Novel tools for the control of soil-transmitted helminthiasis: Drug combinations, diagnostics, and meta-analyses on the effect of sanitation facilities

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Summary

More than half of the world's population is at risk of soil-transmitted helminthiasis, parasitic worm infections most commonly caused by the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*) and the hookworms (*Ancylostoma duodenale* and *Necator americanus*). Together these soil-transmitted helminths cause an estimated burden of 5 million disability-adjusted life years (DALYs), mostly affecting school-aged children living in the least developed settings, lacking clean water and sanitation facilities. To control soil-transmitted helminthiasis annual preventive chemotherapy is given to people at risk of infection. In 2012 a total of 212 million anthelmintic tablets were administered to school age-children. The most commonly used anthelmintics are albendazole and mebendazole. Both are highly effective in treating *A. lumbricoides,* while only albendazole has satisfactory efficacy against hookworm. Both drugs have only low efficacy against *T. trichiura.* Therefore an alternative treatment for *T. trichiura* infections is urgently required. However, even with an effective treatment, re-infection is common in endemic settings. Therefore other interventions besides preventive chemotherapy should be considered.

The primary goal of this PhD thesis was to find an alternative, effective treatment against *T. trichiura* and concomitant soil-transmitted helminth infections. Two compounds with potentially high trichuricidal activity are nitazoxanide and oxantel pamoate. Both of these compounds were tested on their efficacy and safety within two separate clinical trials conducted on Pemba Island, Tanzania in 2011 and 2012, respectively. Both drugs were evaluated alone as well as in combination with albendazole to broaden their spectrum of activity against concomitant soil-transmitted helminths. In a third clinical trial conducted in 2013, the drug combination albendazole-

oxantel pamoate was compared to two other promising drug combinations (i.e. albendazole-ivermectin and albendazole-mebendazole) which were identified in recently conducted randomised controlled trials. Additional objectives were attached to these three randomised controlled trials which included the assessment of the prevalence of intestinal protozoa and the comparison of the performance of different diagnostic approaches. Further we conducted two systematic reviews and meta-analyses to generate evidence on the protective effect of sanitation facilities against soil transmitted helminths and intestinal protozoa infections.

In the first clinical trial conducted in 2011 we found no effect for single nitazoxanide (1,000 mg) against *T. trichiura* infections. Furthermore, children receiving nitazoxanide reported significantly more mild adverse events than children receiving a placebo. Hence we cannot recommend nitazoxanide for the treatment of *T. trichiura* and concomitant soil-transmitted helminths. The prevalence of intestinal protozoa assessed within this clinical trial was relatively high. In detail, 70% of the assessed children were infected with at least one of the (potentially) pathogenic intestinal protozoa *Giardia intestinalis, Entamoeba histolytica*/*E. dispar* and *Blastocystis hominis.* Nitazoxanide (1,000 mg) revealed moderate efficacy against intestinal protozoa, comparable to that of albendazole (400 mg). Within the same clinical trial, the sensitivity of a single, duplicate and quadruplicate Kato-Katz thick smear and the ether-concentration method was assessed for diagnosing soil-transmitted helminths. Quadruplicate Kato-Kato thick smears (duplicate Kato-Katz from two stool samples) revealed the highest sensitivity in general. We could further show that in clinical trials, relatively sensitive diagnostic approaches should be chosen (multiple stool samples); otherwise the efficacy of treatments might be overestimated.

In the second randomised controlled trial conducted in 2012, we examined the efficacy and safety of single oxantel pamoate (20 mg/kg) alone and in combination with albendazole (400 mg). Oxantel pamoate and albendazole were given on subsequent days. Oxantel pamoate was significantly more effective against *T. trichiura* compared to the standard drugs albendazole and mebendazole. The albendazole-oxantel pamoate combination was effective against all three soil-transmitted helminths. Therefore this promising combination was further evaluated within a third clinical trial.

In the third randomised controlled trial conducted in 2013 on Pemba Island, Tanzania, we identified two drug combinations (i.e. albendazole [400 mg]-oxantel pamoate [20 mg/kg], and albendazole [400 mg]-ivermectin [200 µg/kg]) with significantly higher efficacy against *T. trichiura* compared to the standard treatment mebendazole (500 mg). Additionally the albendazole-oxantel pamoate combination had superior efficacy compared to the albendazole-ivermectin treatment. We could not confirm the trichuricidal activity of the albendazole-mebendazole combination. Albendazole-ivermectin is already a commonly used drug combination which also has the advantage that ivermectin is the current standard treatment against *Strongyloidiasis* (probably the most neglected of the neglected tropical diseases) and lymphatic filariasis. But especially for settings highly endemic for *T. trichiura*, the albendazole-oxantel pamoate combination should be further investigated (i.e. dose finding-, safety-, pharmacokinetic-studies) to overcome the current lack of effective treatments and to expand the armamentarium against soil-transmitted helminths.

Prevalence often remains high even though regular preventive chemotherapy with an effective drug is conducted. In our meta-analyses we showed that the implementation of sanitation facilities is an important strategy for the control of soil-transmitted helminthiasis . Moreover we revealed that sanitation facilities and water treatment also

have a positive effect on other diarrheal diseases (i.e. protozoa infections). Therefore, for successful control of soil-transmitted helminths, preventive chemotherapy programmes should be conducted with effective drugs and be accompanied by interventions involving sanitation facilities and safe water.

Pictograph of our clinical trials in laymen terms

Preparation (incomplete):
- Study protocol was written
- Ethical approval was received from country of investigator and country where the trial was conducted
- Insurance for participants was obtained

Study site selection: Pemba Island
- Annual/biannual mass treatment against intestinal worms since the 1990`s
- High prevalence of intestinal worms
- Good infrastructure, skilled team

Parents and teachers were informed:
- Study rationale
- Participation voluntary
- Potential risks
- Potential benefits

Consent:
- Written consent was signed by parents
- Children assented orally

Diagnostic (baseline):
- Two stool samples per child were collected
- Two faecal smears (Kato-Katz thick smears) were prepared from each stool sample and microscopically examined for intestinal worm eggs
- Children positive for the parasite of interest (i.e. *Trichuris trichiura*) were randomly allocated to one of the treatment arms

Treatment: Different stations during day of treatment (from left to right): (i) A nurse assessed children's weight and asked questions about general well being; (ii) two physicians examined children (e.g. heart rate, lung function, enlargement of organs) and decided if child can continue study based on inclusion/exclusion criteria; (iii) children received treatment together with a cup of water. Adverse events were assessed 3 and 24 hours after treatment (not shown on picture).

Diagnostic (follow-up):
- Children were diagnosed for intestinal worms 3 weeks after treatment based on four faecal smears produced from two stool samples (see diagnostic baseline).
- Efficacy of the different treatment arms was calculated based on baseline and follow-up results
- Final results were presented within a peer-reviewed scientific manuscript

Source map of Zanzibar: http://www.genesisofadventure.com/tours/zanzibar-tours.html, assessed 13. May 2014

Table of Abbreviations

CI	Confidence interval
CR	Cure rate
DALY	Disability-adjusted life years
EKBB	Ethical committee of Basel
EPG	Eggs per gram of stool
ERR	Egg reduction rate
ID	Identification number
ITT	Intention-to-treat
MDG	Millennium development goal
N (or n)	Sample size
NPV	Negative predictive value
NTD	Neglected tropical disease
OR	Odds ratio
PHL–IdC	Public Health Laboratory–Ivo de Carneri
PPV	Positive predictive value
SAF	Sodium acetate-acetic acid formalin
SD	Standard deviation
STH	Soil-transmitted helminth
Swiss TPH	Swiss Tropical and Public Health Institute
WHO	World Health Organization
ZAMREC	Zanzibar Medical Research and Ethics Committee

Chapter 1

General Introduction

General Introduction

1 Soil-transmitted helminthiasis

Intestinal nematode infections remain a public health challenge mainly in tropic and subtropics regions. Globally, an estimated 465 million people were infected with the intestinal nematode species *Trichuris trichiura* in 2010 [1]. Additionally, an estimated 819 and 439 million people were infected with *Ascaris lumbricoides* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*), respectively [1], and approximately 100 million people were infected with *Strongyloides stercoralis* [2]. Together these intestinal nematodes are known as soil-transmitted helminths and in total, they infect an estimated 1.5 billion people worldwide. The most affected are school-aged children living in the least developed settings, lacking clean water and sanitation facilities [3–12]. Infections with soil-transmitted helminths cause diarrhoea, abdominal cramps, general malaise, growth stunting, impaired memory and awareness, as well as reduced physical fitness [3,13–15]. The worldwide disease burden due to soil-transmitted helminths has been calculated to be as high as 5 million disability-adjusted life years (DALYs) [1]. Since soil-transmitted helminthiasis mainly affect the poorest and because the vast majority of DALYs are mostly lost due to morbidity, it is difficult to raise the attention of pharmaceutical companies and policy makers [3,16]. Therefore soil-transmitted helminthiasis belong to the so called neglected tropical diseases [17].

