12	0.170
Finished secondary school or higher	**0.29**	**0.16–0.53**	**<0.001**
Employment (adults age 18 + years only)[c]			
Employed – farmer	**1.66**	**1.16–2.38**	**0.005**
Employed – other job	1.58	0.85–2.95	0.152
Household sanitation variables			
Water available to clean self after defecating	**1.43**	**1.00–2.03**	**0.050**
Random effects variance (95% CI)			
Community	1.01 (0.51–2.02)		
Household	1.65 (1.20–2.26)		
Participant	2.22 (1.66–2.97)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]aged 1–5 years; [c]never went to school; [d]not employed outside the home.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

Table 4
Results of final multivariable mixed effects logistic regression examining risk factors for *Ascaris* spp. infection (n = 2333).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.61**	**0.44–0.87**	**0.001**
Follow-up 3	**0.35**	**0.24–0.51**	**<0.001**
Follow-up 4	**0.37**	**0.24–0.55**	**<0.001**
Age group[b]			
6–11 years	0.92	0.63–1.36	0.681
12–17 years	0.70	0.42–1.18	0.184
18–64 years	**0.37**	**0.23–0.61**	**<0.001**
65+ years	**0.37**	**0.19–0.71**	**0.003**
Male sex	1.07	0.82–1.41	0.608
Individual socioeconomic variables			
Education level (adults age 18 + only)[c]			
Didn't finish primary school	1.39	0.77–2.53	0.329
Finished primary but not secondary school	**2.07**	**1.18–3.63**	**0.011**
Finished secondary school or higher	1.81	0.81–4.03	0.197
Household socioeconomic variables			
More than 6 people living in dwelling	**1.70**	**1.18–2.43**	**0.004**
Household sanitation variables			
Household has shared toilet – six months previously	**2.29**	**1.22–4.32**	**0.010**
Household water variables			
Household main water source[d]			
Tube well/borehole	**3.69**	**1.26–10.78**	**0.017**
Unprotected spring/dug well	1.07	0.62–1.88	0.795
Protected spring	1.38	0.55–3.50	0.492
Surface water	1.46	0.61–3.48	0.396
Random effects variance (95% CI)			
Community	5.09 (2.64–9.84)		
Household	0.85 (0.51–1.42)		
Participant	0.58 (0.25–1.37)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]aged 1–5 years; [c]never went to school; [d]household main water source is piped water.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

WASH variables, using household water from tube wells or boreholes was associated with significantly higher odds of infection (aOR 3.7, 95% CI 1.3–10.8). In terms of WASH variables identified 6 months previously, higher odds of infection were observed among those who used a toilet shared between two or more households (aOR 2.3, 95% CI 1.2–4.3). No other WASH variables were significantly associated with *Ascaris* spp. infection.

3.2.3. Factors associated with undifferentiated STH infection

Results of the final adjusted multivariable model for undifferentiated STH infection are shown in Table 5. Mirroring the results for individual STH species, increasing follow-up time was associated with lower odds of infection. All age groups had higher odds of infection compared with children aged 1–5 years, while adults who finished secondary school or higher had lower odds of infection compared with those who never went to school (aOR 0.40, 95% CI 0.24–0.65). In terms of concurrent WASH variables, those using household water from tube wells or boreholes were again found to have higher odds of infection (aOR 2.5, 95% CI 1.3–4.8). Variables that were associated with lower odds of infection were having a water source more than 15 min walk from the household (aOR 0.74, 95% CI 0.59–0.94) and handwashing before eating (aOR 0.80, 95% CI 0.66–0.96). No other WASH variables were significantly associated with undifferentiated STH infection.

A summary of all significant predictors of infection with *N. americanus*, *Ascaris* spp., and undifferentiated STH infection is provided in Table 6.

Table 5
Results of final multivariable mixed effects logistic regression examining risk factors for undifferentiated soil-transmitted helminth infection ($n = 2333$).

Covariate	aOR	95% CI	*P* value
General variables			
Follow-up number[a]			
Follow-up 2	**0.44**	**0.35–0.55**	**<0.001**
Follow-up 3	**0.31**	**0.25–0.39**	**<0.001**
Follow-up 4	**0.21**	**0.76–0.28**	**<0.001**
Age group[b]			
6–11 years	**2.08**	**1.54–2.80**	**<0.001**
12–17 years	**2.72**	**1.86–3.98**	**<0.001**
18–64 years	**3.62**	**2.57–5.09**	**<0.001**
65+ years	**1.94**	**1.26–2.97**	**0.002**
Male sex	**2.34**	**1.90–2.87**	**<0.001**
Individual socioeconomic variables			
Education level (adults age 18 + only)[c]			
Didn't finish primary school	0.87	0.61–1.25	0.454
Finished primary but not secondary school	0.89	0.64–1.25	0.512
Finished secondary school or higher	**0.40**	**0.24–0.65**	**<0.001**
Individual hygiene variables			
Washes hands before eating	**0.80**	**0.66–0.96**	**0.020**
Household water variables			
Household main water source[d]			
Tube well/borehole	**2.54**	**1.34–4.84**	**0.004**
Unprotected spring/dug well	1.09	0.79–1.52	0.594
Protected spring	1.72	0.88–3.37	0.112
Surface water	1.20	0.75–1.93	0.447
Water source ≥ 15 min walk from household	**0.74**	**0.59–0.94**	**0.014**
Random effects variance (95% CI)			
Community	1.83 (0.97–3.49)		
Household	1.06 (0.78–1.44)		
Participant	1.37 (1.01–1.86)		

aOR, adjusted odds ratio; CI, confidence interval.
Reference categories are as follows: [a]follow-up 1; [b]age 1–5 years; [c]never went to school; [d]household main water source is piped water.
Bold values indicate those reaching statistical significance, defined as $P < 0.05$.

4. Discussion

This risk factor analysis was conducted to further investigate the role of WASH on STH infections in the context of the WASH for WORMS intervention trial. A novel component of our analysis was that we examined WASH-related risk factors both concurrently and 6 months prior, at the time of the previous deworming round. In a previous cross-sectional analysis conducted at the study baseline, few WASH variables were associated with STH infections (Campbell et al., 2016). The analysis presented here included data from four study follow-up time points over a 2 year period, with more diverse WASH conditions compared with the study baseline. Nonetheless, only a small number of WASH-related variables were found to be associated with STH infections in this analysis. The odds of infection with both *N. americanus* and *Ascaris* spp. decreased significantly with increasing follow-up time, reflecting the impact of regular, community-wide deworming.

Although water contamination is not thought to play a crucial role in the STH transmission cycle, meta-analysis has demonstrated that piped water is protective against multiple STH species (Strunz et al., 2014), and the recent WASH Benefits study in Kenya found that an intervention aimed at improving water quality was protective against *Ascaris* spp. (Pickering et al., BioRxiv 464917 preprint, cited earlier). Our results also provide some evidence that water source affects the risk of STH infection, lending support to suggestions that contaminated water may play a more important role in transmission than previously recognised (Pickering et al., BioRxiv 464,917 preprint, cited earlier). Using water from a tube well or borehole was associated with significantly higher odds of *Ascaris* spp. and undifferentiated STH infection, while those using a water source more than 15 min walk from the household had lower odds of undifferentiated STH infection. Tube wells and boreholes are considered protected water sources; however, in study communities, these were predominantly used when a community's usual water source was unavailable (i.e., in the dry season). Our results suggest that water contamination may have occurred at the source or during collection. Similarly, the protective effect of water sources located further from the household likely represents decreased faecal contamination.

In terms of sanitation, previous meta-analyses have found that sanitation access and use are protective against both *A. lumbricoides* (Ziegelbauer et al., 2012; Strunz et al., 2014) and hookworm (Ziegelbauer et al., 2012), although results are not consistent across all studies, and these findings have not been mirrored in community level sanitation intervention studies (Clasen et al., 2014; Patil et al., 2014). In our analysis, using a shared latrine (shared between two or more households) 6 months previously was associated with higher odds of *Ascaris* spp. infection, possibly due to increased faecal contamination. Surprisingly, having water available for personal cleaning after defecation was marginally associated with higher odds of *N. americanus* infection. This may

Table 6
Summary of factors associated with *Necator americanus*, *Ascaris* spp. and undifferentiated soil-transmitted helminth infection in final adjusted multivariable models.

Variable domain	*N. americanus*	*Ascaris* spp.	Undifferentiated STH infection
General variables	*Study follow-up number* Age groups 6–11, 12–17, 18–64 and 65 + years[a] Male sex	*Study follow-up number* *Age groups 18–64 and 65 + years*[a]	*Study follow-up number* Age groups 6–11, 12–17, 18–64 and 65 + years[a] Male sex
Individual socioeconomic variables	*Did not finish primary school*[b] Employed as farmer[c]	Finished primary but not secondary school[b]	*Did not finish primary school*[b]
Household socioeconomic variables	–	More than 6 people living in household	
Household sanitation variables	Water available to clean self after defecating	Household has shared toilet	
Household water variables	–	Tube well/borehole as main water source[d]	Tube well/borehole as main water source[d] *Water source ≥ 15 minutes' walk from household*
Individual hygiene variables	–	–	*Washes hands before contact with food*

Italics indicates that variable is associated with decreased odds of infection.
Reference categories are as follows: [a]aged 1–5 years; [b]never went to school; [c]not employed outside the home; [d]household main water source piped water.

represent a chance finding, or may relate to skin contact with water containing infective filariform hookworm larvae (that can penetrate the skin) while washing after defecation. No other sanitation-related variables emerged as significant predictors in our analysis. The lack of association between most sanitation variables and STH infections is likely due to persistent open defecation by a proportion of individuals throughout the study period. Ongoing open defecation within the community allows environmental contamination and transmission to be maintained, such that even those who regularly use a latrine can still be exposed to contaminated soil and become reinfected with STH.

A protective effect of handwashing behaviour on STH has been previously identified in both observational (Strunz et al., 2014) and intervention studies (Mahmud et al., 2015). Our analysis identified lower odds of any STH infection in individuals who reported handwashing before eating, but this protective effect was not seen for individual STH species, and other handwashing variables were not significant. Given our reliance on self-reported hygiene behaviours, this may reflect a degree of measurement error. Previous work has shown that participants may exaggerate responses that reflect more desirable WASH practices (Manun'Ebo et al., 1997), and indeed, hygiene behaviours such as handwashing with soap and handwashing after defecation were reported by more than 75% of participants in our study at the baseline (Nery et al., 2019). A more reliable method of measuring handwashing behaviours may detect a more consistent protective effect against STH. However, structured observations can also lack validity (Ram et al., 2010). Identifying accurate, unbiased methods for measuring WASH behaviour remains a significant challenge.

Demographic and socioeconomic factors were strongly associated with STH infections. Consistent with known age- and sex-related patterns of STH infection (Brooker et al., 2004; Bethony et al., 2006), *N. americanus* infections were highest among males and adults, while *Ascaris* spp. infections were higher among children. Aligned with findings at the study baseline (Campbell et al., 2016), adults with higher levels of education had lower odds of *N. americanus* infection, reflecting that STH disproportionately affect the most disadvantaged people (Holland et al., 1988). Individuals living in a dwelling with more than six people had increased odds of *Ascaris* spp. infection, implicating a role of overcrowding in environmental contamination and transmission. Similar findings have been described previously (Holland et al., 1988; Scolari et al., 2000). Importantly, those working as farmers had increased odds of *N. americanus* infection. This finding can be explained by occupational exposure to contaminated soil (Brooker et al., 2004), and suggests that an educational component tailored specifically to adults working in high risk occupations could be of benefit in community level WASH interventions.

A limitation of this observational analysis is the likelihood of residual confounding. Although we included a wide range of WASH and sociodemographic variables in our analysis, it is likely that other, unmeasured factors contributed to the risk of STH infections. A further limitation is that most sanitation and hygiene variables were self-reported by participants, and in the case of young children, by their parents. As discussed above, self-reported data are prone to bias. This potential source of measurement error decreases the reliability of detected associations (or the lack thereof) between WASH and STH. Finally, it is possible that the highly sensitive diagnostic technique used to detect STH infections may have led to weakening of associations between WASH and STH, due to the detection of very light-intensity infections that would be missed by more conventional microscopy-based approaches (Campbell et al., 2016).

In summary, this risk factor analysis found that only a small number of WASH variables were associated with STH infections, with regular deworming, socioeconomic variables, and demographic factors representing the major predictors of infection. Compared with a cross-sectional analysis conducted at the study baseline (Campbell et al., 2016), the current analysis identified more WASH associations in the context of more diverse WASH conditions. However, many WASH variables were not associated with STH infection, and identified WASH-related risk and protective factors were not consistent across STH species. These findings align with the overall study results of the WASH for WORMS trial, in which no additional impact of the community-based WASH intervention on STH infections was seen, compared with deworming alone (Nery et al., 2019).

STH life cycles rely on individuals being exposed to a faecally-contaminated environment; thus, a link between WASH and STH transmission is undisputable. In practice, however, generating evidence for the impact of WASH interventions, access and practices on STH infections remains challenging. Contributing factors include the complexity of implementing WASH interventions in low income settings, the challenge of achieving sustained behavioural change, and the difficulties in accurately measuring WASH behaviours (Campbell et al., 2018).

Despite these challenges, further research should be undertaken to elucidate the impact of WASH on STH. The focus should be on generating evidence to inform policymakers and program implementers in the WASH and NTD sectors regarding what kinds of interventions, in what settings, and over what period of time, could be expected to have an impact on STH infections. Particularly important research priorities include investigating the role of WASH in settings with low STH transmission, once regular deworming has ceased (Coffeng et al., 2018), and research into simple, reproducible strategies to measure WASH behaviours, including strategies that involve evaluating environmental contamination with STH infective stages (Gyawali et al, 2016; Steinbaum et al, 2017). Beyond research, fostering collaboration between the WASH and NTD sectors remains crucial as global efforts towards sustainable control continue.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpara.2018.12.006.

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Chapter 6
qPCR for STH diagnosis

6.1 Chapter context

This chapter addresses the final thesis research objective, namely to further investigate the role of qPCR in monitoring STH control program impact, by comparing qPCR with sodium nitrate flotation and investigating variability in DNA detection. This chapter is presented as a peer-reviewed journal article, published in the *American Journal of Tropical Medicine & Hygiene* (Paper 7).

Previous chapters have focused on evidence gaps relating to implementation of STH control measures. However, monitoring and evaluation also represents a crucial component of STH control programs, and this depends upon appropriate diagnostic techniques to detect and quantify STH infections. As control programs continue to be scaled up and transmission interruption becomes a possibility in some settings, the ability to detect light-intensity infections is crucial to inform decisions regarding cessation, continuation or re-commencement of preventive chemotherapy programs. Quantitative polymerase chain reaction (qPCR) is a molecular technique that has demonstrated great potential as a highly sensitive diagnostic test for STH.

Currently, the use of qPCR for STH diagnosis is predominantly limited to research, with several important evidence gaps precluding its recommendation for use in monitoring STH control programs. These evidence gaps relate mainly to determining infection intensity. Intensity of STH infection is strongly linked with morbidity, and is a key determinant of transmission levels; therefore, it is vital to measure during monitoring and evaluation of STH control programs. qPCR provides a cycle threshold (Ct) value, representing the number of DNA amplification cycles before a signal exceeding background

level was detected; this can be converted to a measure of DNA intensity that reflects underlying infection intensity. However, the applicability of these values to STH control remains to be elucidated.

In the work presented in this chapter, firstly, STH prevalence and intensity obtained using qPCR and sodium nitrate flotation were compared, using samples collected from children in the (S)WASH-D for Worms pilot study. Secondly, repeated qPCR assays were conducted on a single *N. americanus*-positive stool sample over a six-month period, to examine the stability of Ct values over time. This is important because stool samples collected in the field must be preserved and transported to a central laboratory, leading to delays in processing and examination. The findings of these analyses contribute towards ongoing efforts to determine the capacity of qPCR to provide an accurate, quantitative measure of STH infection intensity, in the context of a pressing need for sensitive diagnostic techniques.

6.2 Paper 7

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Quantitative Polymerase Chain Reaction for Diagnosis of Soil-Transmitted Helminth Infections: A Comparison with a Flotation-Based Technique and an Investigation of Variability in DNA Detection

Naomi E. Clarke,[1]* Stacey Llewellyn,[2] Rebecca J. Traub,[3] James McCarthy,[2,4] Alice Richardson,[1] and Susana V. Nery[1,5]

[1]*Research School of Population Health, Australian National University, Canberra, Australia;* [2]*Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia;* [3]*Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Australia;* [4]*School of Medicine, University of Queensland, Brisbane, Australia;* [5]*Kirby Institute, University of New South Wales, Sydney, Australia*

Abstract. Appropriate diagnostic techniques are crucial to global soil-transmitted helminth (STH) control efforts. The recommended Kato–Katz method has low sensitivity in low-transmission settings. Quantitative polymerase chain reaction (qPCR) is a highly sensitive alternative diagnostic option. However, little is known about the variability in qPCR results, and there are few published comparisons between qPCR and other microscopy-based techniques such as sodium nitrate flotation (SNF). Using 865 stool samples collected from 571 individuals, we compared SNF and qPCR in terms of diagnostic sensitivity and infection intensity measurements. In addition, we conducted repeated examinations on a single *Necator americanus*–positive stool sample over a 6-month period. Results showed good diagnostic agreement between SNF and qPCR for *Ascaris* spp. ($\kappa = 0.69$, $P < 0.001$), and moderate agreement for hookworm ($\kappa = 0.55$, $P < 0.001$) and *Trichuris* spp. ($\kappa = 0.50$, $P < 0.001$). Quantitative polymerase chain reaction demonstrated higher sensitivity than SNF for *Ascaris* spp. (94.1% versus 68.1%) and hookworm (75.7% versus 66.9%) but not for *Trichuris* spp. (53.1% versus 81.3%), which had very low prevalence. Sodium nitrate flotation and qPCR infection intensity measurements were strongly correlated for *Ascaris* spp. ($\rho = 0.82$, $P < 0.001$) and moderately correlated for hookworm ($\rho = 0.58$, $P < 0.001$). Repeated examinations using qPCR showed that *N. americanus* cycle threshold values decreased significantly at 1 month and remained stable thereafter. Results confirm the high diagnostic sensitivity of qPCR for *Ascaris* spp. and hookworm, particularly for light-intensity infections, which is ideal for settings approaching transmission elimination. Results support the potential for qPCR to be used as a quantitative assay for STH. Further research is needed in settings where *Trichuris trichiura* is endemic.

INTRODUCTION

Soil-transmitted helminth (STH) infections—encompassing roundworms (*Ascaris lumbricoides*), hookworms (*Necator americanus*, *Ancylostoma duodenale*, and *Ancylostoma ceylanicum*), and whipworms (*Trichuris trichiura*)—are the most prevalent of the neglected tropical diseases, estimated to infect more than a billion people worldwide[1] and causing a global disease burden of approximately 3.5 million disability-adjusted life years.[2] In recent years, a global focus on the control and elimination of neglected tropical diseases has led to significant scaling up of STH control programs.[3] These programs focus on delivering regular deworming treatments to high-risk population groups, mostly children, and aim to reduce morbidity by reducing the proportion of high-intensity infections.[4,5] Recently, there has been increasing interest in interrupting STH transmission, through expanding drug administration programs community-wide, such that regular drug treatment is no longer required.[6,7]

Mapping and monitoring the prevalence and intensity of STH infections in endemic areas is critical for planning mass drug administration programs, assessing program impact, and observing for emerging benzimidazole resistance in humans.[8] Diagnostic tests that can accurately and sensitively diagnose infections and classify their intensity are, therefore, essential to the success of mass deworming programs, and represent a crucial component in achieving STH control.

The Kato–Katz technique is most commonly used for STH diagnosis, as per World Health Organization (WHO) guidelines.[9] This microscopy-based technique provides a measure of infection intensity in eggs per gram (EPG) of feces, and benefits from being an affordable, relatively simple test that can be carried out in the field.[10] A limitation of the Kato–Katz technique is that multiple samples per individual should be examined, given the low sensitivity of a single examination.[11] Even when multiple samples are examined, the Kato–Katz technique has poor diagnostic sensitivity in areas of low STH transmission.[12] A further limitation is the rapid degradation of hookworm eggs after slide preparation, meaning that examination must occur within 30 minutes of preparation.[13]

Polymerase chain reaction (PCR)–based techniques are increasingly used to diagnose STH infections. This sensitive molecular approach detects very small quantities of DNA, with previous work demonstrating higher sensitivity compared with the Kato–Katz technique.[14–19] Real-time quantitative PCR (qPCR) techniques can also be used to provide quantitative measures of infection intensity.[15,19,20] Polymerase chain reaction–determined infection intensity results have demonstrated good correlation with EPG counts obtained using the Kato–Katz technique for both *Ascaris* spp. and hookworm.[15,18,19,21]

Despite the increasing use of molecular techniques for STH diagnostics, there are limited published data relating to variability in DNA detection using qPCR. In practice, stool samples are generally preserved for transportation from field sites to a laboratory equipped to perform qPCR, where they are stored before DNA extraction. Therefore, understanding the variability in DNA detection over time is crucial for accurate interpretation of qPCR results.[22] A previous report, in which a human stool sample was spiked with *N. americanus* eggs

* Address correspondence to Naomi E. Clarke, Research School of Population Health, Australian National University, Canberra ACT 0200, Australia. E-mail: naomi.clarke@anu.edu.au

isolated from hamster stool and preserved for up to 60 days, found that quantitation cycle values (also known as cycle threshold [Ct] values) remained relatively constant over time when stored at 4°C, whereas more substantial variation occurred when samples were stored at 32°C.[22] However, no studies have examined samples stored at room temperature (21–22°C), and importantly, none have used samples obtained from infected humans to reflect samples collected during field trials.

Furthermore, there are limited published data comparing qPCR results with other microscopy-based diagnostic methods that may be more sensitive than the Kato–Katz technique. Sodium nitrate flotation (SNF) is a simple technique widely used in veterinary parasitology and has previously demonstrated higher sensitivity for detecting hookworm eggs compared with the Kato–Katz technique.[16] Although PCR-based techniques have been shown in two studies to have a higher diagnostic sensitivity than SNF,[16,20] there are no published data comparing intensity measurements obtained from SNF and qPCR. A previous attempt to compare quantitative results from these techniques revealed no significant statistical relationship and data were not presented.[20]

The objectives of this study were 1) to compare the diagnostic performance of SNF and qPCR for STH infections, in terms of both diagnostic sensitivity and infection intensity measurements, using samples from a field trial; and 2) to examine variation in qPCR infection intensity results (Ct values), by conducting repeated analysis on a stool sample positive for *N. americanus*, preserved in 5% potassium dichromate over a 6-month period.

MATERIALS AND METHODS

Ethics and informed consent. The research protocol for the field study in which samples were collected for the SNF and qPCR comparison (the [S]WASH-D for Worms pilot study) was approved by the Human Research Ethics Committees at the Australian National University (2015/111) and the Timor-Leste Ministry of Health (2015/196). Written consent was provided by parents of all participating children.

The stool sample used for investigating DNA extraction and PCR variability was obtained from an adult donor at the QIMR Berghofer Medical Research Institute in Brisbane, Australia, who was known to be infected with *N. americanus* and who provided written informed consent.

Field study area and sample collection. The stool samples used to compare SNF and qPCR were collected from children attending six primary schools in the Aileu and Manufahi municipalities of Timor-Leste. Samples were collected between May 2015 and June 2016 in the (S)WASH-D for Worms pilot study.[23] Children were given labeled stool containers, instructed on how to provide a sample, and requested to collect an early morning sample the following day and bring it to school. Immediately on sample receipt, the research team prepared two aliquots of 2–3 g of stool from each participant. One was preserved in 8 mL of 10% formalin and the other in 5 mL of 5% (w/v) potassium dichromate. The formalin-fixed samples were transported at room temperature to the University of Melbourne, Victoria, Australia, for analysis using SNF. The potassium dichromate–fixed samples were transported at room temperature to the QIMR Berghofer Medical Research Institute, Brisbane, Australia, for analysis using qPCR.

Approximately 5–6 months after baseline sample collection, a single dose of 400 mg albendazole was given to all children under direct observation. Follow-up stool samples were collected and processed using the same procedures 6 months after treatment.

Sodium nitrate flotation procedure. Formalin-fixed samples were examined at the University of Melbourne, between 1 and 3 months after sample collection, using a technique that has been described previously.[16] The samples were centrifuged for 2 minutes at 3,000 × *g*, formalin poured off, and the sample thoroughly mixed with distilled water. The suspension was then strained through two layers of surgical gauze, poured into a fresh 10 mL centrifuge tube, and centrifuged for 2 minutes at 3,000 × *g*. The supernatant was carefully removed using a pipette, leaving a fecal pellet between 100 and 300 mg in size. The volume of the fecal pellet was estimated using graduated lines on the centrifuge tube. Sodium nitrate solution at a specific gravity of 1.20 was added to the centrifuge tube and a positive meniscus created. A coverslip was placed over the centrifuge tube for 10 minutes and then transferred to a microscope slide, which was examined in its entirety at ×100 magnification by one of three trained microscopists for STH egg enumeration. Finally, the EPG for each STH was obtained by multiplying the number of eggs counted as required, depending on the size of the filtered fecal pellet examined (e.g., for a 200 mg sample, the number of eggs was multiplied by 5).

One coverslip was examined for most samples; however, for quality control, two coverslips were prepared separately from the same sample and read by individual microscopists for 10% of samples. In these cases, a positive reading by either microscopist was considered a positive result, and when necessary, the two EPG readings were averaged to give a single EPG.

Quantitative PCR procedure. The potassium dichromate–fixed samples were analyzed at the QIMR Berghofer Medical Research Institute using a multiplex real-time PCR procedure as previously published.[20] Following the removal of the preservative, DNA was extracted from stool samples using the PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA) with minor modifications. DNA extractions were carried out between 6 weeks and 6 months after sample collection. The extracted DNA was run in a multiplex real-time PCR reaction that was a quantitative assay for *Ascaris* spp., *N. americanus*, *Ancylostoma* spp., and *Trichuris* spp.[20] A known amount of equine herpesvirus (EHV) plasmid was added as a positive PCR control. Details of all primers and probes are shown in Supplemental Table 1.

The multiplex qPCR assays were run using the Rotor-Gene 6000 (Qiagen, Melbourne, Victoria, Australia), with reactions set up as per previous descriptions.[14,20] Cycling conditions consisted of 15 minutes at 95°C followed by 40 cycles of 95°C for 9 seconds and 60°C for 60 seconds. Each qPCR assay returned a Ct value for each STH, representing the cycle number at which a signal exceeding background level was detected. The maximum Ct value considered to represent a positive result was 31 for *Ascaris* spp. and 35 for all other species, consistent with previous studies.[20] For each qPCR assay, two Ct values were generated by performing two reaction runs, and these were averaged to give a single Ct value.

The final Ct value for each sample was then converted to a measure of infection intensity in relative fluorescence units (RFU), calculated using the following formula provided by the Rotor-Gene Q software: infection intensity = $10^{-0.298*Ct}$ + 9.81 RFU.

Assessment of DNA extraction and PCR variability. Separate from the examination of samples from the field trial, to explore variability in Ct values obtained from qPCR analysis in preserved samples over time, repeated examinations were conducted on a single stool sample. A stool sample was obtained from a donor known to be infected with *N. americanus*. The sample was homogenized and then separated into aliquots of 1 g each, which were preserved in 5% (w/v) potassium dichromate within several hours of sample collection. The aliquots were stored at room temperature. DNA extraction and subsequent qPCR analysis was performed at five time points: 2 days following preservation, at monthly intervals for 3 months, and then at 6 months after preservation. At each of these time points, three separate aliquots were subjected to triplicate DNA extractions of 200 µg, each of which was examined using the qPCR assay for *N. americanus*. This gave a total of 15 aliquots and 45 PCR replicates from the original stool sample. Equine herpesvirus was included as an extraction and PCR control.

Statistical analysis. Diagnostic agreement between SNF and qPCR was examined using Kappa agreement statistics. The Wilcoxon rank sum test was used to compare the Ct values of qPCR-positive samples that were SNF-positive versus SNF-negative. Spearman's rank correlation coefficients were used to examine the association between PCR intensity and EPG values. The sensitivities of qPCR and SNF were estimated by considering an individual as "true positive" if they had a positive result by either method, creating a diagnostic pseudo-"gold standard." Specificity was assumed to be 100%.

For the analysis of Ct value variation, a linear mixed model was used to examine the impact of time on Ct values and the variability between and within individual aliquots. Time point was included as a categorical fixed effect and sample number as a random effect. All analyses were conducted using Stata Version 14 (StataCorp, College Station, TX).

RESULTS

Field trial participants. Diagnostic results for qPCR and SNF were available for 462 children at baseline and 403 at follow-up, for 865 samples in total, from 571 individuals. The mean age of participating children was 9.2 years (range 4–17 years) at baseline and 9.0 years (range 4–17 years) at follow-up. Slightly more than half (52.1%) of the participants were female.

Soil-transmitted helminth prevalence and intensity. At baseline, *Ascaris* spp. prevalence was 40.0% (185/462 samples) by qPCR and 30.3% (140/462) by SNF. Hookworm prevalence was 12.6% (58/462) by SNF, whereas by qPCR, 13.9% (64/462) of samples were positive for *N. americanus* and 0.9% (4/462) for *Ancylostoma* spp. Prevalence of *Trichuris* spp. was lower at 2.2% (10/462) by qPCR and 2.8% (13/462) by SNF. Prevalence of all infections was lower at follow-up, apart from *Trichuris* spp. by SNF (see Table 1 and Figure 1).

All hookworm and *T. trichiura* infections diagnosed by SNF at baseline were light-intensity infections according to WHO-defined EPG cut-offs.[9] Just 1.4% of baseline *A. lumbricoides* infections were moderate-intensity infections, whereas the remainder were light-intensity. All infections at follow-up were light-intensity. Quality control results for SNF are shown in Supplemental Table 2.

Diagnostic performance of qPCR and SNF. As shown in Table 2, there was substantial agreement between qPCR and SNF for diagnosis of *Ascaris* spp. ($\kappa = 0.69$, $P < 0.001$). Of 186 samples that were positive by SNF, only 16 were negative on qPCR; whereas 87 of 257 qPCR-positive samples were negative on SNF. For hookworm, there was moderate agreement between qPCR and SNF ($\kappa = 0.55$, $P < 0.001$); 45/103 qPCR-positive samples were negative on SNF and 33/91 SNF-positive samples were negative on qPCR. For *Trichuris* spp., there was moderate agreement between qPCR and SNF ($\kappa = 0.50$, $P < 0.001$), with 6/17 qPCR-positive samples negative on SNF and 15/26 SNF-positive samples negative on qPCR.

For *Ascaris* spp., the mean Ct value of qPCR-positive samples that were SNF-positive was 15.75 (95% confidence interval (CI): 15.19–16.32), whereas the mean Ct value of

TABLE 1
Soil-transmitted helminth prevalence and intensity at study time points, as measured by qPCR and SNF

	Prevalence by qPCR (95% CI)	Prevalence by SNF (95% CI)	*P* value*	Mean cycle threshold value (range)	Mean eggs per gram (range)
Baseline (*N* = 462)					
Ascaris spp.	40.0% (35.7–44.6)	30.3% (26.2–34.7)	**0.002**	18.3 (9.1–31.0)	568.2 (4.0–5,244.0)
Hookworm	14.7% (11.8–18.3)	12.6% (9.8–15.9)	0.300	–	26.6 (2.0–180.0)
Necator americanus	13.9% (11.0–17.3)	–		25.1 (24.1–26.0)	–
Ancylostoma spp.	0.9% (0.3–2.3)	–		22.4 (20.5–24.4)	–
Trichuris spp.	2.2% (1.2–4.0)	2.8% (1.6–4.8)	0.547	31.7 (25.7–35.0)	21.6 (4.4–54.6)
Coinfections	6.1% (4.2–8.7)†	5.8% (4.0–8.4)‡	0.851	–	–
Follow-up (*N* = 403)					
Ascaris spp.	17.9% (14.4–21.9)	11.4% (8.7–14.9)	**0.010**	22.6 (11.2–31.0)	350.8 (2.9–3,373.0)
Hookworm	8.7% (6.3–11.9)	8.2% (5.9–11.3)	0.800	–	21.2 (2.2–128.6)
N. americanus	8.7% (6.3–11.9)	–		25.4 (18.6–31.8)	–
Anyclostoma spp.	0	–		–	–
Trichuris spp.	1.7% (0.8–3.6)	3.2% (1.9–5.5)	0.174	29.7 (21.2–34.1)	11.1 (2.5–36.7)
Coinfections	2.2% (1.2–4.3)§	3.2% (1.9–5.5)¶	0.387	–	–

CI = confidence interval; qPCR = quantitative polymerase chain reaction; SNF = sodium nitrate flotation.
* *P* values comparing prevalence obtained using SNF and qPCR.
† Of 28 coinfections on qPCR at baseline: 21 *Ascaris* spp. + *N. americanus*; six *Ascaris* spp. + *Trichuris* spp; one *Ascaris* spp. + *Ancylostoma* spp.
‡ Of 27 coinfections on SNF at baseline: 19 *Ascaris lumbricoides* + hookworm, five *A. lumbricoides* + *Trichuris trichiura*; two hookworm + *T. trichiura*; one triple infection.
§ Of 9 coinfections on qPCR at follow-up: eight *Ascaris* spp. + *N. americanus*; one *Ascaris* spp. + *Trichuris* spp.
¶ Of 13 coinfections on SNF at follow-up: nine *A. lumbricoides* + hookworm; three *A. lumbricoides* + *T. trichiura;* one triple infection.

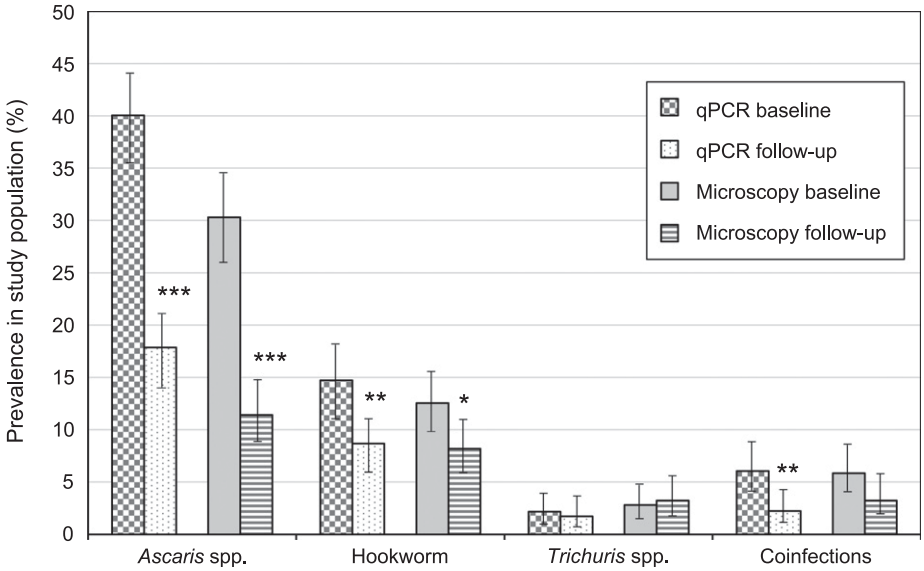

FIGURE 1. Baseline and follow-up prevalence of each soil-transmitted helminth as measured by quantitative polymerase chain reaction (qPCR) and sodium nitrate flotation (microscopy). Difference between baseline and follow-up prevalence: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

qPCR-positive samples that were SNF-negative was significantly higher at 26.78 (95% CI: 25.85–27.61, $P < 0.001$). Similarly, for hookworm, the mean Ct value was higher for SNF-negative samples (27.62, 95% CI: 26.48–28.76) compared with SNF-positive samples (23.10, 95% CI: 22.41–23.78, $P < 0.001$).

The sensitivity of qPCR was 94.1% for *Ascaris* spp. (257/273 samples) and 75.7% for hookworm (103/136 samples), whereas the sensitivity of SNF was lower at 68.1% for *Ascaris* spp. (186/273 samples) and 66.9% for hookworm (91/136 samples). For *Trichuris* spp., the sensitivity of qPCR was 53.1% (17/32 samples) and the sensitivity of SNF was higher at 81.3% (26/32 samples).

Comparison of infection intensity. For *Ascaris* spp., infection intensity values obtained using qPCR were significantly and strongly correlated with EPG values obtained using SNF ($\rho = 0.82$, $P < 0.001$). For hookworm, infection intensity values obtained using qPCR were significantly and moderately correlated with EPG values obtained using SNF ($\rho = 0.58$, $P < 0.001$). Scatter plots of log-transformed EPG and log-transformed infection intensity for *Ascaris* spp. and hookworm are shown in Figure 2.

Correlations between infection intensity values were stronger at follow-up than baseline for both *Ascaris* spp. ($\rho = 0.85$ versus $\rho = 0.77$) and hookworm ($\rho = 0.67$ versus $\rho = 0.55$); see Supplemental Figures 1 and 2. Because of a low number of positive samples, infection intensity comparisons were not performed for *Trichuris* spp.

Variability in DNA detection. Cycle threshold values obtained for each separate aliquot at each time point are depicted in Figure 3 and summarized in Table 3.

As shown in Table 3, the mean Ct value for *N. americanus* obtained from PCR replicates run 2 days after DNA extraction was 17.00 (95% CI: 16.47–17.52), whereas for PCR replicates run at or later than one month, mean Ct values ranged from 10.57 (95% CI: 9.45–11.70) to 11.78 (95% CI: 11.33–12.23). Cycle threshold values for EHV were more consistent across time points, with mean values between 18.37 (95% CI: 18.21–18.53) and 19.57 (95% CI: 19.37–19.78).

Results of the linear mixed model examining the impact of time on *N. americanus* Ct values and the variability within and between different aliquots are shown in Table 4. These results show that Ct values obtained at month 0 were significantly higher than those obtained at month one ($P < 0.001$), whereas there were no significant differences between Ct values obtained at month one and any later time point. There was negligible random effects variance for aliquot, reflecting minimal variability between Ct values obtained from separate aliquots at a given time point. On the other hand, the residual variance was higher at 1.18 (95% CI: 0.78–1.79), reflecting greater variability between separate DNA extractions from the same aliquot.

TABLE 2
Diagnostic agreement of quantitative polymerase chain reaction and sodium nitrate flotation

	PCR result	SNF-positive	SNF-negative	Agreement (%)	Kappa statistic*	*P* value
Ascaris spp.	PCR positive	170	87	762 (88.1)	0.6902	< 0.001
	PCR negative	16	592			
Hookworm	PCR positive	58	45	787 (91.0)	0.5474	< 0.001
	PCR negative	33	729			
Trichuris spp.	PCR positive	11	6	844 (97.6)	0.4997	< 0.001
	PCR negative	15	833			

PCR = polymerase chain reaction; SNF = sodium nitrate flotation.
* Kappa agreement level: < 0.20 Poor; 0.21–0.40 Fair; 0.41–0.60 Moderate; 0.61–0.80 Good; 0.81–1.00 Very good.

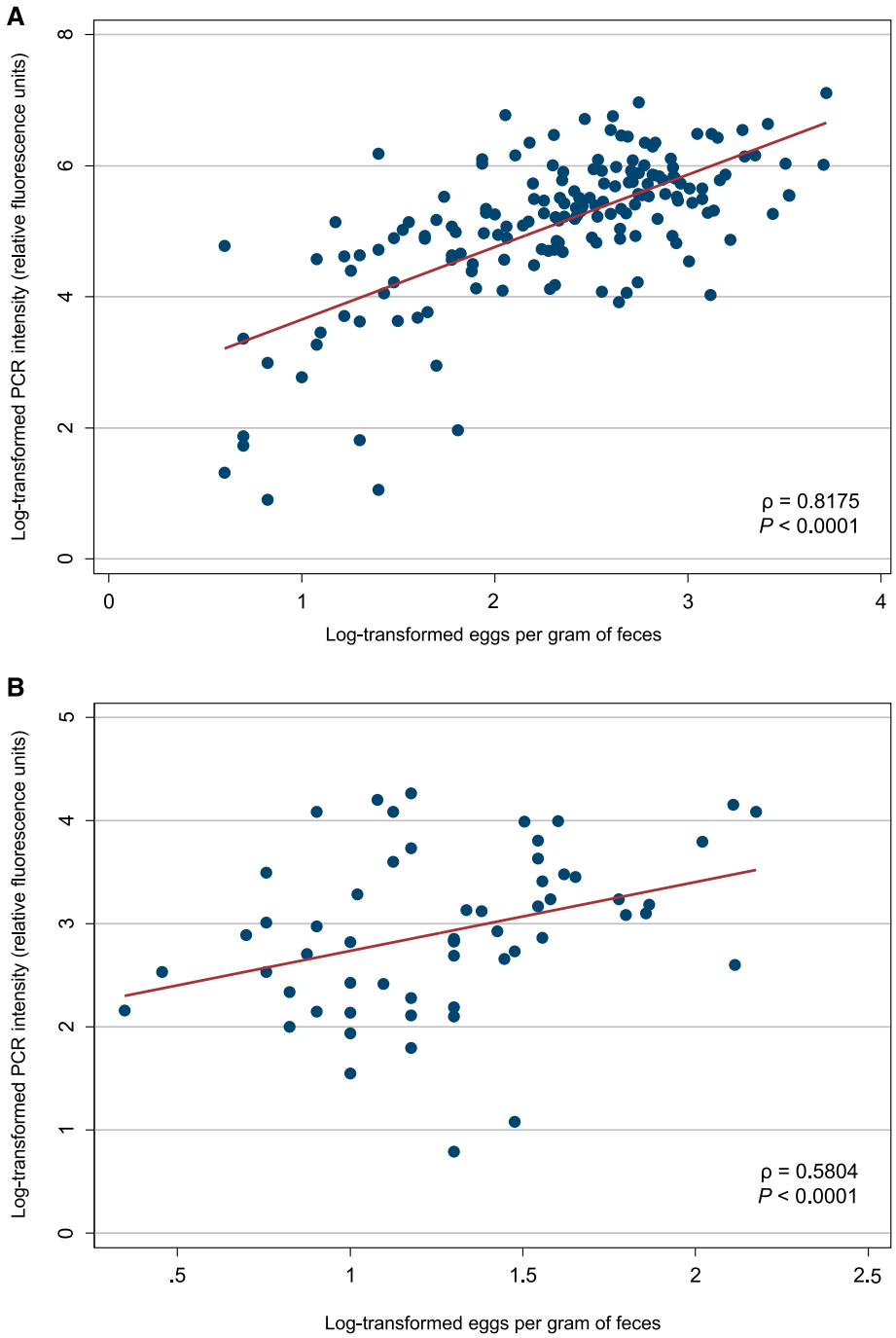

FIGURE 2. Scatter plots showing the relationship between infection intensity measured by sodium nitrate flotation (eggs per gram of feces) and quantitative polymerase chain reaction (PCR) (reactive fluorescence units) on universal $\log_{10}$ transformation, for *Ascaris* spp. (**A**) and hookworm (**B**). This figure appears in color at www.ajtmh.org.

DISCUSSION

We compared the diagnostic performance of SNF and qPCR, both of which have previously demonstrated higher sensitivity compared with the recommended Kato–Katz technique for the diagnosis of STH infections.[15,16] Our results confirm that qPCR is more sensitive than SNF for both *Ascaris* spp. and hookworm.[20] For *Trichuris* spp., qPCR demonstrated lower sensitivity than SNF. Most previous studies comparing qPCR and Kato–Katz have focused on *Ascaris* spp. and hookworm. Only a small number have compared diagnostic sensitivity for *Trichuris* spp.; these showed that qPCR had higher sensitivity than Kato–Katz.[17,18] Our findings highlight the need for further evaluation of qPCR in settings where *T. trichiura* is endemic, both before and after mass drug administration. A more sensitive, species-specific PCR assay for *T. trichiura* may yield improved results.[24]

Notably, for *Ascaris* spp. and hookworm, infections that were detected by qPCR but not by SNF were of significantly lighter intensity than those detected by both techniques,

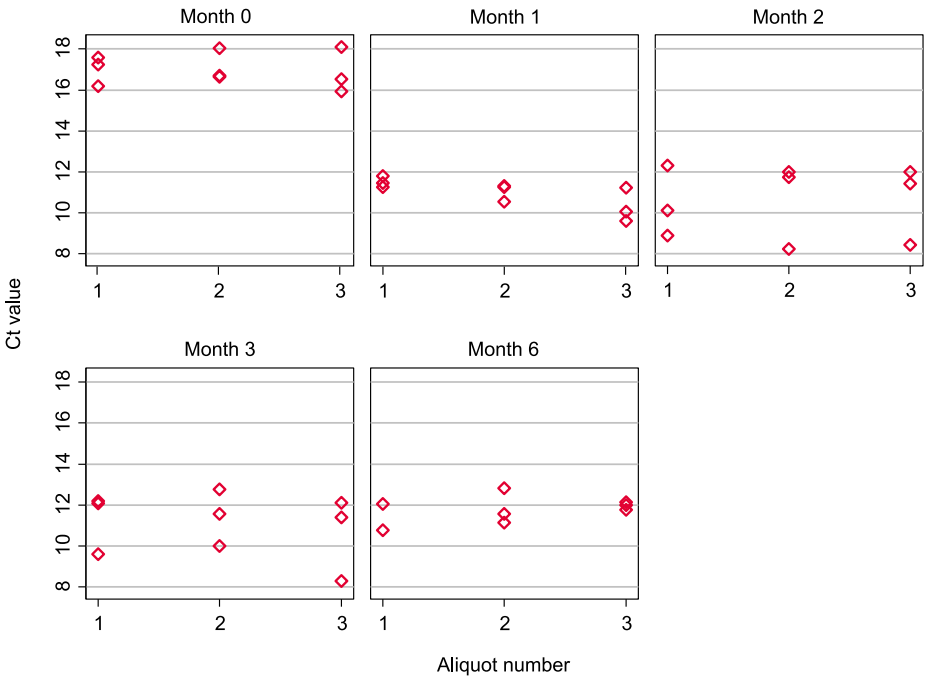

FIGURE 3. Box plots showing *Necator americanus* cycle threshold (Ct) values obtained from individual aliquots measured at five time points following sample preservation. Three aliquots were each subjected to triplicate DNA extractions and quantitative polymerase chain reaction at each time point. This figure appears in color at www.ajtmh.org.

confirming the superior performance of qPCR in detecting light-intensity infections.[14,15] Stool samples are heterogeneous in terms of helminth egg distribution, and even with thorough mixing during the preparation procedures, there is variability in the number of eggs present in prepared slides. Therefore, light-intensity infections may easily be missed using microscopy-based techniques.

Polymerase chain reaction techniques can detect very small quantities of DNA, which explains their greater sensitivity at lower intensities. However, a number of STH eggs were detected on SNF that were negative by qPCR, particularly for hookworm and *Trichuris* spp. This may also be explained by heterogeneous egg distribution. For qPCR, an average 200 mg of feces is used, as opposed to 2 g for SNF, which may explain the discrepancy of results. Microscopy-based techniques may also produce false-positive results if strongyle eggs representing non-hookworm genera, for example *Oesophagostomum* spp. and *Trichostrongylus* spp.,[25,26] or fecal material are mistakenly counted as hookworm eggs.

We demonstrated a strong correlation between intensity results obtained from qPCR and SNF for *Ascaris* spp. and a moderate correlation for hookworm, which provides additional evidence supporting the potential for qPCR as a quantitative technique for STH diagnosis.[15,20] Correlations remained strong at study follow-up, highlighting the capacity of qPCR for evaluating mass drug administration programs at community level. Although qPCR-determined intensity has been shown to correlate with EPG obtained from other microscopy-based techniques,[15,18,19,21] this is the first demonstration of such correlation between qPCR and SNF. A previous study found no relationship between these two techniques and also reported lower diagnostic agreement between SNF and qPCR for hookworm, with more infections missed by microscopy.[20] This demonstrates the importance of skilled microscopists in performing SNF.

TABLE 3

Ct values obtained for *Necator americanus* and equine herpesvirus from the same stool sample, measured at five time points

	Number of aliquots	Number of PCR replicates	Mean (SD) Ct value	95% CI for mean Ct value	Minimum Ct value	Maximum Ct value
N. americanus						
Month 0	3	9	17.00 (0.79)	16.47–17.52	15.94	18.10
Month 1	3	9	10.95 (0.72)	10.47–11.43	9.62	11.80
Month 2	3	9	10.57 (1.67)	9.45–11.70	8.24	12.31
Month 3	3	9	11.11 (1.49)	10.11–12.11	8.28	12.76
Month 6	3	8	11.78 (0.63)	11.33–12.23	10.77	12.83
Equine herpesvirus (positive PCR control)						
Month 0	3	9	19.57 (0.31)	19.37–19.78	19.18	20.00
Month 1	3	9	18.37 (0.26)	18.21–18.53	18.14	18.84
Month 2	3	9	18.78 (0.53)	18.42–19.14	18.30	19.93
Month 3	3	9	19.41 (0.63)	18.99–19.84	18.23	20.39
Month 6	3	8	18.78 (0.51)	18.41–19.14	18.22	19.62

CI = confidence interval; Ct = cycle threshold; PCR = polymerase chain reaction.

TABLE 4

Results of linear mixed model examining impact of time and aliquot on *Necator americanus* cycle threshold values

	Regression coefficient	95% CI	*P* value
Time point			
Month 0	6.05	5.04–7.05	< 0.0001
Month 1	(Ref)	–	–
Month 2	–0.37	–1.38 to 0.63	0.46
Month 3	0.16	–0.84 to 1.16	0.75
Month 6	0.83	–0.20 to 1.87	0.11
Random effects variance (95% CI)			
Aliquot	< 0.0001 (< 0.0001 to < 0.0001)		
Residual	1.18 (0.78–1.79)		

CI = confidence interval.

When performing DNA extraction and qPCR on aliquots of the same stool sample, stored at room temperature in 5% potassium dichromate over a 6-month period, *N. americanus* Ct values decreased significantly between the time of preservation and one month later, and remained relatively stable thereafter. However, results were shown to vary by up to four Ct values at a given time point. This partly reflects heterogeneous egg distribution within stool samples as discussed previously, given the more consistent Ct values obtained for the EHV plasmid positive control. In addition, slight inconsistencies in the volume of stool being added, and in DNA target recovery between replicates, may have contributed to the variability.

The initial decrease in Ct values was likely due to the fact that helminth eggs in the stored stool samples embryonated, causing copy number to exponentially increase. This decrease in Ct values was not seen in a previous study that examined preserved stool samples over a 2-month period.[22] However, that study used hookworm-naïve human stool samples, spiked with *N. americanus* eggs obtained from hamster stool. These eggs may have already embryonated during storage before spiking. To our knowledge, our study is the first to examine variability in DNA detection for STH over time using a stool sample obtained from an infected human, reflecting samples that would be collected in an endemic setting. Our results highlight the importance of time between preservation and DNA extraction on qPCR results, and suggest that to maximize consistency, DNA extraction should occur at least a month after sample preservation because quantitative results appear to stabilize after this time.

Quantitative PCR represents an excellent diagnostic option in scenarios where highly sensitive diagnostic techniques are required to detect light-intensity infections. Such situations include settings approaching STH transmission interruption, or monitoring for STH reemergence following cessation of mass drug administration. Additional benefits of qPCR are its ability to distinguish between hookworm species and its capacity to include assays for other parasitic infections.[15,20]

Polymerase chain reaction–based techniques require specialized equipment generally available only in central laboratories and are expensive compared with microscopy-based methods. This impacts the feasibility of implementing these techniques in low-resource settings where STH are endemic. However, many low- and middle-income countries are currently undertaking molecular diagnostics in central laboratories. Furthermore, a recent economic evaluation suggests that longer-term programmatic benefits may outweigh the higher cost of novel diagnostic tests,[27] particularly in settings approaching STH elimination.

Sodium nitrate flotation represents a less costly alternative diagnostic strategy that could be used in resource-limited settings. This microscopy-based technique, although less sensitive than qPCR, addresses some of the limitations of the Kato–Katz technique, including the need to examine samples within 30 minutes and to examine multiple samples. Our results confirm previous findings that SNF shows good potential as a diagnostic test for STH that could be implemented at scale.[16]

A limitation of our analysis was that we were unable to convert PCR-determined infection intensity to EPG, and therefore, we could not directly compare infection intensity results obtained using qPCR and SNF. This limits the current ability of qPCR in terms of measuring individual-level infection intensity, and is a crucial area for further research. In particular, determining the relationship between qPCR-determined infection intensity and WHO-defined intensity cut-offs should be prioritized. Additional work is also required to investigate the impact of sample preservation and egg development stage on quantitative results.

A further limitation is that samples were stored in formalin for 1–3 months before analysis using SNF. Diagnostic accuracy of flotation techniques for samples stored in formalin may decrease after 15 days for hookworm infections[28]; therefore, SNF sensitivity for detecting hookworm may have been suboptimal.

Finally, our analysis of variability in DNA detection used only one stool sample from one individual, who harbored *N. americanus* only. Further studies should be conducted using multiple samples from multiple infected individuals, including those infected with *A. lumbricoides*, *T. trichiura*, *A. duodenale*, and *A. ceylanicum.*

In conclusion, our study further highlights the sensitivity of qPCR techniques for detecting light-intensity *Ascaris* spp. and hookworm infections, and the potential utility of qPCR for determining STH infection intensity. Further research is required to examine the performance of qPCR for detecting *Trichuris* spp. and to more fully elucidate the ability of qPCR to accurately measure infection intensity. As the global burden of STH decreases and transmission interruption becomes increasingly feasible, ongoing efforts toward incorporating molecular diagnostic methods into STH control efforts should be prioritized.

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Authors' addresses: Naomi E. Clarke and Alice Richardson, Research School of Population Health, Australian National University, Canberra, Australia, E-mails: naomi.clarke@anu.edu.au and alice.richardson@anu.edu.au. Stacey Llewellyn and James McCarthy, Clinical Tropical

Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, Australia, E-mails: stacey.llewellyn@qimrberghofer.edu.au and j.mccarthy@uq.edu.au. Rebecca J. Traub, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Australia, E-mail: rebecca.traub@unimelb.edu.au. Susana V. Nery, Kirby Institute, University of New South Wales, Sydney, Australia, E-mail: snery@kirby.unsw.edu.au.

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Chapter 7
Discussion and conclusion

The preceding five chapters addressed the four key research objectives of this thesis. Each of these chapters and objectives has contributed to the overall goal of this thesis: to generate evidence regarding the potential impact of expanded or alternative STH control strategies, in order to optimise benefits for endemic populations.

Such evidence is critically required in the NTD sector. Current STH control efforts remain strongly focused on the WHO 2020 targets, and with under a year remaining to 2020, now is the time when key discussions must occur, and key decisions must be made, regarding the future of STH control efforts. An additional impetus for these discussions is the global progress towards achieving control targets for lymphatic filariasis, leading to the scaling down of many LF control programs, which collectively deliver a significant proportion of preventive chemotherapy against STH to at-risk populations [1, 2].

The most recently released WHO guideline for STH control has been criticised by some in the NTD research community for failing to address a number of crucial contemporary issues in the STH control sector. Among these issues were all of those addressed in this thesis, namely: (1) the use of drug combinations to enhance anthelminthic efficacy; (2) school-based versus community-wide treatment; (3) the role of complementary interventions such as WASH improvements; and (4) the use of novel diagnostic techniques [3]. This echoes previous calls for urgent re-evaluation of STH control guidelines to consider these highly relevant issues [4, 5].

A key opportunity to address these issues moving forward exists in the discussions that are already underway regarding post-2020 targets and planning for STH control programs [6]. Crucial to these discussions is the body and strength of available evidence that can guide and support policy decisions.

The research presented in this thesis makes a substantial contribution to the evidence base regarding optimising STH control programs, at a time when this evidence base should be rigorously reviewed, evaluated, and incorporated into future guidelines and targets.

This final chapter presents a summary of the main thesis findings and an integrated discussion of the implications of these findings, organised according to the four research objectives. In this chapter, recommendations for NTD policymakers are emphasised, as are important ongoing research priorities that flow from this work. Strengths and limitations of the thesis are then discussed, followed by brief concluding remarks.

7.1 Key thesis findings and implications for policy and research

7.1.1 Anthelminthic drug efficacy

In Chapter 2, the broadest and most comprehensive comparison of anthelminthic treatments for STH to date was presented. Albendazole and mebendazole are currently recommended for use in STH control programs; however, they are known to exhibit poor efficacy against *T. trichiura* [7], which represents one of the major limitations of existing preventive chemotherapy programs. Therefore, a systematic review and meta-analysis was undertaken, comparing the efficacy of 21 different anthelminthic drug treatments in terms of both cure rate and egg reduction rate. To maximise the relevance of this analysis for STH policymakers, all treatments were compared to the current standard treatment (single-dose albendazole), such that study results clearly identified available treatments that could enhance current control efforts.

The results presented in Chapter 2 provide robust evidence that within the limited arsenal of existing anthelminthic drugs, there are options that could enhance STH control efforts by improving efficacy against *T. trichiura*. A number of single-dose drug combinations were found to have significantly greater efficacy against *T. trichiura* than single-dose albendazole, without compromising efficacy against hookworm or *A. lumbricoides*. Both albendazole–ivermectin and albendazole–oxantel pamoate combinations demonstrated superior efficacy against *T. trichiura* compared to single-dose albendazole, in terms of both cure rate and egg reduction rate. Additionally, tribendimidine–oxantel pamoate showed greater efficacy in terms of cure rate, but could not be analysed for egg reduction rate.

These findings are supported by other recent studies highlighting the benefits of drug combinations for STH control. For example, a smaller meta-analysis focused only on albendazole–ivermectin also identified its superior efficacy compared to albendazole alone [8]. Additionally, mathematical modelling has demonstrated that albendazole–ivermectin will improve control of *T. trichiura*, leading to greater reductions in infection prevalence and intensity, and increasing the likelihood of transmission

interruption [9]. The importance of drug combinations in terms of reducing the risk of drug resistance emerging has also been emphasised previously [10, 11]. With very few novel anthelminthics in the development pipeline [12], there is an impetus to identify the best options of those currently available, and make these options available to people living in endemic areas. Therefore, a key recommendation emerging from this thesis for NTD policymakers is to urgently prioritise steps towards including drug combinations in STH control programs where *T. trichiura* is prevalent.

Albendazole–ivermectin is currently most amenable to inclusion in STH control efforts. There are existing guidelines for the co-administration of these drugs in preventive chemotherapy programs [13], a drug donation program for ivermectin is well established [14], and ivermectin was recently included in the WHO Essential Medicines List for STH [15]. An additional advantage of using ivermectin is that, due to its broad-spectrum anti-parasitic activity, it has ancillary population health benefits in terms of controlling other NTDs, including onchocerciasis, LF, scabies, and *Strongyloides stercoralis* [16–18]. Furthermore, there is emerging evidence that ivermectin may have an impact on malaria transmission [19, 20]. On the other hand, results presented in Chapter 2 suggest that albendazole–oxantel pamoate is the most efficacious combination against *T. trichiura*, and that tribendimidine–oxantel pamoate is also promising. Therefore, efforts towards making these combinations available to afflicted populations should also be prioritised.

Additional research is needed to facilitate the incorporation of drug combinations into STH control guidelines. Detailed up-to-date mapping of global *T. trichiura* prevalence will help determine priority areas for preventive chemotherapy with drug combinations. Cost-effectiveness analyses of different drug combinations in different transmission settings should also be conducted to provide additional guidance for policymakers. Furthermore, albendazole–oxantel pamoate and tribendimidine–oxantel pamoate require additional safety and efficacy trials, and ongoing investigation into the optimal doses of tribendimidine and oxantel pamoate for different age groups is required [21, 22]. Identifying a fixed dose for oxantel pamoate would greatly improve the feasibility of its inclusion in STH control programs [23]. In the longer term, research into developing co-formulations of anthelminthic drug combinations is an important goal [1].

Beyond research, global policy efforts should focus on defining criteria for including drug combinations in preventive chemotherapy programs (e.g., based on *T. trichiura* prevalence and/or intensity), determining the number of additional drug doses required, and liaising with pharmaceutical companies regarding the production and procurement of additional doses of ivermectin for use in STH control programs. Identifying potential pharmaceutical manufacturers for oxantel pamoate and tribendimidine, and adding oxantel pamoate and tribendimidine to the WHO Essential Medicines List once sufficient evidence is available are also important priorities.

Finally, although several promising drug combinations have been identified, development of novel anthelminthics, and investigation of novel drug combinations, must remain firmly on researchers' and funders' agenda. Currently, there remains a persistent lack of novel drug development for STH and other NTDs that must be addressed [24]. Although drugs that will improve *T. trichiura* control are most in demand, enhancing efficacy against hookworm is also desirable. Cure rates and egg reduction rates of albendazole and mebendazole are significantly lower for hookworm than *A. lumbricoides* [7], and the comprehensive comparison presented in Chapter 2 identified no superior single-dose option against hookworm. Current candidates warranting further research include moxidectin [25] and triple drug therapy with albendazole, pyrantel pamoate, and oxantel pamoate [26]. Additionally, efforts are continuing towards developing a vaccine for hookworm infection through the Human Hookworm Vaccine Initiative [27, 28], with several phase 1 trials completed and a number of others ongoing [29]. However, STH vaccine development remains an extremely challenging task [28, 30], reinforcing the need for concurrent research into novel anthelminthics.

7.1.2 Community-wide STH control

In Chapters 3 and 4, the first empirical evidence comparing the impact of community-wide and child-targeted strategies for STH control was presented. This work was conducted in the context of existing control efforts strongly focusing on treating children, reflecting the WHO 2020 targets and corresponding drug donations [31–33]. Evidence from mathematical modelling has projected that community-wide drug administration will have a greater impact on STH transmission and morbidity [34–38]; however, the comparative impact of community-wide and child-targeted control strategies on infection prevalence had not been examined either in a synthesis of existing empirical evidence, or in community-based trials.

A systematic review and meta-analysis comparing the impact of child-targeted deworming and community-wide deworming was presented in Chapter 3. The results of this analysis showed that compared to child-targeted deworming, community-wide deworming resulted in significantly greater STH prevalence reduction among school-aged children, for both hookworm and *A. lumbricoides*. Complementing these results, chapter 4 presented the results of the (S)WASH-D for Worms pilot study. This study compared the impact of integrated STH control programs, consisting of both deworming and WASH improvements, when implemented community-wide versus targeted to school-aged children. Results of this pilot study showed preliminary evidence that the program delivered to all community members had a greater impact on STH prevalence in school-aged children, compared to the program delivered only to children.

These findings provide evidence that expanding STH control programs community-wide will rapidly deliver benefits for school-aged children by way of fewer reinfections due to reduced transmission within the community. Recently-released findings of the TUMIKIA trial conducted in Kenya confirm that community-wide albendazole treatment resulted in lower hookworm prevalence and intensity across all age groups, including school-aged children, compared to school-based treatment [39]. Our results also complement mathematical modelling analyses, that predict a greater impact of community-wide control strategies on STH transmission levels, including the potential to interrupt transmission [36–38], leading to improved cost-effectiveness [40, 41]. A community-wide approach has the benefit of reaching all at-risk groups, including young children and women of reproductive age, for whom deworming coverage currently remains low [1]. Therefore, the key recommendation for policymakers based on the findings presented in Chapters 3 and 4 is that STH control guidelines should recommend treatment of all community members, with the exception of children under one year old and pregnant women in the first trimester, in whom safety has not been established [11].

Further field trials are needed to investigate the differential impact of community-wide and child-targeted deworming, in a range of transmission settings. The Deworm3 study is currently underway in Benin, Malawi, and India, investigating the feasibility of interrupting STH transmission using twice-yearly community-wide deworming, compared to yearly school-based deworming [42, 43]. The CoDe-STH trial, to be implemented in Vietnam in 2019–2020, will compare the impact of school-based and community-wide deworming on STH prevalence and intensity among school-aged children (Nery, S., personal communication). Both of these studies, as well as the recently completed TUMIKIA trial, will also compare cost-effectiveness of community-wide and school-based deworming [43, 44]. This is a crucial question for NTD policymakers and represents an important research gap. Although robust cost-effectiveness modelling studies show that community-wide deworming is highly cost-effective compared to child-targeted deworming in the long term [40, 41], a recent systematic review found limited empirical evidence for costs and cost-effectiveness of community-wide deworming [45].

Achieving high treatment coverage and compliance is an important consideration when expanding to community-wide deworming [46]. Existing deworming programs that target children can reach a high proportion of eligible children by delivering drugs through schools and child health days [31, 47], as long as attendance rates are acceptable. On the other hand, treating all age groups will require a community-based drug distribution system, and establishing and maintaining community engagement will be crucial. Operational research should be conducted to identify challenges in reaching all age groups with deworming programs and achieving high coverage and compliance. Such research will help to inform the development of policies and guidelines.

Expanding deworming programs community-wide will increase the selective pressure on parasites and thus increase the likelihood of drug resistance emerging [48]. Mathematical modelling could be used to explore projected timeframes for the emergence of drug resistance in the context of different deworming approaches and treatment coverage levels, and compare these to projected timeframes for achieving transmission interruption. Additionally, given the findings from Chapter 2 regarding the use of drug combinations for STH control, modelling approaches could be used to explore the impact of delivering drug combinations community-wide on STH transmission and the risk of resistance.

To progress towards the goal of community-wide deworming, mirroring the earlier discussion regarding the use of drug combinations, important priorities are to determine the additional number of drug doses required to treat all age groups in endemic areas, and liaise with pharmaceutical companies regarding drug production and procurement. In addition, updated WHO guidelines for implementing and monitoring community-wide deworming programs should be developed. Particularly important priorities, not addressed in the most recent preventive chemotherapy guideline [11], are to determine updated, validated prevalence and/or intensity thresholds at which preventive chemotherapy should be decreased in frequency or stopped; to provide specific guidance for monitoring and evaluation, both during preventive chemotherapy and after its cessation; and to determine thresholds for re-commencing preventive chemotherapy. Such guidance will help to address current deficiencies in parasitological monitoring in endemic countries [1].

To this end, a crucial matter presently facing the WHO and NTD policymakers is the goal of STH control efforts post 2020. Current goals and guidelines focus on deworming of high-risk groups to achieve morbidity control and the arbitrarily defined elimination of STH as a public health problem [11, 32, 49]. However, evidence for the feasibility of STH transmission interruption is growing [36, 50], and a robust framework for identifying priority countries that are in the best position to achieve transmission elimination has been proposed [50]. Thus, the WHO and its partners must give serious consideration to changing the long-term goal of STH control programs to transmission elimination, and supporting priority countries to work towards this goal, starting with national and regional feasibility assessments that include detailed mapping of STH transmission. Additionally, accepted, evidence-based, parasitological definitions of transmission interruption and re-emergence (i.e., in terms of prevalence levels or mean infection intensity) that can be applied to STH control guidelines will be required.

7.1.3 WASH and STH control

Chapter 5 presented two papers reporting results from the WASH for WORMS study, the first cluster RCT examining the impact of a community-based integrated WASH intervention on STH infections. WASH for WORMS was conducted in a highly endemic setting (rural Timor-Leste) in the context of

increasing calls for collaboration between the NTD and WASH sectors to achieve sustainable control of STH [4, 51, 52], and limited, inconsistent experimental evidence for the impact of WASH interventions on STH control [53].

Experimental results of the WASH for WORMS trial showed that, in the context of biannual community-wide deworming in both study arms, the integrated WASH program had no additional impact on STH prevalence, intensity, or morbidity outcomes. Important findings in terms of process evaluation were that there was persistent open defecation in intervention communities (up to 40% of participants at the end of the trial), and that latrine coverage decreased throughout study follow-up after an initial increase following the sanitation intervention. Additionally, despite receiving no study intervention beyond deworming, WASH coverage gradually improved in the control arm over time.

A risk factor analysis was conducted using individual- and household-level data on WASH access and behaviours collected in the WASH for WORMS trial, in an attempt to disentangle the impact of discrete WASH variables and identify priority areas for future research and control efforts. Results of this analysis also showed limited evidence of associations between WASH variables and STH infections. Study follow-up round (reflecting the number of deworming rounds), socioeconomic factors, and demographic variables were most strongly associated with STH infections. There was some evidence that household water source and handwashing behaviour were associated with STH infection risk; however, these findings were not consistent across STH species, and overall, few WASH-related associations were identified. This was likely due to a combination of factors, including the significant impact of regular deworming on STH prevalence, persistent open defecation leading to ongoing environmental exposure for all community members, and difficulties in accurately measuring hygiene behaviours.

The findings presented in Chapter 5 highlight a number of challenges involved in conducting WASH research, including achieving and sustaining high intervention adherence, and accurately measuring WASH behaviours. Findings from both the experimental and observational analyses confirm that deworming is a potent driver of reductions in STH prevalence and intensity; in this context, identifying an additional impact of WASH may not be possible in many settings. Indeed, mathematical modelling suggests that in the context of regular deworming, community-level WASH interventions will have minimal additional impact on STH infection levels in the short term (i.e., within three years) [54]. Other studies of community sanitation interventions have similarly failed to demonstrate an impact on STH [55–57]. The recent WASH Benefits trial, that did not include a deworming component, found some evidence that a combined WASH intervention reduced STH transmission; however, this was seen for *A. lumbricoides* only, and surprisingly appeared to be driven by improved household water quality, with no impact seen from sanitation-only or handwashing-only interventions [58].

Despite challenges, ongoing research to inform WASH and NTD policymakers must remain a priority. Identifying the types of WASH interventions that will facilitate improved STH control, and the required duration and coverage thresholds of those interventions in different transmission settings, remain vital research questions. In Chapter 5, it was seen that a sanitation intervention with 40% residual open defecation after two years had no additional impact on STH prevalence or intensity; however, it is possible that interventions achieving higher coverage over a longer duration may reduce transmission and reinfection. Mathematical modelling has shown that in the long term, STH transmission interruption cannot be achieved in many settings without WASH improvements [34, 59], and that sanitation and hygiene interventions dramatically reduce the risk of transmission re-emergence following cessation of preventive chemotherapy [54]. These findings should be further tested in field-based trials.

A specific question that should be investigated in RCTs is the role of point-of-use water treatment in STH control. Chlorine treatment of household drinking water reduced *A. lumbricoides* prevalence in the WASH Benefits study [58], and the risk factor analysis presented in Chapter 5 showed some evidence for a role of contaminated water in STH transmission. These findings warrant further investigation in other settings. On the other hand, given that RCTs are expensive, time-consuming and logistically challenging, further detailed risk factor analyses are also required to establish priorities for WASH programs tailored towards improving STH control. Identifying improved mechanisms for measuring WASH access and behaviour is an important research priority [60, 61]. This will allow observational studies to more accurately deduce relationships between individual WASH behaviours and risk of STH infection, and also enable improved monitoring of WASH in interventions studies. The development and validation of novel mechanisms for measuring STH environmental contamination [62, 63] provides an indirect, but more objective, measure of behaviour change and may benefit future studies examining the impact of WASH on STH transmission.

The findings presented in Chapter 5 should not be interpreted by WASH and NTD policymakers that WASH interventions are irrelevant to STH control. On the contrary, primary prevention remains a crucial aspect of NTD control efforts [32], and for STH, this involves reducing environmental contamination and transmission through improved sanitation and hygiene [53]. The role of WASH in STH transmission is irrefutable based on the biology of STH lifecycles. The complexities in generating evidence for the role of WASH in STH control must not discourage collaboration between WASH and NTD policymakers and program implementers. WASH interventions may require long-term investment to achieve a detectable impact on STH infections. Such investment, including close collaboration and joint efforts between policymakers and program implementers in the WASH and NTD sectors, is vital for continuing STH control efforts.

As a first step, joint monitoring between the WASH and NTD sectors could encourage future collaboration by improving information sharing and facilitating cross-sectoral planning [64]. A set of basic WASH indicators that could be incorporated into NTD program monitoring was proposed in 2015, including several specific to STH [65]. Including WASH indicators on the London Declaration on NTDs scorecard has been suggested as an important step towards integration of goals and targets [1, 66], as has dedicating proportions of NTD program budgets towards joint planning and monitoring with the WASH sector [67]. Narrowing the focus to STH control, crucial steps forward include incorporating specific WASH guidance into STH strategic plans and operational guidelines [1, 4, 68] and developing country-specific STH control programs that incorporate and support the efforts of the WASH sector [68].

7.1.4 qPCR for STH diagnosis

Finally, in Chapter 6, the first quantitative comparison between qPCR and sodium nitrate flotation for STH diagnosis was presented, along with the first examination of variability in DNA detection from an STH-positive stool sample over time. qPCR is a highly sensitive technique that is commonly used in diagnostics for a wide range of infectious diseases; for STH, its use is predominantly limited to the research sector [69]. This study demonstrated that qPCR is more sensitive than sodium nitrate flotation for the diagnosis of *Ascaris* spp. and hookworm, although surprisingly, not for *Trichuris* spp. It also showed, for the first time, a correlation between measures of infection intensity obtained using qPCR and sodium nitrate flotation, providing further evidence for the validity of qPCR-determined infection intensity measurements. Finally, when stool samples were preserved in 5% potassium dichromate at room temperature, the amount of DNA detected using qPCR increased substantively within the first month, likely due to the embryonation of STH eggs in the sample. Thereafter, DNA detection remained stable for at least six months. These findings highlight the importance of the developmental stage of STH ova when interpreting infection intensity results obtained using qPCR.

The results presented in Chapter 6 contribute to the evidence base documenting the high sensitivity of qPCR in detecting STH infections, and provide new evidence supporting its potential to be used as a quantitative technique to determine infection intensity. However, there are a number of important priorities for ongoing research. Firstly, a more sensitive PCR assay for *T. trichiura* may be required, given that findings in Chapter 6 showed that qPCR had a lower sensitivity for detecting this parasite compared to *Ascaris* spp. and hookworm. In recent years, a number of qPCR assays for STH have been developed [70–75], and only a small number of these have been directly compared [74]. A thorough comparison of available assays is warranted in order to optimise qPCR performance. To this end, an international external quality assessment scheme known as HEMQAS (Helminth External Molecular Quality Assessment Scheme) has recently been developed and piloted by SKML (Dutch Foundation for Quality

Assessment in Medical Laboratories) [76, 77]. This scheme distributes preserved stool specimens to facilitate comparison of qPCR performance between laboratories, and will be available annually starting in 2019 [77].

Future work is needed to determine reliable methods to convert qPCR-determined infection intensity into standard measures of infection intensity (i.e., eggs per gram of faeces) such that it can be applied to existing and future WHO cut-offs and targets for STH control. qPCR has been shown to determine egg intensity more precisely than microscopy-based techniques [72, 78], and Ct values obtained using qPCR correlate extremely strongly with the number of eggs in samples seeded with known quantities of STH ova [72, 79]. However, as shown in Chapter 6 and in other recent work, correlations between qPCR-determined Ct values and microscopy-determined eggs per gram of faeces using field samples, although statistically significant, are somewhat less strong [72]. Furthermore, results presented in Chapter 6 show that the degree of egg embryonation in samples can confound infection intensity measured by qPCR. Therefore, converting Ct values obtained from field samples into eggs per gram of faeces remains an important challenge.

The high sensitivity of qPCR makes it particularly relevant in settings approaching transmission interruption, where the detection of light-intensity infections is crucial [80, 81]. Earlier in this chapter, the need to determine and test parasitological thresholds for transmission interruption and re-emergence was discussed. Given its higher sensitivity, qPCR will be able to more accurately determine transmission interruption compared to the Kato-Katz technique [81]. There is currently no accepted definition of STH transmission interruption; however, mathematical modelling has recently been used to explore this issue [80–83]. One proposed definition for transmission elimination is community-level prevalence (of a given STH species) below 2% using qPCR, two years after cessation of preventive chemotherapy [42, 82]. Further research is required to test this in field-based studies over time.

An additional research priority is examining the cost-effectiveness of using qPCR for monitoring STH control in various transmission settings; it has been suggested that more sensitive diagnostic tests may be more cost-effective than the Kato-Katz technique in the long term [84]. Lastly, an important potential application of qPCR that warrants further investigation is monitoring anthelminthic efficacy and detecting anthelminthic resistance, the importance of which has been discussed throughout this thesis. This is currently being investigated in the Starworms project, as part of its objective to strengthen the monitoring and surveillance of drug efficacy and anthelmintic resistance in STH control programs [85].

The policy recommendation regarding qPCR emerging from this thesis is that qPCR should ultimately be used for monitoring the impact of STH control programs in settings approaching transmission elimination. In addition to the addressing the remaining evidence gaps mentioned above, achieving this will require the development and validation of clear WHO guidelines and protocols for the use of qPCR

in monitoring and evaluation, and provision of support for endemic countries to introduce and implement qPCR technology for STH diagnosis on a large scale.

7.1.5 Operational and economic implications

A common element to the key policy recommendations in this thesis—adding drug combinations to STH control programs, expanding deworming programs to all community members, increased collaboration with the WASH sector, and applying novel diagnostic techniques—is that they have significant operational and economic implications for NTD control program implementers and donors.

A substantial increase in the number of drug doses would be required both to extend preventive chemotherapy programs community-wide, and to incorporate drug combinations into STH control efforts. Existing control efforts depend strongly on established drug donation programs; however, it has recently been highlighted that this approach is unlikely to be sustainable and that generic deworming drugs are likely to become increasingly important [1]. Assuming sufficient quantities could be manufactured and obtained by endemic countries (either through securing additional donations or purchasing generic drugs), delivering drug combinations in existing preventive chemotherapy programs would likely be feasible. On the other hand, conducting community-wide deworming could prove challenging for NTD control programs, which already operate with limited resources. However, the successful implementation of community-wide preventive chemotherapy programs for LF and onchocerciasis control in many countries shows that this approach is both feasible and effective [18, 86, 87]. In countries where they exist, these platforms could be leveraged for the community-wide expansion of STH control programs [2].

Similarly, conducting molecular diagnosis of stool samples on a large scale may be prohibitive in low-income settings in terms of cost, human resources, and logistics of transporting samples. That said, a number of low-income countries are already undertaking PCR-based diagnostics for other infectious diseases in central laboratories [71], and could use existing equipment and training as a starting point for introducing qPCR for STH diagnosis. Careful recommendations regarding circumstances in which qPCR should be utilised could help to reduce financial and logistical burdens.

The costs for STH control programs as a result of increased collaboration with the WASH sector are less clear [68], and could vary considerably depending on the scale of existing WASH programs operating in a given country or district. WASH programs are extremely costly to implement compared to preventive chemotherapy programs for STH control [53]; however, most countries where STH are endemic have government and/or NGO-led WASH programs in place, with funding principally provided by national governments and bilateral donors [52]. The costs to STH control programs of integration with WASH

efforts will likely relate mostly to joint planning and monitoring, rather than the actual implementation of WASH improvements [67].

Although the immediate barriers in terms of costs and logistics are significant, economic analyses suggest that in the long run, strategies that enhance the ability to interrupt STH transmission, including community-wide deworming and using more sensitive diagnostic techniques, will improve cost-effectiveness due to programmatic benefits [40, 41, 84]. This is in addition to the health benefits for NTD-afflicted populations and the economic benefits that flow from improved health. It is also likely that integrating approaches to improving WASH and controlling NTDs will enhance overall cost-effectiveness of these programs [68]. Furthermore, benefits of improved WASH extend far beyond potential impacts on STH control [88], and even beyond health impacts [89]; indeed, access to safe water and sanitation is considered a basic human right [90]. All this considered, there is a strong impetus for the WHO and program donors to support endemic countries in overcoming the logistical, financial, and other barriers involved in implementing the recommendations that have been detailed in this chapter for optimising STH control programs.

7.2 Strengths & limitations

Strengths and limitations of the individual research papers forming this thesis have been detailed in the discussion sections of each paper; a brief overview of the major strengths and limitations of the thesis is provided here.

This thesis employed a number of different study designs and robust statistical methods to address research questions that are highly relevant for NTD researchers and policymakers. A key strength is that for several research questions, multiple study types were used to address the underlying hypothesis. The impact of community-wide deworming was assessed using both meta-analysis and a field study, while the role of WASH in STH control was examined using both an RCT and an observational risk factor analysis.

The rigorous conduct of the systematic reviews presented in Chapters 2 and 3, including meta-analysis using novel, evidence-based statistical techniques, represents a major strength. The systematic reviews adhered to best practice (PRISMA) guidelines, used comprehensive search strategies, and did not restrict publications by year or language. Using a network meta-analytic approach in Chapter 2 facilitated the inclusion of a much broader evidence base and allowed for a comprehensive comparison of available treatments. In Chapter 3, the use of a generalised linear model allowed adjustment for a number of important covariates that, due to heterogeneity in included studies, could not be addressed using conventional meta-analysis. Both meta-analyses used the “inverse variance heterogeneity” model

to assign study weights, a novel approach that has been shown to perform better than the more conventionally employed random effects model [91].

On the other hand, an important limitation of both meta-analyses is heterogeneity among studies in terms of residual confounders, which may decrease the precision of effect estimates. For example, in Chapter 2, studies varied in terms of diagnostic technique, calculation of mean infection intensity (arithmetic vs geometric mean), and timing of efficacy assessment. In Chapter 3, there was heterogeneity between included studies in terms of treatment coverage, diagnostic technique, WASH access, and socioeconomic factors. These issues were addressed using sensitivity analyses where sufficient data were available; however, this was not possible for all identified confounders, and other unmeasured confounders may also have impacted study results.

A particular strength of the community-based studies presented in Chapters 4 and 5 was that they both involved a period of community engagement prior to study commencement. As a result, and with ongoing efforts to maintain engagement during follow-up, participation rates remained high throughout both studies. Furthermore, these studies involved collaborations with established WASH agencies, which had been operating in the WASH sector in rural Timor-Leste for many years and employed local staff to implement the study WASH interventions. This ensured that the interventions were culturally appropriate and tailored to the local context, and, importantly, amenable to implementation outside a trial context. The study findings are therefore highly relevant to WASH implementers and policymakers in Timor-Leste.

The WASH for WORMS study was a rigorously designed cluster RCT of over 2000 individuals, and the first in which an integrated community-based intervention including all individual WASH components (water, sanitation, and hygiene) was implemented. However, as detailed in Paper 5, the WASH intervention—particularly the sanitation component—did not achieve sustained behaviour change among a proportion of community members, which rendered it difficult to detect an impact of WASH on STH. An underestimation of the intra-cluster correlation coefficient (ICC) for *Ascaris* spp. also meant that the study was underpowered to detect even an ambitious 50% difference in prevalence reduction between study arms. The (S)WASH-D for Worms pilot study, by design, also had limited statistical power; as such the detected difference between study arms did not reach significance. Further, due to logistical constraints, communities could not be randomised in the pilot study, which led to several imbalances between study arms, as detailed in Paper 4. Finally, in both the WASH for WORMS and the (S)WASH-D for Worms studies, data regarding WASH access and behaviours were mostly collected through self-report, representing an important potential source of bias. This is discussed in detail in the risk factor analysis presented in Chapter 5.

7.3 Conclusion

Soil-transmitted helminths represent one of the most prevalent infectious diseases worldwide and, along with the other neglected tropical diseases, disproportionately affect the most vulnerable members of society. Over the past decade, significant investments and concerted, collaborative efforts across a range of stakeholders have led to unprecedented achievements in STH control. However, substantial work remains to be done. The World Health Organization and NTD policymakers currently have a vital opportunity to address key issues in STH control, as program targets and guidelines beyond 2020 are discussed and determined. This opportunity must not be wasted; a thorough revision of the increasing evidence base for expanding control strategies beyond the current guidelines must be undertaken. The research presented in this thesis has identified a number of ways in which existing STH control programs could be optimised. It has provided recommendations for policymakers in the NTD sector and identified key areas in which further research is required. The impetus is on researchers and policymakers to sustain global momentum towards controlling STH, and to ensure that guidelines and policies for STH control reflect approaches that will achieve maximal benefits for the hundreds of millions of people infected with STH, and the many more at risk of infection.

7.4 References

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Appendices

The following appendices contain supplementary material associated with each of the published papers presented in the main body of this thesis. A number of additional published works that are relevant to this thesis but do not form part of it are also presented.

Appendix 1
Supplementary material for Paper 1

The following information was published as an online supplement to Paper 1:

Clarke NE, Doi SAR, Wangdi K, Chen Y, Clements ACA, Nery SV. Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis. *Clin Infect Dis* 2019; 68(1): 96–105. http://doi.org/10.1093/cid/ciy423

The material formed part of the manuscript submission and was subjected to peer review.

SUPPLEMENTARY MATERIAL: TABLE OF CONTENTS

Appendix 1. Search strategy: MEDLINE (Ovid)

1. Ascaris/
2. Ascaris lumbricoides/
3. Ascariasis/
4. ascaris.mp.
5. roundworm.mp.
6. Hookworm Infections/
7. Necator/
8. Necator americanus/
9. Ancylostomatoidea/
10. hookworm.mp.
11. necator.mp.
12. ancylostoma.mp.
13. Trichuriasis/
14. Trichuris/
15. trichuris.mp.
16. whipworm.mp.
17. soil-transmitted helminth.mp.
18. (1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17)
19. Albendazole/
20. albendazole.mp.
21. Mebendazole/
22. mebendazole.mp.
23. Pyrantel/
24. Pyrantel Pamoate/
25. pyrantel.mp.
26. Ivermectin/
27. ivermectin.mp.
28. Praziquantel/
29. praziquantel.mp.
30. Levamisole/
31. levamisole.mp.
32. oxantel.mp.
33. nitazoxanide.mp.
34. tribendimidine.mp.
35. Benzimidazoles/
36. benzimidazoles.mp.
37. (19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36)
38. Therapeutics/
39. treatment.mp.
40. efficacy.mp.
41. reinfection.mp.
42. re-infection.mp.
43. cure rate.mp.
44. (38 or 39 or 40 or 41 or 42 or 43)
45. 18 and 37 and 44

Appendix 2. Quality assessment scale

Design bias	
1.	What was the type of design?
	a) randomized and allocation concealed (2 points)
	b) randomized only (1 point)
	c) quasi-experimental (0 points)
2.	Was the method used to generate the sequence of randomization described and appropriate? (1 point)
Selection bias	
3.	Did the inclusion/exclusion criteria remain consistent across the comparison groups of the study? (1 point)
4.	Was the strategy for recruitment into the study the same across comparison groups? (1 point)
5.	Was the interval between the start of intervention and outcome the same across comparison groups? (1 point)
Information bias	
6.	Were the outcome assessors blinded to the nature of intervention or control? (1 point)
7.	Were interventions implemented in the same way across study groups? (1 point)
Confounding bias	
8.	Were the groups similar at baseline in key confounding variables namely:
	8.1 Age (1 point)
	8.2 Sex (1 point)
	8.3 STH infection intensity (1 point)
Analytical bias	
9.	Were effect sizes based on the data available at follow-up, rather than a post hoc portion of the data? (1 point)

Appendix 3. MCMC procedure

The difference in ERR based on mean egg counts was used as the effect size in this network meta-analysis, with the difference given as:

$$diff\_ERR = \left(1 - \frac{post\overline{EC_A}}{pre\overline{EC_A}}\right) - \left(1 - \frac{post\overline{EC_B}}{pre\overline{EC_B}}\right)$$

We computed the standard error of this effect size using a Markov Chain Monte Carlo (MCMC) method as follows. We assumed that the egg counts (EC) pre and post for individuals within study followed a Polya distribution (to account for overdispersion), with the expected value as the arithmetic mean egg count. This is given by:

$$\approx Polya\left(\alpha\beta, \alpha\beta(1+\beta)\right), \qquad (1)$$

where

$$\alpha = \overline{EC}/2 \quad \text{and} \quad \beta = 2 \ .$$

When only the geometric mean was available this was used in lieu of the arithmetic mean and represents a limitation of this approach. However, we conducted a sensitivity analysis excluding all geometric means (see results).

We then sampled 1000 times from the distribution in (1) using a Markov Chain Monte Carlo (MCMC) method for both pre and post mean egg counts in each study arm. A continuity correction of 0.5 was added to each pair of estimates from each arm. The standard error (σ) was computed from the standard deviation of the 1000 sampled estimates divided by the square root of sample size for pre and post egg counts in each arm. We were then able to determine the sampling distribution of the mean EC as follows:

$$\overline{EC} \approx N(\overline{EC}, \sigma^2) \qquad (2)$$

We again sampled 1000 times from the distribution in (2) using a MCMC method for both pre and post mean egg counts in each study arm using the Ersatz software implementation (Epigear International, Noosa, Queensland, Australia) and from results in each iteration computed the ERR and its MCMC based standard error as well as the standard error for the difference in ERR given by the square root of the sum of the ERR variances.

Supplementary Table 1. Studies included in systematic review

						Ascaris lumbricoides		Hookworm		*Trichuris trichiura*	
Author & year	**Reference**	**Study location**	**Study design***	**Diagnostic method(s)†**	**Treatments included in network§**	**Cured/ treated**	**Mean EPG pre/ post treatment**	**Cured/ treated**	**Mean EPG pre/ post treatment**	**Cured/ treated**	**Mean EPG pre/ post treatment**
Abadi, 1985	[1]	Indonesia	QE	Kato-Katz, Harada-Mori	MEB single-dose Placebo	57/61 0/44	215333/2051 249643/234790	41/45 0/43	5322/87 5296/4583	52/67 0/38	34425/2445 21875/19552
Adams et al, 2004	[2]	South Africa	RCT	Other	ALB single-dose ALB multiple-dose	-	-	-	-	8/31 21/39	961/35.9 973/6
Adegnika et al, 2014	[3]	Gabon	RCT	Kato-Katz	ALB single-dose ALB multiple-dose	33/39 34/37	4794/188 4734/180	7/13 14/15	317/360 305/80	20/50 44/53	700/352 415/280
Adugna et al, 2007	[4]	Ethiopia	RCT	Kato-Katz	ALB single-dose MEB multiple-dose	52/62 48/53	45.8/1.7 41.8/1.4	112/133 91/109	29.9/1.5 29.4/1.5	-	-
Albonico et al, 1994	[5]	Tanzania	RCT	Kato-Katz	ALB single-dose MEB single-dose	809/818 714/730	239/0.05 164/0.08	595/1048 226/1011	391/8 423/73	120/1138 156/1095	655/174 658/120
Albonico et al, 2002	[6]	Tanzania	RCT	Kato-Katz	MEB single-dose OX+PP single-dose	105/107 96/100	5/0.1 5/0.1	56/424 56/411	588/193 571/182	102/404 146/382	257/41 213/27
Albonico et al, 2003	[7]	Tanzania	RCT	Kato-Katz	MEB single-dose Placebo	136/141 31/138	114/0.2 96/63	17/224 8/233	447/213 514/432	49/214 11/227	307/52 484/395
Amato Neto et al, 1976	[8]	Brazil	RCT	Willis flotation, Stoll	OX+PP single-dose Placebo	-	-	-	-	18/24 1/26	-
Amato Neto et al, 1983	[9]	Brazil	QE	Willis flotation, Stoll	ALB single-dose MEB multiple-dose	31/34 27/30	-	16/29 11/25	-	15/39 25/35	-
Anonymous, 1983	[10]	China	Not stated	Brine flotation, other	MEB multiple-dose Placebo	-	-	-	-	10/46 0/52	1463/204 638/612
Balasuriya et al, 1990	[11]	Sri Lanka	QE	Kato-Katz	MEB single-dose MEB multiple-dose	61/69 90/95	5849/575 10054/104	13/68 55/72	308/166 397/56	17/91 79/100	232/117 825/38
Bartoloni et al, 1993	[12]	Bolivia	QE	Kato-Katz	ALB single-dose MEB single-dose	6/6 11/11	1900/0 5661.8/0	27/33 5/29	790.9/56.9 1291/485.4	5/15 6/10	148/80.4 348/295.8
Bassily et al, 1984	[13]	Egypt	Not stated	Stoll	ALB single-dose ALB multiple-dose	13/14 12/12	515/25.8 530/0	30/35 31/32	519/109 407/24.4	-	-
Beach et al, 1999	[14]	Haiti	RCT	Stoll	ALB single-dose ALB+IVM single-dose IVM single-dose Placebo	-	284/0 334/0 427/0 352/236.2	-	74/0 67/0 80/6.6 89/45	-	120/69.4 120/38.4 121/69.3 144/100.4
Belizario et al, 2003	[15]	Philippines	RCT	Kato-Katz	ALB single-dose ALB+IVM single-dose IVM single-dose ALB+DEC single-dose	69/96 82/102 80/99 93/119	21656.0/1520.2 41011.4/198.8 36485.5/2072.7 32821.4/1113.3	-	-	54/151 97/146 47/145 30/153	6376.2/2930.8 4948.1/122.5 6340.3/833.9 7544.2/1557.6
Bwibo et al, 1984	[16]	Kenya	RCT	Kato-Katz, McMaster	ALB single-dose Placebo	36/40 5/36	-	30/34 6/34	-	26/31 8/25	-
Cabrera et al, 1980a	[17]	Philippines	RCT	Kato-Katz	MEB single-dose OX+PP single-dose	48/51 57/60	-	15/17 16/22	-	30/66 5/65	-
Cabrera et al, 1980b	[18]	Philippines	QE	Formalin-ether	MEB single-dose MEB multiple-dose	22/28 23/23	-	11/20 11/15	-	21/27 21/27	-

Cabrera et al, 1980c	[19]	Philippines	RCT	Formalin-either	OX+PP single-dose Multiple-dose OX+PP	-	-	-	-	26/31 32/34	3169.3/96.8 2281.2/31.2
Cao et al, 2000	[20]	China	RCT	Kato-Katz, brine flotation	ALB single-dose TRI single-dose	-	-	59/89 81/94	1210/24 1441/15	-	-
Chaia et al, 1971	[21]	Brazil	QE	Stoll, other	MEB multiple-dose Placebo	51/51 0/19	-	25/30 0/33	-	38/43 0/18	-
Charoenlarp et al, 1993	[22]	Thailand	RCT	Kato-Katz, formalin-ether	MEB single-dose Placebo	-	-	4/130 1/127	-	27/71 13/70	-
Chien et al, 1989	[23]	Malaysia	RCT	Direct smear	ALB single-dose Placebo	37/41 12/41	-	34/41 18/41	-	2/41 1/41	-
Choi et al, 1979	[24]	Korea	QE	Stoll	OX+PP single-dose PP single-dose	24/24 18/18	11708/0 5977/0	-	-	-	-
					OX single-dose OX+PP single-dose	-	-	-	-	42/49 18/24	912/63 2758/58
Cruz, 1984	[25]	Mexico	RCT	Stoll	ALB single-dose MEB single-dose	23/26 28/32	33276/47 36536/69	-	-	9/26 24/24	815/158 729/0
De Clercq et al, 1997	[26]	Mali	RCT	Kato-Katz	MEB single-dose Placebo	-	-	8/35 7/31	264.2/281.5 327.9/220.6	-	-
De Silva et al, 1987	[27]	Sri Lanka	QE	Direct smear	OX+PP single-dose MEB single-dose	43/46 20/21	169174/2000 42333/286	10/14 4/6	4428/27 2500/333	9/43 4/14	16581/4907 7071/2214
Dissainaike, 1978	[28]	Malaysia	RCT	Beaver	MEB multiple-dose OX+PP multiple-dose	22/22 21/21	64574/0 54971/0	6/16 10/17	2029/208 2212/141	13/25 16/24	6472/572 7650/422
El-Masry et al, 1983	[29]	Egypt	RCT	Stoll	ALB single-dose Placebo	11/11 0/7	-	17/19 1/23	-	-	-
Excler et al, 1981	[30]	Morocco	RCT	Kato-Katz	ALB single-dose Placebo	-	-	28/30 0/35	-	-	-
Fan et al, 1992	[31]	Taiwan	QE	Formalin-ether, other	MEB single-dose MEB multiple-dose	37/41 17/17	-	17/19 18/19	-	62/68 47/48	-
Fang et al, 2002	[32]	China	RCT	Kato-Katz	TRI single-dose ALB single-dose		-	46/50 37/50	1028/2.0 1336/15.0	-	-
Farahmandian et al, 1972	[33]	Iran	QE	Willis flotation, Stoll	PP single-dose Untreated controls	65/72 5/50	15364/3380.1 15310/13518.7	72/75 8/50	1900/144.4 1661/1239.1	-	-
Farahmandian et al, 1977	[34]	Iran	RCT	Stoll	MEB multiple-dose PP single-dose	51/54 47/48	4516.7/400.2 3194.9/300.3	19/54 43/48	566.7/335.3 580.6/180	-	-
Farid et al, 1977	[35]	Egypt	QE	Stoll	LEV single-dose PP single-dose	20/20 18/20	-	38/41 28/36	-	-	-
Farid et al, 1984	[36]	Egypt	RCT	Stoll	ALB single-dose ALB multiple-dose	13/14 12/12	-	30/35 31/32	-	-	-
Flohr et al, 2007	[37]	Vietnam	RCT	McMaster	MEB single-dose Placebo	-	-	34/90 26/78	263/126 306/181	-	-
					ALB single-dose ALB multiple-dose MEB multiple-dose Placebo	-	-	21/47 34/43 13/50 18/51	1120/230 1173/109 2210/342 1068/930	-	-
Fox et al, 2005	[38]	Haiti	RCT	Stoll	ALB single-dose ALB+DEC single-dose	-	535/1.1 564/39.5	-	66/11.4 83/0.7	-	134/94.9 160/117.1
Gan et al, 1994	[39]	China	Not stated	Other	ALB single-dose ALB multiple-dose	60/60 487/488	23720/0 19377/0	65/76 99/105	-	24/76 371/486	445/45.4 1365/86
Ghadirian et al, 1972	[40]	Iran	QE	Willis flotation, Stoll	PP single-dose Untreated controls	-	-	30/40 0/20	-	-	-

Griffin et al, 1982	[41]	Kenya	QE	Formalin-ether, McMaster	MEB multiple-dose PP single-dose	-	-	12/12 8/11	-	-	-
Hadju et al, 1996	[42]	Indonesia	RCT	Kato-Katz	PP single-dose Placebo	-	27566/908 25375/12264	-	-	-	-
Holzer et al, 1987	[43]	Switzerland	QE	MIF concentration	ALB single-dose MEB single-dose	2/2 6/6	-	41/43 10/45	-	19/21 26/28	-
Ismail et al, 1991	[44]	Sri Lanka	RCT	Kato-Katz	ALB single-dose LEV single-dose MEB single-dose PP single-dose	65/68 63/73 75/77 64/68	19383/54 15045/613 12974/126 11469/119	7/7 7/8 9/10 9/10	283/0 136/12 425/2 642/9	27/85 16/89 34/94 19/84	2561/315 1633/442 2057/442 1415/653
Ismail et al, 1999	[45]	Sri Lanka	RCT	Kato-Katz	ALB+IVM single-dose ALB+DEC single-dose	-	-	-	-	43/53 14/47	1544/78.7 1652.6/195.7
Jongsuksuntigul et al, 1993	[46]	Thailand	RCT	Kato-Katz	ALB single-dose MEB single-dose	13/13 17/17	3710/0 20985.5/0	43/51 16/53	3177.2/127.6 2280.5/674.8	29/43 26/37	415.1/54 719.8/72.7
Kale et al, 1982	[47]	Nigeria	RCT	Stoll	PP single-dose Placebo	42/45 3/50	-	25/45 6/46	1298/296 1020/1100	14/37 12/37	-
Kan, 1984	[48]	Malaysia	QE	Kato-Katz	ALB single-dose OX+PP single-dose	-	-	-	-	11/33 33/50	750/91.5 375/37.5
Karyadi et al, 1996	[49]	Indonesia	QE	Kato-Katz	ALB single-dose Placebo	-	2539/0 2410/1893	-	-	-	163/116 161/123
Katz et al, 1972	[50]	Brazil	QE	Kato-Katz, Stoll, other	PP single-dose Untreated controls	35/35 3/30	7265/0 10326/10127	-	-	5/28 5/33	450/277 745/1013
Kilpatrick et al, 1981	[51]	Egypt	Not stated	MIF concentration	LEV single-dose MEB multiple-dose	7/7 10/10	-	25/25 16/25	-	-	-
Klein, 1972	[52]	Germany	QE	Other	MEB multiple-dose Placebo	-	-	-	-	8/8 0/11	-
Knopp et al, 2010	[53]	Tanzania	RCT	Kato-Katz	ALB single-dose ALB+IVM single-dose MEB single-dose MEB+IVM single-dose	14/14 13/14 14/18 18/18	2680/0 1699/0 2414/5 553/0	23/39 20/30 12/34 9/35	67/4 74/3 61/13 58/29	13/132 53/140 26/138 76/138	154/92 127/11 146/49 160/5
Krepel et al, 1993	[54]	Togo	QE	Other	ALB single-dose PP single-dose	-	-	41/67 30/95	-	-	-
Lara-Aguilera et al, 1984	[55]	Mexico	Not stated	Kato-Katz, Harada-Mori	ALB single-dose ALB multiple-dose	54/57 23/24		-	-	38/57 22/24	-
Lee et al, 1978	[56]	Korea	QE	Kato-Katz, Stoll	MEB multiple-dose OX+PP single-dose OX+PP multiple-dose	20/20 10/10 -	-	-	-	- 7/10 9/10	-
Legesse et al, 2002	[57]	Ethiopia	RCT	Kato-Katz	ALB single-dose MEB multiple-dose	232/234 147/153	1843.3/1 668.9/0.3	-	-	34/244 66/190	291/105.9 354.4/26.3
Legesse et al, 2004	[58]	Ethiopia	RCT	Kato-Katz	ALB single-dose MEB multiple-dose	99/107 109/113	6982.2/305.1 8188.9/1833.2	-	-	106/138 106/118	445.7/258 414.1/91.4
Lim et al, 1987	[59]	Korea	QE	Stoll	OX single-dose OX+PP single-dose	1/43 73/75	-	-	-	45/50 58/75	-
Ma et al, 2001	[60]	China	RCT	Kato-Katz	ALB multiple-dose MEB multiple-dose	31/33 27/29	650.5/39.4 440.8/1.4	-	-	71/98 104/107	68.6/2.3 76.5/1.2
Mawdsley et al, 1975	[61]	Mauritius	RCT	Formalin-ether	PP single-dose Placebo	-	-	7/26 0/10	-	-	-
Mekonnen et al, 2013	[62]	Ethiopia	RCT	McMaster	ALB single-dose MEB single-dose ALB multiple-dose MEB multiple-dose	-	-	-	-	-	1193/843.5 1075/430 1262/334.4 1122/144.7

Miller et al, 1978	[63]	USA	QE	Beaver	LEV single-dose PP single-dose	26/26 16/17	37200/0 54100/5400	-	-	-	-
Morgan et al, 1983	[64]	Malawi	RCT	Kato-Katz	LEV single-dose Placebo	-	-	3/30 0/25	571.2/206.4 472.8/494.4	-	-
Moser et al, 2015	[65]	Tanzania	RCT	Kato-Katz	OX single-dose Placebo	7/25 3/20	2172/331 2781/1368	6/18 3/14	75/17 89/51	11/49 0/49	519/12 611/400
Moser et al, 2017	[66]	Tanzania & Cote d'Ivoire	RCT	Kato-Katz	ALB+OX single-dose TRI single-dose TRI+IVM single-dose TRI+OX single-dose	73/78 71/72 75/76 66/70	4826.2/0.6 2817.2/0.03 4153.7/0.03 2663.4/0.5	71/148 81/151 134/154 77/148	194.6/7.8 183.1/6.1 165.7/0.8 192.1/6.8	82/99 8/97 35/104 63/95	753.9/1.3 771.3/362 642.8/23.1 715/3.8
Muchiri et al, 2001	[67]	Kenya	Cluster-RCT	Kato-Katz	ALB single-dose MEB single-dose	13/14 25/28	3891/3 2736/2	21/27 26/57	130/2 251/22	3/4 6/10	68/3 99/8
Musgrave et al, 1979	[68]	Australia	RCT	Stoll	MEB multiple-dose Placebo	-	-	13/13 0/11	-	36/48 1/60	-
Namwanje et al, 2011	[69]	Uganda	RCT	Kato-Katz	MEB single-dose ALB+MEB single-dose ALB single-dose ALB multiple-dose	-	-	-	-	10/90 41/89 5/83 14/79	191.4/86.4 171.1/11.6 186.4/116.9 194/65.6
Nanivadekar et al, 1984a	[70]	India	RCT	Kato-Katz	MEB multiple-dose PP single-dose	106/120 124/131	-	222/258 248/272	-	31/33 25/43	-
Nanivadekar et al, 1984b	[71]	India	RCT	Kato-Katz	MEB multiple-dose PP single-dose	204/232 198/213	-	-	-	29/30 12/15	-
					LEV single-dose PP single-dose	164/192 183/199	-	-	-	15/19 8/11	-
Ndyomugyenyi et al, 2008	[72]	Uganda	RCT	Kato-Katz	ALB single-dose ALB+IVM single-dose	-	-	170/178 147/188	653.5/237.1 629.5/227.5	4/12 12/17	-
Nokes et al, 1992	[73]	Jamaica	RCT	Kato-Katz	ALB multiple-dose Placebo	-	36012/2 24298/25725	-	64/0 46/23	49/62 1/41	10897/94 8239/8665
Nontasut et al, 1989	[74]	Thailand	RCT	Kato-Katz	ALB single-dose MEB single-dose	-	-	31/48 6/54	1272/17 1460/61	-	-
Nontasut et al, 1997	[75]	Thailand	QE	Kato-Katz	ALB single-dose MEB multiple-dose	15/18 33/33	-	22/24 27/33	-	9/27 31/33	-
Nunez-Fernandez et al, 1989	[76]	Cuba	Not stated	Kato-Katz, Harada-Mori	MEB single-dose MEB multiple-dose	-	-	20/23 9/10	538/56 460/24	45/51 46/48	303/44 229/36
Ortiz et al, 2002	[77]	Peru	RCT	Kato-Katz	ALB single-dose NIT	32/35 25/28	1291/1 1978/1	-	-	16/18 11/19	335/5 412/1
Ovedoff, 1984	[78]	Philippines	RCT	Kato-Katz	ALB single-dose Placebo	16/16 0/12	-	14/15 3/13	-	20/29 2/26	-
Oyediran et al, 1982	[79]	Nigeria	RCT	Kato-Katz	ALB single-dose Placebo	23/27 2/24	-	14/26 4/29	-	11/29 3/30	-
Phuvanandh et al, 1994	[80]	Thailand	QE	Kato-Katz	ALB single-dose MEB single-dose MEB multiple-dose PP single-dose	17/20 25/25 9/9 15/15	-	113/138 16/59 33/35 24/49	-	48/111 36/75 24/32 12/57	-
Pugh et al, 1986	[81]	Malawi	RCT	Kato-Katz	ALB single-dose Placebo	-	-	22/26 0/25	564/28.8 472.8/494.4	-	-
					ALB single-dose MEB multiple-dose	-	-	13/15 10/19	319.8/1.8 481.5/8.8	-	-
Rim et al, 1981	[82]	Korea	RCT	Stoll, formalin-ether	OX+PP single-dose Placebo	29/30 3/31	6336/327 3790/2640	20/21 7/21	193/5 328/127	18/33 3/36	278/40 328/279

Sacko et al, 1999	[83]	Mali	RCT	Kato-Katz	ALB single-dose	-	-	31/37	174.5/4.1	-	-
					MEB single-dose			18/35	185.3/58.3		
					PP single-dose			14/37	158.4/86.3		
					Placebo			6/36	187.8/260.8		
Sargent et al, 1974	[84]	USA	RCT	McMaster	MEB multiple-dose	-	-	-	-	18/29	8888/497.7
					Placebo					0/29	6950/3343
Sargent et al, 1975	[85]	USA	RCT	McMaster	MEB multiple-dose	-	-	-	-	21/32	4160/1268.8
					Placebo					1/10	1720/1840.4
Seo et al, 1983	[86]	Korea	QE	Stoll	MEB single-dose	-	-	-	-	5/12	-
					MEB multiple-dose					34/101	
Silber et al, 2017	[87]	Ethiopia & Rwanda	RCT	Kato-Katz	MEB single-dose	72/86	17610.1/366.7	-	-	42/124	647.8/260.7
					Placebo	9/81	16959.6/13706.7			9/119	584.5/523
Sinniah et al, 1981	[88]	Malaysia	RCT	Beaver	LEV single-dose	59/64	36666/1540.0	22/42	5415/557.7	12/55	5116/1713.9
					PP single-dose	68/71	44600/713.6	11/41	4445/1595.8	20/62	4980/1399.4
					MEB multiple-dose	64/67	35173/351.7	-		-	-
					OX+PP single-dose	82/84	48285/386.3	11/41	2212/659.2	30/63	6870/948.1
					OX+PP multiple-dose	-	-	39/47	6472/634.3	38/48	7652/420.9
Sinniah et al, 1990	[89]	Malaysia	RCT	Beaver	ALB single-dose	51/56	80553/644.4	16/16	2614/0	22/52	21635/6230.9
					MEB single-dose	31/63	120256/962.0	7/12	2500/277.5	13/35	23314/3147.4
					PP single-dose	40/47	107958/13062.9	3/8	3150/900.9	6/52	3271/1570.1
					OX+PP single-dose	38/40	45363/181.5	5/8	2875/333.5	27/40	8025/1324.1
Sirivichayakul et al, 2001	[90]	Thailand	RCT	Kato-Katz	ALB single-dose	-	-	-	-	1/34	622.6/791.8
					ALB multiple-dose					6/34	651.5/119.7
Sorensen et al, 1996	[91]	Sri Lanka	RCT	Kato-Katz	ALB single-dose	69/71	7414.1/27.7	46/59	371.1/17.2	22/84	477/237
					MEB single-dose	82/84	10945.1/34.5	24/67	283.4/72.2	23/88	535.4/205.1
Soukhathammavong et al, 2012	[92]	Lao PDR	RCT	Kato-Katz	ALB single-dose	26/28	1567/0	32/89	859.1/63	26/39	94.1/75
					MEB single-dose	28/30	1584/0	15/82	707/147.3	31/43	65.2/48
Souza et al, 1972	[93]	Brazil	RCT	Stoll	LEV single-dose	29/30	-	-	-	-	-
					PP single-dose	30/30					
Speich et al, 2012	[94]	Tanzania	RCT	Kato-Katz	ALB single-dose	9/9	-	9/11	-	19/131	164/89
					NIT	5/8		8/12		9/136	145/125
					ALB+NIT single-dose	5/5		12/14		21/131	152/69
					Placebo	0/7		5/9		12/135	154/127
Speich et al, 2014	[95]	Tanzania	RCT	Kato-Katz	ALB single-dose	69/75	2426/1	67/112	108/4	3/114	853/469
					MEB single-dose	62/68	1876/1	19/109	109/45	13/110	813/203
					OX single-dose	8/79	3452/2472	12/113	127/78	30/114	874/59
					ALB+OX single-dose	67/71	1967/1	56/109	136/6	35/112	769/31
Speich et al, 2015	[96]	Tanzania	RCT	Kato-Katz	MEB single-dose	42/44	1035/0.36	10/41	79/32	9/107	479/199
					ALB+IVM single-dose	49/50	2023/0.04	21/42	108/5	30/109	489/27
					ALB+MEB single-dose	39/40	1074/0.29	22/46	137/10	9/107	409/198
					ALB+OX single-dose	46/47	1378/0.22	25/55	88/8	74/108	491/4
Steinmann et al, 2008	[97]	China	RCT	Kato-Katz	ALB single-dose	50/50	14415/0	32/46	251/121	7/60	540/265
					TRI single-dose	36/39	8805/2128	24/46	462/118	0/48	434/299
Steinmann et al, 2011	[98]	China	RCT	Kato-Katz	ALB single-dose	75/78	8442/0.1	38/55	69/2	22/65	58/14
					ALB multiple-dose	61/63	6485/0.2	46/50	90/0.3	27/48	68/4
					MEB single-dose	66/71	7955/0.5	18/58	73/12	25/63	47/8
					MEB multiple-dose	67/72	8435/0.2	38/65	86/3	41/58	55/1
Stothard et al, 2009	[99]	Tanzania	RCT	Kato-Katz	ALB single-dose	5/12	-	-	-	5/20	-
					LEV single-dose	7/12				7/20	
Thienpont et al, 1969	[100]	Indonesia	RCT	McMaster	LEV single-dose	20/23	8260/700	18/21	710/150	-	-
					Placebo	3/20	18170/28870	6/27	850/970		