2 Life cycle

Soil-transmitted helminth eggs are eliminated in large numbers with human faeces where they reach the soil via open defecation (Figure 1). Within the soil, eggs of *T. trichiura* and *A. lumbricoides* as well as larvae of hookworm and *S. stercoralis* can develop until they

reach their infective stage. This is crucial for their reproduction since these parasites (with the exception of *S. stercoralis*) are not able to reproduce within the host [3,18].

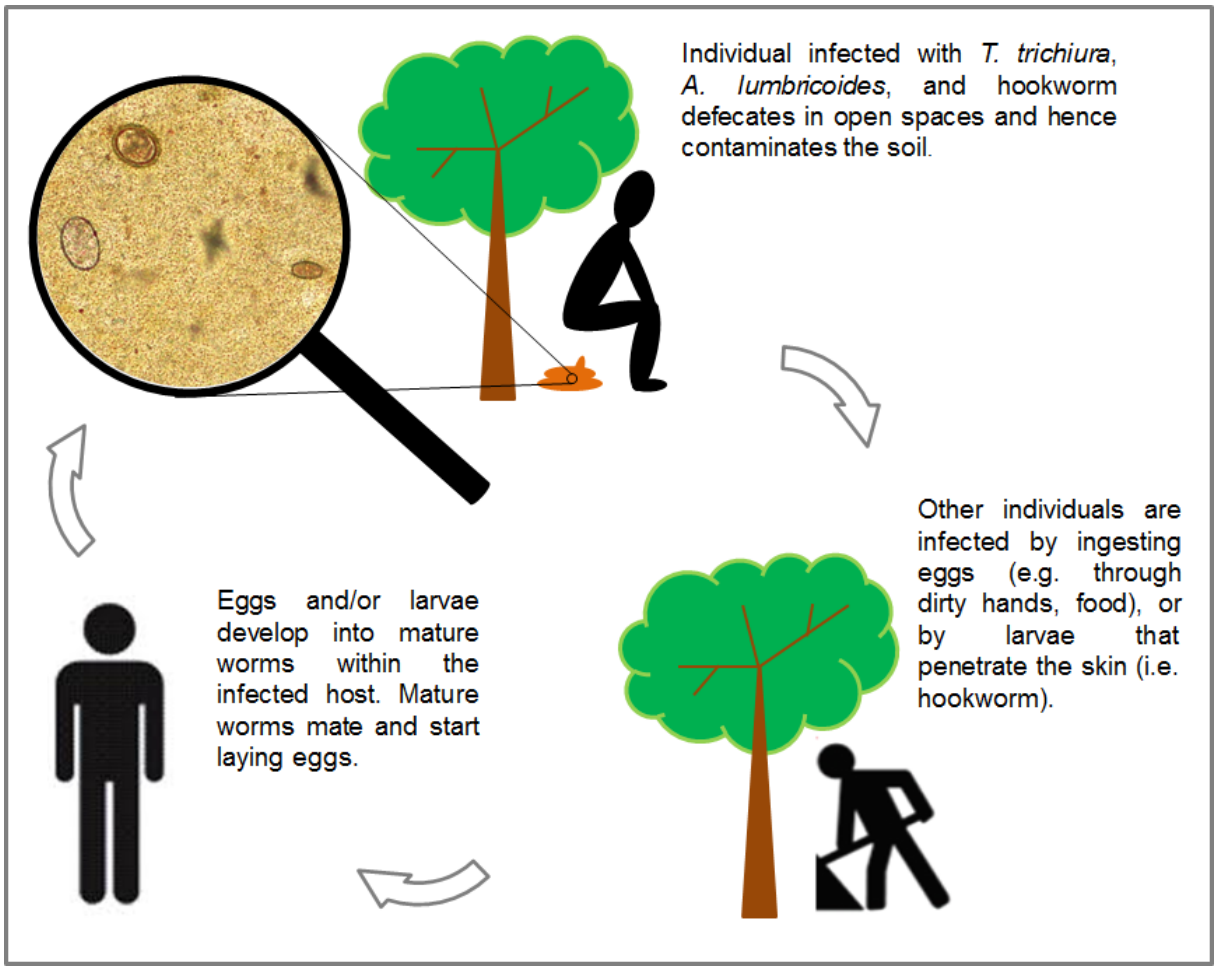

Figure 1. Life-cycle of soil-transmitted helminth infections. Adapted from [19]

T. trichiura has the most direct life cycle of the common soil-transmitted helminths. Its transmission occurs via ingestion of fully developed eggs [18]. This might happen by eating contaminated food or by oral contact with contaminated fingers, especially in smaller children, but also in areas where eating by hand is common. After swallowing *T. trichiura* eggs, the larvae moult three times, travel to the colon and burrow themselves into the epithelia. After 12 weeks, the development into a 30-50 mm long adult whipworm is completed and the female parasite starts producing approximately 3,000-5,000 eggs daily [3,18,20,21].

A. lumbricoides is transmitted via the intake of fully developed eggs as well. After ingestion of the eggs, larvae penetrate the intestinal mucosa and migrate extra-intestinally to the liver and lungs. Finally, via the epiglottis, they re-enter into the gastrointestinal tract and develop to mature worms. Adult worms are 150-400 mm long and start laying eggs 9-11 weeks post-infection. A single female worm can produce around 200,000 eggs per day [3,18,20,21].

Hookworm eggs hatch in the soil where they moult twice to become infectious third-stage larvae [18]. These larvae seek higher ground to enhance the chances of getting in contact with human skin and penetrate through it. They enter the host's afferent circulation via subcutaneous venules and lymphatic vessels. Subsequently, they become trapped in the pulmonary capillaries and enter the lungs, from where they pass over to the epiglottis and finally migrate into the gastrointestinal tract where the female worm produces eggs 5-9 weeks post-infection [18,21]. Adult hookworms are approximately 7-13 mm long. A female *A. duodenale* produces around 25,000 - 30,000 eggs daily, whereas a female *N. americanus* lays approximately 9,000 - 10,000 eggs per day [3,18,20,21]. Of note, *A. duodenale* is also orally infective in contrast to *N. americanus.* As for hookworm, free living infective third-stage larvae of *S. stercoralis* also invade through the skin. However, in contrast to the three most common soil-transmitted helminths, *S. stercoralis* infections can be prolonged by autoinfection [18,22,23].

3 Disease and symptoms

The severity of disease caused by infections with soil-transmitted helminths is directly related to the infection intensity which is usually expressed as eggs per gram (EPG) detected in one gram of stool [24]. At present there is still no clear evidence if light infections of *T. trichiura* and *A. lumbricoides* cause morbidity [1]. Moderate or heavy infections with *T. trichiura* increase the risk of obtaining *Trichuris* dysentery syndrome

(resulting in chronic dysentery and rectal prolapse), or a colitis [3,19]. Hookworm larvae can cause ground itch and a local rash might appear at the site of entry through the skin. The main symptoms of heavy hookworm infections are micronutrient deficiencies, including anaemia, and lack of proteins due to loss of blood [3,25–27]. During migration of *A. lumbricoides* larvae, antigens can cause a strong inflammatory response within the lungs. Further consequences of an infection with *A. lumbricoides* include: loss of appetite and reduced food uptake, lactose intolerance, vitamin A malabsorption, intestinal obstruction and hepatopancreatic ascariasis [3,19]. Common symptoms of soil-transmitted helminthiasis include diarrhoea, abdominal pain, general malaise and weakness. These symptoms may negatively affect the learning capacity of children but also the working capacity of adults [28,29]. In a study from Zanzibar, it was shown that school children showedhad increased growth rates after treatment with anthelminthics [30], while Yap and colleagues could demonstrate that *T. trichiura* infections are associated with lower physical fitness [15]. In general it is difficult to allocate an exact burden to soil-transmitted helminthiasis as infections are often chronic and subtle. Therefore the range of DALY`s lost annually varies from 4.7 up to 39 million [3,31].

4 Preventive chemotherapy

In the 1970s the first safe, orally active and highly efficacious anthelminthics were launched [32]. In the past years, mass disposal of anthelminthics mainly to school-aged children in endemic settings was implemented. This approach (termed “preventive chemotherapy”) is considered as the most cost-effective strategy to control soil-transmitted helminths, particularly in settings where they co-exists with other neglected tropical diseases [33]. The World Health Organization (WHO) recommends implementing annual preventive chemotherapy, without prior diagnosis, when soil-transmitted helminth

prevalence is between 20-50%, and bi-annual treatment when the prevalence exceeds 50% (Figure 2) [24].