Tian et al, 2011	[101]	China	RCT	Kato-Katz	ALB single-dose TRI single-dose	- 	- 	22/23 23/24	-	-	-
Upatham et al, 1989	[102]	Thailand	RCT	Kato-Katz	ALB single-dose Placebo	74/78 10/75	9311/67 12066/6521	119/260 7//287	1516/716 6883/5928	49/146 16/175	655/266 1117/389
Vakil et al, 1975	[103]	India	QE	Stoll	MEB single-dose MEB multiple-dose	10/10 27/28	-	-	-	-	-
Wang et al, 1987	[104]	China	RCT	Flotation unspecified	ALB single-dose ALB multiple-dose MEB multiple-dose PP single-dose	39/41 34/38 34/42 40/42	-	-	-	-	-
Watkins et al, 1996	[105]	Guatemala	RCT	Kato-Katz	ALB single-dose Placebo	-	38485/10000 37442/45984	-	-	-	1840/1370 1114/1088
Wen et al, 2003	[106]	China	RCT	Kato-Katz, brine flotation	ALB single-dose IVM single-dose	34/34 34/34	9330/0 9597/0	26/34 6/34	2573/18 4473/787.2	16/34 23/34	277/6.9 281/12.1
Wen et al, 2008	[107]	China	RCT	Kato-Katz	ALB single-dose IVM single-dose	101/102 102/102	7438/111.6 7286/0	71/102 34/102	1994/199.4 2176/435.2	69/102 68/102	298/37.8 276/38.1
Wesche et al, 1994	[108]	Papua New Guinea	RCT	Other	MEB multiple-dose Untreated controls	-	-	20/22 2/22	-	-	-
Wu et al, 2006	[109]	China	RCT	Kato-Katz, brine flotation	ALB single-dose TRI single-dose	91/92 114/117	-	60/85 85/95	1063/51 1047/18	23/41 25/75	-
Xia et al, 1992	[110]	China	RCT	Kato-Katz	IVM single-dose PP single-dose	42/44 42/44	-	6/51 16/54	-	26/34 12/38	-
Xu et al, 2014	[111]	China	RCT	Kato-Katz	MEB single-dose TRI single-dose	1/13 4/14	73.5/15.1 90.9/26.3	0/18 14/22	77.8/27.5 53.7/11.9	0/14 3/13	82.5/29.9 45.8/10.1
Yap et al, 2013	[112]	China	RCT	Kato-Katz	ALB multiple-dose Placebo	86/94 3/87	15850/1.3 19101/21001	58/60 8/59	130.4/1.2 121.5/62.2	18/92 0/91	216.3/24.3 284.4/304.7
Zhang et al, 1998	[113]	China	RCT	Brine flotation	ALB single-dose PP single-dose	491/491 343/343	-	282/593 169/439	1519/83 1353/123	148/553 84/380	-
Zu et al, 1992	[114]	China	RCT	Kato-Katz	ALB multiple-dose OX+PP multiple-dose	58/58 60/61	-	-	-	40/60 50/56	-
					ALB single-dose OX+PP multiple-dose	-	-	41/43 29/30	-	-	-
					ALB single-dose ALB multiple-dose	47/47 56/57	-	-	-	11/39 16/39	-
					ALB multiple-dose PP single-dose	-	-	23/24 15/18	-	-	-

* QE = quasi-experimental with control group; RCT = randomized controlled trial

† MIF = Merthiolate-iodine-formaldehyde

§ ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 2. Treatments included in network meta-analysis

Treatment group	Included treatments (number of studies)*		
	Ascaris lumbricoides	**Hookworm**	***Trichuris trichiura***
Single-dose albendazole	ALB 400mg (40)[3-5,9,12-16,23,25,29,36,38,39,44,46,49,53,55,57,58,75,77-80,89,91,92,94,95,98,99,102,104,105,109,113,114] ALB 400mg; half dose in children (2)[32,97] ALB 600mg (2)[43,67] ALB 6.7mg/kg (1)[107] ALB 7mg/kg; half dose in children (1)[106]	ALB 400mg (38)[3-5,9,12-14,16,20,23,29,30,36-39,44,46,53,54,72,74,75,78-81,83,89,91,92,94,95,98,102,109,113,114] ALB 400mg; half dose in children (3)[32,97,101] ALB 600mg (2)[43,67] ALB 6.7mg/kg (1)[107] ALB 7mg/kg; half dose in children (1)[106]	ALB 400mg (41)[2,3,5,9,12,14-16,23,25,38,39,44,46,48,49,53,55,57,58,62,69,72,75,77-80,89-92,94,95,98,99,102,105,109,113,114] ALB 400mg; half dose in children (1)[97] ALB 600mg (3)[43,67] ALB 6.7mg/kg (1)[107] ALB 7mg/kg; half dose in children (1)[106]
Multiple-dose albendazole	ALB 400mg daily for 2 days (1)[114] ALB 400mg daily for 3 days (3)[3,73,98,112] ALB 300mg daily for 2 days (1)[60] ALB 200mg daily for 2 days (1)[39] ALB 200mg daily for 3 days (2)[13,36] ALB 200mg twice daily for 1 day (1)[104] ALB 200mg twice daily for 3 days (1)[55]	ALB 400mg daily for 3 days (4)[3,37,73,98,112] ALB 200mg daily for 2 days (1)[39] ALB 200mg daily for 3 days (2)[13,36]	ALB 400mg daily for 2 days (2)[62,114] ALB 400mg daily for 3 days (6)[2,3,73,90,98,112] ALB 400mg twice daily for 1 day (1)[69] ALB 300mg daily for 2 days (1)[60] ALB 200mg daily for 2 days (1)[39] ALB 200mg twice daily for 3 days (1)[55]
Single-dose mebendazole	MEB 500mg (15)[1,5-7,11,44,46,53,80,87,91,92,95,96,98] MEB 400mg (4)[12,89,103,111] MEB 600mg (6)[17,18,25,27,31,67] MEB 1000mg (1)[43]	MEB 500mg (19)[1,5-7,11,22,26,37,44,46,53,76,80,83,91,92,95,96,98] MEB 400mg (3)[12,89,111] MEB 600mg (6)[17,18,27,31,67,74] MEB 1000mg (1)[43]	MEB 500mg (19)[1,5-7,11,22,44,46,53,62,69,76,80,87,91,92,95,96,98] MEB 400mg (3)[12,89,111] MEB 600mg (7)[17,18,25,27,31,67,86] MEB 1000mg (1)[43]
Multiple-dose mebendazole	MEB 100mg twice daily for 3 days (15) [4,9,11,18,28,34,51,56-58,70,71,75,80,88] MEB 100mg twice daily for 4 days (1)[60] MEB 200mg twice daily for 3 days (1)[104] MEB 200mg twice daily for 2 days (2)[21,103] MEB 200mg daily for 3 days (1)[31] MEB 500mg daily for 3 days (1)[98]	MEB 100mg twice daily for 3 days (12)[4,9,11,18,28,34,51,70,75,80,81,108] MEB 100mg twice daily for 3 days; half dose in children (1)[76] MEB 100mg twice daily for 4 days (1)[68] MEB 200mg twice daily for 2 days (1)[21] MEB 200mg daily for 3 days (1)[31] MEB 300mg BD for 3 days (1)[41] MEB 500mg daily for 3 days (2)[37,98]	MEB 100mg twice daily for 3 days (14)[9,11,18,28,57,58,60,70,71,75,80,84-86] MEB 100mg twice daily for 3 days; half dose in children (1)[76] MEB 100mg twice daily for 4 days (1)[68] MEB 200mg twice daily for 2 days (1)[21] MEB 200mg twice daily for 3 days (1)[10] MEB 200mg daily for 3 days (1)[31] MEB 400mg daily for 4 days (1)[52] MEB 500mg daily for 2 days (1)[62] MEB 500mg daily for 3 days (1)[98]
Single-dose pyrantel pamoate	PP 5 mg/kg (1)[24] PP 10mg/kg (13)[33,34,42,44,50,70,71,80,88,89,93,110,113] PP 11mg/kg (1)[63] PP 20mg/kg (1)[47] PP 500mg (1)[104] PP 750mg (1)[35]	PP 10mg/kg (11)[33,34,40,44,54,70,80,88,89,110,113] PP 12.5mg/kg (1)[83] PP 20mg/kg (1)[47] PP 750mg (2)[35,41] PP 1.1g (1)[61]	PP 10mg/kg (9)[44,50,70,71,80,88,89,110,113] PP 20mg/kg (1)[47]
Single-dose ivermectin	IVM 200µg/kg (2)[15,110] IVM 200µg/kg; half dose in children (1)[106] IVM 200-400µg/kg (1)[14] IVM 100µg/kg (1)[107]	IVM 200µg/kg (2)[107,110] IVM 200µg/kg; half dose in children (1) IVM 200-400µg/kg (1)[14]	IVM 200µg/kg (3)[15,107,110] IVM 100µg/kg; half dose in children (1) IVM 200-400µg/kg (1)[14]
Single-dose levamisole	LEV 2.5mg/kg (3)[44,99,100] LEV 2.5-5mg/kg (1)[63] LEV 100mg (1)[88] LEV 150mg (3)[35,51,93] LEV 150mg; reduced in children (1)[71]	LEV 2.5mg/kg (2)[44,100] LEV 100mg (1)[88] LEV 120mg; 80mg in children (1)[64] LEV 150mg (2)[35,51]	LEV 2.5mg/kg (2)[44,99] LEV 100mg (1)[88] LEV 150mg; reduced in children (1)[71]

Single-dose oxantel pamoate	OX 10mg/kg (1)[59] OX 20mg/kg (2)[65,95]	OX 20mg/kg (2)[65,95]	OX 15mg/kg (2)[24,59] OX 20mg/kg (2)[65,95]
Nitazoxanide	NIT 1000mg (1)[94] NIT 200mg BD for 3/7; half dose in younger children (1)[77]	NIT 1000mg (1)[94]	NIT 1000mg (1)[94] NIT 200mg BD for 3/7; half dose in younger children (1)[77]
Single-dose tribendimidine	TRI 400mg (3)[66,109,111] TRI 400mg; half dose in children (1)[97] TRI 300mg; half dose in children (1)[32]	TRI 400mg (5)[20,66,101,109,111] TRI 400mg; half dose in children (2)[32,97]	TRI 400mg (3)[66,109,111] TRI 400mg; half dose in children (1)[97]
Single-dose albendazole + ivermectin	ALB 400mg + IVM 200µg/kg (3)[15,53,96] ALB 400mg + IVM 200-400µg/kg (1)[14]	ALB 400mg + IVM 200µg/kg (3)[53,72,96] ALB 400mg + IVM 200-400µg/kg (1)[14]	ALB 400mg + IVM 200µg/kg (5)[15,45,53,72,96] ALB 400mg + IVM 200-400µg/kg (1)[14]
Single-dose mebendazole + ivermectin	MEB 500mg + IVM 200µg/kg (1)[53]	MEB 500mg + IVM 200µg/kg (1)[53]	MEB 500mg + IVM 200µg/kg (1)[53]
Single-dose albendazole + mebendazole	ALB 400mg + MEB 500mg (1)[96]	ALB 400mg + MEB 500mg (1)[96]	ALB 400mg + MEB 500mg (2)[69,96]
Single-dose albendazole + DEC	ALB 400mg + DEC 6mg/kg (2)[15,38]	ALB 400mg + DEC 6mg/kg (1)[38]	ALB 400mg + DEC 6mg/kg (3)[15,38,45]
Single-dose albendazole + nitazoxanide	ALB 400mg + NIT 1000mg (1)[94]	ALB 400mg + NIT 1000mg (1)[94]	ALB 400mg + NIT 1000mg (1)[94]
Single-dose albendazole + oxantel pamoate	ALB 400mg + OX 20mg/kg (2)[95,96] ALB 400mg + OX 25mg/kg (1)[66]	ALB 400mg + OX 20mg/kg (2)[95,96] ALB 400mg + OX 25mg/kg (1)[66]	ALB 400mg + OX 20mg/kg (2)[95,96] ALB 400mg + OX 25mg/kg (1)[66]
Single-dose oxantel pamoate + pyrantel pamoate	OX+PP 10mg/kg (6)[6,17,59,82,88,89] OX+PP 20mg/kg (3)[24,27,56]	OX+PP 10mg/kg (5)[6,17,82,88,89] OX+PP 20mg/kg (1)[27]	OX+PP 10mg/kg (7)[6,17,48,59,82,88,89] OX+PP 20mg/kg (5)[8,19,24,27,56]
Multiple-dose oxantel pamoate + pyrantel pamoate	OX+PP 10-20mg/kg daily for 3 days (1)[28] OX+PP 150mg twice daily for 2 days (1)[114]	OX+PP 10mg/kg daily for 3 days (1)[88] OX+PP 10-20mg/kg daily for 3 days (1)[28] OX+PP 150mg twice daily for 2 days (1)[114]	OX+PP 10mg/kg daily for 3 days (1)[88] OX+PP 10-20mg/kg daily for 3 days (1)[28] OX+PP 15mg/kg twice daily for 1 day (1)[19] OX+PP 15mg/kg daily for 2 days (1)[56] OX+PP 150mg twice daily for 2 days (1)[114]
Single-dose tribendimidine + oxantel pamoate	TRI 400mg + OX 25mg/kg (1)[66]	TRI 400mg + OX 25mg/kg (1)[66]	TRI 400mg + OX 25mg/kg (1)[66]
Single-dose tribendimidine + ivermectin	TRI 400mg + IVM 200µg/kg (1)[66]	TRI 400mg + IVM 200µg/kg (1)[66]	TRI 400mg + IVM 200µg/kg (1)[66]
Placebo / no treatment	Placebo (20)[1,7,14,16,21,23,29,42,47,49,65,78,79,82,87,94,100,102,105,112] Untreated controls (2)[33,50]	Placebo (25)[1,7,14,16,21-23,26,29,30,37,47,61,64,65,68,78,79,81-83,94,100,102,112] Untreated controls (3)[33,40,108]	Placebo (25)[1,7,8,10,14,16,21-23,47,49,52,65,68,73,78,79,82,84,85,87,94,102,105,112] Untreated controls (1)[50]

* ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 3. Direct, indirect and final results comparing relative risk of cure for different treatments for *A. lumbricoides*

ID	Comparison[a]	Active	Control	RR of cure	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	0.197	0.115	0.337
2	ALB2-PLB	ALB2	PLB	26.532	8.712	80.799
3	ALB2-ALB1	ALB2	ALB1	1.003	0.984	1.023
4	MEB1-PLB	MEB1	PLB	4.946	2.045	11.962
5	MEB2-PLB	MEB2	PLB	39.615	2.565	611.729
6	MEB2-MEB1	MEB2	MEB1	1.051	0.987	1.120
7	PP-MEB1	PP	MEB1	0.988	0.904	1.080
8	MEB1-ALB1	MEB1	ALB1	0.991	0.979	1.003
9	MEB2-ALB1	MEB2	ALB1	0.994	0.924	1.070
10	MEB2-ALB2	MEB2	ALB2	0.991	0.869	1.131
11	PP-PLB	PP	PLB	10.246	5.902	17.787
12	PP-MEB2	PP	MEB2	1.062	1.021	1.105
13	PP-ALB1	PP	ALB1	1.000	0.995	1.004
14	LEV-ALB1	LEV	ALB1	1.400	0.615	3.187
15	LEV-MEB2	LEV	MEB2	0.982	0.788	1.224
16	LEV-PP	LEV	PP	0.961	0.917	1.008
17	LEV-PLB	LEV	PLB	10.000	2.635	37.951
18	IVM-ALB1	IVM	ALB1	1.011	0.986	1.035
19	IVM-PP	IVM	PP	1.000	0.913	1.095
20	OXA-PLB	OXA	PLB	1.867	0.552	6.310
21	OXA-ALB1	OXA	ALB1	0.110	0.057	0.213
22	OXA-OXP1	OXA	OXP1	0.024	0.003	0.166
23	OXP1-ALB1	OXP1	ALB1	1.043	0.936	1.163
24	OXP1-MEB1	OXP1	MEB1	0.985	0.946	1.025
25	OXP2-ALB2	OXP2	ALB2	0.984	0.940	1.030
26	OXP2-MEB2	OXP2	MEB2	0.999	0.915	1.091
27	OXP1-MEB2	OXP1	MEB2	1.015	0.959	1.074
28	OXP1-PP	OXP1	PP	1.006	0.917	1.104
29	OXP1-PLB	OXP1	PLB	9.989	3.401	29.340
30	NIT-ALB1	NIT	ALB1	0.943	0.622	1.429
31	TRI-ALB1	TRI	ALB1	0.977	0.922	1.037
32	TRI-MEB1	TRI	MEB1	3.714	0.475	29.060
33	ALBIVM-ALB1	ALBIVM	ALB1	0.931	0.768	1.129
34	ALBMEB-ALBIVM	ALBMEB	ALBIVM	0.995	0.934	1.060
35	ALBOX-MEB1	ALBOX	MEB1	1.029	0.970	1.092
36	ALBDEC-ALBIVM	ALBDEC	ALBIVM	0.972	0.849	1.113
37	TRIOX-ALBOX	TRIOX	ALBOX	1.007	0.928	1.093
38	TRIIVM-TRI	TRIIVM	TRI	1.001	0.964	1.039
39	MEBIVM-MEB1	MEBIVM	MEB1	1.276	0.983	1.657
Indirect estimates (source IDs)						
40	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	5.214	1.513	17.971
41	Indirect MEB1 vs ALB1 (4, 1)	MEB1	ALB1	0.972	0.345	2.736
42	Indirect MEB2 vs ALB1 (5, 1)	MEB2	ALB1	7.784	0.478	126.709
43	Indirect MEB2 vs ALB1 (6, 8)	MEB2	ALB1	1.042	0.977	1.111
44	Indirect PP vs ALB1 (7, 8)	PP	ALB1	0.979	0.895	1.072
45	Indirect MEB2 vs ALB1 (10, 3)	MEB2	ALB1	0.994	0.871	1.136
46	Indirect PP vs ALB1 (11, 1)	PP	ALB1	2.013	0.931	4.356
47	Indirect PP vs ALB1 (12, 9)	PP	ALB1	1.056	0.972	1.147
48	Indirect LEV vs ALB1 (15, 9)	LEV	ALB1	0.976	0.774	1.232
49	Indirect LEV vs ALB1 (16, 13)	LEV	ALB1	0.961	0.916	1.007
50	Indirect LEV vs ALB1 (17, 1)	LEV	ALB1	1.965	0.466	8.283
51	Indirect IVM vs ALB1 (19, 13)	IVM	ALB1	1.000	0.912	1.095
52	Indirect OXA vs ALB1 (20, 1)	OXA	ALB1	0.367	0.097	1.390
53	Indirect OXA vs ALB1 (22, 23)	OXA	ALB1	0.025	0.004	0.174
54	Indirect OXP1 vs ALB1 (24, 8)	OXP1	ALB1	0.976	0.936	1.018
55	Indirect OXP2 vs ALB1 (25, 3)	OXP2	ALB1	0.987	0.940	1.038

56	Indirect OXP2 vs ALB1 (26, 9)	OXP2	ALB1	0.993	0.886	1.114
57	Indirect OXP1 vs ALB1 (27, 9)	OXP1	ALB1	1.009	0.920	1.107
58	Indirect OXP1 vs ALB1 (28, 13)	OXP1	ALB1	1.006	0.917	1.104
59	Indirect OXP1 vs ALB1 (29, 1)	OXP1	ALB1	1.963	0.588	6.550
60	Indirect TRI vs ALB1 (32, 8)	TRI	ALB1	3.682	0.471	28.808
61	Indirect ALBMEB vs ALB1 (34, 33)	ALBMEB	ALB1	0.926	0.756	1.135
62	Indirect ALBOX vs ALB1 (35, 8)	ALBOX	ALB1	1.020	0.960	1.084
63	Indirect ALBDEC vs ALB1 (36, 33)	ALBDEC	ALB1	0.905	0.715	1.146
64	Indirect TRIIVM vs ALB1 (38, 31)	TRIIVM	ALB1	0.978	0.912	1.049
65	Indirect MEBIVM vs ALB1 (39, 8)	MEBIVM	ALB1	1.265	0.974	1.643
66	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	0.038	0.012	0.115
67	Indirect PLB vs ALB1 (4, 8)	PLB	ALB1	0.200	0.083	0.485
68	Indirect PLB vs ALB1 (5, 9)	PLB	ALB1	0.025	0.002	0.388
69	Indirect MEB1 vs ALB1 (6, 9)	MEB1	ALB1	0.946	0.859	1.041
70	Indirect MEB1 vs ALB1 (7, 13)	MEB1	ALB1	1.012	0.925	1.106
71	Indirect PLB vs ALB1 (11, 13)	PLB	ALB1	0.098	0.056	0.169
72	Indirect MEB2 vs ALB1 (12, 13)	MEB2	ALB1	0.941	0.905	0.979
73	Indirect PLB vs ALB1 (20, 21)	PLB	ALB1	0.059	0.015	0.236
74	Indirect ALB2 vs ALB1 (10, 9)	ALB2	ALB1	1.003	0.863	1.166
75	Indirect MEB2 vs ALB1 (15, 14)	MEB2	ALB1	1.425	0.608	3.341
76	Indirect PP vs ALB1 (16, 14)	PP	ALB1	1.456	0.639	3.320
77	Indirect PLB vs ALB1 (17, 14)	PLB	ALB1	0.140	0.029	0.671
78	Indirect PP vs ALB1 (19, 18)	PP	ALB1	1.011	0.920	1.111
79	Indirect OXP1 vs ALB1 (22, 21)	OXP1	ALB1	4.607	0.595	35.673
80	Indirect MEB1 vs ALB1 (24, 23)	MEB1	ALB1	1.059	0.944	1.189
81	Indirect MEB2 vs ALB1 (27, 23)	MEB2	ALB1	1.028	0.909	1.162
82	Indirect PP vs ALB1 (28, 23)	PP	ALB1	1.036	0.899	1.195
83	Indirect PLB vs ALB1 (29, 23)	PLB	ALB1	0.104	0.035	0.308
84	Indirect MEB1 vs ALB1 (32, 31)	MEB1	ALB1	0.263	0.034	2.061
85	Indirect TRIOX vs ALB1 (37, 62)	TRIOX	ALB1	1.028	0.928	1.138
Final estimates from all evidence (source IDs)						
	PLB (1, 66, 67, 68, 71, 73, 77, 83)	PLB	ALB1	0.121	0.076	0.193
	ALB2 (3, 40, 74)	ALB2	ALB1	1.004	0.797	1.264
	MEB1 (8, 41, 69, 70, 80, 84)	MEB1	ALB1	0.992	0.980	1.004
	MEB2 (9, 42, 43, 45, 72, 75, 81)	MEB2	ALB1	0.977	0.923	1.034
	PP (13, 44, 46, 47, 76, 78, 82)	PP	ALB1	1.000	0.986	1.013
	LEV (14, 48, 49, 50)	LEV	ALB1	0.963	0.920	1.009
	IVM (18, 51)	IVM	ALB1	1.010	0.987	1.034
	OXA (21, 52, 53)	OXA	ALB1	0.121	0.032	0.456
	OXP1 (23, 54, 57, 58, 59, 79)	OXP1	ALB1	0.991	0.959	1.025
	OXP2 (55, 56)	OXP2	ALB1	0.988	0.944	1.034
	NIT (30)	NIT	ALB1	0.943	0.622	1.429
	TRI (31, 60)	TRI	ALB1	0.978	0.318	3.010
	ALBIVM (33)	ALBIVM	ALB1	0.931	0.768	1.129
	ALBMEB (61)	ALBMEB	ALB1	0.926	0.756	1.135
	ALBOX (62)	ALBOX	ALB1	1.020	0.960	1.084
	ALBDEC (63)	ALBDEC	ALB1	0.905	0.715	1.146
	TRIOX (85)	TRIOX	ALB1	1.028	0.928	1.138
	TRIIVM (64)	TRIIVM	ALB1	0.978	0.912	1.049
	MEBIVM (65)	MEBIVM	ALB1	1.265	0.974	1.643
Network H = 1.181						

RR = relative risk; LCI = lower confidence interval; HCI = higher confidence interval
[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBDEC = albendazole + diethylcarbamazine; ALBIVM = albendazole + ivermectin; ALBMEB = albendazole + mebendazole; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine; TRIOX = tribendimidine + oxantel pamoate; TRIIVM = tribendimidine + ivermectin

Supplementary Table 4. Direct, indirect and final results comparing difference in egg reduction rate for different treatments for *A. lumbricoides*

ID	Comparison[a]	Active	Control	dERR	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	-0.756	-1.149	-0.363
2	ALB2-PLB	ALB2	PLB	1.091	1.045	1.136
3	ALB2-ALB1	ALB2	ALB1	0.000	-0.016	0.016
4	MEB1-PLB	MEB1	PLB	0.912	0.734	1.091
5	ALB1-MEB1	ALB1	MEB1	0.000	-0.001	0.001
6	MEB2-MEB1	MEB2	MEB1	0.088	0.086	0.090
7	MEB2-ALB1	MEB2	ALB1	-0.004	-0.247	0.239
8	MEB1-ALB1	MEB1	ALB1	0.000	-0.079	0.079
9	PP-PLB	PP	PLB	0.557	0.233	0.881
10	PP-MEB2	PP	MEB2	-0.005	-0.009	-0.002
11	PP-LEV	PP	LEV	0.012	-0.063	0.086
12	IVM-ALB1	IVM	ALB1	0.000	-0.021	0.021
13	IVM-PLB	IVM	PLB	0.670	0.646	0.694
14	OX-PLB	OX	PLB	0.339	0.325	0.354
15	OX-MEB1	OX	MEB1	-0.715	-0.723	-0.708
16	OXPP1-PLB	OXPP1	PLB	0.645	0.635	0.655
17	OXPP1-MEB1	OXPP1	MEB1	-0.005	-0.005	-0.005
18	OXPP1-PP	OXPP1	PP	0.001	-0.161	0.162
19	OXPP1-MEB2	OXPP1	MEB2	0.002	0.002	0.002
20	OXPP2-MEB2	OXPP2	MEB2	0.000	0.000	0.000
21	ALBIVM-ALB1	ALBIVM	ALB1	0.000	-0.001	0.001
22	ALBIVM-IVM	ALBIVM	IVM	0.052	0.051	0.052
23	ALBDEC-ALB1	ALBDEC	ALB1	0.033	-0.107	0.172
24	NIT-ALB1	NIT	ALB1	0.000	0.000	0.001
25	ALBOX-ALB1	ALBOX	ALB1	0.000	0.000	0.000
26	ALBOX-MEB1	ALBOX	MEB1	0.000	0.000	0.001
27	TRI-ALB1	TRI	ALB1	-0.242	-0.245	-0.239
28	TRI-MEB1	TRI	MEB1	-0.083	-0.160	-0.005
Indirect estimates (source IDs)						
29	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	0.334	-0.061	0.730
30	Indirect MEB1 vs ALB1 (4, 1)	MEB1	ALB1	0.156	-0.275	0.588
31	Indirect MEB2 vs ALB1 (6, 8)	MEB2	ALB1	0.088	0.009	0.167
32	Indirect PP vs ALB1 (9, 1)	PP	ALB1	-0.199	-0.709	0.311
33	Indirect PP vs ALB1 (10, 7)	PP	ALB1	-0.009	-0.253	0.234
34	Indirect IVM vs ALB1 (13, 1)	IVM	ALB1	-0.086	-0.480	0.308
35	Indirect OXA vs ALB1 (14, 1)	OXA	ALB1	-0.417	-0.810	-0.023
36	Indirect OXA vs ALB1 (15, 8)	OXA	ALB1	-0.715	-0.795	-0.636
37	Indirect OXP1 vs ALB1 (16, 1)	OXP1	ALB1	-0.111	-0.504	0.282
38	Indirect OXP1 vs ALB1 (17, 8)	OXP1	ALB1	-0.005	-0.084	0.074
39	Indirect OXP1 vs ALB1 (19, 7)	OXP1	ALB1	-0.002	-0.245	0.241
40	Indirect OXP2 vs ALB1 (20, 7)	OXP2	ALB1	-0.004	-0.247	0.239
41	Indirect ALBIVM vs ALB1 (22, 12)	ALBIVM	ALB1	0.052	0.031	0.073
42	Indirect ALBOX vs ALB1 (26, 8)	ALBOX	ALB1	0.000	-0.079	0.079
43	Indirect TRI vs ALB1 (28, 8)	TRI	ALB1	-0.083	-0.193	0.028
44	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	-1.091	-1.139	-1.042
45	Indirect PLB vs ALB1 (4, 8)	PLB	ALB1	-0.912	-1.107	-0.717
46	Indirect MEB1 vs ALB1 (6, 7)	MEB1	ALB1	-0.092	-0.335	0.151
47	Indirect PLB vs ALB1 (13, 12)	PLB	ALB1	-0.670	-0.702	-0.638
48	Indirect IVM vs ALB1 (22, 21)	IVM	ALB1	-0.052	-0.053	-0.051
49	Indirect MEB1 vs ALB1 (26, 25)	MEB1	ALB1	0.000	-0.001	0.000
50	Indirect MEB1 vs ALB1 (28, 27)	MEB1	ALB1	-0.159	-0.237	-0.082
51	Indirect PLB vs ALB1 (4, 5)	PLB	ALB1	-0.912	-1.091	-0.734
52	Indirect OXP1 vs ALB1 (17, 5)	OXP1	ALB1	-0.005	-0.006	-0.004
53	Indirect TRI vs ALB1 (28, 5)	TRI	ALB1	-0.083	-0.160	-0.005
54	Indirect LEV vs ALB1 (11, 32)	LEV	ALB1	-0.211	-0.726	0.305
Final estimates from all evidence (source IDs)						
	PLB (1, 44, 45, 47, 51)	PLB	ALB1	-0.802	-1.194	-0.410
	ALB2 (3, 29)	ALB2	ALB1	0.001	-0.369	0.370
	MEB1 (8, 5, 30, 46, 49, 50)	MEB1	ALB1	0.000	-0.003	0.002
	MEB2 (7, 31)	MEB2	ALB1	0.079	0.004	0.154
	PP (32, 33)	PP	ALB1	-0.044	-0.264	0.175
	LEV (54)	LEV	ALB1	-0.211	-0.726	0.305
	IVM (12, 34, 48)	IVM	ALB1	-0.052	-0.121	0.017
	OXA (35, 36)	OXA	ALB1	-0.704	-1.004	-0.404
	OXP1 (37, 38, 39, 52)	OXP1	ALB1	-0.005	-0.006	-0.004
	OXP2 (40)	OXP2	ALB1	-0.004	-0.247	0.239

ALBIVM (21, 41)	ALBIVM	ALB1	0.000	-0.070	0.071
ALBDEC (23)	ALBDEC	ALB1	0.033	-0.107	0.172
NIT (24)	NIT	ALB1	0.000	0.000	0.001
ALBOX (25, 42)	ALBOX	ALB1	0.000	0.000	0.000
TRI (27, 43, 53)	TRI	ALB1	-0.241	-0.452	-0.031
Network H = 3.452					

dERR = difference in egg reduction rate; LCI = lower confidence interval; HCI = higher confidence interval
[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBDEC = albendazole + diethylcarbamazine; ALBIVM = albendazole + ivermectin; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 5. Direct, indirect and final results comparing relative risk of cure for different treatments for hookworm

ID	Comparison[a]	Active	Control	RR of cure	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	0.261	0.100	0.683
2	ALB2-PLB	ALB2	PLB	3.096	0.917	10.454
3	ALB2-ALB1	ALB2	ALB1	1.149	1.056	1.251
4	MEB1-PLB	MEB1	PLB	1.364	0.526	3.536
5	MEB2-PLB	MEB2	PLB	15.039	5.032	44.945
6	MEB2-MEB1	MEB2	MEB1	1.238	0.754	2.033
7	PP-PLB	PP	PLB	5.564	3.441	8.997
8	PP-ALB1	PP	ALB1	0.758	0.457	1.258
9	PP-ALB2	PP	ALB2	0.870	0.696	1.087
10	PP-MEB1	PP	MEB1	1.093	0.531	2.248
11	MEB1-ALB1	MEB1	ALB1	0.435	0.273	0.695
12	MEB2-ALB1	MEB2	ALB1	1.015	0.843	1.221
13	PP-MEB2	PP	MEB2	1.074	0.452	2.551
14	LEV-MEB2	LEV	MEB2	1.545	1.149	2.079
15	LEV-PP	LEV	PP	1.180	0.872	1.597
16	LEV-PLB	LEV	PLB	3.214	0.086	120.163
17	IVM-ALB1	IVM	ALB1	0.432	0.198	0.942
18	IVM-PP	IVM	PP	0.397	0.169	0.935
19	OX-ALB1	OX	ALB1	0.178	0.102	0.310
20	OX-PLB	OX	PLB	1.556	0.470	5.149
21	OXPP1-OXPP2	OXPP1	OXPP2	0.323	0.192	0.545
22	OXPP1-MEB1	OXPP1	MEB1	0.927	0.746	1.152
23	OXPP1-ALB1	OXPP1	ALB1	0.630	0.371	1.067
24	OXPP1-PLB	OXPP1	PLB	2.857	1.549	5.271
25	OXPP2-MEB2	OXPP2	MEB2	1.569	0.743	3.312
26	OXPP2-ALB1	OXPP2	ALB1	1.014	0.923	1.113
27	NIT-ALB1	NIT	ALB1	0.815	0.500	1.327
28	TRI-ALB1	TRI	ALB1	1.134	0.964	1.335
29	TRI-MEB1	TRI	MEB1	23.957	1.527	375.844
30	ALBNIT-PLB	ALBNIT	PLB	1.543	0.828	2.875
31	ALBIVM-ALB1	ALBIVM	ALB1	0.832	0.584	1.184
32	MEBIVM-MEB1	MEBIVM	MEB1	0.729	0.353	1.503
33	ALBMEB-ALBIVM	ALBMEB	ALBIVM	0.957	0.624	1.466
34	TRIOX-TRI	TRIOX	TRI	0.951	0.768	1.176
35	TRIIVM-ALBOX	TRIIVM	ALBOX	1.622	1.382	1.904
36	ALBOX-MEB1	ALBOX	MEB1	2.512	1.617	3.902
Indirect estimates (source IDs)						
37	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	0.808	0.171	3.812
38	Indirect MEB1 vs ALB1 (4, 1)	MEB1	ALB1	0.356	0.092	1.379
39	Indirect MEB2 vs ALB1 (5, 1)	MEB2	ALB1	3.927	0.915	16.861
40	Indirect MEB2 vs ALB1 (6, 11)	MEB2	ALB1	0.539	0.273	1.066
41	Indirect PP vs ALB1 (7, 1)	PP	ALB1	1.453	0.496	4.257
42	Indirect PP vs ALB1 (9, 3)	PP	ALB1	0.999	0.787	1.269
43	Indirect PP vs ALB1 (10, 11)	PP	ALB1	0.476	0.201	1.124
44	Indirect PP vs ALB1 (13, 12)	PP	ALB1	1.089	0.450	2.640
45	Indirect LEV vs ALB1 (14, 12)	LEV	ALB1	1.568	1.105	2.225
46	Indirect LEV vs ALB1 (15, 8)	LEV	ALB1	0.895	0.496	1.613
47	Indirect LEV vs ALB1 (16, 1)	LEV	ALB1	0.839	0.020	35.575
48	Indirect IVM vs ALB1 (18, 8)	IVM	ALB1	0.301	0.111	0.814
49	Indirect OX vs ALB1 (20, 1)	OX	ALB1	0.406	0.087	1.886
50	Indirect OXPP1 vs ALB1 (21, 26)	OXPP1	ALB1	0.328	0.193	0.557
51	Indirect OXPP1 vs ALB1 (22, 11)	OXPP1	ALB1	0.404	0.241	0.676
52	Indirect OXPP1 vs ALB1 (24, 1)	OXPP1	ALB1	0.746	0.239	2.333
53	Indirect OXPP2 vs ALB1 (25, 12)	OXPP2	ALB1	1.591	0.737	3.437
54	Indirect TRI vs ALB1 (29, 11)	TRI	ALB1	10.431	0.639	170.244
55	Indirect ALBNIT vs ALB1 (30, 1)	ALBNIT	ALB1	0.403	0.128	1.266

56	Indirect MEBIVM vs ALB1 (32, 11)	MEBIVM	ALB1	0.317	0.134	0.751
57	Indirect ALBMEB vs ALB1 (33, 31)	ALBMEB	ALB1	0.795	0.457	1.385
58	Indirect TRIOX vs ALB1 (34, 28)	TRIOX	ALB1	1.078	0.825	1.410
59	Indirect ALBOX vs ALB1 (36, 11)	ALBOX	ALB1	1.094	0.575	2.080
60	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	0.371	0.110	1.258
61	Indirect PLB vs ALB1 (4, 11)	PLB	ALB1	0.319	0.110	0.922
62	Indirect PLB vs ALB1 (5, 12)	PLB	ALB1	0.067	0.022	0.205
63	Indirect MEB1 vs ALB1 (6, 12)	MEB1	ALB1	0.820	0.483	1.392
64	Indirect PLB vs ALB1 (7, 8)	PLB	ALB1	0.136	0.068	0.274
65	Indirect OXPP2 vs ALB1 (21, 23)	OXPP2	ALB1	1.947	0.927	4.090
66	Indirect MEB1 vs ALB1 (22, 23)	MEB1	ALB1	0.679	0.384	1.201
67	Indirect MEB2 vs ALB1 (25, 26)	MEB2	ALB1	0.646	0.304	1.372
68	Indirect ALB2 vs ALB1 (9, 8)	ALB2	ALB1	0.872	0.502	1.516
69	Indirect MEB1 vs ALB1 (10, 8)	MEB1	ALB1	0.694	0.287	1.675
70	Indirect MEB2 vs ALB1 (13, 8)	MEB2	ALB1	0.706	0.259	1.924
71	Indirect PP vs ALB1 (18, 17)	PP	ALB1	1.089	0.342	3.466
72	Indirect PLB vs ALB1 (20, 19)	PLB	ALB1	0.114	0.030	0.427
73	Indirect PLB vs ALB1 (24, 23)	PLB	ALB1	0.220	0.098	0.495
74	Indirect MEB1 vs ALB1 (29, 28)	MEB1	ALB1	0.047	0.003	0.746
75	Indirect TRIIVM vs ALB1 (35, 59)	TRIIVM	ALB1	1.774	0.915	3.441
Final estimates from all evidence (source IDs)						
	PLB (1, 60, 61, 62, 64, 72, 73)	PLB	ALB1	0.182	0.121	0.274
	ALB2 (3, 37, 68)	ALB2	ALB1	1.141	1.049	1.241
	MEB1 (11, 38, 63, 66, 69, 74)	MEB1	ALB1	0.582	0.403	0.840
	MEB2 (12, 39, 40, 67, 70)	MEB2	ALB1	0.961	0.539	1.713
	PP (8, 41, 42, 43, 44, 71)	PP	ALB1	0.940	0.772	1.145
	LEV (45, 46, 47)	LEV	ALB1	1.351	0.886	2.060
	IVM (17, 48)	IVM	ALB1	0.377	0.204	0.696
	OX (19, 49)	OX	ALB1	0.195	0.116	0.330
	OXPP1 (23, 50, 51, 52)	OXPP1	ALB1	0.452	0.322	0.636
	OXPP2 (26, 53, 65)	OXPP2	ALB1	1.031	0.593	1.793
	NIT (27)	NIT	ALB1	0.815	0.500	1.327
	TRI (28, 54)	TRI	ALB1	1.143	0.109	12.009
	ALBNIT (55)	ALBNIT	ALB1	0.403	0.128	1.266
	ALBIVM (31)	ALBIVM	ALB1	0.832	0.584	1.184
	MEBIVM (56)	MEBIVM	ALB1	0.317	0.134	0.751
	ALBMEB (57)	ALBMEB	ALB1	0.795	0.457	1.385
	TRIOX (58)	TRIOX	ALB1	1.078	0.825	1.410
	TRIIVM (75)	TRIIVM	ALB1	1.774	0.915	3.441
	ALBOX (59)	ALBOX	ALB1	1.094	0.575	2.080
Network H = 1.148						

RR = relative risk; LCI = lower confidence interval; HCI = higher confidence interval

[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBDEC = albendazole + diethylcarbamazine; ALBIVM = albendazole + ivermectin; ALBMEB = albendazole + mebendazole; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine; TRIOX = tribendimidine + oxantel pamoate; TRIIVM = tribendimidine + ivermectin

Supplementary Table 6. Direct, indirect and final results comparing difference in egg reduction rate for different treatments for hookworm

ID	Comparison[a]	Active	Control	dERR	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	-0.395	-1.222	0.431
2	ALB2-PLB	ALB2	PLB	0.501	0.467	0.534
3	ALB2-ALB1	ALB2	ALB1	0.091	-0.017	0.199
4	MEB1-PLB	MEB1	PLB	0.680	0.137	1.223
5	ALB1-MEB1	ALB1	MEB1	0.119	0.022	0.216
6	MEB2-MEB1	MEB2	MEB1	0.149	-0.088	0.386
7	MEB2-ALB1	MEB2	ALB1	-0.011	-0.019	-0.003
8	MEB2-PLB	MEB2	PLB	0.716	0.697	0.735
9	PP-PLB	PP	PLB	0.712	0.518	0.906
10	PP-ALB1	PP	ALB1	-0.036	-0.038	-0.035
11	PP-MEB1	PP	MEB1	0.001	-1.127	1.128
12	PP-MEB2	PP	MEB2	0.281	0.258	0.305
13	LEV-PLB	LEV	PLB	0.844	0.593	1.095
14	LEV-ALB1	LEV	ALB1	-0.089	-0.120	-0.059
15	IVM-ALB1	IVM	ALB1	-0.135	-0.203	-0.067
16	IVM-PLB	IVM	PLB	0.420	0.340	0.501
17	OX-PLB	OX	PLB	0.344	0.244	0.444
18	OX-MEB1	OX	MEB1	-0.200	-0.237	-0.163
19	OXPP1-PLB	OXPP1	PLB	0.360	0.327	0.393
20	OXPP1-MEB1	OXPP1	MEB1	0.043	-0.083	0.168
21	OXPP1-PP	OXPP1	PP	0.086	-0.035	0.207
22	OXPP2-MEB2	OXPP2	MEB2	0.039	0.031	0.047
23	OXPP2-LEV	OXPP2	LEV	0.005	0.002	0.008
24	ALBIVM-ALB1	ALBIVM	ALB1	0.000	-0.007	0.008
25	ALBOX-ALB1	ALBOX	ALB1	-0.006	-0.015	0.003
26	ALBOX-ALBIVM	ALBOX	ALBIVM	-0.045	-0.064	-0.027
27	TRI-ALB1	TRI	ALB1	0.013	-0.005	0.030
28	TRI-MEB1	TRI	MEB1	0.129	0.038	0.219
29	ALBOX-TRI	ALBOX	TRI	-0.007	-0.012	-0.001
Indirect estimates (source IDs)						
30	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	0.105	-0.721	0.932
31	Indirect MEB1 vs ALB1 (4, 1)	MEB1	ALB1	0.285	-0.704	1.273
32	Indirect MEB2 vs ALB1 (8, 1)	MEB2	ALB1	0.320	-0.506	1.147
33	Indirect PP vs ALB1 (9, 1)	PP	ALB1	0.317	-0.532	1.165
34	Indirect PP vs ALB1 (12, 7)	PP	ALB1	0.270	0.246	0.295
35	Indirect LEV vs ALB1 (13, 1)	LEV	ALB1	0.448	-0.415	1.312
36	Indirect IVM vs ALB1 (16, 1)	IVM	ALB1	0.025	-0.805	0.855
37	Indirect OX vs ALB1 (17, 1)	OX	ALB1	-0.052	-0.884	0.780
38	Indirect OXPP1 vs ALB1 (19, 1)	OXPP1	ALB1	-0.036	-0.863	0.791
39	Indirect OXPP1 vs ALB1 (21, 10)	OXPP1	ALB1	0.050	-0.071	0.171
40	Indirect OXPP2 vs ALB1 (22, 7)	OXPP2	ALB1	0.028	0.016	0.039
41	Indirect OXPP2 vs ALB1 (23, 14)	OXPP2	ALB1	-0.084	-0.115	-0.054
42	Indirect ALBOX vs ALB1 (26, 24)	ALBOX	ALB1	-0.045	-0.065	-0.025
43	Indirect ALBOX vs ALB1 (29, 27)	ALBOX	ALB1	0.006	-0.012	0.024
44	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	-0.410	-0.523	-0.296
45	Indirect MEB1 vs ALB1 (6, 7)	MEB1	ALB1	-0.160	-0.398	0.077
46	Indirect PLB vs ALB1 (9, 10)	PLB	ALB1	-0.748	-0.943	-0.554
47	Indirect PLB vs ALB1 (13, 14)	PLB	ALB1	-0.933	-1.186	-0.680
48	Indirect PLB vs ALB1 (8, 7)	PLB	ALB1	-0.727	-0.748	-0.706
49	Indirect MEB1 vs ALB1 (11, 10)	MEB1	ALB1	-0.037	-1.165	1.091
50	Indirect MEB2 vs ALB1 (12, 10)	MEB2	ALB1	-0.318	-0.341	-0.294
51	Indirect PLB vs ALB1 (16, 15)	PLB	ALB1	-0.555	-0.660	-0.450
52	Indirect ALBIVM vs ALB1 (26, 25)	ALBIVM	ALB1	0.039	0.019	0.060
53	Indirect TRI vs ALB1 (29, 25)	TRI	ALB1	0.000	-0.010	0.011
54	Indirect MEB1 vs ALB1 (28, 27)	MEB1	ALB1	-0.116	-0.208	-0.024
55	Indirect PLB vs ALB1 (4, 5)	PLB	ALB1	-0.799	-1.351	-0.247
56	Indirect MEB2 vs ALB1 (6, 5)	MEB2	ALB1	0.031	-0.226	0.287
57	Indirect PP vs ALB1 (11, 5)	PP	ALB1	-0.118	-1.250	1.014
58	Indirect OX vs ALB1 (18, 5)	OX	ALB1	-0.319	-0.423	-0.215
59	Indirect OXPP1 vs ALB1 (20, 5)	OXPP1	ALB1	-0.076	-0.235	0.083
60	Indirect TRI vs ALB1 (28, 5)	TRI	ALB1	0.010	-0.123	0.143
Final estimates from all evidence (source IDs)						
	PLB (1, 44, 46, 47, 48, 51, 55)	PLB	ALB1	-0.713	-0.984	-0.441
	ALB2 (3, 30)	ALB2	ALB1	0.091	-0.016	0.199
	MEB1 (5, 31, 45, 49, 54)	MEB1	ALB1	-0.119	-0.183	-0.055
	MEB2 (7, 32, 50, 56)	MEB2	ALB1	-0.044	-0.423	0.334

PP (10, 33, 34, 57)	PP	ALB1	-0.035	-0.457	0.387
LEV (14, 35)	LEV	ALB1	-0.089	-0.516	0.338
IVM (15, 36)	IVM	ALB1	-0.134	-0.201	-0.067
OX (37, 58)	OX	ALB1	-0.315	-0.418	-0.212
OXPP1 (38, 39, 59)	OXPP1	ALB1	0.003	-0.093	0.099
OXPP2 (40, 41)	OXPP2	ALB1	0.014	-0.123	0.151
ALBIVM (24, 52)	ALBIVM	ALB1	0.005	-0.042	0.051
ALBOX (25, 42, 43)	ALBOX	ALB1	-0.009	-0.039	0.020
TRI (27, 53, 60)	TRI	ALB1	0.004	-0.005	0.012
Network H = 6.871					

dERR = difference in egg reduction rate; LCI = lower confidence interval; HCI = higher confidence interval
[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBIVM = albendazole + ivermectin; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 7. Direct, indirect and final results comparing relative risk of cure for different treatments for *T. trichiura*

ID	Comparison[a]	Active	Control	RR of cure	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	0.292	0.205	0.416
2	ALB2-PLB	ALB2	PLB	33.713	6.850	165.927
3	ALB2-ALB1	ALB2	ALB1	1.738	1.371	2.205
4	ALB2-MEB2	ALB2	MEB2	0.745	0.657	0.846
5	MEB1-PLB	MEB1	PLB	3.522	1.753	7.078
6	MEB2-PLB	MEB2	PLB	21.615	8.233	56.747
7	MEB2-MEB1	MEB2	MEB1	1.123	0.784	1.608
8	PP-PLB	PP	PLB	1.169	0.678	2.017
9	MEB1-PP	MEB1	PP	2.520	1.579	4.023
10	MEB2-PP	MEB2	PP	1.392	1.047	1.851
11	MEB1-ALB1	MEB1	ALB1	1.153	0.946	1.404
12	MEB2-ALB1	MEB2	ALB1	1.346	0.760	2.384
13	PP-ALB1	PP	ALB1	0.826	0.654	1.043
14	LEV-ALB1	LEV	ALB1	1.400	0.533	3.678
15	LEV-PP	LEV	PP	0.893	0.659	1.209
16	IVM-ALB1	IVM	ALB1	1.015	0.815	1.264
17	IVM-PP	IVM	PP	2.422	1.463	4.008
18	OX-PLB	OX	PLB	23.000	1.393	379.803
19	OX-ALB1	OX	ALB1	10.000	3.141	31.838
20	OXPP1-PLB	OXPP1	PLB	8.630	3.258	22.860
21	OXPP2-OXPP1	OXPP2	OXPP1	1.247	0.932	1.669
22	OXPP1-ALB1	OXPP1	ALB1	1.721	1.263	2.344
23	OXPP1-MEB1	OXPP1	MEB1	1.313	0.164	10.507
24	OXPP1-OX	OXPP1	OX	0.863	0.757	0.985
25	OXPP2-MEB2	OXPP2	MEB2	1.282	0.800	2.053
26	OXPP2-ALB2	OXPP2	ALB2	1.339	1.096	1.637
27	NIT-ALB1	NIT	ALB1	1.157	0.313	4.279
28	TRI-ALB1	TRI	ALB1	0.570	0.091	3.563
29	TRI-MEB1	TRI	MEB1	7.500	0.424	132.580
30	ALBIVM-ALB1	ALBIVM	ALB1	3.219	1.842	5.625
31	MEBIVM-MEB1	MEBIVM	MEB1	2.923	2.004	4.264
32	ALBMEB-ALBIVM	ALBMEB	ALBIVM	0.306	0.152	0.613
33	ALBMEB-MEB1	ALBMEB	MEB1	4.146	2.217	7.754
34	ALBNIT-PLB	ALBNIT	PLB	1.803	0.925	3.515
35	ALBOX-MEB1	ALBOX	MEB1	4.398	1.455	13.292
36	ALBDEC-ALBIVM	ALBDEC	ALBIVM	0.319	0.243	0.419
37	TRIIVM-TRI	TRIIVM	TRI	4.081	1.993	8.354
38	TRIOX-ALBOX	TRIOX	ALBOX	0.801	0.676	0.948
Indirect estimates (source IDs)						
39	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	9.848	1.925	50.383
40	Indirect ALB2 vs ALB1 (4, 12)	ALB2	ALB1	1.004	0.559	1.802
41	Indirect MEB1 vs ALB1 (5, 1)	MEB1	ALB1	1.029	0.471	2.249
42	Indirect MEB2 vs ALB1 (6, 1)	MEB2	ALB1	6.314	2.259	17.648
43	Indirect MEB2 vs ALB1 (7, 11)	MEB2	ALB1	1.294	0.859	1.950
44	Indirect PP vs ALB1 (8, 1)	PP	ALB1	0.342	0.178	0.654
45	Indirect MEB1 vs ALB1 (9, 13)	MEB1	ALB1	2.082	1.234	3.511
46	Indirect MEB2 vs ALB1 (10, 13)	MEB2	ALB1	1.150	0.796	1.662
47	Indirect LEV vs ALB1 (15, 13)	LEV	ALB1	0.737	0.503	1.081
48	Indirect IVM vs ALB1 (17, 13)	IVM	ALB1	2.000	1.148	3.485
49	Indirect OX vs ALB1 (18, 1)	OX	ALB1	6.719	0.398	113.435
50	Indirect OXPP1 vs ALB1 (20, 1)	OXPP1	ALB1	2.521	0.894	7.106
51	Indirect OXPP2 vs ALB1 (21, 22)	OXPP2	ALB1	2.146	1.403	3.281
52	Indirect OXPP1 vs ALB1 (23, 11)	OXPP1	ALB1	1.514	0.187	12.225
53	Indirect OXPP1 vs ALB1 (24, 19)	OXPP1	ALB1	8.634	2.691	27.694
54	Indirect OXPP2 vs ALB1 (25, 12)	OXPP2	ALB1	1.726	0.823	3.620
55	Indirect OXPP2 vs ALB1 (26, 3)	OXPP2	ALB1	2.328	1.706	3.177

56	Indirect TRI vs ALB1 (29, 11)	TRI	ALB1	8.645	0.486	153.867
57	Indirect MEBIVM vs ALB1 (31, 11)	MEBIVM	ALB1	3.370	2.200	5.160
58	Indirect ALBMEB vs ALB1 (32, 30)	ALBMEB	ALB1	0.984	0.403	2.400
59	Indirect ALBMEB vs ALB1 (33, 11)	ALBMEB	ALB1	4.779	2.479	9.214
60	Indirect ALBNIT vs ALB1 (34, 1)	ALBNIT	ALB1	0.527	0.248	1.121
61	Indirect ALBOX vs ALB1 (35, 11)	ALBOX	ALB1	5.069	1.648	15.593
62	Indirect ALBDEC vs ALB1 (36, 30)	ALBDEC	ALB1	1.027	0.552	1.912
63	Indirect TRIIVM vs ALB1 (37, 28)	TRIIVM	ALB1	2.325	0.325	16.642
64	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	0.052	0.010	0.258
65	Indirect PLB vs ALB1 (5, 11)	PLB	ALB1	0.327	0.158	0.676
66	Indirect PLB vs ALB1 (6, 12)	PLB	ALB1	0.062	0.020	0.191
67	Indirect MEB1 vs ALB1 (7, 12)	MEB1	ALB1	1.199	0.611	2.355
68	Indirect PLB vs ALB1 (8, 13)	PLB	ALB1	0.706	0.390	1.278
69	Indirect PP vs ALB1 (9, 11)	PP	ALB1	0.457	0.275	0.760
70	Indirect PP vs ALB1 (10, 12)	PP	ALB1	0.967	0.511	1.831
71	Indirect PLB vs ALB1 (18, 19)	PLB	ALB1	0.435	0.021	9.034
72	Indirect PLB vs ALB1 (20, 22)	PLB	ALB1	0.199	0.072	0.554
73	Indirect MEB2 vs ALB1 (4, 3)	MEB2	ALB1	2.332	1.782	3.052
74	Indirect PP vs ALB1 (15, 14)	PP	ALB1	1.568	0.570	4.316
75	Indirect PP vs ALB1 (17, 16)	PP	ALB1	0.419	0.242	0.726
76	Indirect MEB1 vs ALB1 (23, 22)	MEB1	ALB1	1.310	0.160	10.726
77	Indirect OX vs ALB1 (24, 22)	OX	ALB1	1.993	1.425	2.789
78	Indirect MEB1 vs ALB1 (29, 28)	MEB1	ALB1	0.076	0.003	2.293
79	Indirect TRIOX vs ALB1 (38, 61)	TRIOX	ALB1	4.059	1.303	12.643
Final estimates from all evidence (source IDs)						
	PLB (1, 64, 65, 66, 68, 71, 72)	PLB	ALB1	0.302	0.140	0.651
	ALB2 (3, 39, 40)	ALB2	ALB1	1.661	0.693	3.983
	MEB2 (12, 42, 43, 46, 73)	MEB2	ALB1	1.722	1.068	2.774
	MEB1 (11, 41, 45, 67, 76, 78)	MEB1	ALB1	1.219	0.855	1.736
	PP (13, 44, 69, 70, 74, 75)	PP	ALB1	0.690	0.419	1.138
	LEV (14, 47)	LEV	ALB1	0.804	0.458	1.414
	IVM (16, 48)	IVM	ALB1	1.112	0.519	2.386
	OX (19, 49, 77)	OX	ALB1	2.291	0.413	12.693
	OXPP1 (22, 50, 52, 53)	OXPP1	ALB1	1.945	0.723	5.229
	OXPP2 (51, 54, 55)	OXPP2	ALB1	2.201	1.735	2.791
	NIT (27)	NIT	ALB1	1.157	0.313	4.279
	TRI (28, 56)	TRI	ALB1	1.249	0.083	18.722
	ALBIVM (30)	ALBIVM	ALB1	3.219	1.842	5.625
	MEBIVM (57)	MEBIVM	ALB1	3.370	2.200	5.160
	ALBMEB (58, 59)	ALBMEB	ALB1	2.742	0.554	13.572
	ALBNIT (60)	ALBNIT	ALB1	0.527	0.248	1.121
	ALBOX (61)	ALBOX	ALB1	5.069	1.648	15.593
	ALBDEC (62)	ALBDEC	ALB1	1.027	0.552	1.912
	TRIIVM (63)	TRIIVM	ALB1	2.325	0.325	16.642
	TRIOX (79)	TRIOX	ALB1	4.059	1.303	12.643
Network $H = 1.646$						

RR = relative risk; LCI = lower confidence interval; HCI = higher confidence interval
[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBDEC = albendazole + diethylcarbamazine; ALBIVM = albendazole + ivermectin; ALBMEB = albendazole + mebendazole; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine; TRIOX = tribendimidine + oxantel pamoate; TRIIVM = tribendimidine + ivermectin

Supplementary Table 8. Direct, indirect and final results comparing difference in egg reduction rate for different treatments for *T. trichiura*

ID	Comparison[a]	Active	Control	dERR	LCI 95%	HCI 95%
Direct estimates						
1	PLB-ALB1	PLB	ALB1	-0.010	-0.310	0.291
2	ALB2-PLB	ALB2	PLB	1.037	0.927	1.147
3	ALB2-ALB1	ALB2	ALB1	0.038	-0.510	0.586
4	MEB1-PLB	MEB1	PLB	0.782	0.491	1.074
5	ALB1-MEB1	ALB1	MEB1	-0.099	-0.219	0.022
6	MEB2-MEB1	MEB2	MEB1	0.176	-0.141	0.493
7	MEB2-ALB1	MEB2	ALB1	0.310	0.238	0.382
8	MEB2-PLB	MEB2	PLB	0.457	-0.020	0.934
9	ALB2-MEB1	ALB2	MEB1	0.113	0.085	0.141
10	ALB2-MEB2	ALB2	MEB2	-0.089	-0.206	0.028
11	LEV-PP	LEV	PP	0.011	-0.254	0.276
12	IVM-ALB1	IVM	ALB1	-0.015	-0.022	-0.008
13	IVM-PLB	IVM	PLB	0.123	0.078	0.169
14	OX-PLB	OX	PLB	0.631	0.611	0.651
15	OX-MEB1	OX	MEB1	0.182	0.175	0.189
16	OX-OXPP1	OX	OXPP1	-0.048	-0.054	-0.043
17	OXPP1-ALB1	OXPP1	ALB1	0.021	0.010	0.033
18	OXPP1-MEB1	OXPP1	MEB1	0.032	0.026	0.039
19	OXPP1-PP	OXPP1	PP	0.315	0.307	0.323
20	OXPP1-PLB	OXPP1	PLB	0.705	0.664	0.746
21	OXPP2-OXPP1	OXPP2	OXPP1	0.049	-0.016	0.114
22	OXPP2-MEB2	OXPP2	MEB2	0.033	0.030	0.036
23	OXPP2-MEB1	OXPP2	MEB1	0.017	0.010	0.025
24	ALBIVM-ALB1	ALBIVM	ALB1	0.410	0.193	0.627
25	ALBDEC-ALB1	ALBDEC	ALB1	-0.024	-0.057	0.010
26	ALBDEC-IVM	ALBDEC	IVM	-0.075	-0.077	-0.073
27	ALBOX-ALB1	ALBOX	ALB1	0.509	0.499	0.519
28	ALBOX-MEB1	ALBOX	MEB1	0.407	0.395	0.419
29	ALBMEB-ALB1	ALBMEB	ALB1	0.558	0.529	0.586
30	ALBMEB-ALBIVM	ALBMEB	ALBIVM	-0.429	-0.442	-0.415
31	NIT-ALB1	NIT	ALB1	0.006	-0.443	0.456
32	TRI-ALB1	TRI	ALB1	-0.215	-0.243	-0.187
33	TRI-MEB1	TRI	MEB1	0.136	0.035	0.238
Indirect estimates (source IDs)						
34	Indirect ALB2 vs ALB1 (2, 1)	ALB2	ALB1	1.028	0.707	1.348
35	Indirect MEB1 vs ALB1 (4, 1)	MEB1	ALB1	0.773	0.354	1.192
36	Indirect MEB2 vs ALB1 (8, 1)	MEB2	ALB1	0.447	-0.117	1.012
37	Indirect ALB2 vs ALB1 (10, 7)	ALB2	ALB1	0.221	0.083	0.358
38	Indirect IVM vs ALB1 (13, 1)	IVM	ALB1	0.114	-0.191	0.418
39	Indirect OXA vs ALB1 (14, 1)	OXA	ALB1	0.621	0.320	0.923
40	Indirect OXA vs ALB1 (16, 17)	OXA	ALB1	-0.027	-0.040	-0.014
41	Indirect OXP1 vs ALB1 (20, 1)	OXP1	ALB1	0.696	0.392	1.000
42	Indirect OXP2 vs ALB1 (21, 17)	OXP2	ALB1	0.071	0.005	0.137
43	Indirect OXP2 vs ALB1 (22, 7)	OXP2	ALB1	0.343	0.271	0.415
44	Indirect ALBDEC vs ALB1 (26, 12)	ALBDEC	ALB1	-0.090	-0.097	-0.082
45	Indirect ALBMEB vs ALB1 (30, 24)	ALBMEB	ALB1	-0.019	-0.236	0.198
46	Indirect PLB vs ALB1 (2, 3)	PLB	ALB1	-0.999	-1.558	-0.441
47	Indirect MEB1 vs ALB1 (6, 7)	MEB1	ALB1	0.133	-0.192	0.459
48	Indirect MEB1 vs ALB1 (9, 3)	MEB1	ALB1	-0.075	-0.623	0.474
49	Indirect MEB2 vs ALB1 (10, 3)	MEB2	ALB1	0.127	-0.433	0.687
50	Indirect PLB vs ALB1 (8, 7)	PLB	ALB1	-0.147	-0.630	0.336
51	Indirect PLB vs ALB1 (13, 12)	PLB	ALB1	-0.138	-0.184	-0.092
52	Indirect MEB1 vs ALB1 (18, 17)	MEB1	ALB1	-0.011	-0.024	0.002
53	Indirect PP vs ALB1 (19, 17)	PP	ALB1	-0.294	-0.308	-0.280
54	Indirect PLB vs ALB1 (20, 17)	PLB	ALB1	-0.684	-0.727	-0.641
55	Indirect IVM vs ALB1 (26, 25)	IVM	ALB1	0.051	0.018	0.085
56	Indirect MEB1 vs ALB1 (28, 27)	MEB1	ALB1	0.102	0.087	0.118
57	Indirect ALBIVM vs ALB1 (30, 29)	ALBIVM	ALB1	0.986	0.955	1.018
58	Indirect MEB1 vs ALB1 (33, 32)	MEB1	ALB1	-0.351	-0.456	-0.246
59	Indirect PLB vs ALB1 (4, 5)	PLB	ALB1	-0.684	-0.999	-0.369
60	Indirect MEB2 vs ALB1 (6, 5)	MEB2	ALB1	0.275	-0.065	0.614
61	Indirect ALB2 vs ALB1 (9, 5)	ALB2	ALB1	0.212	0.088	0.335
62	Indirect OXA vs ALB1 (15, 5)	OXA	ALB1	0.281	0.160	0.401
63	Indirect OXP1 vs ALB1 (18, 5)	OXP1	ALB1	0.131	0.010	0.252
64	Indirect OXP2 vs ALB1 (23, 5)	OXP2	ALB1	0.116	-0.005	0.236
65	Indirect ALBOX vs ALB1 (28, 5)	ALBOX	ALB1	0.505	0.384	0.627

66	Indirect TRI vs ALB1 (33, 5)	TRI	ALB1	0.235	0.078	0.392
67	Indirect LEV vs ALB1 (11, 53)	LEV	ALB1	-0.283	-0.548	-0.017
Final estimates from all evidence (source IDs)						
	PLB (1, 46, 50, 51, 54, 59)	PLB	ALB1	-0.430	-0.941	0.081
	ALB2 (3, 34, 37, 61)	ALB2	ALB1	0.271	-0.074	0.616
	MEB1 (5, 35, 47, 48, 52, 56, 58)	MEB1	ALB1	0.034	-0.098	0.166
	MEB2 (7, 36, 49, 60)	MEB2	ALB1	0.307	0.238	0.377
	LEV (67)	LEV	ALB1	-0.283	-0.548	-0.017
	PP (53)	PP	ALB1	-0.294	-0.308	-0.280
	IVM (12, 38, 55)	IVM	ALB1	-0.012	-0.095	0.071
	OXA (39, 40, 62)	OXA	ALB1	-0.023	-0.522	0.477
	OXP1 (17, 41, 63)	OXP1	ALB1	0.023	-0.329	0.376
	OXP2 (42, 43, 64)	OXP2	ALB1	0.184	-0.018	0.385
	ALBIVM (24, 57)	ALBIVM	ALB1	0.974	0.206	1.742
	ALBDEC (25, 44)	ALBDEC	ALB1	-0.087	-0.171	-0.002
	ALBOX (27, 65)	ALBOX	ALB1	0.509	0.499	0.519
	ALBMEB (29, 45)	ALBMEB	ALB1	0.548	-0.223	1.319
	NIT (31)	NIT	ALB1	0.006	-0.443	0.456
	TRI (32, 66)	TRI	ALB1	-0.201	-0.796	0.394
Network $H = 4.702$						

dERR = difference in egg reduction rate; LCI = lower confidence interval; HCI = higher confidence interval
[a] ALB1 = single-dose albendazole; ALB2 = multiple-dose albendazole; ALBDEC = albendazole + diethylcarbamazine; ALBIVM = albendazole + ivermectin; ALBMEB = albendazole + mebendazole; ALBOX = albendazole + oxantel pamoate; IVM = ivermectin; LEV = levamisole; MEB1 = single-dose mebendazole; MEB2 = multiple-dose mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; OXPP1 = single-dose oxantel + pyrantel pamoate; OXPP2 = multiple-dose oxantel + pyrantel pamoate; PLB = placebo; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 9. Results using the multivariate frequentist and GPM frameworks for relative risk of cure

	A. lumbricoides				Hookworm				*T. trichiura*			
Treatment[a]	**GPM**		**Multivariate frequentist**		**GPM**		**Multivariate frequentist**		**GPM**		**Multivariate frequentist**	
	RR of cure	**95% CI**	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**
Multiple-dose ALB	1.004	0.797, 1.264	1.003	0.984, 1.022	1.141	1.049, 1.241	1.064	0.794, 1.427	1.661	0.693, 3.983	1.881	1.402, 2.525
Single-dose MEB	0.992	0.980, 1.004	0.990	0.978, 1.002	0.582	0.403, 0.840	0.452	0.364, 0.562	1.219	0.855, 1.736	1.291	1.040, 1.603
Multiple-dose MEB	0.977	0.923, 1.034	0.985	0.966, 1.005	0.961	0.539, 1.713	0.757	0.599, 0.958	1.722	1.068, 2.774	1.801	1.412, 2.297
Single-dose PP	1.000	0.986, 1.013	1.000	0.995, 1.005	0.940	0.772, 1.145	0.768	0.583, 1.010	0.690	0.419, 1.138	0.723	0.511, 1.021
Single-dose IVM	1.010	0.987, 1.034	1.010	0.987, 1.034	0.377	0.204, 0.696	0.351	0.201, 0.614	1.112	0.519, 2.386	1.190	0.780, 1.816
Single-dose LEV	0.963	0.920, 1.009	0.963	0.925, 1.002	1.351	0.886, 2.060	0.929	0.600, 1.437	0.804	0.458, 1.414	0.703	0.398, 1.241
Single-dose OX	0.121	0.032, 0.456	0.120	0.069, 0.209	0.195	0.116, 0.330	0.210	0.096, 0.456	2.291	0.413, 12.693	2.440	1.353, 4.402
NIT (any dose)	0.943	0.622, 1.429	0.943	0.806, 1.103	0.815	0.500, 1.327	0.815	0.336, 1.973	1.157	0.313, 4.279	0.953	0.488, 1.859
Single-dose TRI	0.978	0.318, 3.010	0.978	0.945, 1.012	1.143	0.109, 12.009	1.137	0.809, 1.597	1.249	0.083, 18.722	0.628	0.284, 1.388
Single-dose ALB + IVM	0.931	0.768, 1.129	0.931	0.768, 1.129	0.832	0.584, 1.184	0.946	0.545, 1.642	3.219	1.842, 5.625	4.350	2.285, 8.281
Single-dose MEB + IVM	1.265	0.974, 1.643	1.263	0.972, 1.640	0.317	0.134, 0.751	0.329	0.115, 0.948	3.370	2.200, 5.160	3.774	1.590, 8.961
Single-dose ALB + MEB	0.926	0.756, 1.135	0.926	0.756, 1.135	0.795	0.457, 1.385	0.905	0.328, 2.499	2.742	0.554, 13.572	2.755	1.262, 6.011
Single-dose ALB + DEC	0.905	0.715, 1.146	0.905	0.715, 1.146	-	-	-	-	1.027	0.552, 1.912	1.422	0.590, 3.426
Single-dose ALB + NIT	-	-	-	-	0.403	0.128, 1.266	0.306	0.112, 0.833	0.527	0.248, 1.121	0.488	0.170, 1.406
Single-dose ALB + OX	1.020	0.960, 1.084	1.019	0.959, 1.082	1.094	0.575, 2.080	1.086	0.551, 2.137	5.069	1.648, 15.593	5.865	2.869, 11.989
Single-dose OX + PP	0.991	0.959, 1.025	0.991	0.960, 1.022	0.452	0.322, 0.636	0.467	0.314, 0.696	1.945	0.723, 5.229	1.636	1.146, 2.334
Multiple-dose OX + PP	0.988	0.944, 1.034	0.987	0.945, 1.030	1.031	0.593, 1.793	1.174	0.695, 1.982	2.201	1.735, 2.791	2.274	1.564, 3.532
Single-dose TRI + IVM	1.028	0.928, 1.138	1.026	0.924, 1.141	1.078	0.825, 1.410	1.761	0.638, 4.857	2.325	0.325, 16.642	2.563	0.696, 9.442
Single-dose TRI + OX	0.978	0.912, 1.049	0.978	0.930, 1.030	1.774	0.915, 3.441	1.081	0.477, 2.447	4.059	1.303, 12.643	4.696	1.663, 13.256
Placebo / no treatment	0.121	0.076, 0.193	0.163	0.135, 0.196	0.182	0.121, 0.274	0.198	0.151, 0.261	0.302	0.140, 0.651	0.271	0.193, 0.380

GPM = generalized pair-wise modelling; RR = relative risk; CI = confidence interval

[a] ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 10. Results using the multivariate frequentist and GPM frameworks for difference in egg reduction rates

	A. lumbricoides				Hookworm				*T. trichiura*			
Treatment[a]	**GPM**		**Multivariate frequentist**		**GPM**		**Multivariate frequentist**		**GPM**		**Multivariate frequentist**	
	dERR	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**
Multiple-dose ALB	0.001	-0.369, 0.370	0.072	-0.048, 0.192	0.091	-0.016, 0.199	0.161	-0.016, 0.337	0.271	-0.074, 0.616	0.304	0.145, 0.462
Single-dose MEB	0.000	-0.003, 0.002	-0.012	-0.089, 0.065	-0.119	-0.183, -0.055	-0.166	-0.276, -0.056	0.034	-0.098, 0.166	0.098	-0.019, 0.215
Multiple-dose MEB	0.079	0.004, 0.154	0.009	-0.106, 0.124	-0.044	-0.423, 0.334	0.006	-0.161, 0.174	0.307	0.238, 0.377	0.302	0.138, 0.466
Single-dose PP	-0.044	-0.264, 0.175	-0.009	-0.152, 0.133	-0.035	-0.457, 0.387	0.116	-0.054, 0.286	-0.294	-0.308, -0.280	-0.134	-0.653, 0.384
Single-dose IVM	-0.052	-0.121, 0.017	-0.050	-0.204, 0.105	-0.134	-0.201, -0.067	-0.150	-0.393, 0.093	-0.012	-0.095, 0.071	-0.078	-0.330, 0.175
Single-dose LEV	-0.211	-0.726, 0.305	0.172	-0.018, 0.361	-0.089	-0.516, 0.338	0.097	-0.141, 0.334	-0.283	-0.548, -0.017	-0.066	-0.680, 0.548
Single-dose OX	-0.704	-1.004, -0.404	-0.611	-0.829, -0.392	-0.315	-0.418, -0.212	-0.314	-0.627, -0.001	-0.023	-0.522, 0.477	0.217	-0.079, 0.512
NIT (any dose)	0.000	0.000, 0.001	0.000	-0.291, 0.291	-	-	-	-	0.006	-0.443, 0.456	-0.152	-0.481, 0.177
Single-dose TRI	-0.241	-0.452, -0.031	-0.171	-0.383, 0.042	0.004	-0.005, 0.012	0.041	-0.134, 0.216	-0.201	-0.796, 0.394	0.005	-0.332, 0.343
Single-dose ALB + IVM	0.000	-0.070, 0.071	0.001	-0.175, 0.176	0.005	-0.042, 0.051	0.016	-0.199, 0.232	0.974	0.206, 1.742	0.431	0.212, 0.650
Single-dose ALB + MEB	-	-	-	-	-	-	-	-	0.548	-0.223, 1.319	0.280	-0.067, 0.626
Single-dose ALB + DEC	0.033	-0.107, 0.172	-0.016	-0.222, 0.190	-	-	-	-	-0.087	-0.171, -0.002	-0.088	-0.441, 0.264
Single-dose ALB + OX	0.000	0.000, 0.000	-0.006	-0.215, 0.203	-0.009	-0.039, 0.020	0.000	-0.256, 0.256	0.509	0.499, 0.519	0.507	0.173, 0.841
Single-dose OX + PP	-0.005	-0.006, -0.004	-0.018	-0.161, 0.125	0.003	-0.093, 0.099	0.005	-0.210, 0.220	0.023	-0.329, 0.376	0.181	-0.050, 0.411
Multiple-dose OX + PP	-0.004	-0.247, 0.239	0.009	-0.303, 0.322	0.014	-0.123, 0.151	0.073	-0.258, 0.405	0.184	-0.018, 0.385	0.228	-0.046, 0.501
Placebo / no treatment	-0.802	-1.194, -0.410	-0.833	-0.931, -0.735	-0.713	-0.984, -0.441	-0.602	-0.738, -0.467	-0.430	-0.941, 0.081	-0.394	-0.542, -0.245

GPM = generalized pair-wise modelling; dERR=difference in egg reduction rates; CI = confidence interval

[a] ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine

Supplementary Table 11. Results of network-meta-analysis for relative risk of cure obtained in sensitivity analysis

	Only studies using Kato-Katz diagnostic method (n=66)		Only studies examining efficacy at 14-21 days (n=60)		All studies (n=108)		
Treatment	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**	**RR of cure**	**95% CI**	***H* index**
			Ascaris lumbricoides				
Multiple-dose albendazole	1.002	0.852, 1.178	1.003	0.983, 1.024	1.004	0.797, 1.264	1.845
Single-dose mebendazole	0.991	0.979, 1.003	1.001	0.928, 1.079	0.992	0.980, 1.004	0.892
Multiple-dose mebendazole	1.010	0.964, 1.058	1.014	0.965, 1.065	0.977	0.923, 1.034	1.365
Single-dose pyrantel pamoate	1.021	0.967, 1.078	1.000	0.995, 1.005	1.000	0.986, 1.013	1.012
Single-dose ivermectin	1.011	0.986, 1.035	1.000	0.912, 1.095	1.010	0.987, 1.034	0.227
Single-dose levamisole	1.400	0.615, 3.187	0.968	0.917, 1.022	0.963	0.920, 1.009	0.766
Single-dose oxantel pamoate	0.130	0.064, 0.266	0.115	0.062, 0.212	0.121	0.032, 0.456	1.626
Nitazoxanide (any dose)	0.943	0.622, 1.429	0.643	0.375, 1.104	0.943	0.622, 1.429	1.000
Single-dose tribendimidine	0.978	0.318, 3.009	3.748	0.479, 29.332	0.978	0.318, 3.010	1.263
Single-dose albendazole + ivermectin	0.931	0.768, 1.129	0.931	0.768, 1.129	0.931	0.768, 1.129	1.000
Single-dose mebendazole + ivermectin	1.265	0.974, 1.643	1.287	0.989, 1.676	1.265	0.974, 1.643	1.000
Single-dose albendazole + mebendazole	0.926	0.756, 1.135	0.926	0.756, 1.135	0.926	0.756, 1.135	1.000
Single-dose albendazole + DEC	0.905	0.715, 1.146	-	-	0.905	0.715, 1.146	1.000
Single-dose albendazole + oxantel pamoate	1.020	0.960, 1.084	1.039	0.969, 1.113	1.020	0.960, 1.084	1.000
Single-dose oxantel + pyrantel pamoate	0.976	0.936, 1.018	1.001	0.959, 1.045	0.991	0.959, 1.025	1.001
Multiple-dose oxantel + pyrantel pamoate	0.986	0.930, 1.045	0.987	0.939, 1.038	0.988	0.944, 1.034	0.092
Single-dose tribendimidine + ivermectin	0.978	0.912, 1.049	3.751	0.479, 29.364	0.978	0.912, 1.049	1.000
Single-dose tribendimidine + oxantel pamoate	1.028	0.928, 1.138	1.046	0.940, 1.165	1.028	0.928, 1.138	1.000
Placebo / no treatment	0.119	0.056, 0.254	0.126	0.058, 0.276	0.121	0.076, 0.193	1.284
			Hookworm				
Multiple-dose albendazole	1.338	0.709, 2.525	1.093	0.714, 1.675	1.141	1.049, 1.241	0.751
Single-dose mebendazole	0.565	0.329, 0.971	0.591	0.410, 0.853	0.582	0.403, 0.840	1.200
Multiple-dose mebendazole	1.000	0.833, 1.200	0.784	0.282, 2.183	0.961	0.539, 1.713	1.424
Single-dose pyrantel pamoate	1.090	0.931, 1.277	0.922	0.529, 1.606	0.940	0.772, 1.145	1.005
Single-dose ivermectin	0.432	0.198, 0.942	0.321	0.135, 0.766	0.377	0.204, 0.696	0.561
Single-dose levamisole	1.163	0.748, 1.807	1.203	0.726, 1.993	1.351	0.886, 2.060	1.148
Single-dose oxantel pamoate	0.183	0.109, 0.306	0.187	0.111, 0.313	0.195	0.116, 0.330	0.994
Nitazoxanide (any dose)	0.815	0.500, 1.327	0.815	0.500, 1.327	0.815	0.500, 1.327	1.000

Single-dose tribendimidine	1.143	0.105, 12.446	14.027	0.858, 229.257	1.143	0.109, 12.009	1.304
Single-dose albendazole + ivermectin	0.832	0.584, 1.184	0.832	0.584, 1.184	0.832	0.584, 1.184	1.000
Single-dose mebendazole + ivermectin	0.324	0.136, 0.768	0.427	0.179, 1.015	0.317	0.134, 0.751	1.000
Single-dose albendazole + mebendazole	0.795	0.457, 1.385	0.795	0.457, 1.385	0.795	0.457, 1.385	1.000
Single-dose albendazole + nitazoxanide	0.215	0.088, 0.530	0.257	0.098, 0.675	0.403	0.128, 1.266	1.000
Single-dose albendazole + oxantel pamoate	1.116	0.586, 2.127	1.471	0.769, 2.814	1.094	0.575, 2.080	1.000
Single-dose oxantel + pyrantel pamoate	0.579	0.402, 0.834	0.497	0.326, 0.758	0.452	0.322, 0.636	1.000
Multiple-dose oxantel + pyrantel pamoate	1.014	0.923, 1.113	1.024	0.494, 2.122	1.031	0.593, 1.793	1.000
Single-dose tribendimidine + ivermectin	1.810	0.932, 3.518	2.386	1.223, 4.655	1.078	0.825, 1.410	1.000
Single-dose tribendimidine + oxantel pamoate	1.078	0.825, 1.410	13.334	0.809, 219.711	1.774	0.915, 3.441	1.000
Placebo / no treatment	0.202	0.131, 0.313	0.315	0.155, 0.638	0.182	0.121, 0.274	1.107
Trichuris trichiura							
Multiple-dose albendazole	1.653	0.185, 14.739	1.752	0.695, 4.42	1.661	0.693, 3.983	1.944
Single-dose mebendazole	1.155	1.014, 1.316	1.291	1.004, 1.661	1.219	0.855, 1.736	1.189
Multiple-dose mebendazole	1.366	0.824, 2.262	1.379	0.923, 2.059	1.722	1.068, 2.774	2.134
Single-dose pyrantel pamoate	0.595	0.374, 0.947	0.751	0.323, 1.744	0.690	0.419, 1.138	1.792
Single-dose ivermectin	1.015	0.815, 1.264	2.000	1.148, 3.485	1.112	0.519, 2.386	1.210
Single-dose levamisole	1.400	0.533, 3.678	0.720	0.312, 1.661	0.804	0.458, 1.414	2.227
Single-dose oxantel pamoate	9.428	3.229, 27.531	8.900	3.030, 26.142	2.291	0.413, 12.693	1.928
Nitazoxanide (any dose)	1.157	0.313, 4.279	0.456	0.214, 0.972	1.157	0.313, 4.279	1.000
Single-dose tribendimidine	1.254	0.083, 19.024	8.824	0.491, 158.703	1.249	0.083, 18.722	1.562
Single-dose albendazole + ivermectin	3.219	1.842, 5.625	3.219	1.842, 5.625	3.219	1.842, 5.625	1.000
Single-dose mebendazole + ivermectin	3.404	2.281, 5.080	3.439	2.103, 5.624	3.370	2.200, 5.160	1.000
Single-dose albendazole + mebendazole	2.811	0.557, 14.175	2.647	0.534, 13.127	2.742	0.554, 13.572	2.798
Single-dose albendazole + DEC	1.027	0.552, 1.912	1.182	0.574, 2.432	1.027	0.552, 1.912	1.000
Single-dose albendazole + nitazoxanide	0.520	0.244, 1.109	0.329	0.109, 0.997	0.527	0.248, 1.121	1.000
Single-dose albendazole + oxantel pamoate	5.121	1.681, 15.602	5.174	1.638, 16.343	5.069	1.648, 15.593	1.000
Single-dose oxantel + pyrantel pamoate	1.965	1.176, 3.282	1.654	1.179, 2.319	1.945	0.723, 5.229	1.574
Multiple-dose oxantel + pyrantel pamoate	2.162	1.648, 2.836	2.346	1.679, 3.277	2.201	1.735, 2.791	0.523
Single-dose tribendimidine + ivermectin	2.325	0.325, 16.642	36.008	1.834, 706.818	2.325	0.325, 16.642	1.000
Single-dose tribendimidine + oxantel pamoate	4.100	1.329, 12.652	4.143	1.295, 13.248	4.059	1.303, 12.643	1.000
Placebo / no treatment	0.278	0.147, 0.527	0.278	0.115, 0.671	0.302	0.140, 0.651	1.866

RR = relative risk; CI = confidence interval; DEC = diethylcarbamazine

Supplementary Table 12. Results of network-meta-analysis for difference in egg reduction rate obtained in sensitivity analysis

	Only studies using Kato-Katz diagnostic method (n=56)		Only studies examining efficacy at 14-21 days (n=45)		Excluding studies reporting geometric mean (n=57)		All studies (n=76)		
Treatment	**dERR**	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**	**dERR**	**95% CI**	***H* index**
				Ascaris lumbricoides					
Multiple-dose albendazole	0.001	-0.366, 0.369	0.000	-0.016, 0.016	0.000	-0.314, 0.314	0.001	-0.369, 0.370	1.655
Single-dose mebendazole	0.000	-0.004, 0.003	-0.001	-0.025, 0.024	-0.006	-0.077, 0.065	0.000	-0.003, 0.002	1.888
Multiple-dose mebendazole	0.079	0.004, 0.154	0.031	0.025, 0.037	0.031	0.025, 0.037	0.079	0.004, 0.154	0.704
Single-dose pyrantel pamoate	-0.235	-0.989, 0.520	-0.518	-1.201, 0.166	-0.045	-0.265, 0.176	-0.044	-0.264, 0.175	0.658
Single-dose ivermectin	-0.053	-0.124, 0.019	ND	ND	0.000	-0.021, 0.021	-0.052	-0.121, 0.017	3.482
Single-dose levamisole	-0.265	-1.02, 0.490	-0.545	-0.552, -0.539	-0.211	-0.726, 0.305	-0.211	-0.726, 0.305	1.000
Single-dose oxantel pamoate	-0.704	-1.004, -0.404	-0.730	-0.883, -0.578	ND	ND	-0.704	-1.004, -0.404	1.459
Nitazoxanide (any dose)	0.000	0.000, 0.001	ND	ND	ND	ND	0.000	0.000, 0.001	1.000
Single-dose tribendimidine	0.000	0.000, 0.000	-0.111	-0.167, -0.055	-0.242	-0.245, -0.239	-0.241	-0.452, -0.031	3.469
Single-dose albendazole + ivermectin	-0.241	-0.452, -0.031	-0.001	-0.001, 0	ND	ND	0.000	-0.070, 0.071	4.731
Single-dose albendazole + DEC	0.036	0.036, 0.037	ND	ND	ND	ND	0.033	-0.107, 0.172	1.000
Single-dose albendazole + oxantel pamoate	-0.000	0.000, 0.000	0.000	-0.078, 0.078	ND	ND	-0.000	0.000, 0.000	0.011
Single-dose oxantel + pyrantel pamoate	0.000	-0.086, 0.086	-0.011	-0.082, 0.06	-0.011	-0.082, 0.059	-0.005	-0.006, -0.004	0.305
Multiple-dose oxantel + pyrantel pamoate	ND	ND	ND	ND	-0.004	-0.248, 0.241	-0.004	-0.247, 0.239	1.000
Placebo / no treatment	-1.076	-1.305, -0.847	-0.968	-1.084, -0.853	-1.058	-1.186, -0.931	-0.802	-1.194, -0.410	7.160
				Hookworm					
Multiple-dose albendazole	0.082	-0.59, 0.755	0.128	-0.031, 0.286	0.112	0.103, 0.121	0.091	-0.016, 0.199	0.034
Single-dose mebendazole	-0.119	-0.186, -0.052	-0.029	-0.323, 0.266	-0.187	-0.278, -0.096	-0.119	-0.183, -0.055	0.442
Multiple-dose mebendazole	-0.011	-0.019, -0.003	-0.049	-0.41, 0.312	0.056	-0.581, 0.694	-0.044	-0.423, 0.334	14.114
Single-dose pyrantel pamoate	0.249	-0.421, 0.918	-0.035	-0.414, 0.344	-0.036	-0.490, 0.417	-0.035	-0.457, 0.387	14.109
Single-dose ivermectin	-0.135	-0.203, -0.067	ND	ND	-0.135	-0.203, -0.067	-0.134	-0.201, -0.067	0.376
Single-dose levamisole	-0.089	-0.119, -0.059	-0.124	-0.381, 0.134	0.165	-0.463, 0.794	-0.089	-0.516, 0.338	1.220
Single-dose oxantel pamoate	-0.314	-0.428, -0.201	-0.480	-0.75, -0.211	ND	ND	-0.315	-0.418, -0.212	0.624
Single-dose tribendimidine	0.004	-0.005, 0.012	0.000	-0.01, 0.01	0.013	-0.005, 0.030	0.004	-0.005, 0.012	0.844
Single-dose albendazole + ivermectin	0.019	-0.015, 0.054	0.019	-0.015, 0.054	0.001	-0.009, 0.012	0.005	-0.042, 0.051	3.458

Single-dose albendazole + oxantel pamoate	-0.007	-0.031, 0.017	-0.010	-0.051, 0.031	ND	ND	-0.009	-0.039, 0.02	2.767
Single-dose oxantel + pyrantel pamoate	-0.110	-0.218, -0.001	-0.026	-0.212, 0.16	-0.028	-0.066, 0.010	0.003	-0.093, 0.099	0.877
Multiple-dose oxantel + pyrantel pamoate	ND	ND	-0.084	-0.115, -0.054	-0.084	-0.115, -0.054	0.014	-0.123, 0.151	6.785
Placebo / no treatment	-0.771	-0.828, -0.713	-0.808	-0.95, -0.666	-0.397	-1.212, 0.419	-0.713	-0.984, -0.441	2.630
Trichuris trichiura									
Multiple-dose albendazole	0.297	-0.106, 0.701	0.218	0.104, 0.332	0.360	-0.517, 1.238	0.271	-0.074, 0.616	2.801
Single-dose mebendazole	0.034	-0.099, 0.166	0.109	-0.458, 0.676	0.193	-0.25, 0.637	0.034	-0.098, 0.166	5.595
Multiple-dose mebendazole	0.305	0.235, 0.375	0.312	0.242, 0.382	0.312	0.241, 0.382	0.307	0.238, 0.377	0.474
Single-dose pyrantel pamoate	ND	ND	-0.176	-0.391, 0.039	-0.294	-0.308, -0.28	-0.294	-0.308, -0.28	1.000
Single-dose ivermectin	-0.015	-0.022, -0.008	ND	ND	-0.015	-0.022, -0.008	-0.012	-0.095, 0.071	2.737
Single-dose levamisole	ND	ND	-0.165	-0.507, 0.176	-0.283	-0.548, -0.017	-0.283	-0.548, -0.017	1.000
Single-dose oxantel pamoate	0.306	-0.126, 0.738	0.685	0.157, 1.212	-0.027	-0.04, -0.014	-0.023	-0.522, 0.477	4.595
Nitazoxanide (any dose)	0.006	-0.443, 0.456	-0.318	-0.357, -0.28	-	-	0.006	-0.443, 0.456	1.000
Single-dose tribendimidine	-0.206	-0.716, 0.304	0.243	0.006, 0.481	-0.215	-0.243, -0.187	-0.003	-0.299, 0.294	11.658
Single-dose albendazole + ivermectin	0.974	0.206, 1.742	0.980	0.024, 1.936	0.410	0.181, 0.638	0.974	0.206, 1.742	5.160
Single-dose albendazole + mebendazole	0.548	-0.223, 1.319	0.552	-0.405, 1.51	-	-	0.548	-0.223, 1.319	5.160
Single-dose albendazole + DEC	-0.090	-0.097, -0.082	ND	ND	-0.090	-0.097, -0.082	-0.087	-0.171, -0.002	3.785
Single-dose albendazole + oxantel pamoate	0.509	0.499, 0.519	0.509	0.499, 0.519	-	-	0.482	0.174, 0.791	11.471
Single-dose oxantel + pyrantel pamoate	0.022	0.01, 0.033	0.139	-0.076, 0.354	0.022	-0.886, 0.931	0.023	-0.329, 0.376	3.316
Multiple-dose oxantel + pyrantel pamoate	0.038	0.026, 0.05	0.124	-0.091, 0.339	0.191	-0.046, 0.428	0.184	-0.018, 0.385	3.954
Placebo / no treatment	-0.383	-1.002, 0.235	0.057	-0.571, 0.685	-0.667	-1.341, 0.007	-0.430	-0.941, 0.081	7.827

CI = confidence interval; DEC = diethylcarbamazine; dERR = difference in egg reduction rate

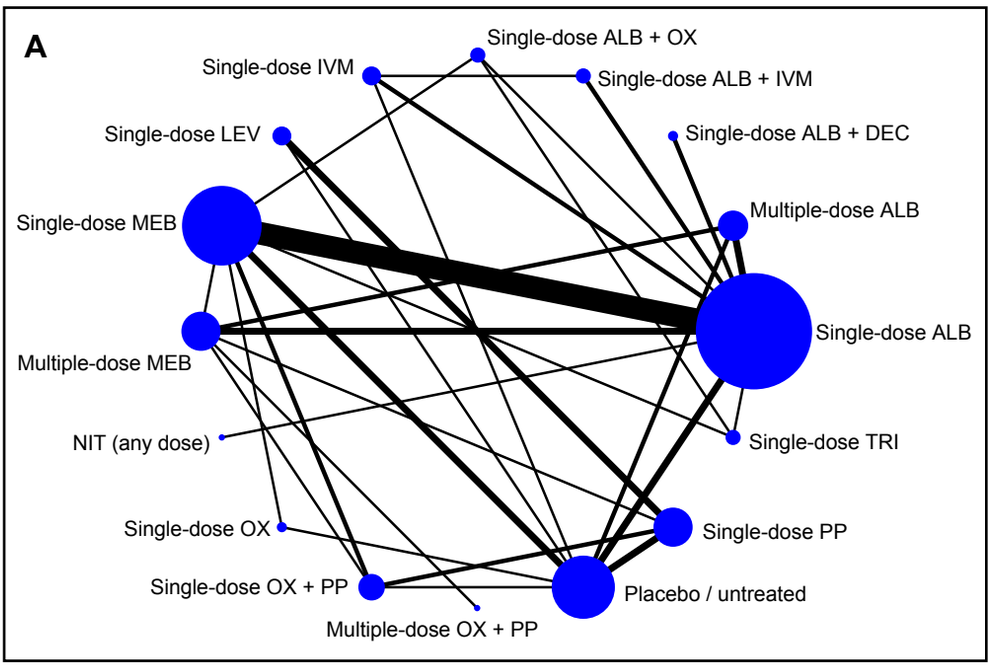
Supplementary Table 13. Quality assessment of studies included in meta-analysis

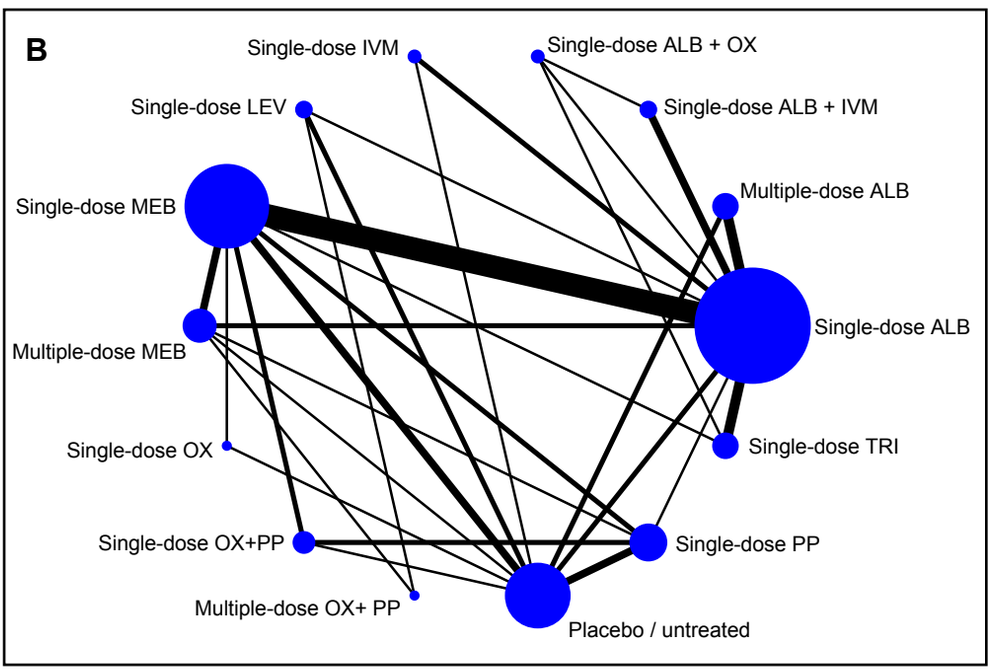
Author & year	Study design (max score 2)	Randomization method	Inclusion/ exclusion criteria	Recruitment strategy	Interval between treatment and assessment	Blinding of outcome assessors	Intervention consistency	Confounding variables at baseline (max score 3)	Data for effect sizes	**TOTAL SCORE (max 12)**
Abadi, 1985	0	0	1	1	1	1	1	1	1	7
Adams et al, 2004	2	1	1	1	1	1	1	1	1	10
Adegnika et al, 2014	2	1	1	1	1	1	1	3	1	12
Adugna et al, 2007	1	0	1	1	1	0	1	2	1	8
Albonico et al, 1994	2	1	1	1	1	0	1	1	1	9
Albonico et al, 2002	2	1	1	1	1	1	1	3	1	12
Albonico et al, 2003	2	1	1	1	1	1	1	2	1	11
Amato Neto et al, 1976	2	0	1	1	1	0	1	0	1	6
Amato Neto et al, 1983	1	0	1	1	1	0	1	1	1	7
Anonymous, 1983	0	0	1	1	1	0	1	0	1	5
Balasuriya et al, 1990	0	0	1	1	1	0	1	0	1	5
Bartoloni et al, 1993	0	0	1	1	1	0	1	0	1	5
Bassily et al, 1984	0	0	0	0	1	0	1	1	1	4
Beach et al, 1999	2	1	1	1	1	1	1	1	1	10
Belizario et al, 2003	2	0	1	1	1	0	1	1	1	8
Bwibo et al, 1984	2	0	1	1	1	1	1	1	1	9
Cabrera et al, 1980a	1	0	1	1	1	0	1	0	1	6
Cabrera et al, 1980b	0	0	1	1	1	0	1	0	1	5
Cabrera et al, 1980c	1	0	1	1	1	0	1	2	1	7
Cao et al, 2000	2	0	1	1	1	0	1	0	1	7
Chaia et al, 1971	0	0	0	0	1	0	1	0	1	3
Charoenlarp et al, 1993	1	0	1	1	1	1	1	3	1	10
Chien et al, 1989	1	0	1	1	1	0	1	1	1	7
Choi et al, 1979	0	0	0	0	1	0	1	0	1	3
Cruz, 1983	1	0	1	1	1	0	1	1	1	7

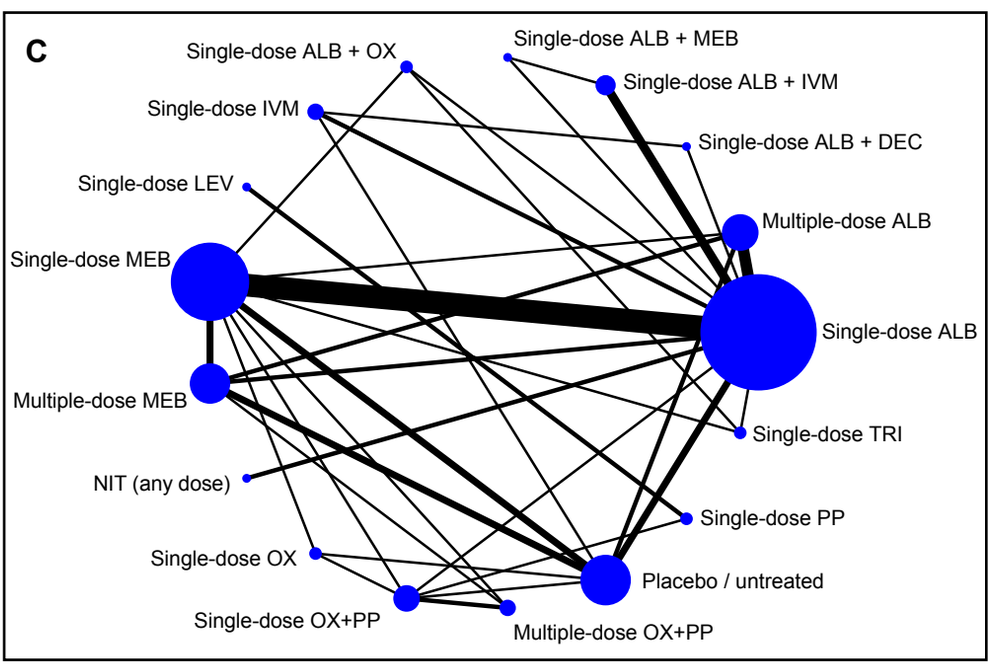
De Clercq et al, 1997	2	1	1	1	1	0	1	3	1	11
De Silva et al, 1987	0	0	0	0	1	0	1	0	1	3
Dissainaike, 1978	1	0	1	1	1	0	1	2	1	8
El-Masry et al, 1983	2	0	1	1	1	1	1	2	1	9
Excler et al, 1981	2	1	1	1	1	0	1	1	1	9
Farahmandian et al, 1972	0	0	0	0	1	0	0	1	1	3
Farahmandian et al, 1977	1	0	1	1	1	0	0	2	1	7
Fan et al, 1992	0	0	1	1	1	0	1	0	1	5
Fang et al, 2002	2	0	1	1	1	1	1	0	1	8
Farid et al, 1977	0	0	1	1	1	0	1	3	1	8
Farid et al, 1984	2	0	1	1	1	1	1	1	1	9
Flohr et al, 2007 (two trials)	2	1	1	1	1	1	1	3	1	12
	1	1	1	1	1	1	1	2	1	10
Fox et al, 2005	2	1	1	1	1	1	1	3	1	12
Gan et al, 1994	0	0	1	1	1	0	1	0	1	5
Ghadirian et al, 1972	0	0	1	1	1	0	0	0	1	4
Griffin et al, 1982	0	0	1	1	1	0	1	0	1	5
Hadju et al, 1996	2	0	1	1	1	0	1	3	1	10
Holzer et al, 1987	0	0	1	1	1	1	1	3	1	9
Ismail et al, 1991	1	1	1	1	1	0	1	1	1	8
Ismail et al, 1999	1	0	1	1	1	0	1	1	1	7
Jongsuksuntigul et al, 1993	1	0	1	1	1	1	1	0	1	7
Kale et al, 1982	1	0	1	1	1	0	1	1	1	7
Kan, 1984	0	0	1	1	1	0	1	0	1	5
Karyadi et al, 1996	2	0	1	1	1	0	1	1	1	8
Katz et al, 1972	0	0	0	0	1	0	1	0	1	3
Kilpatrick et al, 1981	0	0	1	0	1	0	1	1	1	5
Klein, 1972	0	0	1	1	1	0	1	0	1	5
Knopp et al, 2010	2	1	1	1	1	1	1	3	1	12
Krepel et al, 1993	0	0	1	1	1	0	1	0	1	5

Lara-Aguilera et al, 1984	0	0	1	1	1	1	1	0	1	6
Lee et al, 1978	0	0	1	1	1	0	1	0	1	5
Legesse et al, 2002	1	1	1	1	1	0	1	0	1	7
Legesse et al, 2004	1	1	1	1	1	0	1	3	1	10
Lim et al, 1978	0	0	0	0	1	0	1	0	1	3
Ma et al, 2001	2	1	1	1	1	0	1	0	1	8
Mawdsley et al, 1975	1	1	1	1	1	0	1	1	1	8
Mekonnen et al, 2013	1	1	1	1	1	0	1	3	1	10
Miller et al, 1978	0	0	1	1	1	0	1	0	1	
Morgan et al, 1983	2	0	1	1	1	0	1	2	1	9
Moser et al, 2015	2	1	1	1	1	1	1	3	1	12
Moser et al, 2017	2	1	1	1	1	1	1	3	1	12
Muchiri et al, 2001	2	0	1	1	1	1	1	3	1	11
Musgrave et al, 1979	1	0	1	1	1	0	1	0	1	6
Namwanje et al, 2011	1	0	1	1	1	0	1	1	1	7
Nanivadekar et al, 1984a	1	0	1	1	1	1	1	3	1	10
Nanivadekar et al, 1984b	1	0	1	1	1	1	1	3	1	10
Ndyomugyenyi et al, 2008	1	0	1	1	1	0	1	2	1	8
Nokes et al, 1992	2	0	1	1	1	0	1	2	1	9
Nontasut et al, 1989	1	0	1	1	1	0	1	3	1	9
Nontasut et al, 1997	0	0	1	1	1	0	1	0	1	5
Nunez-Fernandez et al, 1989	0	0	1	0	1	0	1	2	1	6
Ortiz et al, 2002	1	0	1	1	1	0	1	3	1	9
Ovedoff, 1984	2	0	1	1	1	0	1	0	1	7
Oyediran et al, 1982	2	0	1	1	1	0	1	2	1	9
Phuvanandh et al, 1994	0	0	0	0	1	0	1	0	1	3
Pugh et al, 1986	2	0	1	1	1	1	1	2	1	10
Rim et al, 1981	2	0	1	1	1	0	1	3	1	10
Sacko et al, 1999	2	0	1	1	1	0	1	3	1	10
Sargent et al, 1974	2	0	1	1	1	0	1	1	1	9

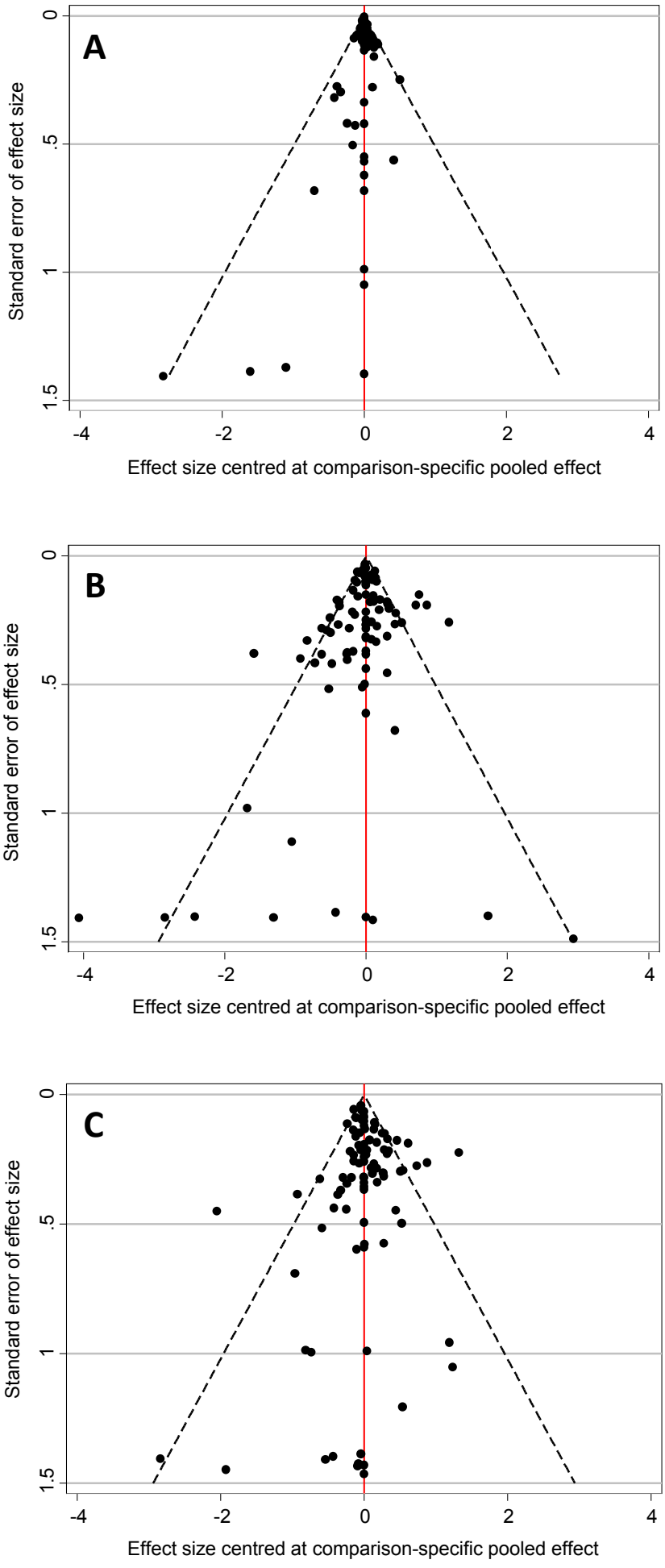
Sargent et al, 1975	2	0	1	1	1	0	1	3	1	10
Seo et al, 1983	0	0	0	0	1	0	1	1	1	4
Silber et al, 2017	2	1	1	1	1	1	1	3	1	12
Sinniah et al, 1981	1	0	1	1	1	0	1	1	1	7
Sinniah et al, 1990	1	0	1	1	1	0	1	0	1	6
Sirivichayakul et al, 2001	1	0	1	1	1	1	1	3	1	10
Sorensen et al, 1996	1	0	1	1	1	1	1	1	1	8
Soukhathammavong et al, 2012	1	1	1	1	1	1	1	3	1	11
Souza et al, 1972	1	0	1	1	1	0	1	2	1	8
Speich et al, 2012	2	1	1	1	1	0	1	3	1	11
Speich et al, 2014	2	1	1	1	1	1	1	3	1	12
Speich et al, 2015	2	1	1	1	1	1	1	3	1	12
Steinmann et al, 2008	1	1	1	1	1	1	1	2	1	10
Steinmann et al, 2011	1	1	1	1	1	0	1	1	1	8
Stothard et al, 2009	1	0	1	1	1	0	1	0	1	6
Thienpont et al, 1969	2	0	1	1	1	1	1	1	1	9
Tian et al, 2011	1	1	1	1	1	0	1	2	1	9
Upatham et al, 1989	2	0	1	1	1	0	1	1	1	8
Vakil et al, 1975	0	0	1	1	1	0	1	0	1	5
Wang et al, 1987	1	0	1	1	1	0	1	0	1	6
Watkins et al, 1996	2	0	1	1	1	0	1	3	1	10
Wen et al, 2003	2	0	1	1	1	1	1	0	1	8
Wen et al, 2008	2	0	1	1	1	1	1	3	1	11
Wesche et al, 1994	1	0	1	1	1	1	1	0	1	7
Wu et al, 2006	2	0	1	1	1	1	1	0	1	8
Xia et al, 1992	0	0	1	1	1	0	1	3	1	8
Xu et al, 2014	1	1	1	1	1	1	1	3	1	11
Yap et al, 2013	2	1	1	1	1	1	1	3	1	12
Zhang et al, 1998	1	1	1	1	1	0	1	2	1	9
Zu et al, 1992	1	0	1	1	1	0	1	0	1	6



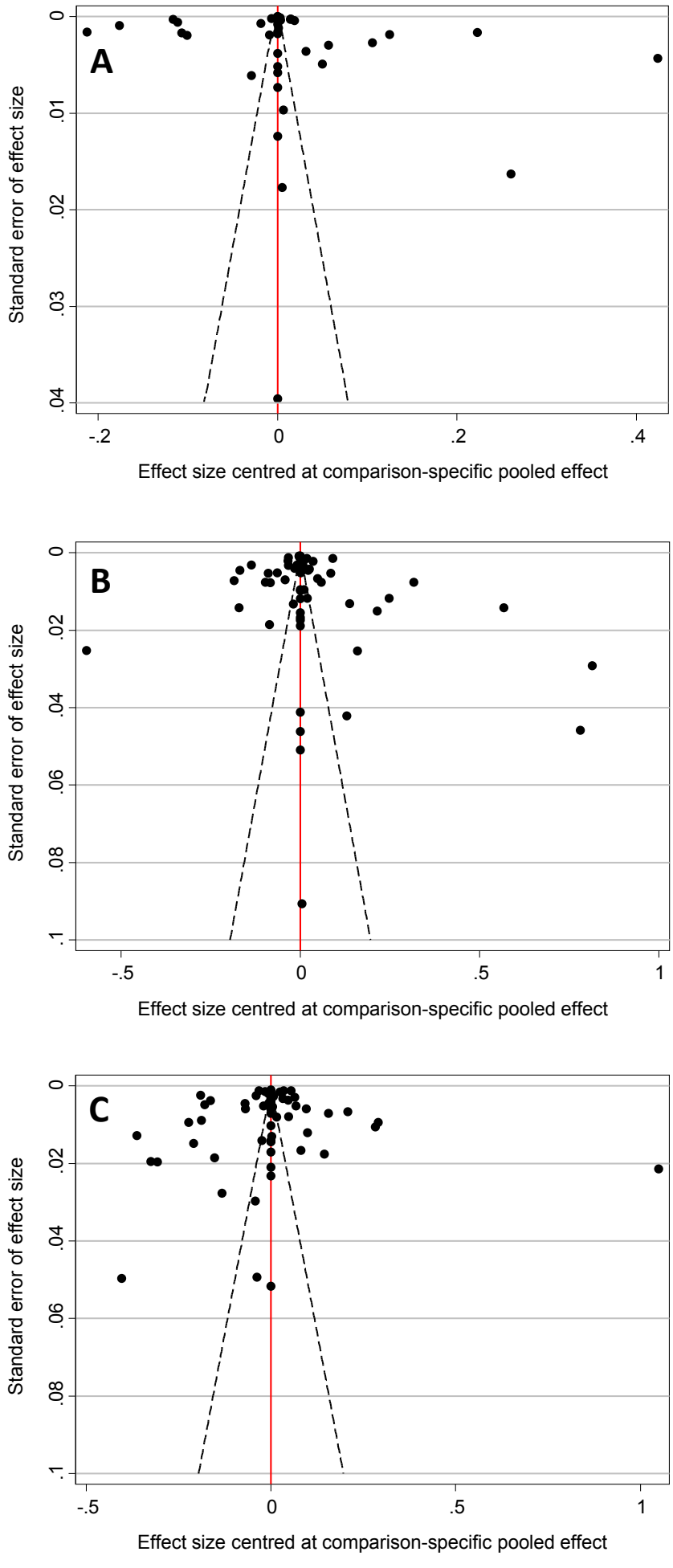




Supplementary Figure 1. Network plots showing the comparison groups for difference in egg reduction rate for *A. lumbricoides* (A), hookworm (B) and *T. trichiura* (C). Circle size is proportional to number of study arms; line width is proportional to number of pairs. ALB = albendazole; DEC = diethylcarbamazine; IVM = ivermectin; LEV = levamisole; MEB = mebendazole; NIT = nitazoxanide; OX = oxantel pamoate; PP = pyrantel pamoate; TRI = tribendimidine



Supplementary figure 2. Comparison-adjusted funnel plots for relative risk of cure for *A. lumbricoides* (A), hookworm (B) and *T. trichiura* (C)



Supplementary figure 3. Comparison-adjusted funnel plots for difference in egg reduction rate for *A. lumbricoides* (A), hookworm (B) and *T. trichiura* (C)

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Appendix 2
Supplementary material for Paper 2

The following information was published as an online supplement to Paper 2:

Clarke NE, Clements ACA, Doi SA, Wang D, Campbell SJ, Gray DJ, Nery SV. Differential effect of mass deworming and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis. *Lancet* 2017; 389(10066): 287–297. http://doi.org/10.1016/S0140-6736(16)32123-7

The material formed part of the manuscript submission and was subjected to peer review.