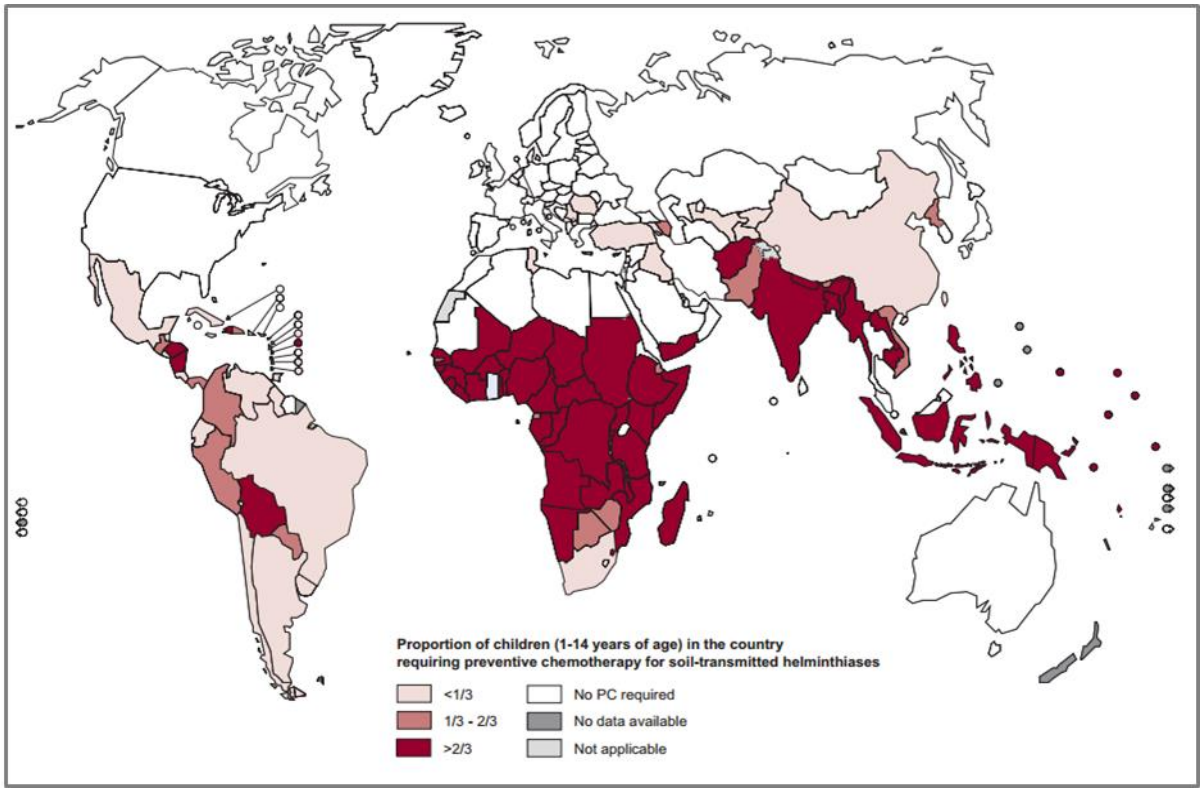

Figure 2. Proportion of children that require preventive chemotherapy (PC) by country as assessed by the World Health Organization in 2009 [19]**.**

The two benzimidazoles, albendazole and mebendazole, as well as pyrantel and levamisole are the four main treatments against soil-transmitted helminths, which are listed in the WHO essential drug list [34,35]. All of these drugs were developed between 1966 (pyrantel) and 1980 (albendazole). Today the cost of one dose of a generic tablet of albendazole (400 mg) or mebendazole (500 mg) is as low as US$ 0.02, or they are even donated by the manufacturer; hence they are widely used during mass drug administration campaigns [4,24,34,36]. Therefore drug resistant soil-transmitted helminths remains a huge threat [37–40] as it occurred already in the veterinary medicine [41]. Preventive chemotherapy is cost-effective and reduces soil-transmitted helminth infections [42,43]; for example in South America, the risk for being infected with soil-transmitted helminths were substantially reduced since 2005 [44]. However, as it is

difficult to achieve cure with a single dose of the current drugs and as reinfections occur quickly, the current goal of the WHO is not cure itself, but to reduce the rate of illness from infection with soil-transmitted helminths in school-age children to below a level that would be considered a public health problem (i.e., to reduce soil-transmitted helminth infections of moderate and high intensity among school-age children to <1%) [24]. While albendazole as well as mebendazole have high cure rates against *A. lumbricoides,* only albendazole reveals high efficacy against hookworm; for the treatment of *T. trichiura* both drugs have low efficacy, and henceforth, reaching the goal stated by the WHO seems unrealistic with the current standard drugs [45,46]. These results show the current need for new anthelminthic treatments, and yet, no drugs are in the development pipeline [32].

5 Promising drug alternatives

Two possible alternative treatments against *T. trichiura* and concomitant soil-transmitted helminth infections are mentioned here, as these drugs were extensively studied during this PhD thesis. The first compound is the 5-nitrothiazole derivate, nitazoxanide [47]. This drug was marketed for the treatment of intestinal protozoan infections but showed also very high cure rates against *A. lumbricoides, T. trichiura* and even against *A. duodenale* when the drug was given in multiple doses [48–51]. Furthermore adverse events were rare, mild and could most likely be attributed to the infection of the children and the dying of parasites [50]. Moreover, nitazoxanide had also high activity against pathogenic intestinal protozoa which are often neglected due to their difficult diagnosis [48,49,51,52]. Thus, treatment with nitazoxanide against soil-transmitted helminthiases could potentially also reduce morbidity attributed to intestinal protozoa. Laboratory studies at the Swiss Tropical and Public Health Institute (Swiss TPH) revealed that nitazoxanide has high activity *in vitro* against *T. muris* but lacks activity against *A. ceylanicum* [53]. *In vivo*

follow-up studies failed to confirm the promising activity of nitazoxanide against *T. muris* [54].

The second drug which showed high activity against *T. trichiura* is oxantel pamoate, a pyrimidine derivative which was introduced on the market in 1974 [55,56]. It was developed from pyrantel because of its activity against *T. trichiura* and belongs to the group of cholinergic agonists, which are fast acting anthelminthics [57]. These drugs "produce spastic paralysis of the nematode by selectively gating acetylcholine receptor ion-channels on nerve and muscle" [58]. Several exploratory studies confirmed the high efficacy of oxantel pamoate against *T. trichiura* having only minor side effects, comparable to the current standard drugs. Of note, oxantel pamoate is absorbed very poorly, which lowers the risk of systemic adverse events, comparable to the other standard drugs [59–61]. Lee and colleagues discovered in 1976 that 93.3% of patients were cured after treating them with 20 mg/kg of oxantel pamoate and that a lower dose of 15 mg/kg still showed high efficacy [62]. In the same year Garcia found that 10 out of 10 *T. trichiura*-infected individuals were cured after a single dose of 20 mg/kg oxantel pamoate [59]. Lee and colleagues (1976) monitored the effect of twice daily 10 mg/kg oxantel pamoate over a 3-day course and observed a large reduction in worm burden [63]. However, since drugs for treating soil-transmitted helminth infections are mostly administered in the framework of preventive chemotherapy programmes it is important to administer single doses of these drugs. Recent *in vitro* and *in vivo* tests found that oxantel pamoate is already active at much lower doses than the nowadays used drugs [64,65]. Regarding the current lack of effective treatments against *T. trichiura,* oxantel and nitazoxanide could become important alternative treatment regimens against *T. trichiura* and concomitant soil-transmitted helminth infections. The observed efficacies of nitazoxanide and oxantel pamoate are listed within Table 1.

Table 1: Cure rates end egg reduction rates of oxantel-pamoate and nitazoxanide against soil-transmitted helminth infections.