SUPPLEMENTARY MATERIAL: TABLE OF CONTENTS

Appendix 1. Search strategy: MEDLINE (OVID)

1. Helminthiasis/
2. Helminths/
2. helminth.mp.
3. soil-transmitted helminth.mp.
4. STH.mp.
5. geohelminth.mp.
6. Nematode Infections/
7. nematode.mp.
8. Ascaris/
9. Ascaris lumbricoides/
10. Ascariasis/
11. ascaris.mp.
12. roundworm.mp.
13. Hookworm Infections/
14. Necator/
15. Necator americanus/
16. Ancylostoma/
17. hookworm.mp.
18. necator.mp.
19. ancylostoma.mp.
20. Trichuriasis/
21. Trichuris/
22. trichuris.mp.
23. whipworm.mp.
24. (1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23)
25. Drug Therapy/
26. chemotherapy.mp.
27. Anthelmintics/
28. antihelminthic.mp.
29. anthelmintic.mp.
30. deworming.mp
31. Albendazole/
32. albendazole.mp.
33. Mebendazole/
34. mebendazole.mp.
35. Benzimidazoles/
36. benzimidazole.mp.
37. reinfection.mp.
38. re-infection.mp.
39. mass drug administration.mp.
40. (25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39)
41. 24 and 40

Table S1. Studies included in systematic review

Author & year	Reference	Study location	Drug distribution strategy	STH studied†	Dosing schedule and number of doses prior to follow-up‡	Follow-up time after last dose‡
Albonico et al, 1996* Shamlaye et al, 2003	1 2	Seychelles	Targeted	A, H, T	3 doses, 4 months apart	3 months
Belizario et al, 2015*	3	Philippines	Both	A, H, T	6 doses, 6 months apart (targeted)	3-4 months
Beltramino et al, 2003*	4	Argentina	Targeted	A, T	3 doses, 6-9 months apart	6 months
Bina et al, 1977*	5	Brazil	Mass	A, H, T	1 dose	5 months
Bird et al, 2014*	6	Zanzibar	Targeted	A, H, T	1 dose	8 months
Boia et al, 2006*	7	Brazil	Mass	A, H, T	1 dose	12 months
Bordignon et al, 2003	8	Nepal	Targeted	STH overall	Unclear	Not stated
Bradley et al, 1993*	9	Zimbabwe	Mass	H	3 doses, 10-11 months apart	12 months
Bundy et al, 1990	10	Montserrat	Targeted	A, T	4 doses, 4 months apart	3-4 months
Cleary et al, 2007*	11	Peru	Mass	A	4 doses, 3 months apart	3 months
Curtale et al, 2003*	12	Egypt	Targeted	A, T	2 doses, 12 months apart	6 months
de Moira et al, 2013*	13	Uganda	Targeted	H	1 dose	6 months
De Rochars et al, 2004*	14	Haiti	Mass	A, H, T	2 doses, annually	9 months
de Silva et al, 2003	15	Sri Lanka	Mass	A, H, T	1 dose	2-3 months
Fallah et al, 2002*	16	Iran	Mass	A, T	8 doses, 3 months apart	4 months
Fernando et al, 2001	17	Sri Lanka	Targeted	A, H, T	2 doses, 12 months apart	12 months
Freeman et al, 2013*	18	Kenya	Targeted	A, H, T	2 doses, 8 months apart	10 months
Gunawardena et al, 2008*	19	Sri Lanka	Mass	A, H, T	4 doses, annually	2-3 months
Guyatt et al, 2001*	20	Tanzania	Targeted	H	1 dose	15 months
Hodges et al, 2012*	21	Sierra Leone	Targeted	A, H, T	1 dose	6 months
Hung et al, 2005*	22	Vietnam	Mass	A, H, T	3 doses, 8-9 months apart	15 months
Idris et al, 2001*	23	Oman	Both	A, H, T	4 doses, annually	6 months
Jancloes et al, 1981*	24	Zaire (Democratic Republic of the Congo)	Mass	A, H, T	4 doses, 3 months apart	3 months
Kightlinger et al, 1995*	25	Madagascar	Targeted	A, H, T	1 dose	12 months
Knopp et al, 2009	26	Zanzibar	Both	A, H, T	16 doses: annually for 6 years (targeted), then annually for 2 years (mass), then annually (mass) and 6 monthly (targeted) for 4 years	6 months
Koukounari et al, 2006 Zhang et al, 2007	27 28	Uganda	Both	A, H, T	2 doses, 12 months apart	Variable
Li et al, 2011	29	China	Mass	A, H, T	3 doses, once a year	Not stated
Longfils et al, 2005*	30	Cambodia	Targeted	H	2 doses, 6 months apart	6 months
Machado et al, 1996*	31	Brazil	Mass	A, H, T	1 dose	6 months

Massa et al, 2009a*	32	Tanzania	Both	A, H, T	2 doses (one mass, one targeted), 8 months apart	8 months (mass); 4 months (targeted)
Massa et al, 2009b*	33	Tanzania	Targeted	A, H, T	2 doses, 3 months apart	9 months
Monse et al, 2013	34	Philippines	Targeted	STH overall	2 doses, 6 months apart	5 months
Mwinzi et al, 2012*	35	Kenya	Mass	A, H, T	1 dose	6 months
Nikolay et al, 2015	36	Kenya	Targeted	A, H, T	2 doses	7 to 13 months
Njenga et al, 2014*	37	Kenya	Both	A, H, T	1 dose (targeted)	8 months
Oqueka et al, 2005 Supali et al, 2013	38 39	Indonesia	Mass	A, H, T	6 doses, 12 months apart	10, 22 and 34 months
Peterson et al, 2011	40	Kenya	Targeted	A, H, T	Unclear number of doses, 3 months apart	3 months
Phommasack et al, 2008	41	Laos	Targeted	A, H, T	1-2 doses, 6-12 months apart	6-12 months
Pion et al, 2015*	42	Republic of Congo	Mass	A, H, T	2 doses, 6 months apart	6 months
Rajendran et al, 2003* Mani et al, 2004 Rajendran et al, 2006 Sunish et al, 2015	43 44 45 46	India	Mass	A, H, T	1 dose	6 months
Saathoff et al, 2004*	47	South Africa	Targeted	A, H, T	2 doses, 6 months apart	6 months
Sanza et al, 2013* Belizario et al, 2014	48 49	Philippines	Targeted	A, T	3 doses, 6 months apart	6 months
Sinuon et al, 2003*	50	Cambodia	Targeted	A, H, T	4 doses, 6 months apart	6 months
Speare et al, 2006	51	Tuvalu	Mass	H, T	3 doses, annually	6 months
Staudacher et al, 2014*	52	Rwanda	Targeted	A, H, T	1 dose	3 months
Steinmann et al, 2015*	53	China	Mass	A, H, T	Group 1: 4 doses, 12 months apart Group 2: 4 doses, 6 months apart	Group 1: 12 months Group 2: 6 months
Stoltzfus et al, 1997* Albonico et al, 1999	54 55	Zanzibar	Targeted	A, H, T	Group 1: 2 doses, 6 months apart Group 2: 3 doses, 4 months apart	Group 1: 6 months Group 2: 4 months
Stothard et al, 2009*	56	Zanzibar	Targeted	A, H, T	2 doses, 12 months apart	10-11 months
Tun et al, 2013	57	Myanmar	Both	A, H, T		
Urbani et al, 2003*	58	Cambodia	Targeted	A, H, T	4 doses, 6 months apart	6 months
Wu et al, 1999*	59	China	Mass	A, H, T	3 doses, 12 months apart	3 months
Yang et al, 2003	60	China	Mass	STH overall	1 dose	2 months
Yao et al, 2012	61	China	Mass	A, H, T	4 doses, over 3 years	Not stated
Zhou et al, 2011	62	China	Mass	H	3 or 4 doses, over 3 years	Not stated
Ziem et al, 2006a*	63	Ghana	Mass	H	4 doses, 6 months apart	6 months
Ziem et al, 2006b	64	Ghana	Mass	H	2 doses, 12 months apart	6 months

* Studies included in meta-analysis

† A = *A. lumbricoides*, H = hookworm, T = *T. trichiura*

‡ For prevalence measurement used in meta-analysis, if applicable

Table S2. Studies excluded from systematic review

Author & year	Reference	Reason for exclusion
Ahmed et al, 2012a	Parasit Vectors 5: 119	Only treated positive cases; no prevalence given at follow-up
Ahmed et al, 2012b	Parasitology 139(6): 802-808	Only treated positive cases; no prevalence given at follow-up
Albonico et al, 1994	Trop Geogr Med 46(3): 142-146	Only random selection of households included treated; prevalence given in figure format only
Albonico et al, 1995	Trans R Soc Trop Med Hyg 89(5): 538-541	Only treated students who returned a stool sample
Albright et al, 2006	Southeast Asian J Trop Med Public Health 37(1): 48-57	Only treated positive cases
Al-Delaimy et al, 2014	Parasit Vectors 7: 214	Only treated positive cases
Al-Mekhlafi et al, 2014	Parasit Vectors 7: 367	Only study subjects treated; not entire school
Al-Mekhlafi et al, 2008	Acta Trop 107(2): 200-204	Positive cases retreated 12-14 days after initial treatment
Appleton et al, 2009	Ann Trop Med Parasitol 103(3): 249-261	Positive cases retreated 30 days after initial treatment
Awasthi et al, 2013	Lancet 381(9876): 1478-1486	Baseline prevalence not given; only treated preschool-aged children
Balen et al, 2006	Trans R Soc Trop Med Hyg 100(11): 1039-1048	Only study subjects treated; not entire school
Belizario et al, 2004	Southeast Asian J Trop Med Public Health 35(Supp 1): 123-139	Only children in grades 3-4 treated
Bieri et al, 2013	New Engl J Med 368(17): 1603-1612	Only study subjects (grades 4-5) treated
Bradley et al, 1990	Trans R Soc Trop Med Hyg 84(6): 826-828	Only a random sample of inhabitants treated; prevalence not given (intensity only)
Cabrera et al, 1980	Southeast Asian J Trop Med Public Health 11(4): 502-506	Only positive cases treated
Campolina et al, 2015	Res Immunol 5: 6-12	Only positive cases treated?? Prevalence results given in figure format only
Chan et al, 1992a	Parasitology 104(2): 371-377	Only subset of population treated (Indian families with 3-7 children between ages of 0-15 years)
Chan et al, 1992b	Southeast Asian J Trop Med Public Health 23(2): 228-234	Only subset of population treated (Indian families with 3-7 children between ages of 0-15 years); duplicate data (from Chan et al, 1992a)
Chao et al, 2012	Chin J Schisto Control 24(5): 585-587	Only treated positive cases, their families and high risk populations
Chhotray et al, 1990	Indian J Med Research 91: 266-269	Only positive cases treated
Chongsuvivatwong et al, 1994	Southeast Asian J Trop Med Public Health 25(4): 745-751	Overall prevalence not given at baseline; follow-up period too short (1-2 months)
Cooper et al, 2008	BMC Immunol 9: 33	Baseline prevalence not given
Cooper et al, 2006	Lancet 367(9522): 1598-1603	Treated all schoolchildren plus their families – not mass or targeted treatment
Cundill et al, 2011	Parasitology 138(11): 1406-1416	Only positive cases treated
Curtale et al, 1995	Panminerva Med 37(4): 214-219	Baseline prevalences not given (grouped with villages that had a different intervention)
Dumba et al, 2013	Afr Health Sci 13(2): 512-517	Only treated preschool-aged children
Ebenezer et al, 2013	Trop Med Int Health 18(8): 942-951	Only children in grade 4 treated
Elkins et al, 1986	Trans R Soc Trop Med Hyg 80(5): 774-792	Not benzimidazole (pyrantel pamoate)
Elkins et al, 1988	Parasitology 96(1): 171-184	Not benzimidazole (pyrantel pamoate)
Fei et al, 2006	Chin J School Doctor 20: 522-523	Only positive cases treated
Feng et al, 2000	Journal of Preventive Medicine of Chinese People's Liberation Army 18: 210-211	Only positive cases treated
Gu et al, 2001	Chin J Parasitol Parasit Dis 19(4): inside back cover	Positive cases treated with higher dose than negative

Gungoren et al, 2007	Trans R Soc Trop Med Hyg 101(6): 564-569	Only positive cases treated
Gyorkos et al, 2013	PLoS Negl Trop Dis 7(9): e2397	Only children in grade 5 treated
Hadju et al, 2003	In "Controlling Disease due to Helminth Infections", Crompton et al., World Health Organization: Geneva.	No prevalence given at follow-up
Hagel et al, 1999	J Trop Pediatr 45(4): 215-220	Not benzimidazole (oxantel pamaote)
Hagel et al, 2008	J Trop Pediatr 54(5): 354-356	Unclear whether all schoolchildren treated or just study subjects
Halpenny et al, 2013	PLoS Negl Trop Dis 7(2): e2070	Only treated preschool-aged children
Halwindi et al, 2011	Parasitology 138(12): 1578-1585	Only treated preschool-aged children
Han et al, 2003	Chin J School Health 24: 358	Only treated a random sample of students
Haswell-Elkins et al, 1989	Parasitology 98(1): 125-134	Not benzimidazole (pyrantel pamoate)
Henry et al, 1988	Trans R Soc Trop Med Hyg 82(3): 460-464	Not benzimidazole (piperazine phosphate)
Henry et al, 1993	Southeast Asian J Trop Med Public Health 24(2): 307-312	Not benzimidazole (levamisole)
Hong-Chun et al, 2011	Chin J Schisto Control 23(5): 518-523	Prevalence not given for communities that received mass drug administration (combined with communities that received different intervention)
Hurlimann et al, 2014	BMC Infect Dis 14: 411	Only children grades 4-6 treated
Imai et al, 1985	Southeast Asian J Trop Med Public Health 16(3): 441-446	Randomised at individual level to receive pyrantel pamoate or pyrantel pamoate + mebendazole
Kabatereine et al, 2007	Bull World Health Organ 85(2): 91-99	Duplicate data (from Zhang et al, 2007)
Kightlinger et al, 1996	J Parasitol 82(1): 25-33	Duplicate data (from Kightlinger et al, 1995)
King et al, 2013	PLoS Negl Trop Dis 7(6): e2223	No formal benzimidazole administration reported, except in preschool aged children
Kinung'hi et al, 2015	BMC Infect Dis 15:136	Only treated children who were infected with *Schistosoma* spp.
Krause et al, 2015	Parasitology 142(12):1543-1554	Positive cases treated with higher dose than negative; only subset of households included (those whose members were part of an agricultural program)
Liu et al, 2006	J Pathogen Biol 6: 449-451	Not benzamidazole (pyrantel pamoate)
Loymek et al, 2004	Southeast Asian J Trop Med Public Health 35(1): 63-67	Reports only prevalence of "intestinal parasites", not described which species
Magnussen et al, 1997	Trop Med Int Health 2(9): 825-831	No baseline prevalence reported separately for group of schools that received albendazole (rather than levamisole)
Mascie-Taylor et al, 1999	J Parasitol 85(1): 6-11	Only a random sample of households treated
Midzi et al, 2011	BMC Int Health Hum Rights 11: 9	Only positive cases treated
Moraes et al, 2004	Parasitology 129(2): 223-232	Only a random sample of children treated
Muchiri et al, 2001	J Parasitol 87(2): 413-418	No prevalence given at follow-up
Muennoo et al, 1997	Southeast Asian J Trop Med Public Health 28(4): 816-819	Only positive cases treated
Narain et al, 2004	Southeast Asian J Trop Med Public Health 35(3): 512-517	Only a random sample of community treated
Nchito et al, 2009	Trans R Soc Trop Med Hyg 103(3): 229-236	Only positive cases treated
Ndenecho et al, 2002	East Afr Med J 79(8): 442-445	Only positive cases treated
Nkengazong et al, 2010	Afr J Env Sci Tech 4(3): 115-121	Unclear whether all schoolchildren treated or just study subjects
Northrop-Clewes et al, 2001	Am J Clin Nutr 73(1): 53-60	Only preschool-aged children treated
Oothuman et al, 1995	Trop Biomed 12: 159-164	Positive cases retreated 4 weeks after initial treatment

Pan et al, 1954	J Parasitol 40(5): 603-608	Not benzimidazole (hexylresorcinol)
Paul et al, 1998	J Commun Dis 30(4): 245-249	Only positive cases treated
Payne et al, 2007	J Nutr 137(6): 1455-1459	Only treated preschool-aged children
Peng et al, 2001	Chin J Schisto Control 13: 156	Only positive cases treated
Poggensee et al, 2005	Acta Trop 93(2): 131-140	Follow-up period too long
Quinnell et al, 1993	Parasitology 106(4): 379-385	Not benzimidazole (pyrantel pamoate)
Rodriguez-Perez et al, 2011	Res Rep Trop Med 2: 147-153	Only small number of negative cases followed up at weekly intervals; positive cases eliminated at each time point
Rousham et al, 1994	Ann Hum Biol 21(4): 315-324	Only treated preschool-aged children
Saathoff et al, 2002	Trans R Soc Trop Med Hyg 96(5): 485-490	Duplicate data (from Saathoff et al, 2004)
Saldiva et al, 2002	Paediatr Perinat Ep 16(2): 166-171	Infected children re-treated at each follow-up and excluded from study
Shield et al, 1984	Papua New Guinea Med J 27(2): 89-94	Only half the schoolchildren treated at each time point
Soerpito et al, 1991	Southeast Asian J Trop Med Public Health 22(2): 216-221	No baseline prevalence given
Sousa-Figueiredo et al, 2011	J Helminthol 85(3): 325-333	Treated only preschool-aged children and their mothers
Stoltzfus et al, 1998	Am J Clin Nutr 68(1): 179-186	Duplicate data (from Stoltzfus et al, 1997)
Sufiyan et al, 2011	Ann Afr Med 10(1): 6-12	Reports only prevalence of “intestinal helminths”, not described which species; unclear whether all children treated or only study subjects
Tanner et al, 1987	Acta Trop 44(2): 137-174	No baseline prevalence given for community who received albendazole (grouped with community receiving different intervention, then presented separately among treated/untreated individuals within community)
Taylor et al, 2001	Trans R Soc Trop Med Hyg 95(2): 211-216	Stratified random sample of students randomised at individual level to receive one of two deworming regimes or placebo – not all students treated
Thein-Hlaing et al, 1990	Bull World Health Organ 68(6): 747-753	Not benzimidazole (levamisole)
Thompson et al, 2001	Lancet 357(9258): 770-771	Pre-treatment prevalence unclear
Ting-Jun et al, 2011	Chin J Schisto Control 23(5): 490-494	Prevalence not given for communities that received mass drug administration (combined with communities that received different intervention)
Udonsi et al, 1993	Public Health 107(1): 53-60	Only positive cases treated
Ulukanligil, 2008	J Trop Pediatr 54(3): 157-163	Treated schoolchildren, plus women of child-bearing age and younger children, and anyone else on request – not mass or child-targeted treatment
Waikagul et al, 2008	Southeast Asian J Trop Med Public Health 39(6): 1008-1014	Treated all schoolchildren plus positive cases from community – not mass or child-targeted treatment
Xia et al, 1991	Southeast Asian J Trop Med Public Health 22(4): 618-622	Only positive cases treated
Xu et al, 2000	Chin J Parasitol Parasit Dis 19(5): 294-297	Only positive cases treated
Yang et al, 2008	Parasitology 135(14): 1685-1690	Compared mass, selective and targeted treatments. In mass treatment, no baseline prevalence was done. In targeted, treated all positive cases, plus children age 2-12, individuals from endemic areas, peasants and symptomatic individuals

Appendix 2. Description of included studies

Anthelminthic administration

Of the 25 studies reporting on targeted drug administration only, distribution was done through schools in 23 studies, and through communities in three studies,[4,25,33] with one study comparing the two.[33] Most studies (20/25; 80%) treated only primary school aged children. Two studies additionally treated preschool children,[10,41] and one treated children from preschool to secondary school.[1] One study each treated children aged 18 months to ten years,[25] and aged two to 13 years.[4]

Of the 24 studies reporting on mass drug administration, nine studies excluded pregnant women,[7,9,16,31,42,46,51,53,63] while one excluded both pregnant and breastfeeding women,[39] and one excluded women of child-bearing potential.[14] One study excluded children under the age of one year,[7] 11 excluded children under the age of two years,[9,14,16,31,39,42,46,51,53,60,61] four excluded children under three years of age,[29,59,62,63] and one study excluded children under five years of age.[35] One study excluded those over the age of 65.[29] Six studies did not specify any exclusion criteria.[5,15,19,22,24,64]

Drug administration was performed as part of large-scale government campaigns to control STH infections in 30/56 studies (53·6%). In 13 studies of mass delivery, anthelminthics were distributed as part of LF elimination campaigns.[3,14,15,19,26,32,37,39,46,51,57,63,64] In nine studies, anthelminthics were delivered along with a schistosomiasis elimination campaign; all involved targeted delivery,[12,13,20,21,26,32,47,56] except one study in Uganda, in which adults were also treated in areas where *S. mansoni* was highly endemic.[28]

Anthelminthic drug doses

Thirty-six studies used only albendazole, while 15 used only mebendazole, and five studies used both albendazole and mebendazole. Standard doses of 400mg albendazole and/or 500mg mebendazole were used in most studies, with exceptions as follows: two studies used mebendazole 600mg (given over three days),[5,24] one study used albendazole 600mg,[60] one study used albendazole 200mg,[59] one study used albendazole 100mg for children under six years and albendazole 134mg for those over seven years,[61] and one study used mebendazole 100mg.[11] Three studies gave a half dose of albendazole to children under 12 years.[29,60,62]

Twelves studies did not document the drug dose of albendazole (ten studies)[3,12,15,19,35,37,51,56,57,64] and/or mebendazole (two studies),[3,21] but standard doses of 400mg and 500mg respectively were assumed, as all involved national STH and/or LF campaigns, which would administer standard doses recommended by WHO.[65]

Study populations

Parasitological monitoring was conducted only in primary school children in 32 studies, of which 21 studies had implemented targeted drug administration, four studies mass drug administration, and seven studies both mass and targeted drug administration. STH prevalence was monitored across all age groups in 20 studies, 19 of which implemented mass drug administration. Only four of these reported age-stratified data. One study each assessed prevalence in children aged 0-16,[22] children aged 2-13 years,[4] children age 0-10 years,[25], and children from preschool through to secondary school.[1]

The majority of included studies represented repeated cross-sectional surveys, with independent samples at each time point. Only 16 studies followed up the same cohort of participants over time, and one study followed the same cohort but also added additional participants at each follow-up.[56] Of the remaining 40 studies, 35 conducted baseline and follow-up assessments in the same villages or schools, and therefore in many cases there is likely to have been some overlap of participants included in baseline and follow-up surveys.

Table S3. Additional interventions administered alongside anthelminthic drugs in included studies

	Number of studies with references		
	Targeted anthelminthic delivery	**Mass anthelminthic delivery**	**Both mass and targeted anthelminthic delivery**
Additional medications			
Praziquantel	10 studies ▪ All schoolchildren (6 studies)[12,13,21,33,47,56] ▪ All schoolchildren in areas with high prevalence of schistosomiasis (2 studies)[20,58] ▪ Only schoolchildren infected with *S. mansoni* (1 study)[18] ▪ Only schoolchildren with visible haematuria (1 study)[55]	2 studies ▪ All community members over 5 years age (1 study)[35] ▪ Any community member infected with *H. nana* (1 study)[22]	4 studies ▪ To schoolchildren only (3 studies)[26,32,37] ▪ To schoolchildren in all areas, and to adults in areas with *S. mansoni* prevalence >50% (1 study)[28]
DEC	No studies	6 studies All community members[14,15,19,39,46,51]	3 studies All community members[3,37,57]
Ivermectin	No studies	3 studies ▪ All community members (2 studies)[63,64] ▪ Any individual positive for *S. stercoralis* (1 study)[14]	2 studies All community members[26,32]
Other	2 studies ▪ Tiabendazole to students positive for *S. stercoralis* (1 study)[4] ▪ Pyrimethamine/sulfadoxine to febrile children (1 study)[55]	1 study ▪ Pyrantel pamoate to all community members over 2 years of age[61]	No studies
Water, sanitation and hygiene interventions			
Water and/or sanitation infrastructure	1 study School-based water treatment technology and sanitation infrastructure, with control group[18]	6 studies ▪ Improvements to both water and sanitation (4 studies)[22,24,29,61] ▪ Sanitation improvements, with control group (1 study)[53] ▪ Latrine promotion (1 study)[9]	1 study Latrine promotion and improved water supply at community level[23]
Health education and/or hygiene promotion	6 studies ▪ At school only (4 studies)[8,12,20,41] ▪ At school, with control group (1 study)[18] ▪ At schools and in community (1 study)[1]	7 studies ▪ In community (5 studies)[9,29,46,53,61] ▪ At schools only (1 study)[15] ▪ At schools and in community (1 study)[22]	1 study[28]
Supervised handwashing	1 study[34]	No studies	No studies
Other			
Nutritional supplements	5 studies ▪ Iron supplements to all schoolchildren (1 study)[12] ▪ Iron supplements to anaemic children only (2 studies)[6,54] ▪ Iron-folic acid supplements to grade one children; vitamin A to all schoolchildren; vitamin B to children with angular gingivitis (1 study)[30] ▪ School feeding program (2 studies)[8,21]	1 study Multivitamins with iron to all community members[11]	No studies
Provision of shoes/sandals	1 study To all schoolchildren, with control group[6]	1 study To all children under 17[22]	No studies

Table S4. Quality assessment of studies included in meta-analysis

Author & year	External validity				Internal validity				
	The target population was clearly described and was a close representation of the general population* in relation to relevant variables, e.g. age and sex	The sampling frame was clearly described and was a true or close representation of the target population	Some form of random selection was used to select the sample, or a census was undertaken	Response rate was ≥75%, or analysis showed no significant difference in relevant demographic characteristics between responders & nonresponders	The STH detection method was clearly described and was reliable and valid	Same method of STH diagnosis was used for all subjects in the study	Subjects were selected or recruited from the same or similar populations at both time points, or the same cohort was followed longitudinally	The sampling method was well described and the same method was used at both time points, or the same cohort was followed longitudinally	The distribution strategy of deworming medication was clearly described and delivered to at least 75% of the target population
Albonico et al, 1996	Y	Y	Y	N	Y	Y	Y	Y	Y
Belizario et al, 2015	Y	Y	Y	N	Y	Y	Y	N	N
Beltramino et al, 2003	Y	Y	Y	Y	Y	Y	Y	Y	Y
Bina et al, 1977	Y	Y	Y	Y	Y	Y	Y	N	N
Bird et al, 2014	Y	Y	Y	Y	Y	Y	Y	Y	N
Boia et al, 2006	Y	Y	Y	N	Y	Y	Y	Y	Y
Bradley et al, 1993	N	Y	Y	N	Y	Y	Y	N	N
Cleary et al, 2007	Y	Y	Y	N	N	Y	Y	Y	N
Curtale et al, 2003	Y	Y	Y	N	Y	Y	Y	Y	N
de Moira et al, 2013	Y	Y	Y	N	Y	Y	Y	Y	N
De Rochars et al, 2004	Y	N	N	N	Y	Y	Y	N	Y
Fallah et al, 2002	N	N	N	N	Y	Y	Y	N	Y
Freeman et al, 2013	Y	Y	Y	Y	Y	Y	Y	Y	N
Gunawardena et al, 2008	N	Y	Y	Y	Y	Y	Y	N	N
Guyatt et al, 2001	N	Y	Y	N	Y	Y	Y	N	N
Hodges et al, 2012	Y	Y	Y	N	Y	Y	Y	N	N
Hung et al, 2005	Y	Y	Y	N	Y	Y	Y	Y	N
Idris et al, 2001	Y	N	Y	N	Y	Y	Y	Y	Y
Jancloes et al, 1981	Y	Y	Y	Y	Y	Y	Y	Y	N

Kightlinger et al, 1995	Y	Y	Y	Y	Y	Y	Y	Y	Y
Longfils et al, 2005	N	Y	Y	N	Y	Y	Y	Y	N
Machado et al, 1996	Y	Y	Y	Y	Y	Y	Y	Y	N
Massa et al, 2009a	Y	Y	Y	Y	Y	Y	Y	Y	Y
Massa et al, 2009b	Y	Y	Y	N	Y	Y	Y	Y	Y
Mwinzi et al, 2012	Y	Y	Y	N	Y	Y	Y	Y	N
Njenga et al, 2014	Y	Y	Y	N	Y	Y	Y	Y	Y
Pion et al, 2015	Y	Y	Y	N	Y	Y	Y	Y	Y
Rajendran et al, 2003	Y	Y	Y	N	Y	Y	Y	Y	N
Saathoff et al, 2004	Y	Y	Y	N	Y	Y	Y	Y	N
Sanza et al, 2013	Y	Y	N	N	Y	Y	Y	Y	Y
Sinuon et al, 2003	N	N	N	N	Y	Y	Y	N	N
Staudacher et al, 2014	Y	N	N	Y	Y	Y	Y	Y	N
Steinmann et al, 2015	Y	Y	Y	N	Y	Y	Y	Y	N
Stoltzfus et al, 1997	N	Y	Y	Y	Y	Y	Y	Y	Y
Stothard et al, 2009	N	Y	Y	N	Y	Y	Y	Y	N
Urbani et al, 2003	N	N	N	N	N	N	N	N	N
Wu et al, 1999	N	N	Y	N	Y	Y	Y	N	Y
Ziem et al, 2006a	Y	Y	Y	N	Y	Y	Y	Y	N

* Population under study for STH prevalence (either school-aged children or whole community). For studies of school-aged children, if only grade 3 children (or children age 9-10) were sampled, this is considered representative given WHO guidelines for monitoring of STH control programmes.[65,66]

Table S5. Pooled effect sizes in sensitivity analyses

Selection criteria	6 months or less: pooled effect size (95% CI)	Greater than 6 months: pooled effect size (95% CI)
***A. lumbricoides* – targeted studies**		
Influential studies excluded (n=16)	0·382 (0·118–0·567)	-0·036 (-0·676–0·359)
Conducted in Africa (n=12)	0·342 (-0·097–0·606)	-0·006 (-0·447–0·300)
Conducted in Asia (n=4)	0·476 (0·264–0·628)	ND
Kato-Katz diagnostic method (n=15)	0·386 (0·117–0·573)	-0·036 (-0·676–0·359)
Studies without water/sanitation improvements (n=17)	0·382 (0·118–0·567)	-0·006 (-0·447–0·300)
Prevalence reduction truncated (n=17; affects 3 studies)	0·382 (0·118–0·567)	0·012 (-0·375–0·290)
All studies (n=17)	0·382 (0·118–0·567)	-0·006 (-0·447–0·300)
***A. lumbricoides* – mass studies**		
Influential studies excluded (n=11)	0·653 (0·116–0·864)	0·229 (0·017–0·395)
Conducted in Africa (n=2)	0·331 (-125·760–1·000)	ND
Conducted in Asia (n=4)	0·450 (0·225–0·610)	0·240 (-0·196–0·516)
Kato-Katz diagnostic method (n=7)	0·372 (0·259–0·467)	0·240 (-0·196–0·516)
Studies without water/sanitation improvements (n=11)	0·517 (-0·074–0·783)	0·229 (0·017–0·395)
Prevalence reduction truncated (n=12; affects 0 studies)	0·522 (-0·095–0·792)	0·229 (0·017–0·395)
All studies (n=12)	0·522 (-0·095–0·792)	0·229 (0·017–0·395)
Hookworm – targeted studies		
Influential studies excluded (n=16)	0·517 (0·338–0·648)	0·296 (-0·207–0·589)
Conducted in Africa (n=15)	0·102 (-0·193–0·324)	0·296 (-0·207–0·589)
Conducted in Asia (n=3)	0·370 (-0·090–0·636)	ND
Kato-Katz diagnostic method (n=16)	0·103 (-0·195–0·327)	0·312 (-0·257–0·623)
Studies without water/sanitation improvements (n=18)	0·107 (-0·195–0·332)	0·296 (-0·207–0·589)
Prevalence reduction truncated (n=18; affects 1 study)	0·107 (-0·195–0·332)	0·341 (-0·062–0·591)
All studies (n=18)	0·107 (-0·195–0·332)	0·296 (-0·207–0·589)
Hookworm – mass studies		
Influential studies excluded (n=14)	0·720 (0·514–0·839)	0·742 (0·532–0·858)
Conducted in Africa (n=6)	0·694 (0·570–0·782)	0·649 (0·516–0·746)
Conducted in Asia (n=6)	0·759 (0·212–0·926)	0·947 (0·735–0·989)
Kato-Katz diagnostic method (n=12)	0·716 (0·538–0·825)	0·659 (0·465–0·782)
Studies without water/sanitation improvements (n=10)	0·691 (0·587–0·769)	0·755 (0·658–0·825)
Prevalence reduction truncated (n=15; affects 0 studies)	0·720 (0·514–0·839)	0·666 (0·467–0·791)
All studies (n=15)	0·720 (0·514–0·839)	0·666 (0·467–0·791)
***T. trichiura* – targeted studies**		
Influential studies excluded (n=11)	0·189 (-0·171–0·438)	0·128 (-0·081–0·297)
Conducted in Africa (n=8)	0·100 (-0·091–0·257)	0·123 (0·088–0·157)
Conducted in Asia (n=4)	0·089 (-0·013–0·181)	ND
Kato-Katz diagnostic method (n=11)	0·116 (-0·109–0·297)	0·140 (-0·118–0·339)
Studies without water/sanitation improvements (n=13)	0·116 (-0·109–0·297)	0·128 (-0·081–0·297)
Prevalence reduction truncated (n=13; affects 1 study)	0·116 (-0·109–0·297)	0·129 (-0·074–0·294)
All studies (n=13)	0·116 (-0·109–0·297)	0·128 (-0·081–0·297)
***T. trichiura* – mass studies**		
Influential studies excluded (n=9)	0·191 (-0·220–0·464)	0·228 (-0·489–0·600)
Conducted in Africa (n=3)	0·144 (-0·063–0·559)	ND
Conducted in Asia (n=4)	0·148 (-0·360–0·466)	0·081 (-0·141–0·260)
Kato-Katz diagnostic method (n=8)	0·144 (-0·222–0·401)	0·081 (-0·141–0·260)
Studies without water/sanitation improvements (n=9)	0·142 (-0·136–0·352)	0·228 (-0·489–0·600)
Prevalence reduction truncated (n=10; affects 0 studies)	0·144 (-0·222–0·401)	0·228 (-0·489–0·600)
All studies (n=10)	0·144 (-0·222–0·401)	0·228 (-0·489–0·600)

ND=no data

Table S6. Odds ratio for selected covariates, stratified by STH (random effects weighted logit-linear regression with robust error variance)

Covariate	Odds ratio	95% CI	*p* value	R^2
	A. lumbricoides			
Mass vs targeted treatment	8·681	1·541–48·890	0·0164	0·434
Baseline prevalence*	0·162	0·002–14·402	0·4103	
Number of drug doses	1·523	0·813–2·854	0·1793	
Follow-up time	0·559	0·366–0·854	0·0093	
	Hookworm			
Mass vs targeted treatment	3·920	1·715–8·958	0·0022	0·331
Baseline prevalence*	0·147	0·024–0·909	0·0399	
Number of drug doses	1·130	0·765–1·670	0·5266	
Follow-up time	0·979	0·838–1·143	0·7781	
	T. trichiura			
Mass vs targeted treated	1·245	0·062–25·147	0·8799	0·132
Baseline prevalence*	0·476	0·007–31·520	0·7142	
Number of drug doses	1·015	0·536–1·920	0·9617	
Follow-up time	0·725	0·359–1·464	0·3492	

* Baseline prevalence data were entered into the model on a scale of 0–1

Table S7. Meta-analysis results (using random effects weights) synthesising non-truncated prevalence reduction estimates from individual studies, shown separately for mass and targeted studies for each STH, stratified by follow-up time

Delivery method	Follow-up time	*PReduc** (95% CI)	Cochran's Q	*p* value (Cochran's Q)	Number of study datasets
		A. lumbricoides			
Mass	6 months or less	0·636 (0·344–0·798)	86·931	<0·0001	9
	Greater than 6 months	0·229 (0·017–0·395)	0·351	0·8390	3
Targeted	6 months or less	0·514 (0·383–0·617)	243·562	<0·0001	11
	Greater than 6 months	0·141 (-0·056–0·302)	41·748	<0·0001	6
		Hookworm			
Mass	6 months or less	0·767 (0·611–0·860)	14·094	0·0495	8
	Greater than 6 months	0·720 (0·578–0·815)	12·349	0·0546	7
Targeted	6 months or less	0·402 (0·298–0·490)	336·406	<0·0001	10
	Greater than 6 months	0·520 (0·289–0·677)	246·377	<0·0001	8
		T. trichiura			
Mass	6 months or less	0·164 (-0·065–0·344)	15·815	0·0148	7
	Greater than 6 months	0·364 (-0·093–0·630)	10·055	0·0066	3
Targeted	6 months or less	0·317 (0·220–0·402)	294·930	<0·0001	9
	Greater than 6 months	0·143 (-0·003–0·267)	16·468	0·0003	3

* *PReduc* = 1 – *PRatio*

Table S8. Pooled effect sizes in sensitivity analyses using random effects weights

Selection criteria	6 months or less: pooled ES (95% CI)	Greater than 6 months: pooled ES (95% CI)
***A. lumbricoides* – targeted studies**		
Influential studies excluded (n=17)	0·514 (0·383–0·617)	0·141 (-0·056–0·302)
Conducted in Africa (n=12)	0·539 (0·345–0·676)	0·141 (-0·056–0·302)
Conducted in Asia (n=4)	0·476 (0·264–0·628)	ND
Kato-Katz diagnostic method (n=15)	0·542 (0·412–0·644)	0·253 (-0·077–0·482)
Studies without water/sanitation improvements (n=17)	0·514 (0·383–0·617)	0·141 (-0·056–0·302)
Prevalence reduction truncated (n=17; affects 3 studies)	0·514 (0·383–0·617)	0·182 (0·009–0·325)
All studies (n=17)	0·514 (0·383–0·617)	0·141 (-0·056–0·302)
***A. lumbricoides* – mass studies**		
Influential studies excluded (n=12)	0·636 (0·344–0·798)	0·229 (0·017–0·395)
Conducted in Africa (n=2)	0·887 (-4·758–0·998)	ND
Conducted in Asia (n=4)	0·450 (0·225–0·610)	0·240 (-0·196–0·516)
Kato-Katz diagnostic method (n=7)	0·378 (0·265–0·471)	0·240 (-0·196–0·516)
Studies without water/sanitation improvements (n=11)	0·517 (-0·074–0·783)	0·229 (0·017–0·395)
Prevalence reduction truncated (n=12; affects 0 studies)	0·636 (0·344–0·798)	0·229 (0·017–0·395)
All studies (n=12)	0·636 (0·344–0·798)	0·229 (0·017–0·395)
Hookworm – targeted studies		
Influential studies excluded (n=18)	0·402 (0·298–0·490)	0·520 (0·289–0·677)
Conducted in Africa (n=15)	0·401 (0·281–0·501)	0·520 (0·289–0·677)
Conducted in Asia (n=3)	0·419 (0·050–0·645)	ND
Kato-Katz diagnostic method (n=16)	0·397 (0·287–0·490)	0·567 (0·327–0·722)
Studies without water/sanitation improvements (n=18)	0·402 (0·298–0·490)	0·520 (0·289–0·677)
Prevalence reduction truncated (n=18; affects 1 study)	0·402 (0·298–0·490)	0·534 (0·340–0·671)
All studies (n=18)	0·402 (0·298–0·490)	0·520 (0·289–0·677)
Hookworm – mass studies		
Influential studies excluded (n=15)	0·767 (0·611–0·860)	0·720 (0·578–0·815)
Conducted in Africa (n=6)	0·694 (0·570–0·782)	0·658 (0·553–0·749)
Conducted in Asia (n=6)	0·837 (0·516–0·945)	0·947 (0·735–0·989)
Kato-Katz diagnostic method (n=12)	0·747 (0·599–0·841)	0·694 (0·540–0·797)
Studies without water/sanitation improvements (n=10)	0·691 (0·587–0·769)	0·755 (0·658–0·825)
Prevalence reduction truncated (n=15; affects 0 studies)	0·767 (0·611–0·860)	0·720 (0·578–0·815)
All studies (n=15)	0·767 (0·611–0·860)	0·720 (0·578–0·815)
***T. trichiura* – targeted studies**		
Influential studies excluded (n=13)	0·317 (0·220–0·402)	0·143 (-0·003–0·267)
Conducted in Africa (n=8)	0·249 (0·142–0·342)	0·123 (0·088–0·157)
Conducted in Asia (n=4)	0·483 (0·216–0·659)	ND
Kato-Katz diagnostic method (n=11)	0·310 (0·213–0·396)	0·206 (0·020–0·357)
Studies without water/sanitation improvements (n=13)	0·317 (0·220–0·402)	0·143 (-0·003–0·267)
Prevalence reduction truncated (n=13; affects 1 study)	0·317 (0·220–0·402)	0·146 (0·004–0·267)
All studies (n=13)	0·317 (0·220–0·402)	0·143 (-0·003–0·267)
***T. trichiura* – mass studies**		
Influential studies excluded (n=10)	0·164 (-0·065–0·344)	0·364 (-0·093–0·630)
Conducted in Africa (n=3)	0·298 (-0·184–0·584)	ND
Conducted in Asia (n=4)	0·076 (-0·454–0·413)	0·081 (-0·141–0·260)
Kato-Katz diagnostic method (n=8)	0·164 (-0·065–0·344)	0·081 (-0·141–0·260)
Studies without water/sanitation improvements (n=9)	0·148 (-0·047–0·306)	0·364 (-0·093–0·630)
Prevalence reduction truncated (n=10; affects 0 studies)	0·164 (-0·065–0·344)	0·364 (-0·093–0·630)
All studies (n=10)	0·164 (-0·065–0·344)	0·364 (-0·093–0·630)

ND=no data

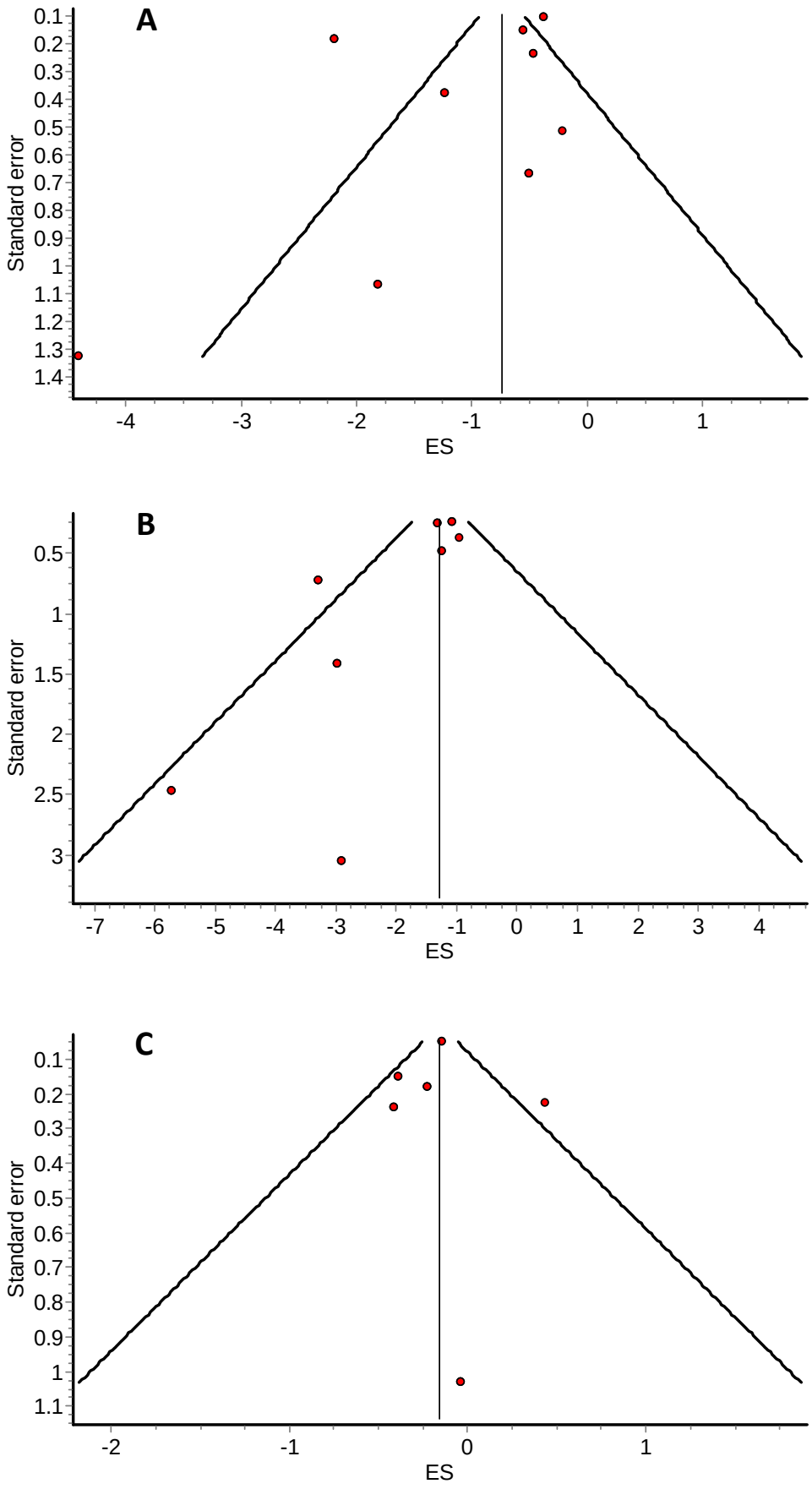

Figure S1. Funnel plots for mass studies with less than 6 months follow-up for *A. lumbricoides* (A), hookworm (B), and *T. trichiura* (C)

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Appendix 3
Supplementary material for Paper 3

The following information was published as an online supplement to Paper 3:

Clarke NE, Clements ACA, Bryan S, McGown J, Gray D, Nery SV. Investigating the differential impact of school and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for Worms pilot study protocol. *Pilot Feasibility Stud* 2016; 2: 69. http://doi.org/10.1186/s40814-016-0109-4

The material formed part of the manuscript submission and was subjected to peer review.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

Table S1: SPIRIT 2013 Checklist – Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	1-20
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	19
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	20
	5b	Name and contact information for the trial sponsor	20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	20
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	3-6
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6-7

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	13-14
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11

Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	6, Fig 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	N/A
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A

Methods: Assignment of interventions (for controlled trials)

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	N/A
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	14-16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16-17
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16-17
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	20
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	14, 20
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	14-16
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	10

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

Appendix 4
Supplementary material for Paper 4

The following information was published as an online supplement to Paper 4:

Clarke NE, Clements ACA, Amaral S, Richardson A, McCarthy JS, McGown J, Bryan S, Gray D, Nery SV. (S)WASH-D for Worms: a pilot study investigating the differential impact of school- versus community-based integrated control programs for soil-transmitted helminths. *PLoS Negl Trop Dis* 2018; 12(5): e0006389. http://doi.org/10.1371/journal.pntd.0006389

The material formed part of the manuscript submission and was subjected to peer review.

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	✓	2
		• Structured abstract recommended	✓	2
		• Information on target population or study sample	✓	2
Introduction				
Background	2	• Scientific background and explanation of rationale	✓	3-4
		• Theories used in designing behavioral interventions	✓	6
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	✓	5
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	✓	5
		• Recruitment setting	✓	5
		• Settings and locations where the data were collected	✓	6-7
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	✓	6
		○ Content: what was given?	✓	5-6
		○ Delivery method: how was the content given?	✓	5-6
		○ Unit of delivery: how were the subjects grouped during delivery?	✓	5-6
		○ Deliverer: who delivered the intervention?	✓	5-6
		○ Setting: where was the intervention delivered?	✓	5-6
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	✓	5-6
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	✓	6
		○ Activities to increase compliance or adherence (e.g., incentives)	N/A	
Objectives	5	• Specific objectives and hypotheses	✓	4
Outcomes	6	• Clearly defined primary and secondary outcome measures	✓	Box 1
		• Methods used to collect data and any methods used to enhance the quality of measurements	✓	6-7
		• Information on validated instruments such as psychometric and biometric properties	N/A	
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	N/A	
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	✓	5
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	✓	5
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	✓	8

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	✓	6-7
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	✓	8
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)	✓	8
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	✓	8
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	✓	8
		• Methods for imputing missing data, if used	N/A	
		• Statistical software or programs used	✓	8
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	✓	Fig 1
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	✓	Fig 1
		○ Assignment: the numbers of participants assigned to a study condition	✓	Fig 1
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	N/A	
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	✓	Fig 1
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	✓	Table 2; S1 Table
		• Description of protocol deviations from study as planned, along with reasons	✓	11
Recruitment	13	• Dates defining the periods of recruitment and follow-up	✓	5-6
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	✓	Table 1
		• Baseline characteristics for each study condition relevant to specific disease prevention research	✓	Page 9; Table 1
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	N/A	
		• Comparison between study population at baseline and target population of interest	N/A	
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	✓	Page 9; Table 1

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	✓	S1 and S2 Table
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	N/A	
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	✓	Figs 2-3; S1 and S2 Table
		• Inclusion of null and negative findings	✓	Figs 2-3
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	N/A	
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	N/A	
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	N/A	
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	✓	16-17
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	✓	17
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	✓	17-18
		• Discussion of research, programmatic, or policy implications	✓	18
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	N/A	
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	✓	18

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/

Register a trial

Request number	369077
Current page	Review

Trial registered on ANZCTR

Trial ID	ACTRN12615001012561
Ethics application status	Approved
Date submitted	4/09/2015
Date registered	28/09/2015
Type of registration	Retrospectively registered

Titles & IDs

Public title	Should integrated deworming and water, sanitation and hygiene (WASH) programs for soil-transmitted helminth (STH) control be delivered in schools or the community? A pilot study
Scientific title	A pilot study comparing the impact of school- and community-based integrated water, sanitation and hygiene (WASH) and deworming programmes on soil-transmitted helminth infections in school-aged children in Timor-Leste
Secondary ID [1]	OPP1119041 (Grant number from Bill and Melinda Gates Foundation)
Universal Trial Number (UTN)	U1111-1172-9719
Trial acronym	(S)WASH-D for Worms pilot
Linked study record	

Health condition

Health condition(s) or problem(s) studied:
Soil-transmitted helminth infection - Trichuris trichiura, Ascaris lumbricoides, hookworms (Necator americanus and Ancylostoma duodenale)
Stunting
Wasting
Anaemia
Intestinal protozoa (Giardia duodenalis, Entamoeba histolytica, Strongyloides spp., Cryptosporidium spp.)

Condition category	Condition code
Infection	Other infectious diseases
Public Health	Epidemiology
Oral and Gastrointestinal	Other diseases of the mouth, teeth, oesophagus, digestive system including liver and colon

Intervention/exposure

Study type	Interventional
Description of intervention(s) / exposure	The intervention to be evaluated in this proposal will involve provision of access to improved water and sanitation and improving related hygiene practices, implemented at both a community level and a primary school level. This intervention will be implemented by non-governmental organisation Plan International in Timor-Leste. The sanitation component will involve construction of school latrines by contractors working with Plan International, as well a Community Led Total Sanitation approach. Access to an improved water supply will also be provided, and local partner NGOs will provide house-by-house education on hygiene practices, in particular hand-washing with soap at critical times. Hygiene education including posters relating to handwashing with soap will be provided to schools, and handwashing stations with soap will be constructed as part of the school latrines. Furthermore, communities in the intervention arm of the pilot study will receive mass chemotherapy (distributed to all members of the community) with one oral tablet of albendazole 400mg, which will be

	administered once 80% of the households have sanitation (as defined by the presence of a household
Intervention code [1]	Prevention
Intervention code [2]	Treatment: Drugs
Intervention code [3]	Behaviour
Comparator / control treatment	Communities in the control group will be provided with access to improved water and sanitation and hygiene promotion implemented only at primary school level. This will be implemented by non-governmental organisation Cruz Vermelha Timor-Leste (CVTL), and will involve construction of school latrines, access to an improved water supply and promotion of hand washing with soap and related hygiene behaviours. This intervention will be similar to that in the intervention arm (although conducted by a different NGO) but will only be delivered to primary school children. Furthermore, communities in the control arm of the pilot study will receive chemotherapy (distributed to school-aged children only) with one oral tablet of albendazole 400mg, which will be administered once the school latrines have been completed. Albendazole intake will be directly observed by the field workers delivering the tablets, who will be working under the supervision of a registered nurse.
Control group	Active

Outcomes

Primary outcome [1]	Cumulative incidence of of infection with A. lumbricoides, T. trichiura, N. americanus and Ancylostoma spp. (undifferentiated) in school aged children - to be assessed by both microscopy and PCR examination of stool
Timepoint [1]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [1]	Proportion of eligible children for whom informed consent is gained - using school records to determine number of eligible children
Timepoint [1]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [2]	Proportion of eligible children for whom stool samples are provided - using school records to determine number of eligible children
Timepoint [2]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [3]	Proportion of eligible children who complete questionnaires - using school records to determine number of eligible children
Timepoint [3]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [4]	Proportion of eligible children who undergo measurement of height, weight and haemoglobin - using school records to determine number of eligible children
Timepoint [4]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [5]	Prevalence of S. stercoralis, G. duodenalis, E. histolytica, and Cryptosporidium spp. (composite outcome) - assessed using laboratory analysis (PCR) of stool samples
Timepoint [5]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [6]	Mean haemoglobin concentration - measured using serum assay on a Hb201 (Hemocue) analyser device
Timepoint [6]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [7]	Anthropometric index weight-for-height Z-score (to identify wasting)
Timepoint [7]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [8]	Anthropometric index weight-for-age Z-score (to identify underweight)
Timepoint [8]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [9]	Anthropometric index height-for-age Z-score (to identify stunting)
Timepoint [9]	At baseline and at follow-up six months after the distribution of albendazole
Secondary outcome [10]	Mean intensity of infection (average number of eggs per gram of faeces)
Timepoint [10]	Six months following distribution of albendazole

Eligibility

Key inclusion criteria	Inclusion criteria for enrollment in the study: - Child enrolled in and attending the primary school - Informed consent obtained from parent/caregiver Selection of communities for inclusion in the study: - Communities were selected for inclusion in this pilot study in consultation with each partner NGO (Plan International and Cruz Vermelha Timor-Leste (CVTL)) - For the intervention clusters, Plan International identified three villages in which they were planning both a school- and community-based WASH programme.

	- For the control clusters, the research team and CVTL identified three schools suitable for a school-
Minimum age	
Maximum age	
Gender	
Can healthy volunteers participate?	Yes
Key exclusion criteria	Exclusion criteria for enrollment in the study: - Not attending the primary school - Informed consent not obtained Exclusion criteria for receiving albendazole (including students enrolled in the study AND other members of communities in the intervention clusters): - Women in the first trimester of pregnancy - Children under the age of 1 year

Study design

Purpose of the study	Prevention
Allocation to intervention	Non-randomised trial
Procedure for enrolling a subject and allocating the treatment (allocation concealment procedures)	All children who are enrolled in and attending the primary school in each of the six communities participating in this pilot study will be eligible for inclusion in the study. Consent will be sought from parents/caregivers at a meeting which will be held at the school. Allocation is not concealed.
Methods used to generate the sequence in which subjects will be randomised (sequence generation)	This pilot project is not randomised. This is because the WASH intervention for each arm of the study is being performed by a different NGO, and communities participating in the study are those in which those NGOs are working.
Masking / blinding	Open (masking not used)
Who is / are masked / blinded?	
Intervention assignment	Parallel
Other design features	
Phase	Not Applicable
Type of endpoint(s)	Efficacy
Statistical methods / analysis	Descriptive statistics will be used to determine the proportion of eligible participants who gave informed consent, provided stool samples, completed questionnaires and underwent measurement of height and weight. Primary and secondary outcomes will be calculated and compared across both arms of the trial using mixed effects multivariate regression models that account for clustering of participants in villages.

Recruitment

Recruitment status	Completed

Date of first participant enrolment			
Anticipated		**Actual**	21/05/2015

Date of last participant enrolment			
Anticipated	7/06/2016	**Actual**	2/07/2016

Date of last data collection			
Anticipated		**Actual**	3/07/2016

Sample size					
Target	475	**Current**		**Final**	557

Recruitment outside Australia	
Country [1]	Timor-Leste
State/province [1]	Aileu and Manufahi Districts

Funding & Sponsors

Funding source category [1]	Charities/Societies/Foundations
Name [1]	Bill and Melinda Gates Foundation - Grand Challenges Explorations

Address [1]	500 Fifth Avenue North Seattle, WA 98109 United States of America
Country [1]	United States of America
Primary sponsor type	Individual
Name	Susana Vaz Nery
Address	ANU College of Medicine, Biology and Environment The Australian National University Building 62 Mills Road Canberra ACT 0200
Country	Australia
Secondary sponsor category [1]	Individual
Name [1]	Darren Gray
Address [1]	ANU College of Medicine, Biology and Environment The Australian National University Building 62 Mills Road Canberra ACT 0200
Country [1]	Australia
Secondary sponsor category [2]	Individual
Name [2]	Archie Clements
Address [2]	ANU College of Medicine, Biology and Environment The Australian National University Building 62 Mills Road Canberra ACT 0200
Country [2]	Australia
Other collaborator category [1]	Individual
Name [1]	Rebecca J Traub, BSc BVMS (Hons) PhD
Address [1]	Faculty of Veterinary Science University of Melbourne Parkville VIC 3052
Country [1]	Australia
Other collaborator category [2]	Individual
Name [2]	James McCarthy
Address [2]	QIMR Berghofer Medical Research Institute University of Queensland Dept. of Infectious Diseases, Royal Brisbane and Womens Hospital Herston Rd Herston QLD 4029
Country [2]	Australia

Ethics approval

Ethics application status	Approved
Ethics committee name [1]	The Australian National University Human Research Ethics Committee
Ethics committee address [1]	The Australian National University Acton ACT 2601
Ethics committee country [1]	Australia
Date submitted for ethics approval [1]	20/03/2015
Approval date [1]	08/05/2015
Ethics approval number [1]	2015/111
Ethics committee name [2]	Cabinet for Ethics and Quality Control - Ministry of Health Timor-Leste
Ethics committee address [2]	Instituto National Saude Comoro Dili
Ethics committee country [2]	Timor-Leste
Date submitted for ethics approval [2]	13/02/2015
Approval date [2]	13/04/2015
Ethics approval number [2]	MS-INS/GDE-Peskija/II/2015/196

Summary

Brief summary	
Trial website	
Trial related presentations / publications	
Public notes	
Private notes	

Contacts

Principal investigator	
Name	Dr Susana Vaz Nery
Address	Research School of Population Health The Australian National University Building 62 Mills Road Canberra ACT 0200
Country	Australia
Phone	+61 2 6125 0155
Fax	
Email	susana.nery@anu.edu.au

Contact person for public queries	
Name	Dr Susana Vaz Nery
Address	Research School of Population Health The Australian National University Building 62 Mills Road Canberra ACT 0200
Country	Australia
Phone	+61 2 6125 0155
Fax	
Email	susana.nery@anu.edu.au

Contact person for scientific queries	
Name	Dr Susana Vaz Nery
Address	Research School of Population Health The Australian National University Building 62 Mills Road Canberra ACT 0200
Country	Australia
Phone	+61 2 6125 0155
Fax	
Email	susana.nery@anu.edu.au

Contact person responsible for updating information	
Title	Dr
Name	Naomi Clarke
Address	Research School of Population Health The Australian National University Building 62 Mills Road Canberra ACT 0200

Country	Australia
Phone	+61 2 6125 0155
Fax	
Email	naomi.clarke@anu.edu.au

Cancer fields

Cancer stage(s)	
Treatment type(s)	
Known and possible side effect(s) for each arm of the trial (if applicable)	
Cost to participants	
Time commitment	
Travel	

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S1 Appendix. STH infection intensity categories

The cycle threshold (Ct) value represents the number of PCR cycles required for the fluorescence signal of the amplified DNA products to cross a set threshold value that exceeds background level. Higher quantities of DNA, reflecting more intense STH infections, will therefore result in lower Ct values. Details of the qPCR technique used in this study have been previously published [1].

The cut-offs used to determine the three infection intensity categories (no infection, lower intensity infection, and higher-intensity infection) are shown in the Table below.

Table. Ct value cut-offs for determining infection intensity categories

	No infection	**Lower-intensity infection***	**Higher-intensity infection***
***Ascaris* spp.**	Greater than 31	Greater than 15.885 and less than or equal to 35	Less than or equal to 15.885
Necator americanus	Greater than 35	Greater than 24.180 and less than or equal to 35	Less than or equal to 24.180

* Cut-off between “lower” and “higher” intensity infections was taken as median of all positive samples at baseline

References

1. Llewellyn S, Inpankaew T, Nery S, Gray D, Verweij J, Clements A, et al. Application of a multiplex quantitative PCR to assess prevalence and intensity of intestinal parasite infections in a controlled clinical trial. PLOS Negl Trop Dis. 2016;10(1): e0004380.

S1 Table. STH infections over time

Variable[a]	Baseline		Follow-up			
	Control *n=372*	**Intervention** *n=110*	**Control** *n=303*	**Intervention** *n=107*	**DID[b]**	***P* value**
Ascaris spp. infections	48.7% (43.6–53.8)	7.6% (3.8–14.4)	23.4% (18.9–28.5)	0.9% (0.1–6.5)	18.6%	0.005
Ascaris spp. higher-intensity infections	27.3% (21.1–30.0)	0.9% (0.1%–6.5%)	4.3% (2.5–7.2)	0	22.1%	0.012
N. americanus infections	13.7% (10.6–17.6)	15.1% (9.4–23.3)	9.9% (7.0–13.8)	5.7% (2.5–12.1)	-5.6%	0.254
N. americanus higher-intensity infections	7.3% (5.0–10.4)	6.6% (3.2–13.3)	4.6% (2.7–7.5)	2.8% (0.9–8.5)	-1.1%	0.832
Ancylostoma spp. infections	1.1% (0.4–2.8)	0	0	0	1.1%	0.367
T. trichiura infections	2.2% (1.1–4.3)	1.9% (0.5–7.3)	2.0% (0.8–4.3)	0.9% (0.1–6.5%)	-0.8%	0.556

[a] All results reported as: proportion (95% confidence interval).

[b] DID = difference in differences between intervention and control arms

S2 Table. Morbidity indicators over time

	Baseline		Follow-up			
	Control	**Intervention**	**Control**	**Intervention**	**DID[a]**	***P* value**
Haematological parameters	*n=382*	*n=116*	*n=324*	*n=112*		
Mean (SD) haemoglobin [g/L]	128.7 (11.2)	130.8 (10.2)	132.3 (10.9)	131.1 (10.9)	-3.3	0.085
Proportion anaemic (95% CI)	12.5% (9.6–16.3)	4.3% (1.8–10.0)	4.9% (3.0–7.9)	4.5% (1.9–10.3)	-7.8%	0.150
Growth (all age groups)	*n=382*	*n=124*	*n=356*	*n=116*		
Mean (SD) height-for-age Z-score	-2.00 (1.18)	-2.16 (1.10)	-1.89 (1.16)	-2.20 (1.09)	-0.15	0.067
Proportion stunting (95% CI)[b]	51.7% (46.6–56.8)	62.1% (53.2–70.2)	47.7% (42.3–53.2)	66.1% (56.9–74.2)	8.0%	0.096
Mean (SD) BMI-for-age Z-score[c]	-1.45 (0.83)	-1.93 (0.99)	-1.72 (0.96)	-2.01 (0.84)	0.19	0.554
Proportion thinness (95% CI)[d]	25.5% (21.3–30.1)	42.7% (34.3–51.6)	34.7% (29.7–40.1)	47.8% (38.9–57.0)	-4.1%	0.762
Growth (age ≤10 years only)	*n=225*	*n=86*	*n=206*	*n=83*		
Mean (SD) weight-for-age Z-score	-1.98 (1.00)	-2.42 (1.03)	-2.11 (0.92)	-2.61 (1.02)	-0.06	0.765
Proportion underweight (95% CI)[e]	53.3% (46.7–59.8)	65.1% (54.4–74.5)	58.4% (51.1–65.3)	76.8% (66.4–84.8)	6.6%	0.484

BMI = body mass index; CI = confidence interval; SD = standard deviation.

[a] DID = difference in differences between intervention and control arms

[b] Stunting defined as greater than 2 standard deviations below the median height-for-age *Z*-score for reference population.

[c] BMI calculated as weight (kg) / height^2 (cm).

[d] Thinness defined as greater than 2 standard deviations below the median BMI-for-age *Z*-score for reference population.

[e] Underweight define as greater than 2 standard deviations below the median weight-for-age *Z*-score for reference population.

Appendix 5
Supplementary material for Paper 5

The following information was published as an online supplement for Paper 5:

Nery SV, Traub RJ, McCarthy JS, **Clarke NE**, Amaral S, Llewellyn S, Weking E, Richardson A, Campbell SJ, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Clements ACA. WASH for WORMS: a cluster-randomized controlled trial of the impact of a community integrated water, sanitation, and hygiene and deworming intervention on soil-transmitted helminth infections. *Am J Trop Med Hyg* 2019; 100(3): 750–761. http://doi.org/10.4269/ajtmh.18-0705

The material formed part of the manuscript submission and was subjected to peer review.

Supplemental Tables

WASH for WORMS, a cluster randomized controlled trial of the impact of a community integrated WASH and deworming intervention on soil-transmitted helminths

Susana Vaz Nery, Rebecca Traub, James S McCarthy, Naomi E Clarke, Salvador Amaral, Stacey Llewellyn, Edmund Weking, Alice Richardson, Darren J Gray, Andrew J Vallely, Gail M Williams, Ross M Andrews, Suzy J Campbell, Archie C A Clements

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Supplemental Table 1. Participation rates over time

	Baseline		Follow-up 1		Follow-up 2		Follow-up 3		Follow-up 4	
	Control	**Intervention**	**Control**	**Intervention**	**Control**	**Intervention**	**Control**	**Intervention**	**Control**	**Intervention**
Households (n)	252	241	253	244	254	244	254	244	254	244
Reported residents (n)	1191	1115	1118	1112	1095	1039	1055	1042	1090	1036
Residents present, n (%)	1109 (92.4%)	991 (88.9%)	975 (87.2%)	907 (81.6%)	880 (80.4%)	869 (83.6	879 (83.3%)	866 (83.1%)	909 (83.4%)	843 (81.4%)
Consented, n (%)*	1077 (97.1%)	939 (94.8%)	937 (96.1%)	874 (96.4%)	871 (99.0%)	814 (93.7%)	840 (95.6%)	779 (90.0%)	882 (97.0%)	771 (91.5%)
Provided stool, n (%)	891 (83.0%)	711 (76.5%)	689 (76.0%)	584 (69.5%)	624 (73.6%)	552 (72.5%)	609 (80.3%)	531 (72.5%)	623 (78.5%)	553 (75.7%)
Provided individual questionnaire, n (%)	1046 (97.1%)	901 (96.0%)	921 (98.3%)	857 (98.1%)	846 (97.1%)	778 (95.6%)	833 (99.2%)	733 (94.1%)	869 (98.5%)	719 (93.3%)
Hemoglobin measured, n (%)†	966 (90.0%)	831 (89.0%)	-	-	634 (73.4%)	607 (77.6%)	-	-	652 (78.5%)	521 (69.7%)
Height and weight measured, n (%)	465 (91.5%)	421 (89.8%)	-	-	321 (78.3%)	338 (86.4%)	-	-	295 (77.0%)	306 (78.3%)
Albendazole distributed, n (%)‡	1019 (94.4%)	913 (94.4%)	915 (97.2%)	863 (97.6%)	839 (96.1%)	762 (90.7%)	832 (96.0%)	791 (94.5%)	850 (94.7%)	715 (88.3%)

* Calculated for the residents who were present and consented to provide a questionnaire. † Height and weight measurements were taken for participants aged under 18 years only.
‡ Denominator is those eligible and present at time. For all other participation indicators denominator is all present over 1 year of age and consented.

Supplemental Table 2. Uptake of the WASH intervention among study participants*

	Baseline % (95%CI)†			Follow-up 1 % (95%CI)†			Follow-up 2 % (95%CI)†			Follow-up 3 % (95%CI)†			Follow-up 4 % (95%CI)†		
	Control	**Intervention**	**p value**	**Control**	**Intervention**	**p value**	**Control**	**Intervention**	**p value**	**Control**	**Intervention**	**p value**	**Control**	**Intervention**	**p value**
Individual sanitation	*n=1046*	*n=901*		*n=921*	*n=857*		*n=846*	*n=778*		*n=833*	*n=733*		*n=869*	*n=719*	
Has toilet in household	20.6% (6.3-31.9)	21.2% (6.7-32.3)	0.968	21.3% (6.5-32.1)	75.8% (63.3-91.1)	**<0.001**	22.8% (8.2-24.9)	73.8% (65.4-86.3)	**<0.001**	25.5% (11.9-37.7)	68.1% (56.1-83.9)	**<0.001**	41.7% (25.2-65.8)	62.9% (46.0-83.7)	0.177
Uses household toilet	20.3% (5.9-30.9)	19.9% (6.3-31.3)	0.964	20.7% (6.4-30.4)	74.9% (62.4-89.9)	**<0.001**	22.1% (7.9-24.0)	72.8% (64.5-85.6)	**<0.001**	22.9% (10.6-32.3)	65.1% (52.4-79.4)	**<0.001**	35.7% (18.6-48.3)	59.4% (47.4-78.0)	**0.010**
Main place of defecation is toilet	19.8% (5.6-28.5)	20.8% (7.4-32.4)	0.735	20.8% (6.6-30.2)	76.2% (64.1-90.5)	**<0.001**	22.2% (7.9-24.0)	73.7% (65.5-86.1)	**<0.001**	23.3% (11.2-32.5)	65.5% (53.4-79.6)	**<0.001**	37.5% (21.9-54.0)	64.5% (51.3-81.7)	**0.015**
Practices open defecation‡	82.1% (72.8-95.6)	82.8% (70.9-94.7)	0.864	79.8% (71.5-93.5)	26.1% (12.0-39.1)	**<0.001**	80.1% (77.3-91.9)	33.8% (22.5-41.8)	**<0.001**	78.3% (69.6-89.8)	40.1% (24.9-52.1)	**<0.001**	66.1% (54.2-80.2)	40.2% (25.3-52.6)	**0.005**
	n=900	*n=735*		*n=791*	*n=700*		*n=725*	*n=634*		*n=715*	*n=594*		*n=758*	*n=591*	
Practices open defecation despite HH toilet; >5yo	2.6% (0.0-8.0)	4.6% (0.0-6.6)	0.801	0.9% (0.0-2.8)	1.7% (0.0-4.4)	0.605	2.3% (0.0-4.8)	4.7% (0.0-10.9)	0.245	2.8% (0.0-4.5)	3.0% (0.0-9.6)	0.310	6.6% (0.0-10.5)	2.4% (0.0-11.0)	0.978
	n=146	*n=166*		*n=130*	*n=157*		*n=121*	*n=144*		*n=118*	*n=139*		*n=111*	*n=128*	
Practices open defecation despite HH toilet; ≤5yo	2.7% (0.0-7.0)	8.6% (1.0-12.1)	0.335	3.1% (0.0-11.6)	9.6% (0.0-22.5)	0.391	7.4% (1.3-12.4)	25.0% (14.1-35.3)	**0.005**	11.9% (4.9-22.6)	32.4% (20.0-44.8)	**0.019**	29.7% (11.3-47.9)	35.2% (19.4-57.8)	0.510
Household sanitation	*n=244*	*n=219*		*n=222*	*n=208*		*n=208*	*n=204*		*n=198*	*n=183*		*n=212*	*n=179*	
Household has toilet	20.9% (9.2-28.7)	21.9% (10.7-31.4)	0.770	22.1% (8.0-32.7)	75.1% (60.1-87.9)	**<0.001**	22.6% (10.2-26.2)	73.5% (65.2-83.7)	**<0.001**	24.7% (12.6-33.8)	66.7% (56.9-81.0)	**<0.001**	41.5% (24.6-60.4)	62.0% (46.8-80.3)	0.100
Household has toilet with slab	6.2% (0.5-9.2)	10.8% (3.3-18.3)	0.170	8.6% (0.0-12.2)	29.8% (17.4-52.5)	**0.002**	12.5% (0.0-15.2)	40.7% (24.2-60.5)	**0.001**	10.6% (0.1-12.9)	40.4% (25.1-59.6)	**<0.001**	18.4% (5.9-30.9)	36.3% (22.0-56.7)	0.059
Household has toilet with water	6.1% (0.5-9.2)	3.3% (0.0-7.1)	0.636	10.8% (2.3-14.1)	44.7% (33.9-62.4)	**<0.001**	13.5% (0.0-15.6)	55.4% (36.0-73.3)	**<0.001**	13.1% (1.2-15.9)	48.1% (34.9-68.6)	**<0.001**	26.9% (12.3-39.8)	44.1% (31.9-64.9)	**0.049**
Household has clean toilet	4.1% (0.0-7.0)	8.2% (0.9-15.1)	0.220	7.2% (0.0-11.5)	14.4% (1.5-26.0)	0.194	5.29% (0.6-8.0)	20.6% (11.3-30.8)	**0.002**	5.6% (0.0-9.1)	15.8% (4.7-26.1)	0.055	10.8% (1.2-18.4)	16.8% (4.7-28.2)	0.363
	n=51	*n=44*		*n=49*	*n=150*		*n=47*	*n=150*		*n=49*	*n=122*		*n=88*	*n=111*	
For households with toilet: toilet has slab	30.6% (6.0-51.8)	54.8% (31.3-81.4)	0.146	38.8% (8.5-58.6)	41.3% (25.1-67.3)	0.466	55.3% (12.8-67.1)	55.3% (32.4-75.7)	0.437	42.9% (6.2-52.6)	60.7% (37.2-76.5)	0.115	44.3% (23.1-61.2)	58.6% (39.5-73.1)	0.281
For households with toilet: toilet has water	29.4% (3.6-53.5)	15.9% (0.0-44.3)	0.698	49.0% (18.1-66.9)	62.0% (51.9-85.9)	0.103	59.6% (12.6-68.7)	75.3% (48.5-91.0)	0.117	53.1% (14.4-65.1)	72.1% (50.8-87.3)	0.086	64.8% (47.8-79.9)	71.2% (58.4-83.9)	0.484
For households with toilet: toilet is clean	19.6% (2.4-32.1)	37.5% (13.9-53.1)	0.203	32.7% (2.3-49.9)	20.00% (2.5-35.1)	0.608	23.4% (4.9-41.0)	28.0% (15.1-41.9)	0.638	22.4% (0.1-33.8)	23.8% (7.9-37.2)	0.653	26.1% (7.7-39.0)	27.0% (10.1-37.2)	0.978
Water	*n=244*	*n=219*		*n=222*	*n=208*		*n=208*	*n=204*		*n=198*	*n=183*		*n=212*	*n=179*	
Main water source is piped water§	0	21.5% (16.0-26.9)	-	0	71.2% (65.0-77.3)	-	34.1% (27.7-40.6)	72.1% (65.9-78.2)	**<0.001**	38.4% (31.6-45.2)	87.4% (82.6-92.2)	**<0.001**	61.8% (55.3-68.3)	81.6% (75.9-87.2)	**<0.001**

Main water source is unprotected§	86.1% (81.7-90.4)	71.7% (65.8-77.7)	**0.020**	96.4% (93.9-98.8)	26.0% (20.0-31.9)	**<0.001**	52.9% (46.1-59.7)	0	-	57.1% (50.0-64.0)	2.2% (0.1-4.3)	**<0.001**	25.9% (20.0-31.8)	1.6% (0.0-3.6)	**<0.001**
Time to water source >15 min	41.0% (12.0-43.0)	27.5% (14.6-47.1)	0.768	29.7% (12.4-57.4)	33.2% (9.4-52.2)	0.795	29.8% (19.9-62.0)	9.3% (0.0-20.6)	**0.014**	30.3% (6.7-38.3)	10.9% (1.3-24.8)	0.333	31.1% (10.0-42.6)	14.0% (3.6-29.0)	0.336
Hygiene	*n=1046*	*n=901*		*n=921*	*n=857*		*n=846*	*n=774*		*n=833*	*n=733*		*n=869*	*n=719*	
Uses soap to wash hands	77.2% (63.5-91.6)	77.4% (56.5-87.7)	0.605	78.5% (56.9-88.0)	61.8% (39.0-75.2)	0.208	80.6% (63.8-95.5)	94.2% (73.2-100.0)	0.451	88.4% (74.9-100.0)	97.8% (84.4-100.0)	0.422	95.7% (32.6-100.0)	97.0 (94.6-100.0)	0.652
Washes hands before contact with food	47.3% (35.0-59.2)	68.6% (52.7-75.6)	**0.048**	45.2% (35.7-58.5)	46.4% (34.6-57.1)	0.879	41.7% (27.6-47.0)	42.6% (32.6-52.5)	0.456	43.1% (27.6-46.4)	41.9% (34.6-54.3)	0.286	47.3% (36.9-60.2)	53.6% (42.4-65.7)	0.508
Washes hands after contact with feces	84.3% (71.8-93.5)	76.5% (57.9-86.2)	0.234	58.3% (34.9-68.4)	68.5% (49.0-80.3)	0.270	78.1% (55.1-89.4)	77.4% (60.0-91.8)	0.757	86.1% (74.6-99.3)	89.1% (68.7-97.6)	0.681	87.7% (74.9-97.5)	88.7% (76.9-98.2)	0.861

CI = confidence interval; HH = household

* Participants defined as those completing study questionnaires. Note that denominator for each variable may be different given that not all participants replied to all questions. † P values and 95% confidence intervals are based on logistic regression models accounting for community-level clustering. ‡ Open defecation defined as any non-use of toilet, regardless of toilet ownership. § Piped water to household, yard or common place in the community (tapstand). Results for main water source could not be adjusted for clustering because there was no variability within clusters.

Supplemental Table 3. Reasons given by household heads for not building or rebuilding a household latrine, at the last follow-up

	Households that never built a latrine			Households that did not rebuild a latrine		
	Control	**Intervention**	**Overall**	**Control**	**Intervention**	**Overall**
Number of respondents	120	53	173	4	15	19
Didn't have time (n, (%))	70 (58.3%)	28 (52.8%)	98 (56.7%)	2 (50.0%)	7 (46.7%)	9 (47.4%)
Didn't have money or access to materials	61 (50.8%)	24 (45.3%)	85 (49.1%)	0	13 (86.7%)	13 (68.4%)
Don't know how	38 (31.7%)	21 (39.6%)	59 (34.1%)	1 (25.0%)	4 (26.7%)	5 (26.3%)
Prefer to defecate elsewhere	35 (29.2%)	10 (18.9%)	45 (26.0%)	1 (12.5%)	5 (33.3%)	6 (31.6%)

The total number of households in the intervention arm at the last follow-up was 212 in the control arm and 179 in the intervention arm. Note: percentages add to greater than 100% because respondents were able to select more than one reason.

Supplemental Table 4. Results of generalized linear mixed models showing effect estimate of the study intervention, age group and gender on STH infection and intensity*

Variable†	*Ascaris* spp. infection			*Ascaris* spp. intensity group‡			*N. americanus* infection			*N. americanus* intensity group‡		
	RR	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**
Intervention at FU1	1.38	0.37-5.11	0.632	1.59	0.21-11.77	0.650	1.06	0.68-1.64	0.795	0.94	0.26-3.35	0.921
Intervention at FU2	1.44	0.35-5.87	0.607	1.58	0.23-10.89	0.643	1.10	0.66-1.85	0.715	1.07	0.37-3.14	0.896
Intervention at FU3	1.49	0.39-5.79	0.560	1.46	0.24-8.85	0.684	1.26	0.72-2.20	0.416	1.94	0.74-5.07	0.178
Intervention at FU4	2.87	0.66-12.48	0.159	4.91	0.77-31.37	0.093	0.99	0.52-1.89	0.987	0.92	0.29-2.95	0.893
Age group:												
6 to 11 years	1.04	0.84-1.28	0.722	0.97	0.61-1.54	0.882	**2.20**	**1.83-2.64**	**<0.001**	**3.24**	**2.37-4.44**	**<0.001**
12 to 17 years	0.95	0.66-1.36	0.779	0.87	0.43-1.78	0.708	**2.71**	**2.01-3.48**	**<0.001**	**5.46**	**3.21-9.28**	**<0.001**
18 to 64 years	**0.75**	**0.57-0.97**	**0.032**	**0.55**	**0.34-0.89**	**0.015**	**2.75**	**2.08-3.65**	**<0.001**	**6.05**	**3.69-9.90**	**<0.001**
65+ years	**0.63**	**0.44-0.90**	**0.012**	**0.44**	**0.23-0.85**	**0.014**	**2.53**	**1.64-3.89**	**<0.001**	**5.01**	**2.25-11.18**	**<0.001**
Male	1.02	0.84-1.34	0.661	1.02	0.86-1.21	0.820	**1.83**	**1.53-2.20**	**<0.001**	**3.47**	**2.50-4.87**	**<0.001**
Random effects variance (95% CI)												
Community	1.83 (1.12-3.00)			3.51 (1.88-6.55)			0.21 (0.12-0.38)			1.04 (0.57-1.91)		
Household	0.05 (0.01-0.36)			0.80 (0.45-1.42)			0.26 (0.14-0.50)			1.39 (0.91-2.12)		
Participant	<0.001			0.42 (0.18-0.98)			<0.001			1.93 (1.27-2.93)		

CI = confidence interval; FU = follow-up; RR = relative risk

* Included 1878 participants in 456 households in 18 clusters. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Intensity group was run as an ordinal model, with the following categories: no infection, lower intensity infection, and higher intensity infection

Supplemental Table 5. Results of mixed effects multilevel models showing effect estimate of the study intervention and other covariates on qPCR intensity (positive samples only)*

Variable†	*Ascaris* spp. PCR intensity (x1000)			*N. americanus* PCR intensity (x1000)		
	Coefficient	**95% CI**	**p value**	**Coefficient**	**95% CI**	**p value**
Intervention at FU1	-176.1	-550.0, 197.8	0.356	-3.5	-8.6, 1.6	0.180
Intervention at FU2	31.4	-388.9, 451.6	0.884	0.9	-5.3, 7.1	0.774
Intervention at FU3	-340.5	-795.7, 114.6	0.143	3.6	-2.9, 10.13	0.278
Intervention at FU4	173.4	-339.6, 686.3	0.508	-1.8	-8.9, 5.3	0.618
Age group:						
6 to 11 years	-91.2	-349.3, -166.9	0.489	0.9	-4.6, 6.3	0.758
12 to 17 years	-409.5	-732.5, -86.4	**0.013**	2.9	-3.1, 9.0	0.344
18 to 64 years	-389.1	-644.2, -134.0	**0.003**	3.6	-1.6, 8.7	0.172
65+ years	-462.7	-937.4, 12.0	0.056	8.3	1.6, 14.9	**0.013**
Male	10.7	-173.3, 194.6	0.910	0.5	-2.2, 3.3	0.702
Random effects variance (95% CI)						
Community	23251 (4373, 123634)			3.7 (0.04, 372.7)		
Household	15146 (895, 256303)			100.4 (66.7, 151.2)		
Participant	<0.001			<0.001		

CI = confidence interval; FU = follow-up

***** Included 358 participants in 157 households in 18 villages. **†** Reference groups are as follows: control group; 1-5 years of age; female.

Supplemental Table 6. Results of generalized linear mixed models showing effect estimate of the study intervention, baseline infection/intensity group, age group and gender on STH infection and intensity*

Variable†	*Ascaris* spp. infection			*Ascaris* spp. intensity group‡			*N. americanus* infection			*N. americanus* intensity group‡		
	RR	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**
Intervention at FU1	1.43	0.37-5.44	0.604	1.73	0.24-12.19	0.583	1.01	0.72-1.42	0.965	0.97	0.32-2.93	0.952
Intervention at FU2	1.33	0.34-5.13	0.683	1.21	0.20-7.44	0.838	1.06	0.65-1.73	0.827	1.14	0.39-3.34	0.815
Intervention at FU3	1.42	0.37-5.46	0.613	1.24	0.20-7.65	0.820	1.28	0.77-2.13	0.338	2.14	0.86-5.35	0.103
Intervention at FU4	3.00	0.63-14.24	0.166	5.46	0.78-38.40	0.088	0.99	0.55-1.77	0.962	1.04	0.36-3.02	0.939
Age group:												
6 to 11 years	0.96	0.77-1.21	0.755	0.75	0.42-1.34	0.334	1.22	0.93-1.60	0.150	1.25	0.80-1.95	0.337
12 to 17 years	0.89	0.69-1.15	0.381	**0.58**	**0.34-0.99**	**0.045**	**1.48**	**1.11-1.98**	**0.008**	**2.19**	**1.20-4.00**	**0.011**
18 to 64 years	0.76	0.56-1.05	0.092	0.52	0.25-1.08	0.080	**1.41**	**1.02-1.95**	**0.039**	**2.00**	**1.18-3.41**	**0.011**
65+ years	0.79	0.45-1.41	0.429	0.60	0.26-1.38	0.230	1.10	0.67-1.79	0.712	1.26	0.54-2.96	0.594
Male	0.97	0.86-1.09	0.614	0.94	0.75-1.18	0.609	**1.60**	**1.33-1.93**	**<0.001**	**2.65**	**1.84-3.84**	**<0.001**
Baseline infection/ intensity group	**1.40**	**1.18-1.66**	**<0.001**	**1.49**	**1.10-2.02**	**0.010**	**3.34**	**2.43-4.60**	**<0.001**	**2.77**	**1.96-3.92**	**<0.001**
Random effects variance (95% CI)												
Community	1.82 (1.14-2.88)			3.22 (1.87-5.54)			0.12 (0.06-0.24)			0.71 (0.36-1.44)		
Household	0.02 (0.00-3.19)			0.66 (0.28-1.58)			0.18 (0.08-0.40)			1.22 (0.69-2.17)		
Participant	<0.001			0.36 (0.05-2.50)			<0.001			2.17 (1.43-3.29)		

CI = confidence interval; FU = follow=up; RR = relative risk

* Included 1339 participants in 416 households in 18 clusters. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Intensity group was run as an ordinal model, with the following categories: no infection, lower intensity infection, and higher intensity infection

Supplemental Table 7. Prevalence and intensity of STH infections over time

	Baseline % (95%CI)*			Follow-up 1 % (95%CI)*			Follow-up 2 % (95%CI)*			Follow-up 3 % (95%CI)*			Follow-up 4 % (95%CI)*		
	Control (n=891)	**Intervention (n=711)**	**p value**	**Control (n=689)**	**Intervention (n=584)**	**p value**	**Control (n=624)**	**Intervention (n=552)**	**p value**	**Control (n=609)**	**Intervention (n=531)**	**p value**	**Control (n=623)**	**Intervention (n=553)**	**p value**
***Ascaris* spp.**															
Prevalence	14.0% (8.1-37.8)	21.9% (7.6-36.6)	0.934	12.8% (2.1-24.5)	17.3% (4.3-30.7)	0.621	10.6% (0.0-21.8)	13.6% (1.9-29.6)	0.557	7.9% (0-20.0)	12.4% (1.0-24.7)	0.688	4.5% (0.0-13.3)	14.3% (2.9-30.3)	0.139
Mean PCR intensity (x1000) (SD)	365.7 (812.4)	768.4 (2208.1)	0.053	767.5 (1224.6)	651.9 (1182.3)	0.509	142.5 (308.1)	265.4 (606.1)	0.137	616.0 (1463.8)	275.1 (1032.8)	0.276	246.0 (467.1)	452.0 (1568.3)	0.495
Prevalence of higher-intensity infections	5.4% (1.9-14.6)	11.1% (4.0-27.2)	0.936	8.0% (2.0-27.0)	10.8% (2.9-32.5)	0.632	4.6% (1.0-18.5)	5.3% (1.0-23.2)	0.599	3.8% (0.7-17.2)	4.5% (1.6-12.4)	0.728	1.6% (0.2-11.1)	5.6% (1.5-19.0)	0.144
Necator americanus															
Prevalence	59.8% (51.9-74.0)	60.5% (51.3-73.4)	0.940	35.3% (26.8-47.6)	33.6% (24.1-44.2)	0.677	22.4% (15.5-32.1)	22.3% (15.3-31.7)	0.958	19.5% (13.4-28.0)	22.0% (15.2-30.7)	0.670	16.9% (11.6-28.2)	15.4% (9.6-24.6)	0.619
Mean PCR intensity (x1000) (SD)	13.3 (35.1)	11.2 (22.8)	0.386	12.6 (30.0)	8.8 (17.3)	0.226	7.5 (15.6)	7.7 (21.0)	0.816	3.0 (10.2)	6.6 (28.5)	0.333	9.3 (31.7)	7.0 (20.6)	0.552
Prevalence of higher-intensity infections	32.3% (24.1-38.2)	31.4% (26.6-36.6)	0.736	17.6% (8.9-31.8)	14.7% (6.5-30.1)	0.656	8.3% (5.1-13.2)	11.1% (5.2-21.9)	0.880	3.4% (2.1-5.5)	5.3% (3.4-8.0)	0.596	6.3% (2.7-13.8)	4.0% (2.3-6.8)	0.598
Other															
Ancylostoma spp. prevalence	4.8% (3.0-7.8)	3.2% (1.6-5.1)	0.155	0.9% (0.0-2.2)	1.0% (0.0-1.9)	0.937	0.5% (0.0-1.1)	1.4% (0.3-2.6)	0.130	1.5% (0.0-4.1)	0.9% (0.0-2.0)	0.381	0.8% (0.0-2.4)	0.4% (0.0-1.2)	0.418
T. trichiura prevalence	0.1% (0.0-0.5)	0.7% (0.0-1.6)	0.283	0.3% (0.0-0.8)	0.7% (0.0-1.5)	0.460	0.2% (0.0-0.5)	0.4% (0.0-0.9)	0.505	0.2% (0.0-0.5)	0.6% (0.0-1.2)	0.284	0.2% (0.0-0.6)	0.7% (0.0-1.6)	0.312
S. stercoralis prevalence	0	0.1% (0-0.4)	-	0.3% (0.0-0.7)	0.3% (0..0-0.8)	0.869	0.2% (0.0-0.5)	0.2% (0.0-0.5)	0.931	0.2% (0.0-0.5)	0	-	0	0	-

CI = confidence intervals; SD = standard deviation

* p values and 95% confidence intervals are based on logistic regression models accounting for community-level clustering.

Supplemental Table 8. Results of generalized linear mixed models showing effect estimate of the study intervention, age group and gender on anemia and growth parameters*

Variable†	Anemia			Stunting‡			Thinness‡			Wasting‡			Underweight‡		
	RR	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**
Intervention (FU2)	0.75	0.43-1.31	0.317	1.28	1.03-1.60	**0.026**	0.76	0.47-1.22	0.256	0.86	0.40-1.87	0.711	0.85	0.58-1.23	0.393
Intervention (FU4)	0.63	0.34-1.14	0.126	1.18	0.92-1.51	0.198	0.75	0.51-1.11	0.151	0.79	0.45-1.38	0.413	1.06	0.84-1.34	0.614
Age group:															
6 to 11 years	0.60	0.47-0.76	**<0.001**	1.1	0.96-1.31	0.147	1.48	1.19-1.83	**<0.001**	-	-	-	-	-	-
12 to 17 years	0.74	0.60-0.91	**0.005**	1.26	1.08-1.46	**0.003**	1.58	1.11-2.26	**0.011**	-	-	-	-	-	-
18 to 64 years	0.80	0.57-1.12	0.188	-	-	-	-	-	-	-	-	-	-	-	-
65+ years	0.93	0.61-1.43	0.750	-	-	-	-	-	-	-	-	-	-	-	-
Age in years (continuous)§	-	-	-	-	-	-	-	-	-	1.00	0.79-1.28	0.974	1.06	1.03-1.08	**<0.001**
Male	1.07	0.85-1.35	0.535	1.41	1.16-1.71	**<0.001**	1.17	0.93-1.47	0.167	1.05	0.74-1.48	0.799	1.24	1.12-1.38	**<0.001**
Random effects variance (95% CI)															
Community	0.099 (0.122-0.823)			0.021 (0.002-0.230)			0.085 (0.037-0.196)			0.135 (0.038-0.478)			0.026 (0.008-0.083)		
Household	0.113 (0.382-1.375)			<0.001			<0.001			<0.001			<0.001		
Participant	<0.001			**			<0.001			<0.001			<0.001		

CI = confidence interval; FU = follow-up; RR = relative risk

* Models included the following numbers of participants in 18 communities: for anemia, 1598 participants in 428 households; for stunting, 789 participants in 304 households; for thinness, 781 participants in 301 households; for wasting, 231 participants in 157 households; for underweight 511 participants in 249 households. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Anthropometric indices defined as < -2SD below the mean of a standard population for the following indicators: stunting = weight-for-age; wasting: weight-for-height; underweight = weight-for-age; thinness = BMI-for-age (BMI calculated as weight (kg) / height^2 (cm). § Age included as a continuous variable in the model for wasting and underweight because data were only available for a small range of ages (wasting: age 1 to 5 years; underweight: age 1 to 10 years). ** Clustering at individual level not included in model due to low variability at the individual level causing non-convergence.

Supplemental Table 9. Results of generalized linear mixed models showing effect estimate of the study intervention on hemoglobin and growth parameters as continuous outcomes*

Variable†	Adjusted hemoglobin			Height-for-age *Z*-score			BMI-for-age *Z*-score			Weight-for-height *Z*-score			Weight-for-age *Z*-score		
	Coef	**95% CI**	**p value**	**Coef**	**95% CI**	**p value**	**Coef**	**95% CI**	**p value**	**Coef**	**95% CI**	**p value**	**Coef**	**95% CI**	**p value**
Intervention (FU2)	1.32	-2.07, 4.71	0.445	-0.40	-0.63, -0.18	**<0.001**	0.20	-0.16, 0.55	0.277	-0.07	-0.53, 0.38	0.756	-0.03	-0.37, 0.31	0.846
Intervention (FU4)	2.30	-1.12, 5.72	0.187	-0.25	-0.48, 0.03	**0.028**	0.04	-0.32, 0.55	0.820	-0.06	-0.42, 0.54	0.810	-0.08	-0.42, 0.27	0.661
Age group:															
6 to 11 years	8.10	6.40, 9.80	**<0.001**	-0.06	-0.19, 0.07	0.356	-0.37	-0.52, -0.22	**<0.001**	-	-	-	-	-	-
12 to 17 years	12.10	9.99, 14.20	**<0.001**	-0.27	-0.44, -0.10	**0.001**	-0.42	-0.61, -0.221	**<0.001**	-	-	-	-	-	-
18 to 64 years	17.78	16.19, 19.37	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-
65+ years	14.65	12.26, 17.04	**<0.001**	-	-	-	-	-	-	-	-	-	-	-	-
Age in years (continuous)‡	-	-	-	-	-	-	-	-	-	-0.14	-0.26, -0.02	**0.019**	-0.07	-0.10, -0.04	**<0.001**
Male	6.27	5.18, 7.37	**<0.001**	-0.36	-0.49, -0.22	**<0.001**	-0.13	-0.27, 0.01	0.071	-0.06	-0.35, 0.22	0.657	-0.28	-0.44, -0.12	**0.001**
Random effects variance (95% CI)															
Community	10.07 (4.47, 22.65)			0.02 (0.00, 0.21)			0.09 (0.04, 0.22)			0.08 (0.02, 0.28)			0.07 (0.02, 0.22)		
Household	16.18 (10.52, 24.88)			0.28 (0.19, 0.40)			0.18 (0.11, 0.31)			<0.001			0.25 (0.15, 0.39)		
Participant	38.65 (28.79, 51.88)			0.57 (0.21, 0.27)			0.29 (0.19, 0.45)			0.29 (0.93, 1.54)			0.40 (0.30, 0.54)		

BMI = body mass index; CI = confidence interval; Coef = regression coefficient; FU = follow=up

* Models included the following numbers of participants in 18 communities: for anemia, 1598 participants in 421 households; for stunting, 793 participants in 304 households; for thinness, 786 participants in 302 households; for wasting, 232 participants in 157 households; for underweight 511 participants in 249 households. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Age included as a continuous variable in the model for weight-for-height and weight-for-age because data were only collected for one or two age groups

Supplemental Table 10. Morbidity indicators over time

	Baseline*			**Follow-up 2***			**Follow-up 4***		
	Control	**Intervention**	**p value**	**Control**	**Intervention**	**p value**	**Control**	**Intervention**	**p value**
Hematological parameters	*n=966*	*n=831*		*n=634*	*n=607*		*n=652*	*n=521*	
Mean (SD) hemoglobin (g/L)	132.22 (15.92)	128.74 (15.75)	**0.019**	130.94 (15.83)	132.01 (14.72)	0.965	128.94 (14.81)	129.20 (14.37)	0.656
Anemic, % (95% CI)	15.4% (11.7-20.5)	21.1% (15.3-25.4)	0.209	15.9% (9.2-20.3)	12.7% (8.2-18.6)	0.726	23.9% (17.8-33.6)	16.3% (8.9-20.1)	**0.022**
Growth	*n=463*	*n=418*		*n=319*	*n=338*		*n=294*	*n=303*	
Mean (SD) height-for-age Z-score (1 to 18 years)	-2.03 (1.25)	-2.38 (1.16)	**0.041**	-1.93 (1.17)	-2.36 (1.07)	**0.007**	-2.12 (1.12)	-2.23 (0.94)	0.500
Stunted, % (95% CI) (1 to 18 years)†	51.9% (41.4-60.4)	64.7% (55.3-72.7)	**0.049**	52.7% (41.0-59.2)	63.9% (56.1-72.2)	**0.026**	56.0% (43.2-64.1)	59.9% (49.4-69.3)	0.440
Mean (SD) BMI-for-age Z score (1 to 18 years)†‡	-1.31 (1.04)	-1.16 (1.05)	0.227	-1.67 (1.30)	-1.14 (1.33)	0.291	-1.67 (1.17)	-1.27 (1.04)	0.374
Thinness, % (95% CI) (1 to 18 years)†	22.5% (16.1-29.5)	17.8% (11.9-23.3)	0.243	40.4% (24.6-46.1)	23.9% (18.3-37.5)	0.307	37.4% (22.7-40.9)	21.6% (14.8-29.4)	0.104
	n=109	*n=119*		*n=84*	*n=99*		*n=68*	*n=86*	
Mean (SD) weight-for-length Z-score (1 to 5 years)	-1.07(0.93)	-0.97 (1.07)	0.399	-1.31 (1.35)	-1.21 (1.29)	0.970	-1.44 (1.30)	-1.21 (1.16)	0.382
Wasted, % (95% CI) (1 to 5 years)†	13.8% (5.6-24.3)	15.1% (5.2-22.5)	0.868	29.8% (15.3-44.6)	22.4% (13.1-40.8)	0.756	30.9% (18.5-42.3)	21.2% (12.0-31.1)	0.254
	n=274	*n=266*		*n=209*	*n=215*		*n=177*	*n=197*	
Mean (SD) weight-for-age Z-score (1 to 10 years)	-2.02 (1.04)	-2.19 (0.96)	0.425	-2.29 (1.20)	-2.13 (1.07)	0.877	-2.35 (1.08)	-2.22 (0.94)	0.672
Underweight, % (95% CI) (1 to 10 years)†	52.0% (42.3-61.2)	60.4% (48.3-66.7)	0.391	63.2% (48.4-73.6)	49.8% (41.4-67.3)	0.474	61.9% (50.4-71.4)	59.2% (48.7-69.0)	0.789

BMI = body mass index; CI = confidence interval; SD = standard deviation

***** p values and confidence intervals are based on logistic regression models accounting for community-level clustering. **†** Computed as greater than 2 standard deviations below the median for reference population, according to WHO guidelines (underweight = weight-for-age; stunting = height-for-age; thinness = BMI-for-age; wasting = weight-for-height). ‡ BMI calculated as weight (kg) / height^2 (cm).

Supplemental Table 11. Results of generalized linear mixed models showing effect estimate of the study intervention on STH prevalence and intensity, including the 23 communities that completed the study*

Variable†	*Ascaris* spp. infection			*Ascaris* spp. intensity group‡			*N. americanus* prevalence			*N. americanus* intensity group‡		
	RR	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**
Intervention at FU1	1.20	0.38-3.83	0.754	1.18	0.21-6.69	0.852	0.99	0.68-1.45	0.979	0.85	0.29-2.47	0.761
Intervention at FU2	1.31	0.39-4.37	0.662	1.36	0.25-7.43	0.724	1.05	0.68-1.63	0.814	1.02	0.41-2.52	0.971
Intervention at FU3	1.46	0.44-4.88	0.539	1.70	0. 33-8.66	0.523	1.25	0.75-2.09	0.391	1.67	0.71-3.90	0.240
Intervention at FU4	1.69	0.46-6.19	0.427	2.19	0.37-13.03	0.390	0.91	0.51-1.60	0.740	0.78	0.29-2.15	0.637
Age group:												
6 to 11 years	1.05	0.84-1.32	0.655	1.03	0.66-1.60	0.893	2.26	1.90-2.69	**<0.001**	3.54	2.65-4.71	**<0.001**
12 to 17 years	1.01	0.77-1.34	0.926	0.93	0.54-1.61	0.800	2.78	2.22-3.48	**<0.001**	5.65	3.57-8.92	**<0.001**
18 to 64 years	0.78	0.61-1.00	**0.048**	0.58	0.39-0.87	**0.008**	3.10	2.34-4.10	**<0.001**	7.36	4.56-11.86	**<0.001**
65+ years	0.54	0.39-0.74	**<0.001**	0.32	0.19-0.54	**0.000**	2.61	1.78-3.83	**<0.001**	5.20	2.64-10.25	**<0.001**
Male	1.02	0.88-1.17	0.837	1.03	0.78-1.36	0.821	1.83	1.78-3.93	**<0.001**	3.44	2.61-4. 53	**<0.001**
Random effects variance (95% CI)												
Community	1.94 (1.34-2.80)			3.72 (2.47-5.59)			0.20 (0.12-0.33)			0.99 (0.59-1.63)		
Household	0.09 (0.04-0.23)			0.90 (0.60-1.35)			0.29 (0.17-0.51)			1.40 (0.96-2.04)		
Participant	<0.001			0.38 (0.21-0.69)			<0.001			1.88 (1.33-2.68)		

CI = confidence interval; FU = follow-up; RR = relative risk

* Included 2376 participants in 569 households in 23 communities. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Intensity group was run as an ordinal model, with the following categories: no infection, lower intensity infection, and higher intensity infection

Supplemental Table 12. Results of generalized linear mixed models showing effect estimate of the study intervention on anemia and growth parameters, including the 23 communities that completed the study*

Variable†	Anemia			Stunting‡			Thinness‡			Wasting‡			Underweight‡		
	RR	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**	**RR**	**95% CI**	**p value**
Intervention (FU2)	0.81	0.48-1.35	0.417	1.18	0.96-1.44	0.111	0.70	0.47-1.07	0.097	0.74	0.38-1.45	0.381	0.88	0.65-1.20	0.432
Intervention (FU4)	0.81	0.48-1.35	0.411	1.09	0.87-1.37	0.437	0.71	0.51-1.00	0.053	0.77	0.47-1.26	0.294	1.00	0.81-1.22	0.972
Age group:															
6 to 11 years	0.55	0.44-0.69	**<0.001**	1.0	0.93-1.21	0.394	1.40	1.17-1.69	**<0.001**	-	-	-	-	-	-
12 to 17 years	0.70	0.56-0.87	**0.003**	1.18	1.03-1.34	**0.017**	1.51	1.13-2.02	**0.005**	-	-	-	-	-	-
18 to 64 years	0.71	0.53-0.96	**0.026**	-	-	-	-	-	-	-	-	-	-	-	-
65+ years	0.96	0.67-1.38	0.822	-	-	-	-	-	-	-	-	-	-	-	-
Age in years (continuous)§	-	-	-	-	-	-	-	-	-	1.04	0.87-1.24	0.694	1.05	1.04-1.07	**<0.001**
Male	1.16	0.95-1.42	0.144	1.34	1.14-1.58	**<0.001**	1.15	0.97-1.38	0.116	1.08	0.81-1.45	0.594	1.22	1.11-1.34	**<0.001**
Random effects variance (95% CI)															
Community	0.13 (0.06-0.29)			0.03 (0.01-0.13)			0.09 (0.05-0.18)			0.10 (0.02-0.46)			0.02 (0.01-0.07)		
Household	0.12 (0.05-0.27)			<0.001			<0.001			<0.001			<0.001		
Participant	<0.001			**			<0.001			<0.001			<0.001		

CI = confidence interval; FU = follow-up; RR = relative risk

* Models included the following numbers of participants in 23 communities: for anemia, 2007 participants in 526 households; for stunting, 1018 participants in 393 households; for thinness, 1010 participants in 390 households; for wasting, 307 participants in 206 households; for underweight, 672 participants in 326 households. † Reference groups are as follows: control group; 1-5 years of age; female. ‡ Anthropometric indices defined as < -2SD below the mean of a standard population for the following indicators: stunting = height-for-age; wasting: weight-for-height; underweight = weight-for-age; thinness = BMI-for-age (BMI calculated as weight (kg) / height^2 (cm). § Age included as a continuous variable in the model for wasting and underweight because data were only available for a small range of ages (wasting: age 1 to 5 years; underweight: age 1 to 10 years). ** Clustering at individual level not included in model due to low variability at the individual level causing non-convergence.

CONSORT 2010 checklist of information to include when reporting a cluster randomised trial

Please note that additional details are included in the previously published protocol paper:
Nery SV, McCarthy JS, Traub R, et al. A cluster-randomised controlled trial integrating a community-based water, sanitation and hygiene programme, with mass distribution of albendazole to reduce intestinal parasites in Timor-Leste: the WASH for WORMS research protocol. *BMJ open* 2015; **5**(12): e009293.

Section/Topic	Item No	Standard Checklist item	Extension for cluster designs	Page No *
Title and abstract				
	1a	Identification as a randomised trial in the title	Identification as a cluster randomized trial in the title	Page 1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)[1,2]	See table 2	Page 2
Introduction				
Background and objectives	2a	Scientific background and explanation of rationale	Rationale for using a cluster design	Page 4-5
	2b	Specific objectives or hypotheses	Whether objectives pertain to the cluster level, the individual participant level or both	Page 4-5
Methods				
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	Definition of cluster and description of how the design features apply to the clusters	Page 4-5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons		N/A
Participants	4a	Eligibility criteria for participants	Eligibility criteria for clusters	Page 7
	4b	Settings and locations where the data were collected		Page 4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	Whether interventions pertain to the cluster level, the individual participant level or both	Page 5-6

Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	Whether outcome measures pertain to the cluster level, the individual participant level or both	Page 9-10
	6b	Any changes to trial outcomes after the trial commenced, with reasons		N/A
Sample size	7a	How sample size was determined	Method of calculation, number of clusters(s) (and whether equal or unequal cluster sizes are assumed), cluster size, a coefficient of intracluster correlation (ICC or *k*), and an indication of its uncertainty	Page 10
	7b	When applicable, explanation of any interim analyses and stopping guidelines		N/A
Randomisation:				
Sequence generation	8a	Method used to generate the random allocation sequence		Page 7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Details of stratification or matching if used	Page 7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Specification that allocation was based on clusters rather than individuals and whether allocation concealment (if any) was at the cluster level, the individual participant level or both	N/A
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Replace by 10a, 10b and 10c	
	10a		Who generated the random allocation sequence, who enrolled clusters, and who assigned clusters to interventions	Page 7

	10b		Mechanism by which individual participants were included in clusters for the purposes of the trial (such as complete enumeration, random sampling)	Page 8
	10c		From whom consent was sought (representatives of the cluster, or individual cluster members, or both), and whether consent was sought before or after randomisation	Page 7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how		N/A
	11b	If relevant, description of the similarity of interventions		N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	How clustering was taken into account	Page 11-12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses		Page 11-12
Results				
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	For each group, the numbers of clusters that were randomly assigned, received intended treatment, and were analysed for the primary outcome	Page 7-8 and Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	For each group, losses and exclusions for both clusters and individual cluster members	Page 7-8 and Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up		Page 12
	14b	Why the trial ended or was stopped		N/A

Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Baseline characteristics for the individual and cluster levels as applicable for each group	Page 12 and Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	For each group, number of clusters included in each analysis	Fig 1, Table 2, Table 3
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Results at the individual or cluster level as applicable and a coefficient of intracluster correlation (ICC or k) for each primary outcome	Table 2, Table 3, Supplemental Table 4, Supplemental Table 6
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended		Table 2, Table 3, Supplemental Tables 4-7
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory		Supplemental Tables 5, 8-9, 11-12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[3])		N/A
Discussion				
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses		21-23
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	Generalisability to clusters and/or individual participants (as relevant)	20-21
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and		20-21

		considering other relevant evidence	
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	4
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	24-25

** Note: page numbers optional depending on journal requirements*

Extension of CONSORT for abstracts[1,2] to reports of cluster randomised trials

Item	Standard Checklist item	Extension for cluster trials
Title	Identification of study as randomised	**Identification of study as cluster randomised**
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	**Eligibility criteria for clusters**
Interventions	Interventions intended for each group	
Objective	Specific objective or hypothesis	**Whether objective or hypothesis pertains to the cluster level, the individual participant level or both**
Outcome	Clearly defined primary outcome for this report	**Whether the primary outcome pertains to the cluster level, the individual participant level or both**
Randomization	How participants were allocated to interventions	**How clusters were allocated to interventions**
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	
Results		
Numbers randomized	Number of participants randomized to each group	**Number of clusters randomized to each group**
Recruitment	Trial status[1]	
Numbers analysed	Number of participants analysed in each group	**Number of clusters analysed in each group**
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	**Results at the cluster or individual participant level as applicable for each primary outcome**
Harms	Important adverse events or side effects	
Conclusions	General interpretation of the results	
Trial registration	Registration number and name of trial register	
Funding	Source of funding	

[1] Relevant to Conference Abstracts

REFERENCES

[1] Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG, et al. CONSORT for reporting randomised trials in journal and conference abstracts. *Lancet* 2008, 371:281-283

[2] Hopewell S, Clarke M, Moher D, Wager E, Middleton P, Altman DG at al (2008) CONSORT for reporting randomized controlled trials in journal and conference abstracts: explanation and elaboration. *PLoS Med* 5(1): e20

[3] Ioannidis JP, Evans SJ, Gotzsche PC, O'Neill RT, Altman DG, Schulz K, Moher D. Better reporting of harms in randomized trials: an extension of the CONSORT statement. *Ann Intern Med* 2004; 141(10):781-788.

Appendix 6
Supplementary material for Paper 6

The following information will be published as an online supplement for Paper 6:

Nery SV*, **Clarke NE***, Richardson A, Traub RJ, McCarthy JS, Gray DJ, Vallely AJ, Williams GM, Andrews RM, Campbell SJ, Clements ACA. Risk factors for infection with soil-transmitted helminths during an integrated community-level WASH and deworming intervention in Timor-Leste. *Int J Parasitol* 2019; 49(5): 389–396. https://doi.org/10.1016/j.ijpara.2018.12.006 (* co-first authors)

The material formed part of the manuscript submission and was subjected to peer review.

Supplementary Data S1. Full list of variables examined as risk factors for STH infection.

Demographic variables*

Age group

Sex

Individual hygiene variables

Washes hands using soap or ash

Washes hands before contact with food

Washes hands after contact with faeces

Washes hands after contact with dirt

Always wears shoes indoors

Always wears shoes outdoors and while toileting

Individual sanitation variables

Main place of defecation is toilet

Practises open defecation

Uses water to clean self after defecation

School sanitation variables

Uses toilet at school (children aged 6-17 years only)

Individual socioeconomic variables*

Education level (adults aged 18+ years only)

Employment (adults aged 18+ years only)

Household sanitation variables

Household has toilet

Household toilet has slab

Household toilet is pour-flush latrine

Household toilet observed to be clean

No water available to clean self after defecating

Household toilet is shared with another household

Child waste disposed of hygienically

Household garbage disposed of in bush

Household garbage disposed of by digging/burying

Household garbage disposed of by burning

Household water variables

Household main water source

Distance to water source is more than 15 minutes

Water always available from main water source

Household water stored in only covered containers

Household water treated

Household socioeconomic variables*

At least one child under 5 years of age in household

More than 6 people living in dwelling

Socioeconomic quintile

* Variables in these domains were examined as concurrent (cross-sectional) risk factors only. All other variables were examined both concurrently (cross-sectional) and six months previously (longitudinal), as risk factors for current infection.