Drug tested	Number of patients	Dose	Cure rate	Egg reduction rate	Reference
Oxantel-pamoate	12-122	10 mg/kg 15 mg/kg 20 mg/kg 25 mg/kg	*T. trichiura*: 57-93%	*T. trichiura*: 90-96%	Lee et al., 1976 [63]
	10-26	10 mg/kg 15 mg/kg 20 mg/kg	*T. trichiura*: 77-100% *A. lumbricoides*: 0%	-	Garcia et al., 1976 [59]
Nitazoxanide	9-144	7.5 mg/kg (500 mg to adults, 200 mg to children under 12 years) every 12 hours for 3 consecutive days	*T. trichiura*: 56-78% *A. lumbricoides*: 48-100%	*T. trichiura*: 99.5-99.6% *A. lumbricoides*: 99.7-100%	Cabello et al., 1997 [48]
	29 *T.t* . -155 *A.l.*	500 mg to adults, 200 mg to children 4 to 11 years, and children 1 to 3 years 100 mg every 12 hours for 3 consecutive days	*T. trichiura*: 86% *A. lumbricoides*: 95% Hookworm: 96% *S. stercoralis*: 94%	-	Abaza et al., 1998 [49]
	18 *T.t* . - 28 *A.l.*	200 mg to children 4 to 11 years, and children 1 to 3 years 100 mg every 12 hours for 3 consecutive days	*T. trichiura*: 89% *A. lumbricoides*: 89% *S. stercoralis*: 83%	*A. lumbricoides*: 99.9% *T. trichiura*: 99.8%	Ortiz et al., 2002 [50]

6 Drug combinations

The lack of concerted efforts in anthelminthic for human use, combined with the growing concerns of emerging drug resistance in the face of large-scale distribution of benzimidazoles and the low cure rates observed in the treatment of *T. trichiura*, implicate an urgent need to develop safe and effective new anthelminthic drugs. Combinations of existing drugs represents an important strategy in the fight against soil-transmitted helminth infections [32,39,66]. Mathematical modelling revealed that the likelihood of anthelminthic resistance development is significantly delayed when drug combinations are administered [67]. Drug combinations are widely used in the treatment of malaria, tuberculosis or HIV [68–70]. Interestingly though, only few human clinical trials have tested combinations of approved anthelminthic drugs. For example ivermectin, the current drug of choice against *S. stercoralis*, was combined with either albendazole or mebendazole and therefore significantly enhanced the efficacy of these drugs against *T. trichiura* and concomitant soil-transmitted helminths [39,41,71–73]. Other drugs which were tested in combinations with mebendazole or albendazole are pyrantel, levamisole and diethylcarbamazine. However, these combinations had only small or no success in increasing cure rates [32,66,74,75]. Combining the two benzimidazoles albendazole and mebendazole resulted in promising cure rates against *T. trichiura* [76]. Because of the narrow profile of activity of oxantel pamoate, it was soon combined with pyrantel pamoate [59,61,63,77–82]. Several studies examined the effect of an oxantel-pyrantel pamoate combinationn against soil-transmitted helminths and compared this regimen against the standard single drugs. Interestingly, to our knowledge, an oxantel pamoate-albendazole combination has not been studied before. One can assume that an oxantel pamoate-albendazole combination has a higher efficacy against hookworm given the superior activity profile of albendazole compared to pyrantel pamoate [45,61]. Hence, one

objective of this PhD thesis was to assess the efficacy of an albendazole-oxantel pamoate combination against *T. trichiura* and concomitant soil-transmitted helminth infections.

7 Limitations of preventive chemotherapy

Besides the observations that preventive chemotherapy reduces morbidity due to soil-transmitted helminth infections and that it is supposed to be cost-effective [42,43], there are a number of limitations which need to be mentioned [11]. The current control strategy focuses largely on school-aged children [33]. Studies on different age groups (i.e. adults or pre-school children) indicate that these individuals are similarly infected as school-aged children [83–88]. The WHO actually recommends the inclusion of the following individuals within control programs: pre-school children, school-aged children, woman of childbearing age (including pregnant women in the second and third trimesters and breast feeding women), and adults in certain high risk occupations [24]. How other individuals besides school-aged children should be reached for mass drug administration remains, to some extent, unclear. Further, it is understandable that mass drug administration is not any more recommended when prevalence is low (i.e. below 20%). The approach how people in endemic settings with prevalence below 20% receive treatment remains vague [11,19]. Another limitation of the current preventive chemotherapy programs is the efficacy of the current drugs. As stated before, albendazole and mebendazole have excellent efficacy against *A. lumbricoides*, while only albendazole achieves high cure rates against hookworm [45]. The cure rate of the two standard drugs against *T. tricuiura* was assessed in a meta-analysis in 2008 conducted by Keiser and Utzinger. The calculated cure rate for albendazole and mebendazole was 28% and 36%, respectively [45]. Nevertheless, the WHO calls the current drugs “effective” referring to the egg reduction rates against *T. trichiura* (i.e. egg reduction rate range for albendazole 53-89%; egg reduction range for mebendazole, 81-90%) [19]. Keiser and Utzinger who are cited in the corresponding

WHO-report [19] found a substantially lower egg reduction range for albendazole (i.e. 0-90%) and a similar range for mebendazole (i.e. 81-93%) which was based on only 3 studies [45]. It is striking that recent trials report markedly lower egg reduction rates for albendazole and mebendazole against *T. trichiura* [39,76,89,90]. Furthermore, reported egg reduction rates are based on geometric means which are likely to overestimate the efficacy [91]. A third limitation of the current mass drug administration programs is that it is not sustainable. Indeed, the two benzimidazoles can significantly reduce infection intensities and cure individuals to some extent. However, a recent meta-analysis conducted by Jia and colleagues demonstrated that prevalence after 12 month are back to the same levels as before treatment especially for *A. lumbrcoides* and *T. trichiura* [92]. With some delay, infection intensities can also reach the same levels as before treatment [92]. Therefore, the long term effect is dependent on the regular administration of preventive chemotherapy. In case those programs are ceased, or resistance to the available drugs would develop, prevalence and infection intensities would rapidly reach pre-treatment levels [11,92–96]. But also with mass drug administration programs in place, prevalence in certain settings remains high. For example in Zanzibar, Tanzania - the setting where the three clinical trials from this PhD thesis were conducted – prevalence for all soil-transmitted helminths remain high in a number of hotspots after decades of mass drug administration [39,97–101]. Hence, for an effective control of soil-transmitted helminth infections, preventive chemotherapy should be accompanied by water, sanitation, and hygiene (WASH) programs [11].

8 Water, sanitation and hygiene

To prevent and control soil-transmitted helminth infections, health education, sanitation facilities (i.e. facilities for the safe disposal of faeces), and safe water supply are required alongside to preventive chemotherapy [3,11,102]. Already 100 years ago, the Rockefeller

Sanitary Commission for the Eradication of Hookworm Disease (founded in 1909) had great success in the struggle against hookworm infections with implementing sanitation facilities together with health education and treatment [103,104]. One reason for the success of the Rockefeller Foundation was that they recognised early the importance of sanitation facilities and hence stated already more than 70 years ago that "Cure alone is almost useless in stamping out hookworm disease, because the patient can go out and immediately pick up more hookworms. The cure should be accompanied by a sanitation campaign for the prevention of soil pollution" [56]. Additionally, access to safe water, which can be used to clean hands and vegetables, is imperative. However, it is important that these water sources and latrines are well maintained and don`t become so called "hookworm-traps" [11,105,106]. Wearing shoes can partially protect from hookworm and *S. stercoralis* infections since the feet are the main entry site for the infectious larvae [3,101,104].

In 2012 an estimated 780 million and 2.5 billion people lacked access to adequate drinking water and sanitation, respectively [107]. The United Nations millennium development goal (MDG) 7c aims to half the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015 [108]. Recognizing sanitation and water as a basic human right and therefore improving access to safe water and sanitation facilities will certainly result in major health benefits such as lower incidences of diarrheal episodes and infant mortality [109,110]. While the goal for access to drinking water will probably be reached by 2015, it is unlikely that the target pertaining to sanitation will be met [107]. It is assumed that sanitation facilities will aid to permanently eliminate soil-transmitted helminth infections in the context of economic development only [83]. However, poverty does not inevitably mean that people lack hygiene and proper sanitation [111], hence WASH programs should be promoted for soil-transmitted helminth control and go hand in hand with preventive chemotherapy. To implement WASH into current soil-transmitted

helminth control programs Campbell and colleagues [11] suggest that (i) the WHO guidelines [19] should include clear statements about which type of sanitation and water facilities and what kind of health education is recommended; and (ii) that collecting indicators pertaining to the MDG could be implemented into soil-transmitted helminth control (i.e. proportion of population using improved sanitation facilities and improved drinking water sources) [112]. Community-Led Total Sanitation (CLTS) could be a possibility how to implement WASH into the WHO guidelines for soil-transmitted helminth control programs [113–115]. The aim of CLTS is that communities actively analyse their defecation behaviour and the consequences, and to build toilets using local designs rather than given engineer designs. Therefore CLTS should only trigger discussions and later information can be provided as how to proceed with facility construction. This is an important difference from early projects that were rendered obsolete: CLTS does not use a top-down design but rather, the people should "decide for themselves" [114]. Further, collecting data on WASH indicators within the mass drug administration programs would generate data on the protective effect of WASH against soil-transmitted helminth infections (e.g. WASH coverage needed in a community to delay or halt re-infection). This would help to overcome the current lack of evidence on how effective and cost-effective WASH interventions are [116,117]. A recent study showed that sanitation facilities are highly cost-effective, however, this study was mostly based on assumptions and concluded that further evidence would be required [116]. Thus, to emphasize the importance of sanitation and access to safe water, an additional goal of this PhD thesis was to evaluate the impact of sanitation facilities and water treatment on soil-transmitted helminth infections and intestinal protozoa infections by conducting two systematic reviews and meta-analysis.

9 Diagnostics

To assess efficacy of drugs within clinical trials, to treat cases accurately and effectively, and to be able to carefully monitor the development of anthelminthic drug resistance, it is important to have a reliable tool for the diagnosis of soil-transmitted helminth infections. In epidemiological surveys, the common tool for detection of *A. lumbricoides, T. trichiura* and hookworm is the Kato-Katz technique which is also recommended by the WHO [19,118]. This copromicroscopic diagnosis examines an average of 41.7 mg stool and enables quantification of infection intensities by counting the eggs for each species (Figure 3). From these counts, the eggs per one gram of stool can be calculated, which allows for the classification of the infection intensities defined by the WHO (i.e. light, moderate, heavy) [19,24]. In general, the Kato-Katz technique shows a reasonable sensitivity when infection intensities are high. However, it becomes insensitive in the diagnosis of low-intensity infections [119–121]. Sensitivity of the Kato-Katz method can be enhanced through multiple examinations either from the same stool sample or ideally from multiple stool samples [119,121–125].

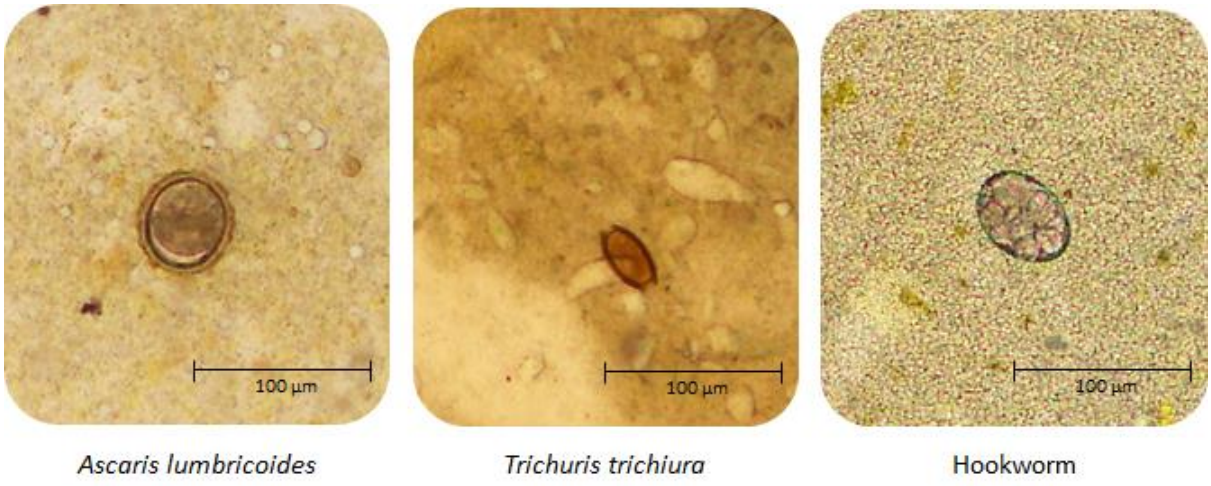

Figure 3: Eggs from the three most common soil-transmitted helminth species. Pictures taken in the Public Health Laboratory – Ivo de Carneri, Pemba, Tanzania, using the Kato-Katz technique.

For the diagnosis of *S. stercoralis* the Baermann technique as well as the Koga-agar plate are commonly used standard methods [126,127]. Both methods have only low to

moderate sensitivity; therefore a combination of both methods examining multiple stool samples is recommended [128]. Since these diagnostic techniques are rarely used, only few epidemiological data about *S. stercoralis* is available. Hence, *S. stercoralis* is nowadays often termed the most neglected of the neglected tropical diseases [2].

The FLOTAC technique showed significantly higher sensitivity than single or multiple Kato-Katz thick smears for the diagnosis of the three most common soil-transmitted helminth infections [100,121,123,129]. In particular, the FLOTAC is highly sensitive in diagnosing low-intensity helminth infections. One possible reason for the higher sensitivity of the FLOTAC method is the higher amount of faeces that is used in the FLOTAC technique (1 g in FLOTAC versus only 41.7 mg in a single Kato-Katz thick smear). The FLOTAC technique takes advantage of the fact that parasitic elements float to the top as soon as flotation takes place in a centrifuge. Subsequently it is relatively straightforward to separate the parasitic elements from the faecal suspension with a horizontal cut [130]. A disadvantage of the FLOTAC technique is that egg counts are significantly lower compared to the Kato-Katz technique [100,129,131]. Further, the FLOTAC technique requires more equipment, is more time consuming and consequently also more expensive than the Kato-Katz method [132,133]. An advanced version of the FLOTAC technique is the recently developed mini-FLOTAC. The mini-FLOTAC revealed also high sensitivity and further simplifies the procedure of preparation. Thus it is less complicated than the original FLOTAC technique [134–136].

The ether-concentration method is often used in European reference laboratories for the diagnosis of intestinal protozoa and soil-transmitted helminth infections [137,138]. Similarly to the FLOTAC technique, the ether-concentration method has the advantage that it can detect soil-transmitted helminth eggs within a large amount of a fixed stool sample [139]. In a centrifugation step, the parasitic eggs are separated from the debris. After decanting the supernatant the sediment can be examined for soil-transmitted

helminth eggs [138–140]. Two studies revealed that multiple Kato-Katz thick smears with fresh stool samples have similar sensitivity compared to one examination with the ether concentration method using a preserved stool sample for the detection of soil-transmitted helminth eggs [8,123]. A disadvantage of the ether-concentration technique is that it is only semi-quantitative, hence no eggs per gram of stools and no egg reduction rates can be calculated [138,140].

As many infected people live in rural underserviced areas, on-spot diagnosis is often not possible, as microscopes and electricity are lacking [3]. An objective of this PhD thesis was to compare the ether-concentration method with the current standard method using Kato-Katz thick smears.

10 Intestinal protozoa

Intestinal protozoa infections are transmitted over the faecal-oral route and also share many other characteristics with soil-transmitted helminths [141,142]. Drinking contaminated water is the most common source of infection with intestinal protozoa; consequently sanitation facilities as well as safe drinking water are important preventive interventions. Infections with intestinal protozoa (i.e. *Cryptosporidium* spp., *Entamoeba histolytica, Giardia intestinalis, Blastocystis hominis*) are responsible for considerable gastrointestinal morbidity, malnutrition, and mortality worldwide [143–146]. For example, amoebiasis, caused by *E. histolytica*, is responsible for an estimated 100,000 deaths per year; hence, amoebiasis is one of the deadliest parasitic infections [143,145]. Epidemiological data on intestinal protozoa is generally scarce [52]. For example on Pemba Island, to our knowledge, the prevalence of intestinal protozoa has only been assessed twice and dates back to 1984 and 1992 [147,148]. As intestinal protozoa share many characteristics with soil-transmitted helminths and since we tested a drug against intestinal protozoa (nitazoxanide) within a randomised controlled trial, the prevalence of

intestinal protozoa at two schools on Pemba islands was evaluated and the efficacy of nitazoxanide and albendazole calculated.

11 Study sites

The field work of this PhD thesis was conducted on Pemba Island, belonging to the Zanzibar archipelago in Tanzania. The Zanzibar archipelago consists of two main islands, Pemba and the southern, more touristic island Unguja, as well as numerous smaller islands. In Europe and the United States of America, the main island Unguja is often termed Zanzibar (Figure 4). The climate of the Zanzibar archipelago is humid and tropical with an average temperature of 27°C. Less than 40% of the inhabitants have sanitation facilities and the human density is high [149]. The regular administration of anthelminthic drugs has been in place since several decades, yet the prevalence of soil-transmitted helminths remains high [39,97,98,101]. As the current standard drugs are in use for many decades in many endemic settings, similarly to the Zanzibar archipelago, these islands with its persistently high prevalence yet well-established infrastructure are an ideal setting for clinical trials and other epidemiological studies. The laboratory work on Pemba Island was performed at the Public Health Laboratory – Ivo de Carneri, located close to the main city Chake Chake. Each year from 2011-2013 a randomised controlled trial was conducted. For the first trial in 2011, children from the school of Wawi (geographical coordinates; 5°15’22’’S latitude, 39°47’28’’E longitude) and Al-Sadik (5°15’42’’S, 39°48’25’’E) were enrolled. The randomised controlled trials in 2012 and 2013 were both conducted at Mchangamdogo school (5°7’29’’ S, 39°48’34’’ E) and Shungi school (5°16’14’’ S, 39°44’16’’ E). The locations of all four schools and the Public Health Laboratory – Ivo de Carneri are marked in Figure 4.