Supplementary Table S1. Participation in the study over time

	Follow-up 1	Follow-up 2	Follow-up 3	Follow-up 4	Overall*
Individuals present (n)	2303	2135	2171	2126	2725
Provided stool sample, n (%)	1620 (70.3%)	1493 (69.9%)	1490 (68.6%)	1462 (68.8%)	2631 (86.7%)
Provided questionnaire, n (%)	2231 (96.9%)	2021 (94.7%)	2010 (92.6%)	1954 (91.2%)	2673 (98.1%)
Provided both stool sample and questionnaire, n (%)	1598 (69.4%)	1458 (68.3%)	1465 (67.5%)	1411 (66.4%)	2333 (85.6%)

* Overall participation was defined as participation at one or more time points

Supplementary Table S2. Prevalence of STH infections among study participants over time

	Proportion of study population infected (95% confidence interval)			
	Follow-up 1 (N=1598)	**Follow-up 2 (N=1459)**	**Follow-up 3 (N=1465)**	**Follow-up 4 (N=1412)**
Ascaris spp.	17.9 (16.1-19.9)	15.1 (13.3-17.0)	12.8 (11.2-14.6)	10.5 (9.0-12.2)
N. americanus	33.6 (31.3-36.0)	21.0 (19.0-23.2)	17.7 (15.8-19.7)	14.6 (12.8-16.5)
Ancylostoma spp.	0.8 (0.5-1.4)	0.9 (0.5-1.5)	1.1 (0.7-1.8)	0.5 (0.2-1.0)
Trichuris spp.	0.9 (0.6-1.6)	0.3 (0.1-0.8)	0.3 (0.1-0.7)	0.6 (0.3-1.1)
Any STH infection	44.2 (41.8-46.7)	32.6 (30.3-35.1)	27.9 (25.7-30.3)	22.8 (20.7-25.1)

Supplementary Table S3. Results of univariable analyses for *N. americanus* infection (*N*=2333), based on mixed-effects multilevel logistic regression models accounting for village, household and individual-level clustering

	Concurrent			Six months previously		
Covariate	**Odds ratio**	**95% CI**	***p* value**	**Odds ratio**	**95% CI**	***p* value**
General variables						
Age group[a]						
6-11 years	**3.80**	**2.51-5.76**	**<0.001**	-	-	-
12-17 years	**6.24**	**3.76-10.33**	**<0.001**	-	-	-
18-64 years	**7.87**	**5.26-11.79**	**<0.001**	-	-	-
65+ years	**5.69**	**3.23-10.04**	**<0.001**	-	-	-
Male sex	**3.45**	**2.68-4.46**	**<0.001**	-	-	-
Individual hygiene variables						
Washes hands using soap or ash	**1.45**	**1.11-1.90**	**0.007**	**0.76**	**0.57-1.02**	**0.064**
Washes hands before contact with food	**0.80**	**0.65-0.98**	**0.033**	0.92	0.71-1.17	0.486
Washes hands after contact with faeces	**1.61**	**1.26-2.06**	**0.001**	**0.84**	**0.63-1.10**	**0.198**
Washes hands after contact with dirt	**1.45**	**1.16-1.81**	**0.001**	1.15	0.89-1.48	0.272
Always wears shoes indoors	0.99	0.80-1.24	0.956	0.99	0.75-1.30	0.959
Always wears shoes outdoors and while toileting	**1.19**	**0.97-1.48**	**0.100**	1.17	0.84-1.62	0.346
Individual sanitation variables						
Main place of defecation is toilet	1.18	0.89-1.55	0.257	1.16	0.85-1.59	0.343
Practises open defecation	**0.73**	**0.56-0.95**	**0.020**	0.94	0.69-1.28	0.691
Uses water to clean self after defecation	1.01	0.77-1.31	0.952	0.85	0.62-1.16	0.303
School sanitation variables (children age 6-17 years only)						
Uses toilet at school	0.90	0.57-1.24	0.639	**0.68**	**0.39-1.18**	**0.167**
Individual socioeconomic variables						
Education level *(adults age 18+ years only)*[b]						
Didn't finish primary school	1.04	0.68-1.56	0.862	-	-	-
Finished primary but not secondary school	0.96	0.64-1.43	0.838	-	-	-
Finished secondary school or higher	**0.43**	**0.23-0.78**	**0.006**	-	-	-
Employment *(adults age 18+ years only)*[c]						
Employed – farmer	**3.28**	**2.37-4.55**	**<0.001**	-	-	-
Employed – other job	**2.70**	**1.48-4.93**	**0.001**	-	-	-
Household sanitation variables						
Household has toilet	1.07	0.80-1.41	0.661	1.10	0.81-1.51	0.523

Household toilet has slab	**1.40**	**0.97-2.00**	**0.070**	0.90	0.55-1.47	0.674
Household toilet is pour-flush latrine	1.24	0.84-1.82	0.277	1.04	0.62-1.76	0.878
Household toilet observed to be clean	**1.43**	**0.98-2.08**	**0.063**	1.06	0.63-1.77	0.824
Water available to clean self after defecating	**1.32**	**0.89-1.98**	**0.162**	1.22	0.69-2.14	0.491
Household toilet is shared with another household	0.83	0.48-1.43	0.495	**0.45**	**0.22-0.90**	**0.025**
Child waste disposed of hygienically	0.98	0.59-1.64	0.943	**0.50**	**0.28-0.88**	**0.017**
Household garbage disposed of in bush	**1.23**	**0.99-1.53**	**0.059**	1.18	0.91-1.52	0.207
Household garbage disposed of by digging/burying	0.95	0.66-1.37	0.785	1.02	0.68-1.55	0.907
Household garbage disposed of by burning	0.89	0.73-1.11	0.321	0.90	0.70-1.15	0.394
Household water variables						
Household main water source[d]						
Tubewell/borehole	1.44	0.70-3.11	0.350	0.76	0.38-1.51	0.427
Unprotected spring/dugwell	0.81	0.57-1.16	0.250	**1.30**	**0.89-1.92**	**0.176**
Protected spring	1.17	0.51-2.68	0.719	1.19	0.48-3.00	0.701
Surface water	1.01	0.60-1.69	0.983	1.10	0.66-1.85	0.706
Distance to water source is more than 15 minutes	**0.72**	**0.56-0.94**	**0.013**	1.14	0.86-1.51	0.366
Water always available from main water source	1.04	0.78-1.41	0.758	**0.80**	**0.57-1.12**	**0.192**
Household water stored in only covered containers	0.78	0.51-1.19	0.251	0.89	0.58-1.35	0.584
Household water treated	1.10	0.89-1.37	0.369	**0.74**	**0.57-0.96**	**0.025**
Household socioeconomic variables						
At least one child under 5 years of age in household	**0.58**	**0.44-0.78**	**<0.001**	-	-	-
More than 6 people living in dwelling	0.82	0.60-1.14	0.237	-	-	-
Socioeconomic quintile[e]						
Quintile 2	**1.27**	**0.91-1.77**	**0.162**	-	-	-
Quintile 3	1.12	0.80-1.56	0.515	-	-	-
Quintile 4	1.01	0.70-1.45	0.957	-	-	-
Quintile 5 (richest)	1.13	0.77-1.65	0.540	-	-	-

Results in bold: Covariates entered in multivariable regression models (p<0.2 in univariable analyses)

Reference categories as follows: [a] Follow-up 1, [b] Age 1-5 years, [c] Never went to school, [d] No employment outside the home, [e] Household water source is piped water, [f] Socioeconomic quintile 1 (poorest)

Supplementary Table S4. Results of univariable analyses for *Ascaris* spp. infection (*N*=2333), based on mixed-effects multilevel logistic regression models accounting for village, household and individual-level clustering

	Concurrent			Six months previously		
Covariate	**Odds ratio**	**95% CI**	***p* value**	**Odds ratio**	**95% CI**	***p* value**
General variables						
Age group[b]						
6-11 years	1.07	0.79-1.48	0.640	-	-	-
12-17 years	0.97	0.65-1.44	0.862	-	-	-
18-64 years	**0.64**	**0.48-0.86**	**0.004**	**-**	**-**	**-**
65+ years	**0.33**	**0.20-0.60**	**<0.001**	**-**	**-**	**-**
Male sex	1.05	0.84-1.31	0.674	-	-	-
Individual hygiene variables						
Washes hands using soap or ash	1.01	0.78-1.31	0.934	0.92	0.69-1.23	0.580
Washes hands before contact with food	**0.84**	**0.68-1.05**	**0.122**	0.92	0.71-1.19	0.529
Washes hands after contact with faeces	1.12	0.87-1.44	0.373	0.95	0.72-1.25	0.704
Washes hands after contact with dirt	0.92	0.74-1.15	0.476	1.06	0.81-1.38	0.669
Always wears shoes indoors	0.94	0.75-1.18	0.598	**0.83**	**0.63-1.09**	**0.182**
Always wears shoes outdoors and while toileting	1.06	0.86-1.32	0.572	0.92	0.66-1.27	0.608
Individual sanitation variables						
Main place of defecation is toilet	1.01	0.74-1.36	0.963	**1.29**	**0.92-1.82**	**0.144**
Practises open defecation	0.90	0.68-1.19	0.459	**0.76**	**0.54-1.08**	**0.123**
Uses water to clean self after defecation	1.18	0.88-1.58	0.269	1.20	0.86-1.66	0.278
School sanitation variables (children age 6-17 years only)						
Uses toilet at school	**1.97**	**1.20-3.22**	**0.007**	0.74	0.41-1.33	0.316
Individual socioeconomic variables						
Education level *(adults age 18+ years only)*[b]						
Didn't finish primary school	1.26	0.80-1.97	0.315	-	-	-
Finished primary but not secondary school	**1.90**	**1.25-2.90**	**0.003**	-	-	-
Finished secondary school or higher	0.99	0.53-1.85	0.975	-	-	-
Employment *(adults age 18+ years only)*[c]						
Employed – farmer	1.03	0.72-1.48	0.862	-	-	-
Employed – other job	1.01	0.52-1.95	0.973	-	-	-
Household sanitation variables						
Household has toilet	0.92	0.68-1.25	0.606	**1.40**	**0.99-1.98**	**0.058**

Household toilet has slab	1.17	0.85-1.62	0.344	1.23	0.79-1.92	0.367
Household toilet is pour-flush latrine	**1.32**	**0.91-1.91**	**0.147**	0.93	0.56-0.52	0.768
Household toilet observed to be clean	**1.24**	**0.89-1.73**	**0.195**	**1.36**	**0.87-2.12**	**0.176**
Water available to clean self after defecating	1.00	0.68-1.49	0.986	0.89	0.51-1.56	0.690
Household toilet is shared with another household	**1.49**	**0.92-2.44**	**0.110**	**1.87**	**1.01-3.44**	**0.046**
Child waste disposed of hygienically	0.98	0.65-1.50	0.938	1.19	0.74-1.92	0.475
Household garbage disposed of in bush	0.98	0.77-1.25	0.880	0.95	0.72-1.25	0.699
Household garbage disposed of by digging/burying	**1.43**	**1.03-1.99**	**0.032**	1.17	0.80-1.72	0.423
Household garbage disposed of by burning	0.92	0.72-1.16	0.457	0.99	0.77-1.30	0.995
Household water variables						
Household main water source[c]						
Tubewell/borehole	**4.54**	**1.76-11.65**	**0.002**	**0.28**	**0.11-0.72**	**0.008**
Unprotected spring/dugwell	1.20	0.76-1.91	0.481	0.78	0.49-1.22	0.274
Protected spring	1.78	0.89-3.61	0.104	**0.29**	**0.10-0.79**	**0.016**
Surface water	1.32	0.66-2.68	0.425	0.67	0.35-1.28	0.228
Distance to water source is more than 15 minutes	**0.79**	**0.57-1.09**	**0.157**	**0.78**	**0.55-1.13**	**0.191**
Water always available from main water source	1.18	0.85-1.62	0.321	1.01	0.69-1.48	0.946
Household water stored in only covered containers	0.96	0.67-1.39	0.832	**0.65**	**0.42-0.99**	**0.046**
Household water treated	**1.22**	**0.96-1.55**	**0.104**	0.95	0.71-1.28	0.754
Household socioeconomic variables						
At least one child under 5 years of age in household	1.10	0.83-1.48	0.478	-	-	-
More than 6 people living in dwelling	**1.44**	**1.07-1.93**	**0.016**	-	-	-
Socioeconomic quintile[d]						
Quintile 2	0.88	0.64-1.21	0.435	-	-	-
Quintile 3	**0.64**	**0.46-0.90**	**0.011**	-	-	-
Quintile 4	0.90	0.61-1.33	0.605	-	-	-
Quintile 5	0.95	0.64-1.43	0.816	-	-	-

Results in bold: Covariates entered in multivariable regression models (p<0.2 in univariable analyses)

Reference categories as follows: [a] Follow-up 1, [b] Age 1-5 years, [c] Never went to school, [d] No employment outside the home, [e] Household water source is piped water, [f] Socioeconomic quintile 1 (poorest)

Supplementary Table S5. Results of univariable analyses for undifferentiated STH infection (*N*=2333), based on mixed-effects multilevel logistic regression models accounting for village, household and individual-level clustering

	Concurrent			Six months previously		
Covariate	**Odds ratio**	**95% CI**	***p* value**	**Odds ratio**	**95% CI**	***p* value**
General variables						
Age group[a]						
6-11 years	**1.99**	**1.47-2.71**	**<0.001**	-	-	-
12-17 years	**2.60**	**1.77-3.81**	**<0.001**	-	-	-
18-64 years	**2.64**	**1.99-3.52**	**<0.001**	-	-	-
65+ years	**1.73**	**1.12-2.66**	**<0.001**	-	-	-
Male sex	**2.31**	**1.89-2.81**	**<0.001**	-	-	-
Individual hygiene variables						
Washes hands using soap or ash	**1.20**	**0.95-1.51**	**0.120**	0.87	0.68-1.10	0.244
Washes hands before contact with food	**0.76**	**0.64-0.90**	**0.002**	0.89	0.73-1.09	0.271
Washes hands after contact with faeces	**1.38**	**1.12-1.69**	**0.002**	0.88	0.69-1.10	0.259
Washes hands after contact with dirt	**1.28**	**1.06-1.53**	**0.009**	**1.27**	**1.03-1.57**	**0.023**
Always wears shoes indoors	0.94	0.78-1.13	0.493	1.03	0.82-1.29	0.806
Always wears shoes outdoors and while toileting	**1.14**	**0.96-1.36**	**0.131**	1.06	0.82-1.38	0.634
Individual sanitation variables						
Main place of defecation is toilet	1.13	0.90-1.42	0.281	**1.38**	**1.06-1.79**	**0.014**
Practises open defecation	**0.80**	**0.64-0.99**	**0.041**	**0.76**	**0.58-0.98**	**0.032**
Uses water to clean self after defecation	1.06	0.86-1.32	0.573	1.03	0.80-1.32	0.845
School sanitation variables (children age 6-17 years only)						
Uses toilet at school	1.26	0.85-1.87	0.247	**0.69**	**0.43-1.10**	**0.114**
Individual socioeconomic variables						
Education level *(adults age 18+ years only)*[b]						
Didn't finish primary school	1.09	0.76-1.56	0.650	-	-	-
Finished primary but not secondary school	1.07	0.76-1.51	0.688	-	-	-
Finished secondary school or higher	**0.53**	**0.32-0.87**	**0.012**	-	-	-
Employment *(adults age 18+ years only)*[c]						
Employed – farmer	**2.24**	**1.70-2.94**	**<0.001**	-	-	-
Employed – other job	**1.92**	**1.14-3.24**	**0.015**	-	-	-
Household sanitation variables						
Household has toilet	1.07	0.85-1.35	0.561	**1.36**	**1.06-1.76**	**0.017**

Household toilet has slab	**1.33**	**1.01-1.77**	**0.044**	1.16	0.81-1.68	0.413
Household toilet is pour-flush latrine	**1.33**	**0.97-1.81**	**0.076**	1.07	0.72-1.58	0.754
Household toilet observed to be clean	**1.34**	**1.00-1.79**	**0.048**	1.27	0.87-1.86	0.211
Water available to clean self after defecating	**1.26**	**0.92-1.74**	**0.151**	1.07	0.70-1.64	0.746
Household toilet is shared with another household	0.91	0.58-1.42	0.677	0.87	0.52-1.45	0.599
Child waste disposed of hygienically	0.81	0.55-1.17	0.260	**0.74**	**0.48-1.13**	**0.160**
Household garbage disposed of in bush	1.00	0.83-1.20	0.994	1.14	0.92-1.40	0.231
Household garbage disposed of by digging/burying	1.18	0.79-1.77	0.420	1.17	0.84-1.63	0.365
Household garbage disposed of by burning	0.97	0.81-1.16	0.746	**0.86**	**0.71-1.06**	**0.157**
Household water variables						
Household main water source[d]						
Tubewell/borehole	**2.51**	**1.32-4.77**	**0.005**	**0.57**	**0.32-1.01**	**0.053**
Unprotected spring/dugwell	0.99	0.73-1.36	0.974	0.93	0.69-1.28	0.640
Protected spring	**1.72**	**0.87-3.38**	**0.128**	0.58	0.27-1.24	0.160
Surface water	1.05	0.67-1.66	0.828	0.86	0.55-1.34	0.502
Distance to water source is more than 15 minutes	**0.77**	**0.62-0.96**	**0.018**	0.99	0.78-1.26	0.959
Water always available from main water source	1.08	0.84-1.38	0.565	**0.83**	**0.63-1.09**	**0.186**
Household water stored in only covered containers	0.83	0.58-1.19	0.315	0.91	0.64-1.30	0.612
Household water treated	1.11	0.93-1.34	0.247	0.87	0.70-1.08	0.209
Household socioeconomic variables						
At least one child under 5 years of age in household	**0.74**	**0.58-0.93**	**0.011**	-	-	-
More than 6 people living in dwelling	0.93	0.72-1.20	0.577	-	-	-
Socioeconomic quintile[e]						
Quintile 2	1.03	0.78-1.36	0.823	-	-	-
Quintile 3	0.87	0.66-1.15	0.344	-	-	-
Quintile 4	0.99	0.73-1.34	0.951	-	-	-
Quintile 5 (richest)	1.00	0.73-1.37	0.995	-	-	-

Results in bold: Covariates entered in multivariable regression models (p<0.2 in univariable analyses)

Reference categories as follows: [a] Follow-up 1, [b] Age 1-5 years, [c] Never went to school, [d] No employment outside the home, [e] Household water source is piped water, [f] Socioeconomic quintile 1 (poorest)

Appendix 7
Supplementary material for Paper 7

The following information was published as an online supplement to Paper 7:

Clarke NE, Llewellyn S, Traub RJ, McCarthy JS, Richardson A, Nery SV. Quantitative polymerase chain reaction for diagnosis of soil-transmitted helminth infections: a comparison with a flotation-based technique and an investigation of variability in DNA detection. *Am J Trop Med Hyg* 2018; 99(4): 1033–1040. http://doi.org/10.4269/ajtmh.18-0356

The material formed part of the manuscript submission and was subjected to peer review.

Supplementary Table 1. Primers and probes used in qPCR

Target	Oligonucletide Sequence 5'—3'		Product size	Gene target	Final conc. nM	GenBank accession #	Source*
Ascaris spp.	Forward	GTAATAGCAGTCGGCGGTTTCTT	88bp	ITS1	60	AB571301.1	Ref 1
	Reverse	GCCCAACATGCCACCTATTC			60		
	Probe	ROX—TTGGCGGACAATTGCATGCGAT—IBRQ			100		
Necator americanus	Forward	CTGTTTGTCGAACGGTACTTGC	101bp	ITS2	200	AJ001599.1	Ref 2
	Reverse	ATAACAGCGTGCACATGTTGC			200		
	Probe	FAM—CTGTACTACGCATTGTATAC—MGBNFQ			100		
Ancylostoma spp.	Forward	GAATGACAGCAAACTCGTTGTTG	71bp	ITS1	100	EU344797.1	Ref 2
	Reverse	ATACTAGCCACTGCCGAAACGT			100		
	Probe	VIC—ATCGTTTACCGACTTTAG—MGBNFQ			100		
Trichuris spp.	Forward	TCCGAACGGCGGATCA	56bp	ITS1	60	FM991956.1	Ref 3
	Reverse	CTCGAGTGTCACGTCGTCCTT			60		
	Probe	CY5.5—TTGGCTCGTAGGTCGTT- BHQ-2			100		
Equine herpesvirus	Forward	GATGACACTAGCGACTTCGA	81bp	gB	40	M26171.1	Ref 4
	Reverse	CAGGGCAGAAACCATAGACA			40		
	Probe	CY5/FAM—TTTCGCGTGCCTCCTCCAG—IBRQ/IBFQ			100		

* References as follows:

1. Wiria A, et al., 2010. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). *BMC Infect Dis 10*, 77.
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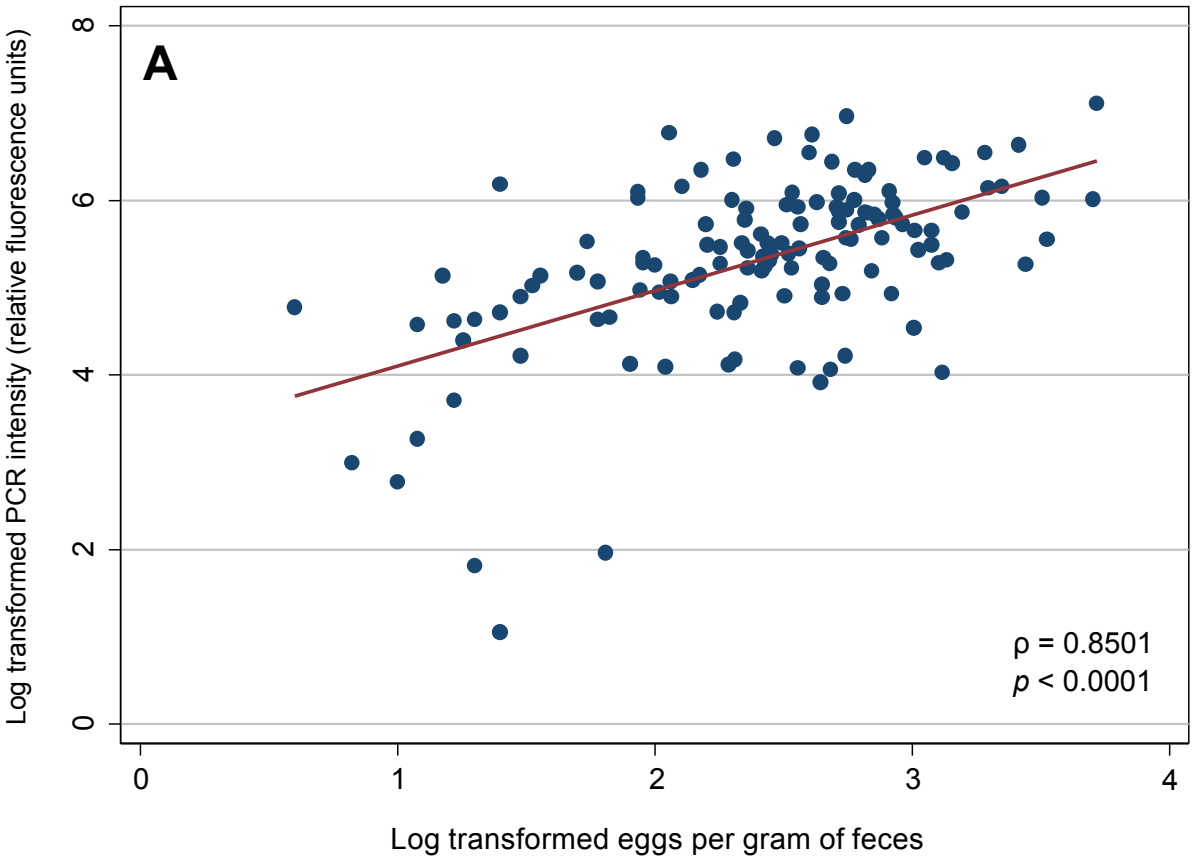
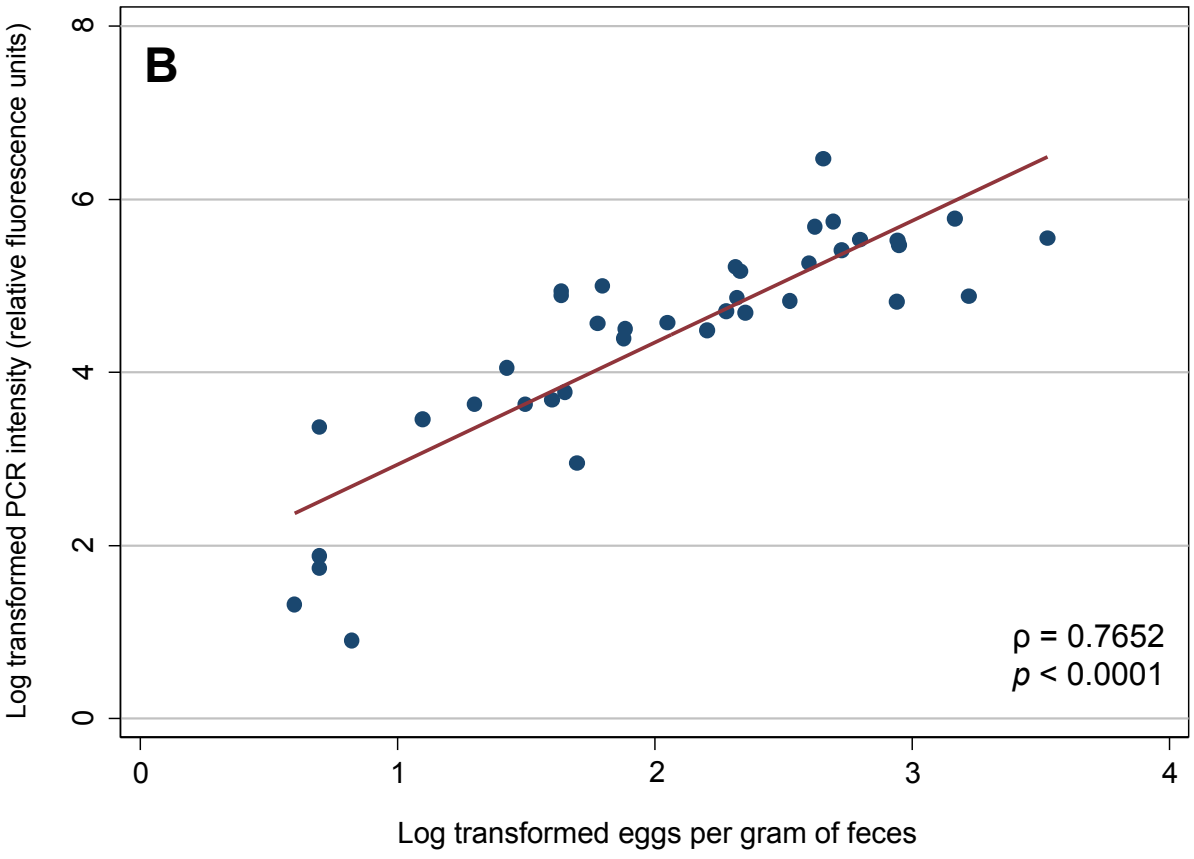
3. Mejia R, Vicuña Y, Broncano N, Sandoval C, Vaca M, Chico M, Cooper PJ, Nutman TB, 2013. A novel, multi-parallel, real-time polymerase chain reaction approach for eight gastrointestinal parasites provides improved diagnostic capabilities to resource-limited at-risk populations. *Am J Trop Med Hyg 88*: 1041–1047.
4. Bialasiewicz S, et al, 2009. A novel gel-based method for self-collection and ambient temperature postal transport of urine for PCR detection of *Chlamydia trachomatis*. *Sex Transm Infect 85*: 102–105.

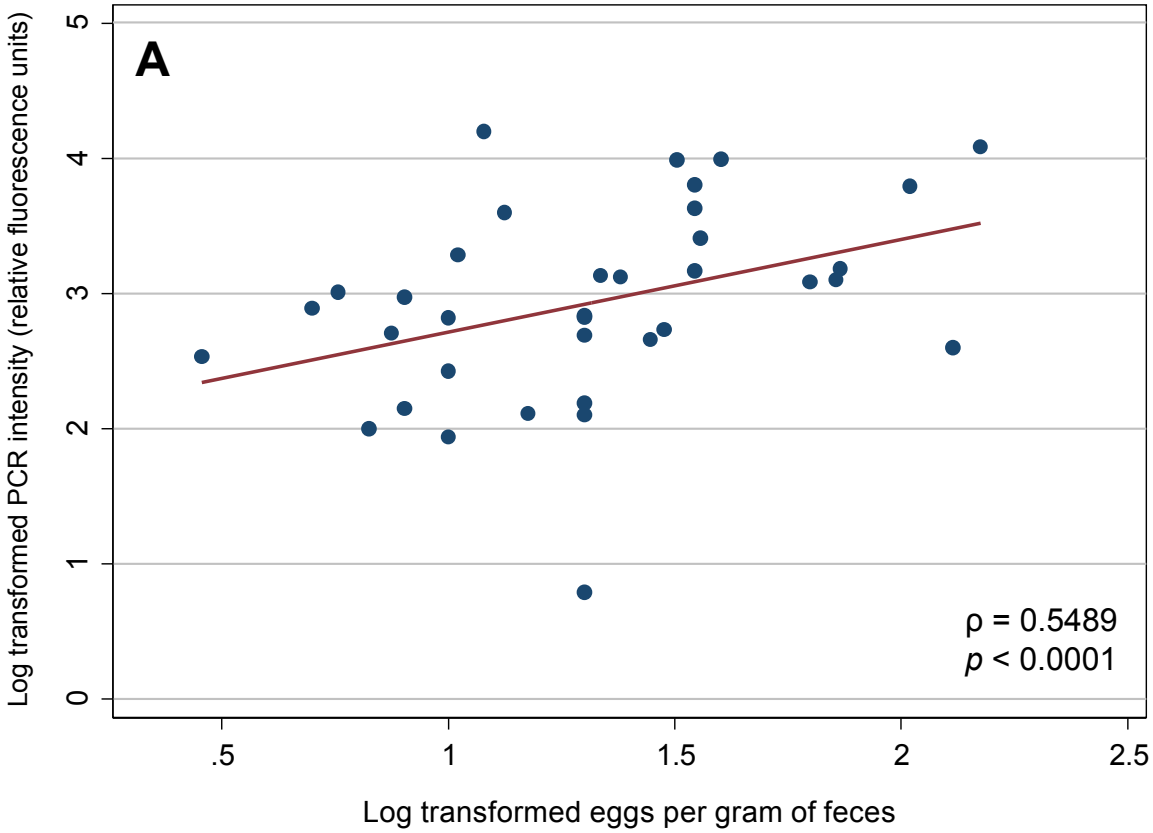
Supplementary Table 2. Quality control results for sodium nitrate flotation demonstrating the agreement between two microscopists

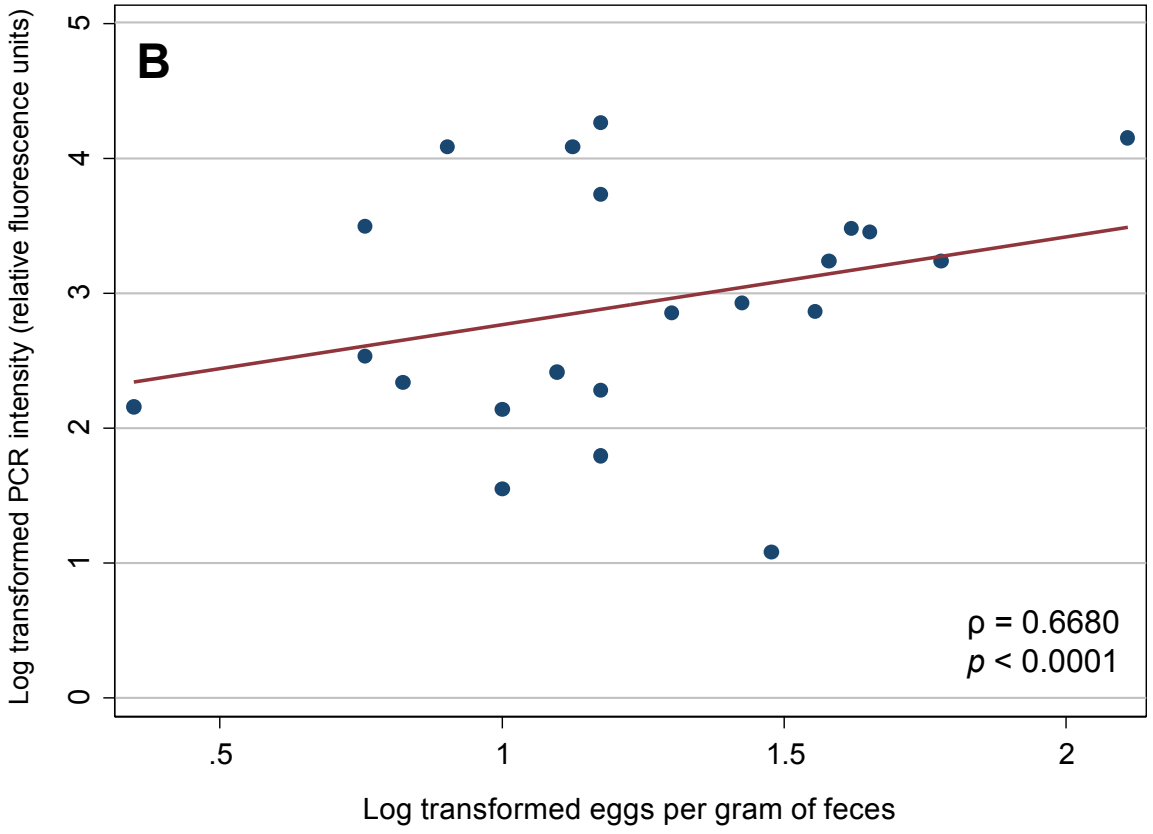
	Agreement	**Kappa statistic**	***p* value**
Ascaris lumbricoides	96.6%	0.8828	<0.001
Hookworm	94.4%	0.5868	<0.001
Trichuris trichiura	97.8%	0.7886	<0.001





Supplementary Figure 1. Scatter plots showing the relationship between infection intensity measured by sodium nitrate flotation (eggs per gram of feces) and qPCR (reactive fluorescence units) upon universal $\log_{10}$ transformation, for *Ascaris* spp. at baseline (panel A) and follow-up (panel B).





Supplementary Figure 2. Scatter plots showing the relationship between infection intensity measured by sodium nitrate flotation (eggs per gram of feces) and qPCR (reactive fluorescence units) upon universal $\log_{10}$ transformation, for hookworm at baseline (panel A) and follow-up (panel B).

Appendix 8
Additional publication (letter)

This appendix contains an author's reply in response to a letter to the editor regarding Paper 2.

Clarke NE, Doi SA, Clements AC, Gray D, Campbell S, Wang D, Nery SV. The expansion of soil-transmitted helminth control strategies – Authors' reply. *Lancet* 2017; 389(10085): 2191. http://doi.org/10.1016/S0140-6736(17)31343-0

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Authors' reply

We thank Gang Qin and Xun Zhuang for their letter regarding our systematic review and meta-analysis published in *The Lancet*.[1]

Qin and Zhuang raise concerns about the heterogeneity of the included studies, reflecting low study quality. However, the heterogeneity in our meta-analysis in terms of prevalence reduction cannot be solely attributed to low study quality. The effect of soil-transmitted helminth (STH) control programmes varies significantly, because of a variety of factors, including environmental conditions, water, sanitation, and hygiene, and underlying transmission intensity.[2]

We disagree that our results show an opposite effect of baseline prevalence for *Ascaris lumbricoides* and hookworm. Our results suggest that baseline prevalence has no effect on *A lumbricoides* prevalence reduction (OR 2·7, 95% CI 0·03–239·7); whereas for hookworm, higher baseline prevalence results in lower odds of prevalence reduction (0·07, 0·01–0·77). Again, these findings are not necessarily attributable to low study quality, given known differences between the parasites in terms of environmental resilience, drug efficacy, and reinfection rates.

Qin and Zhuang are also concerned about the inclusion of studies with low treatment coverage. Many studies did not report treatment coverage, and of the 34 studies that did, 26 (76·5%) reported coverage greater than the WHO target of 75%. The remaining eight studies had coverage between 29·3% and 72·7%, and were all studies of mass drug administration. This would be expected to bias results comparing mass and targeted approaches towards the null.

We agree that cluster-randomised control trials are needed. We have conducted a pilot trial,[3] and several large-scale trials are currently underway, such as the TUMIKIA trials in Kenya.

We also agree that drug resistance is an important concern, potentially more so when school-based control programmes are expanded to be community wide. Mathematical modelling can be used to investigate these issues in different transmission scenarios, and help guide discussions around optimal approaches. Strategies to decrease selective pressure on resistant phenotypes include drug combinations and alternative anthelmintics, which are important research priorities.[4] Although mass drug administration might eventually interrupt STH transmission,[5] the risk of benzimidazole resistance emerging before this is achieved must be carefully considered.

Finally, the examples presented of benzimidazole toxicity occurred after several weeks of two or three times daily treatment. In the high doses used to treat hydatid disease, the potential for severe toxicity, including hepatic dysfunction and bone marrow suppression, is recognised. However, when used for STH control, in single doses at intervals of 6–12 months, albendazole and mebendazole have been repeatedly shown to be extremely safe, with only transient mild gastrointestinal symptoms occurring in around 1% of treated individuals.[6]

We declare no competing interests.

Naomi E Clarke, Suhail A Doi, Archie C Clements, Darren Gray, Suzy Campbell, Dongxu Wang, Susana V Nery
naomi.clarke@anu.edu.au

Research School of Population Health, Australian National University, Acton, ACT 2601, Australia

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For more on the **TUMIKIA Project** see http://www.thiswormyworld.org/tumikia-project

Panos

Appendix 9
Additional publication (opinion piece)

This appendix contains an opinion piece written for "The Conversation" website.

Clarke NE, Nery SV. A new approach for controlling intestinal worm infections could help millions of the world's most vulnerable people. 2016. Available at: http://theconversation.com/a-new-approach-for-controlling-intestinal-worm-infections-could-help-millions-of-the-worlds-most-vulnerable-people-70418.

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THE CONVERSATION
Academic rigour, journalistic flair

Children living in areas with poor sanitation and hygiene account for 60% of people around the world infected with intestinal worms. Marcos Brindicci/Reuters

Authors

Naomi Clarke
PhD candidate in Global Health, Australian National University

Susana Vaz Nery
Research Fellow - Global Health, Australian National University

Expanding the control strategy for intestinal worms to treating adults as well as children could improve the health of millions of people worldwide who are infected or reinfected by these parasites every year.

These intestinal worms – soil-transmitted helminths – are responsible for the most common parasitic disease of humans worldwide. A staggering 1.45 billion people – that's nearly a fifth of the global population – are affected and at risk of the long-term consequences of this largely preventable infection.

Neglected diseases

Soil-transmitted helminthiasis is one of 17 "neglected tropical diseases", a grouping that also includes dengue and chikungunya, rabies, and leprosy. These infectious diseases largely affect the world's most impoverished people, causing a high human and economic toll through chronic disability.

As their name suggests, they have historically received little global interest or research funding when compared to the "big three" diseases on the global health agenda: HIV/AIDS, tuberculosis and malaria.

The good news is that neglected tropical diseases have been rising to prominence since the 2012 London Declaration on Neglected Tropical Diseases. This large public-private partnership is committed to eliminating or controlling ten preventable neglected tropical diseases by 2020, and has attracted substantial investment from government and philanthropic sources.

It's also included unprecedented drug donations from large pharmaceutical companies to combat the five neglected tropical diseases that can be controlled or eliminated with eradication: trachoma, onchocerciasis (river blindness), lymphatic filariasis, schistosomiasis, and soil-transmitted helminthiasis.

Soil-transmitted helminthiasis is by far the most prevalent of all 17 neglected tropical diseases. Transmitted through the accidental ingestion of worm eggs that are released in the faeces of people who are already infected, they thrive in areas with poor sanitation and hygiene, and are endemic across Africa, Southeast Asia, and the Pacific.

Children suffer disproportionately from the consequences of these infections. Due to the nutrient malabsorption and chronic blood loss that infection causes, children with heavy worm infestations can suffer developmental setbacks and fail to reach their full physical and intellectual capacity. This perpetuates the cycle of poverty in which they and their families are entrenched.

As a result of frequent exposure to contaminated environments, over 876 million childrenare currently at risk of infection from these intestinal worms.

Current control efforts

The key public health intervention for controlling soil-transmitted helminthiasis is the large-scale distribution of anthelmintic medication – often referred to as "deworming". This must be repeated regularly as people don't develop long-lasting immunity to intestinal worms, and can soon be reinfected if their environment remains contaminated.

Children are the primary focus of global control efforts for intestinal worms because of the greater impact the disease has on them. World Health Organization guidelines have focused predominantly on deworming school-aged children (five to 14-year-olds), with the goal of preventing complications associated with heavy infections.

WHO guidelines have focused on deworming school-aged children. Noor Khamis/Reuters

Deworming through schools is an efficient and low-cost approach. The drugs are easy to administer and side effects are rare, so children can be treated by their teachers, minimising the costs of both infrastructure and personnel.

Between 2008 and 2013, the number of children treated for intestinal worms globally nearly doubled, and over half a billion children were treated in 2015.

This is astounding progress, and a testament to what can be achieved with concerted, collaborative effort. But it doesn't prevent reinfection and relies on regularly re-administering medication.

A better approach?

The last few years have seen burgeoning interest from researchers in the idea of expanding soil-transmitted helminthiasis control programs beyond school-based deworming.

This interest has centred mainly on the idea that treating all community members, rather than only children, could lead, over time, to "transmission interruption" – elimination of all worms would mean regular deworming is no longer required. This suggestion has been supported by several mathematical modelling studies.

Research that my colleagues and I recently published shows expanded deworming programs may also have direct and, more significantly, immediate benefits for children.

We undertook an analysis of the results from dozens of previous studies of intestinal worm control programs, delivered either to children alone or to whole communities. What we found was that when whole communities are given deworming medication, children are less likely to be reinfected, than when only children are treated in the first instance.

The findings make sense. Expanded deworming programs will reduce the number of people excreting worm eggs into the environment, thereby reducing exposure and infection. But until now, robust evidence to support this idea has been lacking.

We can now be confident that expanding control programs to whole communities will result in children having fewer infections. Although current child-focused efforts are lowering the number of infections and reducing complications, the growing body of evidence for expanding deworming compels us to revisit our current approach.

But community-wide treatment is far from a quick fix. It would require a significant increase in drug donations and other resources. And complicating factors, such as the increased potential for drug resistance, need to be carefully considered. But, as a global community, we must ensure that we are doing our best to promote the health and well-being of vulnerable populations.

Neglected tropical diseases afflict some of the most world's most vulnerable people, and we must maintain the momentum of recent times in controlling these diseases. There's a growing body of evidence that shows we could be doing more for the close to billion children at risk of intestinal worms. We simply cannot afford to ignore it.

Appendix 10
Additional publication (peer-reviewed manuscript)

This appendix consists of a published paper that examines the efficacy of a single dose of albendazole on STH infections in Timor-Leste. This efficacy study was performed as a sub-study of the WASH for WORMS trial, following the first dose of albendazole that was administered after study baseline.

Nery SV, Qi J, Llewellyn S, **Clarke NE**, Traub R, Gray DJ, Vallely AJ, Williams GM, Andrews RM, McCarthy JS, Clements ACA. Use of quantitative PCR to assess the efficacy of albendazole against *Necator americanus* and *Ascaris* spp. in Manufahi District, Timor-Leste. *Parasit Vectors* 2018; 11: 373. http://doi.org/10.1186/s13071-018-2838-0

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Vaz Nery et al. Parasites & Vectors (2018) 11:373
https://doi.org/10.1186/s13071-018-2838-0

Parasites & Vectors

RESEARCH Open Access

Use of quantitative PCR to assess the efficacy of albendazole against *Necator americanus* and *Ascaris* spp. in Manufahi District, Timor-Leste

Susana Vaz Nery[1,6*], Jessica Qi[2], Stacey Llewellyn[3], Naomi E. Clarke[1], Rebecca Traub[4], Darren J. Gray[1], Andrew J. Vallely[5], Gail M. Williams[7], Ross M. Andrews[8], James S. McCarthy[3] and Archie C. A. Clements[1]

Abstract

Background: Soil-transmitted helminths (STHs) including *Ascaris lumbricoides*, *Necator americanus*, *Ancylostoma* spp. and *Trichuris trichiura* are cause of significant global morbidity. To mitigate their disease burden, at-risk groups in endemic regions receive periodic mass drug administration using anthelmintics, most commonly albendazole and mebendazole. Assessing the efficacy of anthelmintic drugs is important for confirming that these regimens are working effectively and that drug resistance has not emerged. In this study we aimed to characterise the therapeutic efficacy of albendazole against *Ascaris* spp. and *N. americanus* in Timor-Leste, using a quantitative polymerase chain reaction (qPCR) method for parasite detection and quantification.

Results: A total of 314 participants from 8 communities in Timor-Leste provided stool samples before and 10–14 days after the administration of a single 400 mg dose of albendazole. Helminth infection status and infection intensity (measured in Ct-values and relative fluorescence units) were determined using qPCR. Efficacy was determined by examining the cure rates and infection intensity reduction rates. Albendazole was found to be highly efficacious against *Ascaris* spp., with a cure rate of 91.4% (95% CI: 85.9–95.2%) and infection intensity reduction rate of 95.6% (95% CI: 88.3–100%). The drug was less efficacious against *N. americanus* with a cure rate of 58.3% (95% CI: 51.4–64.9%) and infection intensity reduction rate of 88.9% (95% CI: 84.0–97.0%).

Conclusions: The observed cure rates and infection intensity reduction rates obtained for *Ascaris* spp. and to a lower extent *N. americanus*, demonstrate the continued efficacy of albendazole against these species and its utility as a mass chemotherapy agent in Timor-Leste. Furthermore, this study demonstrates the usefulness of qPCR as a method to measure the efficacy of anthelminthic drugs. Additional research is necessary to translate Ct-values into eggs per gram in a systematic way.

Trial registration: Australian and New Zealand Clinical Trials Registry 12614000680662 (registered 27 June 2014).

Keywords: Albendazole, Efficacy, *Necator americanus*, *Ascaris lumbricoides*, Hookworm, Soil-transmitted helminths, Anthelminthic drug efficacy

* Correspondence: snery@kirby.unsw.edu.au
[1]Research School of Population Health, Australian National University, 62 Mills Rd, Canberra 2601, ACT, Australia
[6]Present Address: Kirby Institute, University of New South Wales, Wallace Wurth Building, Sydney 2052, NSW, Australia
Full list of author information is available at the end of the article

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Background

More than 1.4 billion people worldwide are estimated to suffer from infection with soil-transmitted helminths (STHs) [1]. The infective stages of these parasites thrive in the warm moist soils of tropical regions, and are transmitted through oral ingestion or skin penetration [2]. These modes of transmission mean that the most affected individuals are from poor communities that lack the adequate water, hygiene and sanitation necessary to prevent transmission and reinfection. *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *Necator americanus* and *Ancylostoma* spp. (hookworms) are the most common STHs. They cause significant morbidity, particularly in children, who are commonly co-infected with multiple species [2]. Chronic infection can retard child growth and development, and infected individuals can suffer malnutrition, growth stunting and reduced cognitive abilities and intellectual capacity [2, 3]. Studies have indicated the significant adverse impact of STH infection on school attendance and performance and future economic productivity, although the health impact of STH infection is being debated [4–6].

The World Health Organization (WHO) has set goals to reduce STH-associated morbidity in children to a level at which it is no longer considered a public health problem [7]. To achieve this, populations at risk in endemic areas, mainly school-age children, are targeted with mass chemotherapy using anthelmintic drugs at either six monthly or yearly intervals depending on infection prevalence [8, 9]. The current recommended drugs are the benzimidazole drugs, albendazole and mebendazole [8], which are highly efficacious against *A. lumbricoides* with a recent meta-analysis indicating pooled cure rates of 95.7 and 96.2%, respectively, and egg reduction rates of 98.5 and 98.0%, respectively [10]. Albendazole is also efficacious against hookworm, with a pooled cure rate of 79.5% and egg reduction rate of 89.6%, compared to a cure rate of 32.5% and egg reduction rate of 61.0% for mebendazole [10]. Both drugs have poor efficacy against *T. trichiura*, with pooled cure rates of 30.7 and 42.1% for albendazole and mebendazole, respectively [10]. The WHO, international partners and pharmaceutical companies are committed to scaling up mass drug administration so that by 2020, 75% of at-risk children are being dewormed [7]. In 2016 alone, over 470 million schoolchildren were treated with anthelmintic drugs in endemic countries, corresponding to 69.5% of children at risk [11].

Greater usage of albendazole entails greater selection pressure of the drug for resistant parasite strains. Therefore, with the scaling up of mass drug administration programs, there are growing concerns over the potential for drug resistance to emerge in humans, similar to what has happened in other animals. In livestock, resistance to benzimidazoles is widespread, having emerged from the large-scale use of the drugs [12–14]. Monitoring the efficacy of anthelmintic drugs in order to detect the potential emergence of resistance in human populations is imperative to ensure that mitigation strategies can be promptly implemented to preserve the effectiveness of mass deworming campaigns [15]. STH are generally diagnosed using microscopy-based methods - most commonly the Kato-Katz method - to detect helminth eggs in stool. However, this method is known to have low sensitivity in lower-intensity and lower transmission settings, and requires examination of multiple samples to improve sensitivity [16]. Recently, quantitative polymerase chain reaction (qPCR)-based methods have been developed for the diagnosis and quantification of STH and validated as more sensitive than the conventional microscopy approaches [17–21].

The aim of this study was to determine the efficacy of a single dose of albendazole against STH infections using quantitative polymerase chain reaction (qPCR) for the detection and quantification of *Ascaris* spp., *N. americanus*, *Ancylostoma* spp. and *T. trichiura*, in the context of the implementation of the WASH for WORMS study, a cluster randomised controlled trial (RCT) in rural communities in Timor-Leste [17, 22]. Timor-Leste is a lower middle income country [23], where malnutrition and infectious diseases (such as pneumonia, diarrhea, malaria, tuberculosis and dengue) remain significant health problems [24]. The WASH for WORMS RCT which included community distribution of albendazole every 6 months for 2 years, at a time when no regular mass deworming was being implemented in the country [22]. The previous "*Lumbriga... Mak Lae Duni*" (Worms, no way!) mass drug administration program was implemented from 2005 to 2008 and was resumed in 2015. To our knowledge, this is the first albendazole efficacy study to be conducted in Timor-Leste and the first efficacy study to use qPCR for the calculation of cure rates and infection intensity reduction rates.

Methods

Study setting and data collection

This efficacy study was conducted from January 2012 to March 2013, in 8 communities in Manufahi municipality of Timor-Leste, which had been enrolled in the WASH for WORMS cluster RCT [22]. All community members were eligible for participation in the efficacy study, excluding women in the first trimester of pregnancy and children under 12 months of age. Baseline stool samples were collected for assessment of infection status and intensity, and individuals were subsequently given a single 400 mg dose of albendazole. Drug distribution was done by trained field workers. Children aged under 2 years were given half the dose. Between 10 and 14 days later, a

second stool sample was collected to again determine infection status and intensity.

Sample size was calculated based on the following current recommendations [25]. Tree-based methods indicated that a minimum of 200 subjects (independent of infection status) is recommended to be able to detect a normal *vs* reduced efficacy based on faecal egg count reduction (FECR) [25]. Furthermore, WHO recommends a sample of 50 positive individuals for each parasite tested [26]. To achieve the necessary sample size and considering a compliance rate of 0.75 at each stool collection time point and estimated prevalences of 30% for *Ascaris* spp. and 50% for hookworm (based on studies in neighbouring Indonesia), we enrolled the first 8 of the 24 communities participating in the WASH for WORMS trial into the efficacy study, corresponding to approximately 500 eligible participants [27–29].

Assessment of STH infection

Once collected, the stool samples were preserved at room temperature in 5% (weight/volume) potassium dichromate and transported to the QIMR Berghofer Medical Research Institute in Brisbane, Australia. The presence and intensity of protozoa and STH infection in stool samples was determined using qPCR methods as described previously [17]. In short, DNA extracted from samples that were spiked with a known amount of the plasmid used as positive control was run in a pentaplex real-time PCR reaction for detection and quantification of *Ascaris* spp., *N. americanus*, *Ancylostoma* spp. and *T. trichiura* [17]. The Rotor-Gene 6000 (Qiagen, Melbourne VIC, Australia) was used for all PCR assays [17]. Cycle threshold (Ct) values obtained using qPCR correspond to the amplification cycle at which the detected signal exceeds the background level. For a stool sample to be considered positive for infection, a limit of detection cut-off was set at 31 for *Ascaris* spp. and 35 for *N. americanus*, *Ancylostoma* spp. and *T. trichiura*, to ensure consistency with previously published PCRs [17]. For each qPCR assay, two runs were performed to generate two Ct-values. The arithmetic mean was taken of these two values to produce a single value. For calculation of intensity reduction rates, Ct-values were then converted to infection intensity measured in Relative Fluorescence Units (RFU) based on an assumed 100% reaction run efficiency, provided by the Rotorgene Q software (Infection intensity as determined by $\text{qPCR} = 10^{-0.2980\text{Ct}+9.81}$ RFU) [17]. Samples which did not record a Ct-value were assigned an infection intensity value of 0.

Statistical analysis

Pre- and post-treatment prevalence were compared using Chi-square test, or Fischer's exact test in cases when frequency values were below 5. Only individuals who were positive at the pre-treatment time point were included in calculations of cure rate and infection intensity reduction rate derived from PCR.

Cure rate was calculated using the following formula:

$$\frac{No.\ of\ individuals\ positive\ pre\text{-}treatment\ and\ negative\ post\text{-}treatment}{No.\ of\ individuals\ positive\ pre\text{-}treatment} \times 100$$

95% binomial exact confidence intervals were calculated for both prevalence and cure rate. Age group (1–5 years; 6–11 years; 12–17 years; 18–64 years; and > 65 years) and sex were examined separately for potential associations with the probability of being cured, using the Wald Chi-square test adjusted for community-level clustering. The impact of baseline prevalence and baseline infection intensity (Ct-values) on cure rate was assessed using a multivariate logistic regression model, adjusted for age and sex, with a robust standard error adjusted for clustering at the community level.

Infection intensity reduction rate was calculated using the following formula as per WHO recommendations [25, 26]:

$$\frac{\left(Arithmetic\ mean\ intensity_{pre\text{-}treatment} - Arithmetic\ mean\ intensity_{post\text{-}treatment}\right)}{Arithmetic\ mean\ intensity_{pre\text{-}treatment}} \times 100$$

Confidence intervals for infection intensity reduction rate were calculated using a bootstrap re-sampling method with 10,000 replicates. The impact of baseline infection intensity (Ct-values) on infection intensity reduction rate was assessed using a multivariate linear regression model, adjusted for age and sex, with a robust standard error adjusted for clustering at the community level.

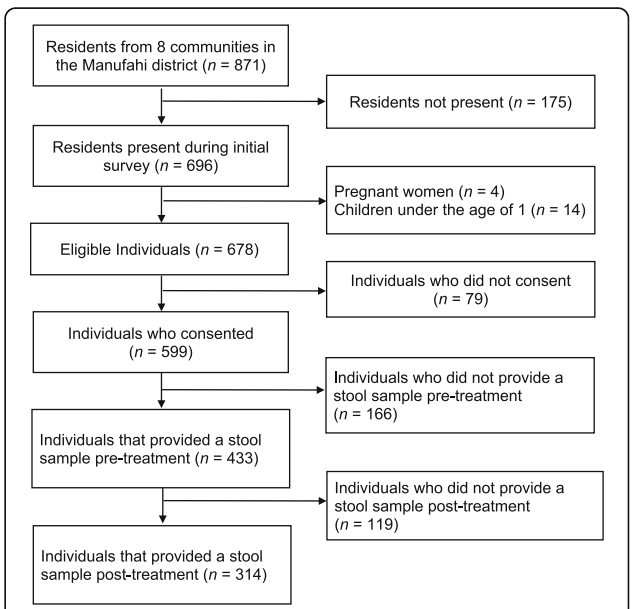

Fig. 1 Efficacy study diagram

Table 1 Cure rates for *N. americanus* and *Ascaris* spp., overall and across sex and age groups

	Necator americanus				*Ascaris* spp.			
	n	Cure rate (%) (95% CI)	χ^2	*P*-value	*n*	Cure rate (%) (95% CI)	χ^2	*P*-value
Overall	218	58.3 (51.4–64.9)			162	91.4 (85.9–95.2)		
Sex			1.77	0.184			2.19	0.139
Male	104	54.8 (44.7–64.6)			74	89.2 (79.8–95.2)		
Female	114	61.4 (51.8–70.4)			88	93.2 (85.7–97.5)		
Age group (years)			1.88	0.758			5.71	0.222
1–5	35	60 (42.1–76.1)			40	92.5 (79.6–98.4)		
6–11	64	62.5 (49.5–74.3)			51	88.2 (76.1–95.6)		
12–17	29	58.6 (38.9–76.5)			22	95.5 (77.2–99.9)		
18–64	75	52 (40.2–63.9)			43	93.0 (80.9–98.5)		
65+	15	66.7 (38.4–88.2)			6	83.3 (35.9–99.6)		

Abbreviations: *n*, number of individuals positive pre-treatment; CI, confidence interval

All analyses were conducted using Stata version 11.0 (College Station, TX, USA). A 5% significance limit was used for all analyses.

Results

Population under study

From the 8 communities enrolled, 678 individuals were present and eligible for study participation, of whom 599 (88.3%) agreed to participate. In total, 314 individuals provided both pre- and post-treatment stool samples and were included in the efficacy analysis presented here (Fig. 1). Participants ranged in age from 1 to 72 years, with a mean of 21 years. Of the study participants 54.8% were females and 45.2% were males.

The most prevalent species was *N. americanus* at 69.4% (95% CI: 64.1–74.2%), followed by *Ascaris* spp. at 51.6% (95% CI: 46.1–57.1%). *Ancylostoma* spp. (2.6%; 95% CI: 1.1–5.0%) and *T. trichuria* (1.3%; 95% CI: 0.3–3.2%) both had a low prevalence in the study population. For *Ascaris* spp., community-level prevalence ranged from 19.3% (95% CI: 10.9–31.8%) to 80.5% (95% CI: 65.3–90.0%), with a mean community-level prevalence of 54.2%. For *N. americanus*, community-level prevalence ranged from 52.5% (95% CI: 40.0–64.5%) to 87.8% (95% CI: 73.6–94.9%), with a mean community-level prevalence of 72.6%.