Figure 4: Map of Pemba Island with the study sites (colored dots) and an overview localization of the island within the Indian Ocean. (Sources: http://news.bbc.co.uk/2/hi/africa/4167807.stm; http://omani.canalblog.com/archives/2006/01/10/1179686.html, accessed 8. May 2014)

12 Aim and objectives

The current standard treatments used in preventive chemotherapy lack efficacy against *T. trichiura.* The main goal of this PhD thesis was to test promising alternative drugs (i.e. nitazoxanide, oxantel-pamoate) alone and in combination with albendazole against *T. trichiura* and concomitant soil-transmitted helminth infections. Further goals were to compare the ether concentration method with the Kato-Katz method and to evaluate the impact of sanitation facilities on infection with soil-transmitted helminths and intestinal protozoa. The following specific objectives were linked to these goals:

1. **Efficacy of albendazole-nitazoxanide**: To assess the efficacy and safety of (i) a nitazoxanide-albendazole combination against *T. trichiura* and concomitant soil-transmitted helminth infections among school aged children on Pemba Island, Tanzania (Chapter 2a). Additionally, to evaluate the prevalence of intestinal protozoa on Pemba Island and the efficacy of the different treatment arms on intestinal protozoa (chapter 2b).

2. **Efficacy of albendazole-oxantel pamoate**: To study the efficacy and safety of an albendazole-oxantel pamoate combination against *T. trichiura* and concomitant soil-transmitted helminth infections (chapter 3).

3. **Efficacy of three promising drug combinations**: To assess the efficacy and safety of (i) albendazole plus ivermectin; (ii) albendazole plus mebendazole and; (iii) albendazole plus oxantel pamoate against *T. trichiura* and concomitant soil-transmitted helminth infections. The standard drug mebendazole was used as a comparator within a fourth treatment arm (chapter 4).

4. **Compare different diagnostic techniques**: To evaluate the sensitivity of the ether-concentration technique compared to the Kato-Katz method for the diagnosis of soil-transmitted helminths, and to further appraise the impact of the diagnostic technique on cure rates within a randomised controlled trial (chapter 5a).

5. **Effect of sanitation facilities and water treatment**: To conduct a systematic review and meta-analysis about the impact of sanitation facilities (i.e. facilities for the safe disposal of faeces) on infections with soil-transmitted helminths (chapter 6a). Further to assess the effect of sanitation facilities and water treatment against intestinal protozoa infection (chapter 6b).

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Chapter 2

Efficacy of nitazoxanide against soil-transmitted helminth and intestinal protozoa infections

Chapter 2a

Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against *Trichuris trichiura* infection: a randomized controlled trial

Chapter 2b

Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide

Chapter 2a

Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against *Trichuris trichiura* infection: a randomized controlled trial

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Efficacy and Safety of Nitazoxanide, Albendazole, and Nitazoxanide-Albendazole against *Trichuris trichiura* Infection: A Randomized Controlled Trial

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Abstract

Background: The currently used anthelmintic drugs, in single oral application, have low efficacy against *Trichuris trichiura* infection, and hence novel anthelmintic drugs are needed. Nitazoxanide has been suggested as potential drug candidate.

Methodology: The efficacy and safety of a single oral dose of nitazoxanide (1,000 mg), or albendazole (400 mg), and a nitazoxanide-albendazole combination (1,000 mg–400 mg), with each drug administered separately on two consecutive days, were assessed in a double-blind, randomized, placebo-controlled trial in two schools on Pemba, Tanzania. Cure and egg reduction rates were calculated by per-protocol analysis and by available case analysis. Adverse events were assessed and graded before treatment and four times after treatment.

Principal Findings: Complete data for the per-protocol analysis were available from 533 *T. trichiura*-positive children. Cure rates against *T. trichiura* were low regardless of the treatment (nitazoxanide-albendazole, 16.0%; albendazole, 14.5%; and nitazoxanide, 6.6%). Egg reduction rates were 54.9% for the nitazoxanide-albendazole combination, 45.6% for single albendazole, and 13.4% for single nitazoxanide. Similar cure and egg reduction rates were calculated using the available case analysis. Children receiving nitazoxanide had significantly more adverse events compared to placebo recipients. Most of the adverse events were mild and had resolved within 24 hours posttreatment.

Conclusions/Significance: Nitazoxanide shows no effect on *T. trichiura* infection. The low efficacy of albendazole against *T. trichiura* in the current setting characterized by high anthelmintic drug pressure is confirmed. There is a pressing need to develop new anthelmintics against trichuriasis.

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Introduction

Infections with the soil-transmitted helminths, *Ascaris lumbricoides*, *Trichuris trichiura*, and the two hookworm species *Ancylostoma duodenale* and *Necator americanus*, are the most common infections of humans, causing an estimated global burden of 39 million disability-adjusted life years lost (DALYs) [1–3]. Globally more than 5 billion people are at risk and at least 1 billion people are currently infected with one or several of these nematodes [1,3,4]. On Pemba, soil-transmitted helminth infections remain of considerable public health importance with particularly high prevalences observed for *T. trichiura* and hookworm [5].

Preventive chemotherapy targeting at-risk communities (i.e., school-aged children) is in place in many countries. These programs aim at morbidity control, and hence intensity of infection is kept below a threshold of disease [6]. The two benzimidazoles, albendazole and mebendazole, as well as pyrantel pamoate and levamisole are recommended drugs against infections with soil-transmitted helminths [7,8]. These drugs have been widely used as they were developed and put on the market between 1966 (pyrantel pamoate) and 1980 (albendazole) [9]. The four drugs exhibit distinct differences in their therapeutic profile, with the exception of considerably high efficacy of all drugs against *A. lumbricoides*. For the treatment of hookworm infection, only albendazole achieves satisfactory cure rates when administered as a single oral dose. Of particular concern is the situation for *T. trichiura*; single doses of mebendazole show the highest cure rates, but these are usually low or only moderate (<50%) [10]. The

Author Summary

More than 5 billion people are at risk of infection with one of the three most common intestinal worms, the roundworm *Ascaris lumbricoides*, the whipworm *Trichuris trichiura*, and two different kinds of hookworms. The global strategy to control these intestinal worm infections is through the regular administration of deworming drugs to school-aged children (albendazole, 400 mg; mebendazole, 500 mg). However, especially against *T. trichiura*, a low treatment response is observed with single doses of both drugs. We tested the antiprotozoal drug nitazoxanide, which had shown promising trichuricidal properties in *in vitro* experiments. A randomized controlled trial was carried out on the island of Pemba in Tanzania. Four treatment arms were included: (i) single albendazole (400 mg), (ii) single nitazoxanide (1,000 mg), (iii) nitazoxanide-albendazole combination (1,000 mg–400 mg) with each drug given separately on two consecutive days, and (iv) placebo. Children were asked for adverse events at several time points after treatment. Nitazoxanide showed no ability to cure *T. trichiura*-infected children and caused significantly more mild adverse events than placebo. Albendazole and the nitazoxanide-albendazole combination showed only a minimal effect against *T. trichiura*. Our results emphasize the urgent need to develop new, safe, and effective anthelmintic drugs against *T. trichiura*.

pressing need for developing new anthelmintic drugs, particularly for *T. trichiura*, cannot be emphasized enough. In addition, the therapeutically useful life span of these drugs is endangered should resistance develop and start to spread [11–13].

The antiprotozoal drug nitazoxanide, which is marketed for the treatment of intestinal parasitic infections (i.e., *Cryptosporidium parvum* and *Giardia intestinalis*) has been reported to also have trichuricidal properties [14,15]. Silbereisen and colleagues recently showed that nitazoxanide is highly active against *Trichuris muris in vitro* [16]. In clinical trials high cure rates were reported against *T. trichiura* as well as *A. lumbricoides* and hookworm, following multiple doses (200 mg for children aged below 12 years and 500 mg for patients aged 12 years and above, twice daily for 3 days) of nitazoxanide [17–19]. Hence, nitazoxanide has been listed as a potential drug candidate for human-soil transmitted helminthiasis and further research was suggested [20].

We report the findings of a randomized, double-blind, placebo-controlled trial, which specifically assessed the efficacy and safety of nitazoxanide given as a single dose (1,000 mg) in the treatment of *T. trichiura* in school-aged children on Pemba, Tanzania. The standard treatment with albendazole (400 mg) served as a benchmark. In addition, one group of children was given nitazoxanide (1,000 mg) on the first day and albendazole (400 mg) on the following day, in order to evaluate if an enhanced efficacy would be observed following this combination chemotherapy. A fourth group of children received placebo (the treatment groups are summarized in Figure 1). Since *A. lumbricoides* and hookworm coexist in the current setting, outcomes on these nematodes are also reported.

Methods

Ethics Statement

Ethical clearance was obtained from the ethics committee of Basel, Switzerland (EKBB, reference no. 225/10) and from the Ministry of Health and Social Welfare in Zanzibar (ZAMREC, reference no. 0001/010). The study is registered at Current Controlled Trials (ISRCTN08336605). Written informed consent was acquired from the children's parents or legal guardians to participate in this trial. Children assented orally. It was emphasized that study participation would be voluntary and withdrawal possible at any time.

Study Setting

Our trial was carried out in June and July 2011 on Pemba, one of the major islands in the Zanzibar archipelago, which belongs to Tanzania. The schools of Wawi (geographical coordinates; 5°15′22″S latitude, 39°47′28″E longitude) and Al-Sadik (5°15′42″S, 39°48′25″E), both located less than 10 km from Chake Chake, the main town of Pemba (5°14′45″S, 39°45′00″E, ~22,000 inhabitants), were selected. In the school year 2010/2011, a total of 1,100 children were registered in Wawi and 437 in Al-Sadik. Both schools were easily accessible by car from the Public Health Laboratory–Ivo de Carneri (PHL–IdC) in Chake Chake.

Sample Size Calculation

To determine the sample size, we assumed a cure rate of 28% for single oral albendazole against *T. trichiura* based on data from a meta-analysis [21]. The efficacy of single oral nitazoxanide against *T. trichiura* is not known. Therefore we assumed that it would be at least similar to that of albendazole (20–30%). Moreover, we hypothesized that an albendazole-nitazoxanide combination would achieve a considerably higher cure rate (50%). Monte Carlo simulations (imperfect test with a sensitivity of 90%) computed a sample size of 95 *T. trichiura*-infected individuals in each arm to detect a difference of single medication treatments *versus* both, the placebo group and the albendazole-nitazoxanide group at a significance level of 5% with 80% power. Allowing for drop outs and assuming an overall *T. trichiura* prevalence of 80%, we targeted approximately 125 children per treatment arm for the baseline screening.

Study Flow and Procedures

Before the onset of the study, the headmasters of Wawi and Al-Sadik were asked for permission to carry out the trial at their schools. The parents of the children were invited to the schools, so that they could be explained the purpose and procedures of the study, including potential risks and benefits. Questions could be asked in a discussion round and clarification was given to parents.

At the first day of enrolment, all children attending standards one to five received an empty stool container together with a consent form; both labeled with unique identification numbers (ID). After recording their name, sex, age, and school grade, children were invited to return the container with a fresh sample of the next morning stool together with the signed consent form. Filled stool containers and signed consent forms were collected from the children and a new empty container was handed out for collection of a second stool sample on the following day.

All children who returned a signed informed consent and had two stool samples were assigned to one of the four treatment groups, regardless of their helminths infection status. Before drug administration, all children were examined by a physician. Exclusion criteria were: (i) presence of any abnormal medical condition, judged by the study physician; (ii) history of acute or severe chronic disease (cancer, diabetes, chronic heart, liver, or renal disease); and (iii) recent use of anthelmintic drugs (within past 4 weeks). Additionally, the weight and height of all children were measured and if there was any indication of fever, axillary temperature was taken using a digital thermometer.

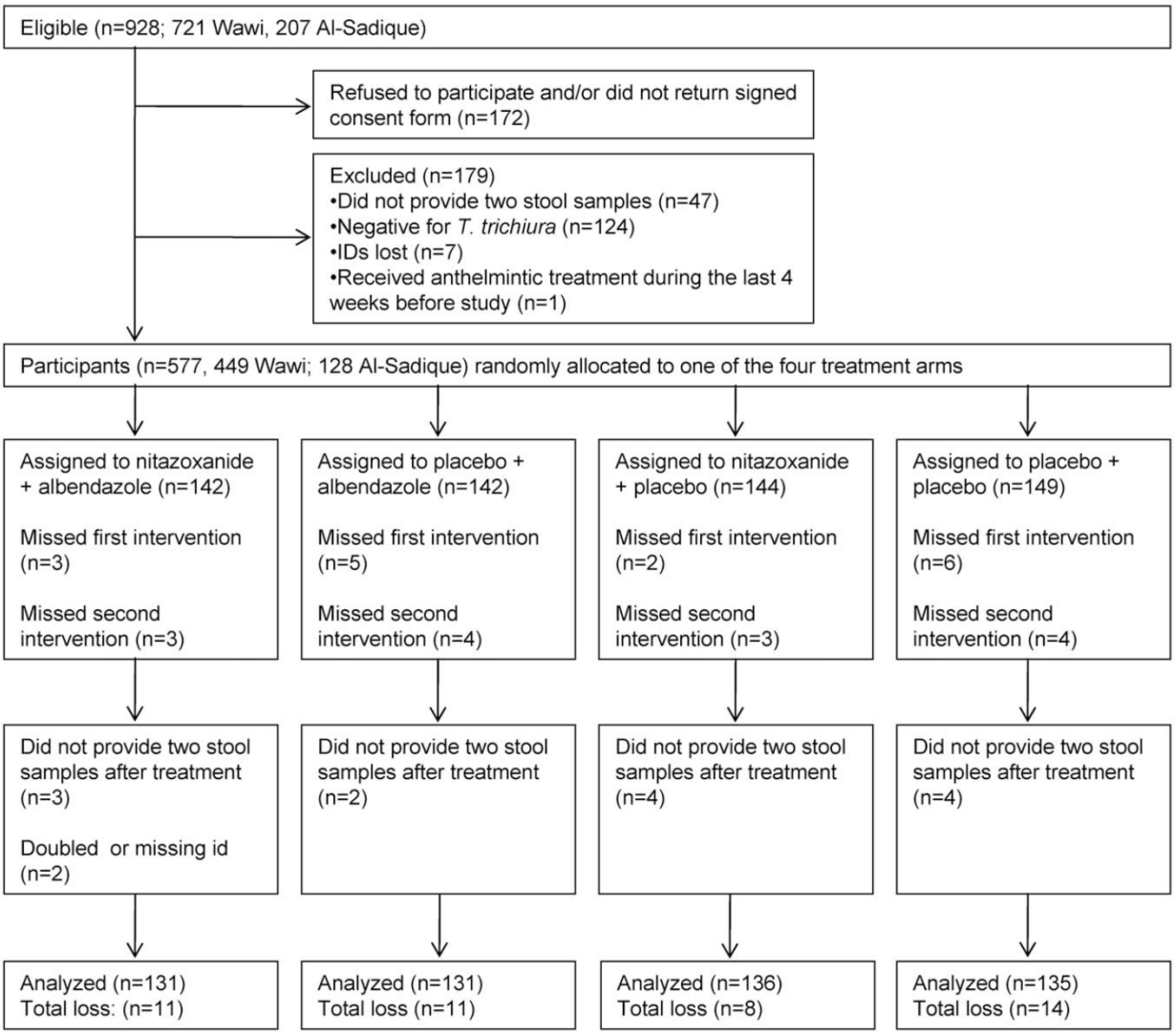

Figure 1. Flow diagram illustrating the compliance in the randomized controlled trial. Flow diagram of the randomized, placebo-controlled trial assessing the efficacy of albendazole, nitazoxanide, and albendazole combined with nitazoxanide, administered separately on two consecutive days, in the treatment of *Trichuris trichiura* infections.
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Three weeks posttreatment, children were asked again for two stool samples collected over consecutive days to determine the efficacy of the different treatments. At the end of the study, all children who were still infected with soil-transmitted helminths were offered a dose of albendazole (400 mg) following national guidelines [7,8].

Randomization, Treatment, and Adverse Events

An independent statistician created a randomization code assigning to each ID a number from 1–4, representing the four treatment arms: (i) nitazoxanide (1,000 mg) plus albendazole (400 mg); (ii) nitazoxanide-matching placebo plus albendazole (400 mg); (iii) nitazoxanide (1,000 mg) plus albendazole-matching placebo; and (iv) two placebos. For blinding purposes, the tablets were packed before treatment into small plastic bags, labeled with the unique IDs. Nitazoxanide (Alinia®) and albendazole (Zentel®) tablets were the products of Romark and GlaxoSmithKline, respectively. Placebos were produced at the Department of Pharmaceutical Sciences, University of Basel by one of the authors (R.A.). During the trial all drugs were stored at room temperature, not exceeding 25°C. Since interactions between nitazoxanide and albendazole have not been studied before, the two drugs were administered on two consecutive days. Hence, on the first day of treatment, children received two tablets of nitazoxanide (500 mg each) or two nitazoxanide-matching placebos. On the second day, children received one tablet of albendazole (400 mg) or an albendazole-matching placebo. Since the trial was double-blinded, neither the child, nor the person giving the treatment knew to which treatment arm the child was allocated to.