Albendazole efficacy - cure rates and reduction in intensity

As shown in Table 1, the cure rate for *Ascaris* spp. was 91.4% (95% CI: 85.9–95.2%), and the cure rate for *N. americanus* was 58.3% (95% CI: 51.4–64.9%). The cure rate for *Ancylostoma* spp. was 100% (95% CI: 68.7–100%) and for *T. trichiura* was 50% (95% CI: 67.6–93.4%); due to the low number of individuals infected with *Ancylostoma* spp. and *T. trichiura,* further analyses were not performed for these helminths.

Cure rates stratified by age group and sex are presented in Table 1. There was no significant difference in cure rate between males and females for either *N. americanus* (54.8% *vs* 61.4%, χ^2 = 1.77, *df* = 1, *P* = 0.18) or *Ascaris* spp. (89.2% *vs* 93.2%, χ^2 = 2.19, *df* = 1, *P* = 0.14). Similarly, age was not associated with being cured, with no statistically significant difference between age groups for either *N. americanus* (χ^2 = 1.88, *df* = 4, *P* = 0.76) or *Ascaris* spp. (χ^2 = 5.71, *df* = 4, *P* = 0.22).

For *N. americanus,* community-level baseline prevalence was negatively and significantly associated with cure: for a 1% increase in baseline prevalence, the odds of being cured decreased by 3% (OR = 0.97, 95% CI: 0.94–0.99, *P* = 0.03). Baseline infection intensity (Ct-value) was not associated with cure. For *Ascaris* spp., neither baseline community-level prevalence nor baseline infection intensity (Ct-value) were associated with cure. Age and sex were not associated with cure for either *N. americanus* or *Ascaris* spp. Full results of the multivariable analysis are shown in Table 2.

Table 2 Results of multivariate logistic regression for infection cure

	Odds ratio	95% CI	*P*-value
Necator americanus			
Baseline prevalence in community (%)	**0.97**	**0.94–0.99**	**0.029**
Baseline infection intensity (Ct-value)	1.04	0.94–1.14	0.474
Age (years)	0.99	0.98–1.01	0.463
Female sex	0.76	0.43–1.32	0.327
Ascaris spp.			
Baseline prevalence in community (%)	0.98	0.95–1.02	0.448
Baseline infection intensity (Ct-value)	1.02	0.94–1.12	0.596
Age (years)	1.00	0.97–1.03	0.975
Female sex	0.56	0.18–1.75	0.321

Abbreviation: CI, confidence interval
Bold indicates statistical significance

Table 3 Infection intensity values before treatment, after treatment and reduction in infection intensity

	Pre-intervention mean infection intensity, RFU (95% CI)	Post-intervention mean infection intensity, RFU (95% CI)	Infection intensity reduction rate (%) (95% CI)
N. americanus (*n* = 218)	18,283 (13,251–23,316)	2024 (505–3,543)	88.9 (84.0–97.0)
Ascaris spp. (*n* = 162)	1,517,583 (1,124,742–1,910,424)	67,696 (0–200,926)	95.5 (88.3–100.0)

Abbreviations: *n*, number of individuals positive pre-treatment; RFU, relative fluorescent units

There was a significant decrease in infection intensity (RFU) for both STH species following treatment, with an infection intensity reduction rate for *Ascaris* spp. of 95.6% (95% CI: 88.3–100%) and 88.9% (95% CI: 83.0–97.0%) for *N. americanus* (see Table 3). The distribution of individual infection intensity reduction rates is shown in Table 4. In short, for both species the large majority of infections were cured or had an infection intensity reduction rate higher than 80%. An increase in infection intensity happened in 6.4% of the cases for *N. americanus* and in 1.2% of the cases for *Ascaris* spp.

For *Ascaris* spp., baseline infection intensity (Ct-value) was not associated with infection intensity reduction rate. For *N. americanus*, a higher baseline infection intensity was associated with a higher intensity reduction rate ($P = 0.04$). There was no association between age or sex and infection intensity reduction rate for either species. Full results of the multivariate linear regression model are shown in Table 5.

Discussion

The findings of this efficacy study are consistent with earlier reports indicating that a single 400 mg dose of albendazole is highly efficacious against *Ascaris* spp. and less efficacious against hookworm [30]. While our cure rate for *Ascaris* spp. is comparable to previous reports, our detected cure rate for *N. americanus* was lower [30]. The lower cure rate obtained for *N. americanus* in this study is likely to be due to the higher diagnostic sensitivity of qPCR as compared to microscopy-based techniques that are generally used in efficacy studies, rather than implicating the existence of emerging benzimidazole resistance. That is, the lower sensitivity of microscopy relative to qPCR could mean that previously reported cure rates are over-estimated, as an individual may be misclassified as cured when in fact their faecal egg count was very low and not detected by microscopy. This is particularly true for hookworm, given that allowing the smear to stand for too long can result in the collapse and disappearance of hookworm eggs but not those of *Ascaris* spp. [31]

While cure rate is usually one of the indicators calculated in efficacy studies, it is not the best measure of drug efficacy, as it depends on baseline intensity and is influenced by the sensitivity of the diagnostic technique [32]. Therefore, current WHO guidelines recommend using a measure of intensity reduction - the faecal egg count reduction (FECR) - as the appropriate indicator of efficacy [26]. WHO guidelines stipulate that the FECR rate should exceed 95% in the case of *A. lumbricoides* and 90% in the case of hookworm [26, 33]. Because we employed qPCR as a diagnostic technique, infection intensity reduction rates were calculated based on PCR intensity values. Given that we were unable to also use microscopy methods in these samples we were not able to measure intensity in eggs per gram, hence the main limitation of this study is that our infection intensity reduction rate values are not directly comparable to FECR rates previously reported in the literature. However, given that both these parameters measure the proportional reduction in parasite load within a sample, and in the absence of a microscopic comparator allowing conversion of Ct to eggs per gram, we feel it is appropriate to apply thresholds pertaining to FECR rates to our results, as a first step in the use of qPCR for drug efficacy

Table 4 Distribution of individual infection intensity reduction rates

Infection intensity reduction rate (%)	Number (%) of individuals	
	N. americanus (*n* = 218)	*Ascaris* spp. (*n* = 162)
100 (cured)	127 (58.3)	148 (91.4)
80–99.9	58 (26.6)	10 (6.2)
60–79.9	12 (5.5)	1 (0.6)
40–59.9	4 (1.8)	0
20–39.9	2 (0.9)	1 (0.6)
0–19.9	1 (0.5)	0
Increase in infection intensity	14 (6.4)	2 (1.2)

Table 5 Results of multivariate linear regression for infection intensity reduction rate (%)

Variable	Regression coefficient	95% CI	*P*-value
Necator americanus			
Baseline infection intensity (Ct-value)	**-31.8**	**-61.5– -2.1**	**0.039**
Age (years)	-0.6	-2.2–0.9	0.371
Female sex	38.7	-6.6–84.1	0.083
Ascaris spp.			
Baseline infection intensity (Ct-value)	-0.20	-0.55–0.16	0.238
Age (years)	0.09	-0.30–0.48	0.594
Female sex	-9.90	-28.87–9.08	0.257

Abbreviation: CI, confidence interval
Bold indicates statistical significance

studies. Our infection intensity reduction rates were above reference efficacy thresholds for *Ascaris* spp. and just under the threshold for *N. americanus*, which suggest the continued efficacy of albendazole against these STH infections. In order for qPCR to be used as a quantitative method in efficacy studies where changes in infection intensity are the designated endpoints, additional research directly comparing infection intensity by microscopy and qPCR is needed. This will allow the conversion of Ct-values into eggs per gram, the establishment of appropriate thresholds for infection intensity reduction rates derived from qPCR to determine drug efficacy, and also the identification of qPCR intensity cut-offs corresponding to low, moderate and high intensity infection. In previous work infection intensity was derived by converting Ct-values in eggs per gram, using standard curves generated by qPCR assays undertaken on fresh hookworm and *Ascaris* spp. eggs. These were then used to interpolate eggs per gram from the PCR Ct-values obtained for the field samples [17]. Since this work was done, a number of potential confounding factors have been identified, including DNA extraction methods and stool preservative. A notable example is that we have observed that hookworm eggs preserved in potassium dichromate, as was the case in our field samples, can embryonate with storage, potentially resulting in an overestimation of infection intensity. Additional work is necessary to take into account storage conditions of field samples as was recently reported by Papaiakovou et al. [34]. Besides increased sensitivity, an additional advantage of the use of qPCR is that allows identification of the different hookworm species present in the population under study, which is not possible with microscopy. This will lead to a more refined understanding of albendazole efficacy against specific hookworm species, each of which can cause different levels and types of morbidity, and in the case of *A. ceylanicum* may require a One Health approach to overall control [35].

Conclusion

As the first albendazole efficacy study to be conducted in Timor-Leste, the results of this study confirm the utility of this drug as a chemotherapeutic agent in the region. Furthermore, it demonstrates that qPCR can be effectively used to determine infection intensity reduction rates. In the future, this study will provide a useful point of comparison to establish whether there is any emerging resistance to albendazole in the context of mass chemotherapy campaigns that have recently restarted in Timor-Leste.

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Availability of data and materials

The data supporting the conclusions of this study are provided within the article. The datasets used and analysed during the current study are available from the corresponding author upon request.

Authors' contributions

SVN was an investigator of this study, responsible for revising and implementing the study protocol, data collection and entry. ACAC was the principle investigator of this study. ACAC, JQ, NEC and SVN designed the analysis. JQ and NEC conducted data analysis, with statistical advice from SVN and ACAC. JQ drafted an initial version of this manuscript. RT, JSM, RMA, DJG, AJV and GMW were study investigators who participated in designing the study. SVN and NEC conducted data cleaning and management of data. SL analyzed qPCR specimens under supervision of JSM and RT. All the authors contributed to editing and revising the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Human Research Ethics Committees at: The University of Queensland (2011000734), Australian National University (2014/311) and the Timorese Ministry of Health (2011/51). Written consent was obtained from all participants aged 18 years or older, and from parents or guardians for those younger than 18 years. Participants aged 12–17 years provided written assent. Consenting individuals were given a single 400 mg dose of albendazole after stool collection. Drug distribution was done by trained field workers. Children aged under 2 years were given half the dose.

Competing interests

The authors declare that they have no competing interests.

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Author details

[1]Research School of Population Health, Australian National University, 62 Mills Rd, Canberra 2601, ACT, Australia. [2]Medical School, Australian National University, Building 42, Canberra, ACT 2601, Australia. [3]Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, 300 Herston Rd, Brisbane QLD 4006, Australia. [4]Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria 3052, Australia. [5]Kirby Institute, University of New South Wales, Wallace Wurth Building, Sydney 2052, NSW, Australia. [6]Present Address: Kirby Institute, University of New South Wales, Wallace Wurth Building, Sydney 2052, NSW, Australia. [7]School of Public Health, The University of Queensland, Corner of Herston Road and Wyndham Street, Brisbane QLD 4006, Australia. [8]Menzies School of Health Research, Charles Darwin University, PO Box 41096, Casuarina NT 0811, Australia.

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Appendix 11
Additional publication (peer-reviewed manuscript)

This appendix consists of a published paper that examines biomarkers for environmental enteropathy and associated risk factors among children in Timor-Leste. This study was performed as a sub-study of the WASH for WORMS trial and was conducted at the final study follow-up in children aged five years and younger.

Nery SV, Bennett I, **Clarke NE**, Lin A, Rahman Z, Rahman M, Clements ACA. Characterisation of environmental enteropathy biomarkers and associated risk factors in children in the context of a WASH trial in Timor-Leste. *Int J Hyg Environ Health* 2018; 221(6): 901–906. http://doi.org/10.1016/j.ijheh.2018.05.012

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Characterisation of environmental enteropathy biomarkers and associated risk factors in children in the context of a WASH trial in Timor-Leste

Susana Vaz Nery[a,*,1], Isaac Bennett[a], Naomi E. Clarke[a], Audrie Lin[b], Ziaur Rahman[c], Mahbubur Rahman[c], Archie C.A. Clements[a,2]

[a] Research School of Population Health, Australian National University, Canberra, Australian Capital Territory, Australia
[b] Division of Epidemiology, School of Public Health, University of California, Berkeley, California, USA
[c] International Centre for Diarrheal Diseases Research, Bangladesh (icddr,b), Mohakhali, Dhaka, Bangladesh

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ABSTRACT

Environmental enteropathy (EE) is characterised by subclinical inflammation and hyperpermeability of the small intestine, hypothesised to be caused by recurrent ingestion of faecal bacteria. It has been suggested that EE may be a contributor to malnutrition and growth delays seen in children living in unsanitary conditions. We measured putative faecal EE markers myeloperoxidase (MPO) (ng/mL) and alpha-1-antitrypsin (AAT) (mg/g) in stool samples collected from 133 children aged 1–5 years in 16 communities enrolled in the WASH for WORMS randomised controlled trial in Timor-Leste. Samples were collected two years after a community-wide water, sanitation and hygiene (WASH) intervention that was integrated with regular deworming. Mixed effects multivariable linear regression models were used to examine the impact of the study intervention and of various WASH and infection-related factors on EE biomarkers. Children who lived in communities that received both the WASH intervention and deworming had similar AAT values as those who lived in communities that received only deworming (regression coefficient -0.14, $p = 0.583$), but they had a trend towards lower MPO values (coeff -0.51, $p = 0.055$). Younger children showed significantly higher MPO levels (coeff: -0.29, $p = 0.002$). No WASH variables or parasitic infections were associated with AAT levels. Household water being stored in covered containers was associated with lower MPO levels (coeff -1.75, $p = 0.046$). We found little evidence that a community-based WASH intervention had an impact on EE over a two-year period.

1. Introduction

1.1. Background

Environmental enteropathy (EE) is a subclinical inflammatory condition of the small intestine whereby the morphology and function of the intestinal barrier is chronically altered. It is characterised by crypt hyperplasia, villous atrophy and lymphocytic infiltration (Cook et al., 1969; Haghighi and Wolf, 1997; Lindenbaum et al., 1966; Menzies et al., 1999). EE has been associated with growth faltering, poor vaccine uptake, gut immune dysfunction and anaemia of inflammation in children from low-income settings without obvious symptoms or diarrhoea (Kosek et al., 2013; Lunn et al., 1991; Ramakrishna et al., 2006; Sullivan et al., 1991).

Although its precise aetiology and pathophysiology are not known, chronic indirect ingestion of faecal bacteria via contaminated food and water has been postulated to cause EE (Prendergast and Kelly, 2012). This suggests a potential association between inadequate water, sanitation and hygiene (WASH) and EE risk, which has been supported by several studies (Exum et al., 2018; George et al., 2016, 2015; Lin et al., 2013; Ngure et al., 2014; Prendergast and Kelly, 2012). Morphological changes to the small intestine have been shown to be reversible when adults were relocated from areas of poor WASH to less contaminated environments (Lindenbaum et al., 1966). To date, there has only been one small, underpowered study assessing the impact of a handwashing intervention on EE (Langford et al., 2011), which found that although the handwashing intervention reduced cases of diarrhoea, there was no improvement in intestinal inflammation or childhood growth.

Other enteric conditions that thrive in poorer regions of world such as soil-transmitted helminth (STH) and neglected enteric protozoal (NEP) infections have also been associated with poor WASH conditions, and often produce symptoms of enteric dysfunction such as diarrhoea,

* Corresponding author. Current address: Kirby Institute, University of New South Wales, Sydney, New South Wales, Australia.
E-mail address: snery@kirby.unsw.edu.au (S. Vaz Nery).
[1] Current affiliation: Kirby Institute, University of New South Wales, Sydney, New South Wales, Australia.
[2] Current address: Faculty of Health Sciences, Curtin University, Western Australia, Australia.

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iron deficiency anaemia and growth faltering (Campbell et al., 2016). EE is almost always seen in areas of high enteric pathogen prevalence (Kelly et al., 2004; Kosek and Investigators, 2017; Prendergast et al., 2015). In light of this, there potentially exist shared risk factors for EE and intestinal parasitic and protozoa infection and infection with these agents may be part of the aetiology of EE (Bartelt et al., 2013).

Measuring EE in children is difficult without impractical and unethical endoscopy procedures (Prendergast et al., 2015; Watanabe and Petri, 2016). The urinary Lactulose-Mannitol test assesses permeability of the small intestine and has been reported as an indirect measure of EE (Kosek et al., 2014). However, a recent systematic review highlighted inconsistencies in the administration, analysis and reporting of the test, often resulting in incomparable measurements across studies (Denno et al., 2014; Keusch et al., 2014). More recently, several faecal biomarkers have been proposed as indirect measures of EE (Kosek et al., 2013). Myeloperoxidase (MPO) is a faecal marker that indicates increased neutrophil activity and inflammation in the intestine (Saiki, 1998). Alpha-1 antitrypsin (AAT) is a biomarker used to detect protein loss in enteropathy, and is a marker of 'nutrient wasting' - a failure to absorb nutrients in the gut caused by hyperpermeability of the small intestine (Sharp, 1976). Measurement of these markers using commercially available, enzyme-linked immunosorbent assays (ELISA), is significantly faster, more affordable and requiring less training and expertise than the Lactulose-Mannitol ratio test (Kosek et al., 2014, 2013). In some contexts these markers have been associated with growth faltering by some authors (George et al., 2015; Lin et al., 2013; Naylor et al., 2015), although recent studies suggest only a weak association between the biomarkers and poor growth (Arndt et al., 2016; Kosek and Investigators, 2017).

1.2. WASH for WORMS

This study of EE biomarkers was performed in the context of the WASH for WORMS trial (Nery et al., 2015). The two-arm cluster randomised controlled trial aimed to assess the impact of a community based WASH intervention, integrated with community deworming, on intestinal parasitic infection (Nery et al., 2018). Twenty-four rural communities of the Manufahi district in Timor-Leste were initially enrolled. Half the communities (intervention arm) received a community-based WASH program focused on improving access to water sources; increasing coverage and use of household sanitation facilities; and promoting appropriate hygiene behaviours focused on promoting handwashing with soap. All intervention and control communities received single dose albendazole every six months for two years. The first deworming round took place two to six months after baseline data collection. For each community in the intervention arm this was done once 80% of households in that community reported having built a latrine.

1.3. Objectives

This study aimed to examine the impact of WASH conditions on EE faecal biomarkers MPO and AAT in children aged between one and five years enrolled in the WASH for WORMS trial. Primary 'intention to treat' analysis aimed to assess the effect of the WASH intervention on EE biomarker levels in children that were dewormed, while secondary analysis explored WASH variables, STH infections, and protozoal infections as potential risk factors for elevated measures of EE in individual children. Finally, we aimed to investigate associations between EE biomarkers and measures of morbidity, including growth indices and anaemia.

2. Material & methods

2.1. Study setting, design and data collection

The WASH for WORMS study collected baseline data between May 2012 and October 2013. Following the WASH intervention, data were collected at four follow-up timepoints, every six months for two years. Data for this study of environmental enteropathy were collected in the first 16 communities enrolled in the WASH for WORMS study, including eight intervention and eight control communities. The majority of data, including the stool samples, used for this study were collected at the final follow-up of the trial, between December 2014 and October 2015. Some WASH survey data from each of the four previous data collection rounds were also used, as described further below in section 2.1 and 2.2.

For measurement of EE biomarkers, single stool samples were collected from children aged over 1 year and below six years (i.e., children aged 1–5 years inclusive). This age group was chosen to reflect a period where physical development is considerable and STH infections start to become prevalent. Samples were frozen on dry ice upon collection in the field and stored without fixative at -20°C until being shipped on dry ice to the International Centre for Diarrhoeal Diseases, Bangladesh (icddr,b). They were analysed for myeloperoxidase [MPO] (ng/mL) and alpha-1 antitrypsin [AAT] (mg/g) using commercial ELISA kits. Samples were diluted 1:500 for myeloperoxidase (Alpco, Salem, NH) and 1:25000 for alpha-1 antitrypsin (BioVendor, Asheville, NC). For STH (*Ascaris* spp., *Necator americanus*) and protozoa (*Giardia duodenalis*) diagnosis, another stool aliquot was fixed in 5% potassium dichromate at room temperature and sent to QIMR Berghofer Medical Research Institute, Brisbane, Australia for analysis by multiplex PCR techniques. Full details of this procedure are published elsewhere (Llewellyn et al., 2016).

Trained field workers conducted detailed WASH surveys that covered a range of WASH and socioeconomic variables at individual and household level. Most of the data were self-reported, but latrines (if present) at household level were inspected by field workers.

Field workers also performed anthropometric measurements on children. For children older than 2 years, weight was measured to the nearest 0.1 kg using electronic scales (CAMRY, ED-301), and height was measured to the nearest 0.1 cm via a portable stadiometer (Wedderburn, WSHRP). Children aged 1–2 years had length measured in a supine position via a measuring mat (Wedderburn, SE210), and weight measured by taring (i.e. with the child held by an adult, and the adult's weight subsequently deducted). Height-for-age (HAZ), weight-for-height (WHZ), BMI-for-age (BMIZ, i.e. weight over height^2-for-age) and weight-for-age (WAZ) z-scores were calculated for all children, and standardised to the international 2006 reference population using the World Health Organization (WHO) Anthro and Anthroplus software package (Campbell et al., 2017a; WHO, 2007, 2015). Children were classified as stunted, underweight, thin or wasted if HAZ, WAZ and BMIZ or WHZ respectively were more than two standard deviations below the reference median of these continuous measurements. Haemoglobin concentration was assessed via a finger-prick blood test using a portable haemoglobinometer (HemoCue, Ängelholm, Sweden); measurements were adjusted for elevation as per WHO guidelines (WHO, 2011). Full details of these procedures are published elsewhere (Campbell et al., 2017a, b; Nery et al., 2015)

2.2. Data analysis

Data were imported into STATA 14 (Stata Corporation, College Station, Texas USA) for statistical analysis. The impact of the intervention on MPO (ng/mL) and AAT (mg/g) levels was assessed using mixed-effects multivariable linear regression, adjusted for age and sex, accounting for clustering at the community level. The MPO and AAT variables were log transformed due to being highly negatively skewed.

Risk factors for increased EE biomarkers were investigated using mixed-effects multivariable linear regression, adjusted for age and sex and accounting for clustering. Risk factors investigated included infection with soil-transmitted helminths and *Giardia duodenalis*, and a large number of WASH variables. Besides WASH variables collected at the last follow-up, three composite WASH variables were created to reflect the cleanliness of a child's environment throughout the course of the WASH for WORMS study and tested in the models. These were: (a) living in an open-defecation free dwelling, (b) main household food preparer washing hands before food preparation, and (c) household drinking water being stored in covered containers. For each of these, binary variables from each of the five timepoints were combined to give composite variable with a value ranging between 0 and 5, with a higher score reflective of a cleaner environment over a longer time period.

Multicollinearity between WASH variables was investigated using the "collin" user-written Stata package. Univariable analysis of infection and WASH variables, including the composite variables described above, was then performed and predictor variables with $p < 0.2$ were retained to be used in a multivariable model for each domain (water, sanitation, hygiene, and enteric infections). These "within-domain" multivariable models were adjusted for age, sex and community level clustering. Variables with $p < 0.1$ from the "within-domain" models were then included in the multivariable model encompassing all domains. The final model was constructed using a sequential backward selection process until only age, sex, and variables with $p < 0.05$ remained.

Finally, the association between EE biomarkers and proxies for malnutrition and anaemia was investigated using mixed-effects logistic and linear regression models, adjusted for age and sex and accounting for clustering at the community level.

2.3. Ethical approval and consent

The WASH for WORMS study protocol, including the EE study, was approved by the University of Queensland (Australia) Human Research Ethics Committee; the Australian National University Human Research Ethics Committee; the Timorese Ministry of Health Research and Ethics Committee; and the University of Melbourne (Australia) Human Research Ethics Committee. Individual written consent was obtained from parents/guardians of children under 18 years after explaining the study aims and procedures to participants (The WASH for WORMS trial is registered in the Australian and New Zealand Clinical Trials Registry ACTRN12614000680662).

3. Results

3.1. Descriptive analysis of key WASH, infection and morbidity variables in the EE cohort

There were 273 eligible children in the 16 participating communities, of which 214 were present for data collection. Of those present, 133 children (62.1%) provided a stool sample for EE biomarkers (75 intervention, 58 control), and of these, there were 124 children that completed the individual WASH questionnaire (via a parent/guardian), 129 children for whom household level WASH information was recorded, 130 children for whom stool samples were analysed for STH and protozoa, 117 children whose height and weight were measured and 106 children whose haemoglobin was measured.

Table 1 depicts demographic, WASH, and clinical characteristics among our study population. The mean age of children in our study was 3.7 years, with slightly more males (53.4%) than females.

Reported handwashing with soap was very common amongst

Table 1
Selected characteristics of study participants.

	All participants	Intervention arm	Control arm	*p* value[a]
Demographics	**n = 133**	**n = 75**	**n = 58**	
Mean age, years (SD)	3.7 (1.4)	3.7 (1.4)	3.7 (1.4)	0.96
Male sex, % (95% CI)	53.4 (44.8, 61.8)	49.3 (38.1, 60.7)	58.6 (45.4, 70.7)	0.29
EE biomarkers	**n = 133**	**n = 75**	**n = 58**	
Mean log MPO, ng/mL (SD)	7.4 (1.6)	7.2 (1.7)	7.7 (1.4)	0.11
Mean log AAT, mg/g (SD)	−2.1 (1.4)	−2.2 (1.5)	−2.0 (1.3)	0.47
WASH practices and conditions	**n = 124**	**n = 70**	**n = 54**	
Washes hands with soap, % (95% CI)	97.6 (92.7, 99.2)	98.6 (90.3, 99.8)	96.3 (86.0, 99.1)	0.47
Always wears shoes inside, % (95% CI)	15.3 (9.9, 22.9)	12.9 (6.8, 23.1)	18.5 (10.1, 31.4)	0.47
Always wears shoes when outside and toileting, % (95% CI)	19.4 (13.3, 27.4)	20.0 (12.1, 31.2)	18.5 (10.1, 31.4)	0.93
Practices open defecation, % (95% CI)	66.9 (58.1, 74.7)	67.1 (55.2, 77.2)	66.7 (52.9, 78.1)	0.58
At least one adult in household practices open defecation, % (95% CI)	37.9 (29.7, 46.9)	44.4 (31.6, 58.0)	32.9 (22.8, 44.8)	0.08
	n = 129	**n = 72**	**n = 57**	
Household has a toilet, % (95% CI)	51.2 (42.5, 59.8)	58.3 (46.5, 69.3)	42.1 (29.9, 55.4)	0.07
STH/protozoal infections	**n = 130**	**n = 73**	**n = 57**	
Ascaris spp. prevalence (95% CI)	20.0 (13.9, 27.9)	20.6 (12.7, 31.5)	19.3 (10.9, 31.9)	0.78
N. americanus prevalence (95% CI)	10.8 (6.4, 17.5)	9.59 (4.6, 19.0)	12.3 (5.9, 23.9)	0.60
G. duodenalis prevalence (95% CI)	32.3 (24.8, 40.9)	31.5 (21. 8, 43.2)	33.3 (22.2, 46.7)	0.83
Morbidity indicators[b]	**n = 117**	**n = 64**	**n = 53**	
Stunting, % (95% CI)	61.5 (52.3, 70.0)	57.8 (45.3, 69.4)	66.04 (52.1, 77.6)	0.67
Underweight, % (95% CI)	54.7 (45.5, 63.6)	53.9 (41.5, 65.7)	55.77 (41.9, 68.8)	0.84
	n = 97	**n = 55**	**n = 42**	
Wasting, % (95% CI)	23.7 (16.2, 33.4)	20.0 (11.3, 33.0)	28.6 (16.8, 44.3)	0.33
	n = 115	**n = 64**	**n = 51**	
Thinness, % (95% CI)	20.0 (13.6, 28.5)	17.2 (9. 7, 28.7)	23.5 (13.7, 37.3)	0.40
	n = 106	**n = 57**	**n = 49**	
Anaemia, % (95% CI)	27.4 (19.6, 36.8)	24. 6 (15.0, 37.6)	30.6 (19.2, 45.1)	0.39
Mean haemoglobin, g/L (SD)	116.2 (12.5)	117.8 (12.8)	114.2 (12.0)	0.08

[a] p values adjusted for clustering at community level.

[b] Anthropometric indices defined as follows: Underweight = weight-for-age *Z*-score > 2 standard deviations (SD) below reference median; Stunting: height-for-age *Z*-score > 2 SD below reference median; Thinness = BMI-for-age *Z*-score > 2 SD below reference median; Wasting = weight-for-height *Z*-score > 2 SD below reference median; Anaemia = defined as per WHO thresholds, adjusted for altitude.

Table 2
Results of mixed effects linear regression examining impact of the WASH intervention on EE biomarker levels.

	Coefficient	95% CI	*p* value
Log transformed MPO (ng/mL)			
Intervention arm	−0.51	−1.02, 0.01	0.055
Age (years)	−0.29	−0.47, −1.02	0.002
Male sex	−0.08	−0.60, 0.44	0.761
Log transformed AAT (mg/g)			
Intervention arm	−0.14	−0.63, 0.35	0.583
Age (years)	−0.16	−0.33, 0.01	0.062
Male sex	0.13	−0.34, 0.59	0.60

participating children from both intervention and control groups (98.6% and 96.3% respectively, $p = 0.47$). Approximately half of the participants surveyed had household latrines. Those who received the WASH intervention had higher coverage of household latrines than compared to those in the control communities (58.3% and 42.1% respectively, $p = 0.07$). Reported open defecation among the young children in our study cohort was common, and there was no difference between intervention and control arms (67.1% and 66.7% respectively, $p = 0.58$; see Table 1). There were fewer children living in a household in which at least one adult practiced open defecation in the WASH arm than in the control arm (32.9% vs 44.4%, $p = 0.08$).

Prevalence of infection with *Ascaris* spp. was 20.6% and 19.3% in the intervention and control arms respectively ($p = 0.78$). *Necator americanus* prevalence was 9.6% and 12.3% in the intervention and control arms, respectively ($p = 0.60$), and *Giardia duodenalis* prevalence was 31.5% and 33.3% respectively ($p = 0.83$; see Table 1).

There were no differences between children in the intervention and control arms with respect to growth parameters or anaemia. More than half of the children were stunted or underweight, approximately a fifth were wasted or thin, and approximately a quarter of were anaemic (see Table 1).

There were no notable differences between all children aged 1–5 that were present at the final follow-up and participated in the WASH for WORMS study (by providing answers to the questionnaire, providing stool for STH and protozoa analysis, or undergoing measurement of haemoglobin and/or height and weight), and the subsample of these children who additionally provided stool for EE analysis (Supplementary Table 1).

3.2. Impact of the intervention on EE biomarkers

Median MPO was 1528.3 ng/mL in the intervention arm and 2046.8 ng/mL in the control arm, with no significant difference between means of log-transformed values ($p = 0.11$). Median AAT was 0.12 mg/g in the intervention arm and 0.16 mg/g in the control arm, also with no significant difference in the means of log-transformed values ($p = 0.47$) (Table 1).

The mixed − effects linear regression model found no significant association between the WASH intervention and faecal AAT levels (coefficient -0.14, $p = 0.583$) (Table 2). There was a trend towards lower MPO levels in children from the intervention arm of the study (coeff -0.51, $p = 0.055$). Increasing age was significantly associated with lower MPO levels (coeff: -0.29, $p = 0.002$), while no significant age association was observed for AAT. Sex was not associated with faecal biomarker levels for MPO or AAT.

3.3. Risk factors for EE

In univariable analysis, we identified several variables with $p < 0.2$ for faecal MPO levels ($p < 0.2$) (Table 3, Supplementary Table 2). Handwashing with soap (coeff -1.58, $p = 0.083$), drying hands hygienically (coeff -2.54, $p = 0.104$) and handwashing before contact with food (coeff-0.89, $p = 0.050$) all showed a trend towards lower MPO levels. Always wearing shoes inside (coeff -0.69, $p = 0.077$), and always wearing shoes both when outside and toileting (coeff-0.63, $p = 0.075$) also showed a trend toward lower MPO levels in univariable analysis. Using water to clean oneself after toileting showed a trend towards a higher MPO level (coeff 0.41, $p = 0.177$). Disposing of household rubbish by burning showed a trend towards lower MPO levels (coeff -0.44, $p = 0.156$), while burying household rubbish disposal showed a trend towards higher levels (coeff 0.66, $p = 0.156$). Disposing of child waste hygienically was associated with lower MPO levels (coeff -1.95, $p = 0.030$), and storing water in covered containers showed a trend towards lower MPO levels (coeff -1.58, $p = 0.081$). Finally, having more than one STH infection was associated with higher MPO levels (coeff -1.06, $p = 0.104$). No other enteric infections had $p < 2.0$ for MPO levels in univariable analysis and were not included in the multivariable model.

There were also several variables associated with faecal AAT in univariable analysis ($p < 0.2$) (Table 3, Supplementary Table 2). Handwashing with soap (coeff -1.06, $p = 0.181$), drying hands hygienically (coeff -2.82, $p = 0.037$) and handwashing before contact with food (coeff-0.60, $p = 0.131$) all showed a trend towards lower AAT levels. Using water to clean oneself after toileting showed a trend towards higher AAT levels (coeff 0.37, $p = 0.159$). No enteric infections reached $p < 0.2$ for AAT levels in univariable analysis.

Results of the final multivariable models are shown in Table 4. Of the WASH and infection variables associated with MPO at univariable level, only storing water in covered containers remained significant and was retained in the final multivariable model, associated with lower MPO levels (coeff -1.75, $p = 0.046$). All of the WASH variables associated with AAT identified at univariable level were not significant in the multivariable model and were not retained in the final model.

Table 3
WASH and infection variables associated with EE biomarkers in univariable analysis ($p < 0.2$).

Variable domain	Log-transformed MPO (ng/mL)	Log-transformed AAT (mg/g)
Individual hygiene variables	**Washes hands with soap**	**Washes hands with soap**
	Dries hands hygienically	**Dries hands hygienically**
	Washes hands before contact with food	**Washes hands before contact with food**
	Always wears shoes when inside	
	Always wears shoes when outside and toileting	
Individual sanitation variables	***Uses water to clean self after toileting***	***Uses water to clean self after toileting***
Household sanitation variables	Household rubbish is disposed of by burning	–
	Household rubbish is disposed of by burying	
	Disposal of child waste hygienically	
Household water variables	Water stored in covered container	–
STH and protozoal infections	*Co-infection with two or more STH*	–

Italics indicates that variable is associated with a higher EE biomarker level. **Bold** indicates variables in common between both univariable outcome measures. Full results of univariable analyses including all *p* values are shown in Supplementary Table 2.

Table 4
Results of the final multivariable models for EE biomarkers, including age, sex and significant risk factors.

Variable*	Coefficient	95% CI	*p value*
Log-transformed MPO (ng/mL)			
Age (years)	−0.28	−0.47, −0.09	0.005
Male sex	−0.16	−0.69, 0.35	0.372
All household drinking water stored in covered containers	−1.75	−3.47, −0.03	0.046
Random effects variance (95% CI)	0.08 (0.004, 1.43)		
Log-transformed AAT (mg/g)			
Age (years)	−0.16	−0.33, 0.01	0.061
Male sex	0.14	−0.33, 0.60	0.561
Random effects variance (95% CI)	0.04 (0.0006, 2.27)		

3.4. EE biomarkers as predictors of morbidity

Finally, we tested for a potential association between EE biomarkers and morbidity outcomes including anthropometric indices (underweight, stunting, thinness and wasting) and haematological parameters (haemoglobin level and anaemia). EE biomarkers were not significantly associated with any of these morbidity indicators in the study population (Supplementary Tables 3 and 4).

4. Discussion

4.1. Discussion of study findings

This study represents the first description of EE biomarkers in the context of a WASH intervention and risk factor analysis in Timor-Leste. Our findings highlight the difficulties in effectively diagnosing EE and in assessing the health impacts of WASH interventions.

To our knowledge there has only been one previous intervention study assessing the impact of a WASH program on indirect EE markers (Langford et al., 2011), which failed to show improvements in intestinal mucosal damage or growth as a result of a handwashing intervention. In our study, we observed no difference in faecal biomarkers between children who received the WASH intervention and those who did not. However, we also observed no differences in WASH behaviours in this age group between study arms, which suggests that the ability of children to adopt the WASH intervention was limited. The high prevalence of open defecation in this age group at the end of the trial suggests faecal contamination was not considerably improved by the WASH intervention. The lower uptake of the WASH intervention in households with children and by children may explain the absence of detectable impact of the WASH intervention in AAT levels and a marginal impact in MPO levels. Of note is the fact that looking at overall WASH outcomes of the trial, including all households in the participating communities, the WASH intervention had success in increasing sanitation use (from 19.9% of participants using a household latrine at study baseline, to 59.4% at the end of the trial) and decreasing prevalence of open defecation (from 82.7%–40.2%) (Nery et al., 2018). This suggests that WASH interventions need to be tailored to be able to induce change in children and households with children.

Although previous studies have found an association between household WASH variables and EE, with sample sizes similar to or bigger than ours, (George et al., 2016, 2015; Lin et al., 2013) our adjusted model identified no significant associations between WASH variables or enteric infections and EE biomarkers, except for disposing of child waste hygienically that was associated with lower MPO levels. This could be due to a lack of statistical power to detect such associations. Additionally, one could expect that if the level of faecal contamination in the environment is high enough then individual behaviours may not confer enough protection against EE. However, we cannot rule out the possibility that there is no association between WASH and EE and that other factors are responsible for this condition.

Because intestinal inflammation and hyperpermeability have been proposed to be associated with impaired growth in children, we hypothesised that EE biomarker levels would be associated with haemoglobin levels and malnutrition indicators. However, we found no such relationship between MPO or AAT levels and concurrent growth indices or haemoglobin. Although several studies have detected relationships between biomarker levels and growth stunting (George et al., 2015; Kosek et al., 2013), more recent reports, that followed more than 200 children over several time points, found that stool biomarkers were only weakly predictive of subsequent growth (Arndt et al., 2016; Kosek and Investigators, 2017).

Observed levels of MPO in our study were more than twice as high as the normal adult reference values of < 2000 ng/mL observed in high-income countries (Saiki, 1998). On the other hand, observed levels of AAT were within the normal adult reference range of < 0.27 mg/g (McCormick et al., 2017; Meyers et al., 1985). Although adult reference values from high-income countries cannot be used as a direct comparison, these findings may suggest that intestinal inflammation is widespread in our study population. Most existing analyses of faecal biomarkers MPO and AAT in low-income countries refer to populations aged 0 − 2 years (Arndt et al., 2016; Kosek et al., 2013; Kosek and Investigators, 2017; McCormick et al., 2017). Our measurements for MPO and AAT in children aged 1–5 years appear to be lower overall than those measured from populations in the MAL-ED cohort study (Kosek et al., 2013), which examined children across eight low- and middle- income countries. Other studies report very similar MPO and AAT levels to those observed in our population (George et al., 2015). Recent analyses of faecal biomarker concentration in children of low-income countries reported that EE biomarker levels are highest in the first year of life, then gradually decrease and stabilise at three years (Colston et al., 2017). Indeed, we observed an inverse relationship between age and MPO (ng/mL), although age was not significantly associated with AAT (mg/g) levels.

4.2. Limitations

Past assessments of MPO and AAT as indirect markers of EE have shown large measurement variability across subjects in a population, and between multiple measurements on individual subjects (McCormick et al., 2017). Therefore, the small study population may have limited our ability to detect an impact of the WASH intervention and WASH risk factors on EE. Furthermore, given that there are no defined cut-off values for any of the measured EE biomarkers (or the composite of multiple markers) that would allow a binary treatment of these variables, we analysed EE biomarkers as continuous variables and were unable to diagnose participants with EE. An 'EE index' is yet to be standardised; therefore, it is difficult to establish the clinical significance of faecal biomarker levels without these cut-off categories (Kosek et al., 2013).

An additional limitation is that WASH data were collected through self-reporting. Survey-based study designs relying on self-reported WASH data introduce potential for bias. This is particularly true when an element of embarrassment or shame may be associated with truthfully reporting personal or household sanitation and hygiene practices.

Our analysis of EE and morbidity indicators was cross-sectional, and therefore focused on concurrent, rather than subsequent growth. Thismay have limited our ability to detect an association between EE biomarkers and growth indices. Additionally, we were unable to account for dietary factors, diarrhoeal episodes or birthweight of children in the study population, all of which are important factors influencing child growth. Biomarker concentration is also subject to interference from factors such as recent breast milk intake, which has been shown to elevate AAT (mg/g) and MPO (ng/mL) levels (McCormick et al., 2017). We were unable to control for such of this variability in our analysis.

5. Conclusions and future directions

We report the first characterisation of faecal EE biomarkers in Timor-Leste. Young age was associated with higher MPO levels. We did not see a significant impact of a WASH intervention on faecal biomarkers although children in the WASH arm tended to exhibit lower levels of MPO. The only WASH variable that was associated with increased EE markers was disposing child faeces hygienically, but only for MPO.

In order to further investigate the impact of improved WASH conditions on chronic intestinal damage, we suggest interventions with large study populations, conducted over several years and including younger children, with a rigorous monitoring of WASH improvement and environmental faecal contamination. This will enable the study population to adapt to practical use of WASH facilities, and importantly also generate meaningful attitude change regarding appropriate WASH behaviours within the community. Furthermore, sanitation interventions promoting construction of household latrines with the aim of ending open defecation need to include strategies tailored to young children who are not toilet trained.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijheh.2018.05.012.

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Supplementary Table 1. Selected characteristics of all children aged between one and five years present in the study communities, and those who gave stool for EE biomarker analysis

	Children present who did not participate in EE analysis	Children in EE analysis	*p* value
	n = 214	**n = 133**	0.89
Mean age in years (SD)	3.7 (0.08)	3.7 (0.12)	
Male sex, % (95% CI)	53.7 (47.0, 60.4)	53.4 (44.8, 61.8)	0.95
WASH variables, % (95% CI)	**n = 193**	**n = 124**	
Washes hands with soap	98.5 (95.3, 99.5)	97.6 (92.7, 99.2)	0.58
Always wears shoes inside	17.1 (12.4, 23.1)	15.3 (9.9, 22.9)	0.68
Always wears shoes both when outside and toileting	22.2 (16.9, 28.8)	19.4 (13.3, 27.4)	0.54
Currently practices open defecation	70.5 (63.6, 76.5)	67.0 (58.1, 74.7)	0.51
	n = 199	**n = 129**	
Household has a toilet	51.8 (44.8–58.7)	51.2 (42.5, 59.8)	0.91
STH and protozoa infection	**n = 164**	**n = 130**	
Ascaris spp. prevalence (95% CI)	22.0 (16.2, 29.0)	20.0 (13.9, 27.9)	0.68
Necator americanus prevalence (95% CI)	10.4 (6.5, 16.1)	10.8 (6.4, 17.5)	0.91
Giardia duodenalis prevalence (95% CI)	35.4 (28.4 , 43.1)	32.3 (24.8, 40.9)	0.58
Morbidity indicators			
Stunting, % (95% CI)	**n = 172** 61.5 (53.9, 68.6)	**n = 117** 61.5 (52.3, 70.0)	1.00
Underweight, % (95% CI)	**n = 135** 51.7 (44.2, 59.2)	**n = 97** 54.7 (45.5, 63.6)	0.62
Wasting, % (95% CI)	**n = 167** 20.0 (14.0–27.7)	**n = 115** 23.7 (16.2 , 33.4)	0.50
Thinness, % (95% CI)	**n = 157** 17.4 (12.3, 24.0)	**n = 106** 20 (13.6, 28.4)	0.58
Anaemia, % (95% CI)	**n = 152** 25.5 (19.2, 33.0)	**n = 106** 27.4 (19.6, 36.8)	0.73
Mean haemoglobin (SD)	**n = 152** 116.5 (1.0)	**n = 106** 116.2 (1.2)	0.59

[a] Anthropometric indices defined as follows: Underweight = weight-for-age *Z*-score >2 standard deviations (SD) below reference median; Stunting: height-for-age *Z*-score >2 SD below reference median; Thinness = BMI-for-age *Z*-score >2 SD below reference median; Wasting = weight-for-height *Z*-score >2 SD below reference median; Anaemia = defined as per WHO thresholds, adjusted for altitude

Supplementary Table 2. Univariable analysis of risk factors for EE biomarkers

			Log transformed MPO (ng/mL)			Log transformed AAT (mg/g)		
	Observations (n)	**Proportion of respondents (%)**	**Coefficient**	**95% CI**	***p* value[a]**	**Coefficient**	**95% CI**	***p* value[a]**
Domain: Demographic								
Age	133	-	-0.287	-0.473, -1.00	**0.003**	-0.163	-0.331, 0.004	**0.055**
Male sex	133	53.4	0.033	-0.499, 0.566	0.902	0.171	-0.297, 0.639	0.474
Poorest socioeconomic quintile (relative to wealthiest)	129	37.2	-0.079	-1.062, 0.905	0.875	0.177	-0.716, 1.070	0.698
Domain: Individual hygiene								
Washes hands with soap	124	97.6	-1.575	-3.354, 0.203	**0.083**	-1.065	-2.624, 0.495	**0.181**
Dries hands hygienically	124	0.8	-2.537	-5.595, 0.521	**0.104**	-2.823	-5.473, -1.737	**0.037**
Washes hands before any contact with food	124	10.5	-0.887	-1.776, 0.002	**0.050**	-0.605	-1.389, 0.179	**0.131**
Washes hands after any contact with faeces	124	91.1	0.340	-0.631, 1.312	0.493	0.488	-0.363, 1.341	0.262
Washes hands after any contact with dirty objects	124	68.6	-0.424	-1.017, 0.169	**0.161**	0.134	-0.398, 0.666	0.622
Always wears shoes when inside	124	15.3	-0.690	-1.457, 0.077	**0.078**	0.058	-0.624, 0.74	0.868
Always wears shoes both when outside and toileting	124	19.4	-0.632	-1.327, 0.063	**0.075**	-0.126	-0.749, 0.497	0.692
Domain: Individual sanitation								
Main place of defecation is toilet	124	47.6	0.068	-0.491, 0.627	0.813	0.264	-0.229, 0.758	0.294
Currently practices open defecation	124	66.9	-0.074	-0.665, 0.517	0.807	-0.315	-0.832, 0.201	0.232
Uses water to clean self after toileting	124	66.1	0.410	-0.17, 0.989	**0.166**	0.372	-0.145, 0.889	**0.159**
Domain: Household sanitation								
Household has toilet	129	51.2	0.094	-0.461, 0.649	0.739	0.305	-0.173, 0.783	0.211
Household toilet has slab	66	31.0	-0.002	-0.32, 0.316	0.990	-0.010	-0.701, 0.682	0.978
Household toilet has water	66	34.1	-0.020	-0.33, 0.29	0.898	-0.248	-0.959, 0.463	0.494
Household toilet is clean	129	12.4	-0.015	-0.417, 0.386	0.941	0.127	-0.22, 0.474	0.473
Household toilet is shared between dwellings	129	7.0	0.025	-0.419, 0.47	0.911	0.195	-0.19, 0.579	0.321
Child faeces is disposed of hygienically	99	3.0	-1.950	-3.726, -0.174	**0.031**	-0.945	-2.501, 0.612	0.234
Household rubbish is disposed of by burning	129	28.7	-0.436	-1.039, 0.167	**0.156**	0.198	-0.347, 0.743	0.476
Household rubbish is disposed of in bushes/ground	129	64.3	0.371	-0.205, 0.947	0.207	0.035	-0.476, 0.545	0.894
Household rubbish is disposed of by burying	129	10.1	0.661	-0.252, 1.575	**0.156**	0.115	-0.698, 0.928	0.781

Domain: Household water								
Main source of water is improved	129	86.1	-0.264	-1.091, 0.563	0.532	0.220	-0.522, 0.961	0.562
Main household water source always available	129	89.9	-0.603	-1.547, 0.341	0.211	-0.181	-0.997, 0.634	0.663
All water stored in covered containers	125	97.6	-1.580	-3.351, 0.192	**0.081**	0.993	-0.567, 2.553	0.212
Domain: Household hygiene								
Household has food garden	129	98.5	1.039	-1.162, 3.239	0.355	0.435	-1.478, 2.349	0.656
Human faeces is used on food garden	129	1.6	-0.001	-0.003, 0.001	0.355	0.0004	-0.002, 0.001	0.656
Domain: Composite environmental cleanliness (over time)								
Living in open defecation free household	133	-	0.006	-0.202, 0.215	0.955	0.082	-0.097, 0.262	0.368
Main food preparer washes hands before food preparation	133	-	0.044	-0.272, 0.36	0.786	-0.043	-0.32, 0.234	0.761
Household drinking water stored in covered containers	133	-	0.138	-0.162, 0.439	0.367	0.099	-0.162, 0.36	0.458
Domain: Household socioeconomic								
More than 6 people in dwelling	133	51.9	0.131	-0.404, 0.667	0.631	-0.031	-0.502, 0.439	0.897
Households share a dwelling	133	24.8	0.367	-0.255, 0.99	0.247	0.258	-0.289, 0.805	0.355
Domain: STH and protozoa infection								
Ascaris spp.	130	20.0	-0.261	-0.939, 0.418	0.451	-0.082	-0.679, 0.516	0.789
Necator americanus	130	10.8	0.105	-0.763, 0.972	0.813	0.413	-0.35, 1.176	0.289
Ancylostoma spp.	130	0.8	-1.000	-4.07, 2.07	0.523	-0.884	-3.596, 1.827	0.523
Trichuris trichiura	130	0.8	-1.290	-4.346, 1.765	0.408	-1.114	-3.819, 1.591	0.420
Any STH infection	130	26.9	0.029	-0.584, 0.642	0.926	0.135	-0.403, 0.673	0.623
STH coinfection	130	4.6	-1.060	-2.337, 0.218	**0.104**	-0.166	-1.303, 0.971	0.775
Giardia duodenalis	130	32.3	-0.151	-0.722, 0.419	0.603	0.138	-0.367, 0.644	0.592

Variables grouped by domain for ease of reporting

Bold indicates significant (p<0.2) variables retained for multivariable analysis

[a] p values adjusted for clustering at the community level

Supplementary Table 3. Mixed effects logistic regression examining the association of EE biomarkers with binary morbidity outcomes, adjusted for age and sex

Anthropometric index[a]	Underweight	Severe underweight	Stunting	Severe stunting	Thinness	Severe thinness	Wasting	Severe wasting	Anaemia
Observations (n)	117	117	117	117	115	115	97	98	106
Proportion (%)	54.7	19.7	61.5	26.5	20.0	3.5	23.7	8.2	27.4
Log transformed MPO (ng/mL)									
Odds ratio	1.00	1.20	0.90	0.87	1.13	1.58	1.17	1.22	0.95
95% CI	0.78, 1.27	0.88, 1.64	0.68, 1.20	0.65, 1.17	0.83, 1.54	0.73, 3.43	0.85, 160	0.74, 2.03	0.70, 1.29
p value[b]	0.981	0.246	0.472	0.3511	0.437	0.250	0.341	0.440	0.741
Log transformed AAT (mg/g)									
Odds ratio	1.13	1.29	0.93	0.99	1.00	1.20	0.90	0.92	0.90
95% CI	0.85, 1.50	0.89, 1.88	0.67, 1.29	0.71, 1.40	0.71, 1.42	0.51, 2.77	0.63, 1.28	0.53, 1.60	0.64, 1.27
p value[b]	0.390	0.176	0.655	0.969	0.988	0.678	0.544	0.768	0.552

[a] Anthropometric indices defined as follows: Underweight = weight-for-age *Z*-score >2 standard deviations (SD) below reference median; Severe underweight = weight-for-age *Z*-score >3 SD below reference median; Stunting: height-for-age *Z*-score >2 SD below reference median; Severe stunting = height-for-age *Z*-score >3 SD below reference median; Thinness = BMI-for-age *Z*-score >2 SD below reference median; Severe thinness = BMI-for-age *Z*-score >3 SD below reference median; Wasting = weight-for-height *Z*-score >2 SD below reference median; Severe wasting = weight-for-height *Z*-score >3 SD below reference median; Anaemia = defined as per WHO thresholds, adjusted for altitude

[b] *p* values adjusted for clustering at the community level

Supplementary Table 4. Mixed effects linear regression examining the association of EE biomarkers with continuous morbidity outcomes, adjusted for age and sex

	Weight for age *Z*-score	Height for age *Z*-score	BMI[a] for age *Z*-score	Weight for length *Z*-score	Haemoglobin (g/L)
Observations (n)	117	117	115.00	98	106
Mean (95%CI)	-2.12 (-2.33, -1.91)	-2.25 (-2.46, -2.03)	-0.86 (-1.09, -0.62)	-0.91(-1.29, -0.52)	116.15 (113.74, 118.56)
Log transformed MPO (ng/mL)					
Coefficient	0.019	0.104	-0.02	-0.056	0.704
95% CI	-0.108, 0.146	-0.026, 0.234	-0.163, 0.123	-0.291, 0.179	-0.635, 2.044
p value[b]	0.771	0.118	0.783	0.640	0.303
Log transformed AAT (mg/g)					
Coefficient	-0.037	0.07	-0.044	-0.028	0.745
95% CI	-0.183, 0.108	-0.085, 0.225	-0.208, 0.12	-0.303, 0.248	-0.759, 2.249
p value[b]	0.616	0.377	0.595	0.845	0.332

[a] BMI = body mass index, calculated as $(\text{weight in kg})^2/(\text{height in metres})$

[b] *p* values adjusted for clustering at the village level

Appendix 12
List of conference presentations arising from this work

This final appendix contains a list of conference talks and posters that I presented during the course of my PhD candidature, in chronological order.

Clarke NE, Clements ACA, Gray D, McCarthy J, Nery SV. Investigating school- and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for WORMS pilot study (Oral presentation). Australian Society for Medical Research New Investigator Forum, Canberra Australia, June 2016.

Clarke NE, Clements ACA, Gray D, McCarthy J, Nery SV. Investigating school- and community-based integrated control programmes for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for WORMS pilot study (Oral presentation). Canberra Health Annual Research Meeting, August 2016.

Clarke NE, Clements ACA, Doi SA, Wang D, Campbell SJ, Gray DJ, Nery SV. Differential impact of mass and targeted deworming campaigns for soil-transmitted helminth control in children: a systematic review and meta-analysis (Oral presentation). International Congress for Tropical Medicine & Malaria, Brisbane Australia, September 2016.

Clarke NE, Clements ACA, Traub R, McCarthy J, Gray DJ, Nery SV. Investigating the differential impact of school and community-based integrated control programs for soil-transmitted helminths: the (S)WASH for WORMS pilot study (Oral presentation). International Congress for Tropical Medicine & Malaria, Brisbane Australia, September 2016.

Clarke NE, Clements ACA, Doi SA, Wang D, Campbell SJ, Gray DJ, Nery SV. Differential impact of mass and targeted deworming for soil-transmitted helminth control in children: a systematic review and meta-analysis (Oral presentation). American Society for Tropical Medicine & Hygiene annual meeting, Atlanta USA, November 2016.

Clarke NE, Clements ACA, Traub R, Gray D, McCarthy J, Nery SV. Investigating the differential impact of school and community-based integrated control programs for soil-transmitted helminths in Timor-Leste: the (S)WASH-D for WORMS pilot study (Poster presentation). American Society for Tropical Medicine & Hygiene annual meeting, Atlanta USA, November 2016.

Clarke NE, Doi SA, Wangdi K, Chen Y, Clements ACA, Nery SV. Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis (Poster presentation). Australian Society for Medical Research New Investigator Forum, Canberra Australia, June 2017. *Awarded best poster presentation.*

Clarke NE, Doi SA, Wangdi K, Chen Y, Clements ACA, Nery SV. Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis (Oral presentation). Australian Society for Parasitology Annual Conference, Katoomba Australia, July 2017.

Clarke NE, Doi SA, Wangdi K, Chen Y, Clements ACA, Nery SV. Efficacy of anthelminthic drugs and drug combinations against soil-transmitted helminths: a systematic review and network meta-analysis (Poster presentation). Canberra Health Annual Research Meeting, Canberra Australia, August 2017. *Awarded best poster presentation.*

Clarke NE, Llewellyn S, Traub RJ, McCarthy J, Richardson A, Nery SV. Further investigations of quantitative PCR for diagnosis of soil-transmitted helminth infections (Oral presentation). Australian Society for Medical Research New Investigator Forum, Canberra Australia, June 2018.

Clarke NE. Waging war on worms (Oral presentation). Canberra Health Annual Research Meeting, August 2018. *Awarded runner-up for best 3 minute presentation*.

Clarke NE, Llewellyn S, Traub RJ, McCarthy JS, Richardson A, Nery SV. Quantitative PCR for diagnosis of soil-transmitted helminth infections: a comparison with a flotation-based technique and an investigation of variability in DNA detection (Oral presentation). International Congress of Parasitology, Daegu South Korea, August 2018.