Before treatment, children were asked if they suffer from any adverse events. Drugs were then administered with a cup of water and each child received a small snack. Three hours after treatment, adverse events were actively assessed by interviewing

each child. On the following day, before receiving the second treatment, adverse events were investigated again. The same procedures were repeated 3 and 24 hours after the second treatment.

Parasitological Analysis

Stool samples were transferred to PHL–IdC. Duplicate Kato-Katz thick smears, using 41.7 mg templates, were prepared from each stool sample [22]. Kato-Katz thick smears were examined under a microscope by experienced laboratory technicians within 20–40 min after preparation, as recommended to avoid over clearing of hookworm eggs [23]. All hookworm eggs were counted. Subsequently, the slides were re-examined for *T. trichiura* and *A. lumbricoides*, with parasite eggs counted and recorded separately. To ensure high quality of the diagnosis, 10% of the slides, selected at random, were re-examined. In cases of discordant results, slides were read a third time and results discussed until agreement was reached.

Statistical Analysis

Parasitological data and reported adverse events were double entered into an Excel spreadsheet and cross-checked. In case of discrepancy, the original files were consulted to correct the data entry. All statistical analyses were performed with Stata 10.1 software (StataCorp).

Cure rates were calculated as the proportion of egg-positive children at baseline, who became egg-negative after treatment. Egg counts from the four Kato-Katz thick smears were added up for each species and multiplied by a factor 6 and expressed as eggs per gram of stool (EPG). Infection intensity was classified using pre-defined cut-offs by the World Health Organization (WHO; *T. trichiura* light, 1–999 EPG; moderate, 1,000–9,999; and heavy, ≥10,000) [24]. Differences among treatment arms concerning cure rates and observed adverse events were analyzed with logistic regressions.

Geometric mean egg counts were calculated for the different treatment arms before and after drug intervention to calculate the respective egg reduction rates (ERR). We also calculated the average dose (mg/kg) of each drug that the children received per treatment arm and analyzed with a logistic regression if the dose of treatment had an influence on the cure rates.

We used two different types of analyses: (i) per-protocol analysis, including only those children who had complete data records (quadruplicate Kato-Katz results before and after treatment and being treated); and (ii) available case analysis (which is sometimes erroneously referred to as an intention-to-treat analysis [25]) based on the treatment intent, hence analyzing data from all individuals who were assigned to one of the four treatment arms and had primary outcome data. A greater emphasis is given in our manuscript on the per-protocol analysis (available case results summarized in Table S1) since a bias might have been introduced in the available case analysis given that children absent on the first treatment day (nitazoxanide) were shifted to a later starting treatment period, while this was not possible for children missing the second treatment day (albendazole).

Results

Adherence

From the 928 children invited to participate in the study, 172 refused to participate or did not return a signed consent form. Another 47 children failed to provide two stool samples and from seven children the IDs on the stool samples were lost and hence they had to be excluded from the trial. One child received anthelmintic treatment less than 4 weeks before the onset of our trial and was therefore excluded. The remaining 701 children (549 from Wawi and 152 from Al-Sadik) were randomly assigned to one of the four treatment arms independently of their parasitological status. Of these, 124 were *T. trichiura*-negative and therefore excluded from the final analysis (Figure 1). Fifteen children missed the first treatment, and 14 children were absent on the second day of treatment. At follow-up, 13 children provided no or only a single stool sample and two children were excluded due to other reasons. In total 44 *T. trichiura*-infected children were lost during treatment and follow-up. Hence, 533 children were included in the per-protocol analysis. The loss of participants was distributed equally over the different treatment arms; the double placebo group was characterized by loss of the most participants (n = 14). Of the 29 participants not receiving treatment, all 14 who missed the second treatment provided primary end point data and could therefore be followed up; hence 547 children were included in the available case analysis.

Baseline Characteristics

Most of the 701 children subjected to multiple Kato-Katz thick smears readings were diagnosed positive for *T. trichiura* (n = 577, 82%). Infections were mainly of light intensity (94%) and only one heavy *T. trichiura* infection was identified. Prevalence of hookworm and *A. lumbricoides* were 7% and 5%, respectively, and most of these infections were of light intensity. An infection with all three helminth species was diagnosed in 11 children (1.5%). The mean age of the 701 children was 10 years (range 7–15 years). Mean age, weight, and height were comparable in all four treatment arms (Table 1). There was a similar number of boys (n = 348) and girls (n = 353) participating in the study.

Efficacy against *T. trichiura*

Only very low cure rates were observed regardless of whether the sequentially administered nitazoxanide-albendazole combination, albendazole, or nitazoxanide were administered. In more detail, using the per-protocol analysis, nitazoxanide combined with albendazole achieved a cure rate of 16.0% (95% confidence interval (CI), 9.7–22.4%), whereas single doses of albendazole or nitazoxanide resulted in cure rates of 14.5% (95% CI, 8.4–20.6%), and 6.6% (95% CI, 2.4–10.8%), respectively. Children receiving placebo showed an apparent cure rate of 8.9% (95% CI, 4.0–13.8%) (Table 2). Similar results were observed using the available case analysis (Table S1). Comparing the treatment outcomes using a logistic regression revealed that albendazole had a significant effect on infections with *T. trichiura* (odds ratio (OR), 0.47; 95% CI, 0.27–0.81; p = 0.007) while nitazoxanide showed no effect (OR, 1.04; 95% CI, 0.61–1.78; p = 0.89). There was some indication of an interaction between the two drugs (OR, 0.64; 95% CI, 0.21–1.98), however, this result was not significant (p = 0.44).

The ERR for *T. trichiura* was 54.9% (bootstrap 95% CI, 37.7–67.9%) for the nitazoxanide-albendazole combination, 45.6% (95% CI, 25.9–61.0%) for albendazole alone, 13.4% (95% CI, 0.0–33.7%) for nitazoxanide alone, and 17.6% (95% CI, 0.0–36.7%) for the placebo-controlled treatment arm (Table 2). Large confidence intervals indicate a high variance in ERRs. Therefore the only significant difference between the treatment arms (defined by non-overlapping bootstrap CI), was found between nitazoxanide-albendazole compared to the nitazoxanide monotherapy treatment group and the placebo-controlled treatment arm. However, even though CIs are overlapping, there seems to be a trend that albendazole alone resulted in higher ERRs.

The amount of milligrams of drug administered per kilogram of body weight ranged from 22.7 to 64.9 mg/kg for nitazoxanide and

Table 1. Baseline characteristics of school-aged children included in the trial.

Characteristic	Overall	Nitazoxanide+albendazole	Only albendazole	Only nitazoxanide	Only placebo
N	701	165	177	180	179
Age mean (±SD), years	9.7 (1.6)	9.7 (1.6)	9.8 (1.7)	9.4 (1.6)	9.9 (1.6)
No. of boys/girls	348/353	82/83	85/92	94/86	87/92
No. of participants Wawi/Al Sadique	549/152	127/38	142/35	139/41	141/38
Weight mean (±SD), kg[a]	26 (5.6)	26 (5.6)	26 (5.9)	26 (5.3)	26 (5.4)
Height mean (±SD), cm[b]	130 (9.2)	130 (9.4)	130 (9.3)	129 (9.3)	130 (8.8)
Infected with *T. trichiura*					
No. infected children (%)	577 (82.3)	142 (86.1)	142 (80.2)	144 (80.0)	149 (83.2)
Geometric mean, EPG	153	147	162	148	156
Infection intensity, no (%) of infected children					
Light (1–999 EPG)	541 (93.7)	133 (93.0)	131 (92.3)	137 (95.1)	140 (94.6)
Moderate (1,000–9,999 EPG)	35 (6.1)	10 (7.0)	10 (7.0)	7 (4.9)	8 (5.4)
Heavy (≥10,000 EPG)	1 (0.2)		1 (0.7)		
Infected with hookworm					
No. infected children (%)	48 (6.8)	15 (9.0)	11 (6.2)	13 (7.2)	9 (5.0)
Geometric mean EPG*	36	35	37	39	35
Infected with *A. lumbricoides*					
No. infected children (%)	31 (4.4)	6 (3.6)	9 (5.1)	8 (4.4)	8 (4.5)
Geometric mean EPG	507	686	207	435	1,297
Infection intensity, no (%) of infected participants					
Light (1–4,999 EPG)	29 (93.6)	5 (83.3)	9 (100)	8 (100)	7 (87.5)
Moderate (5,000–49,000 EPG)	2 (6.6)	1 (6.7)	0	0	1 (12.5)

[a]684 datasets.
[b]683 datasets.
*all infections with hookworms were classified as light.
doi:10.1371/journal.pntd.0001685.t001

from 7.7 to 27.2 mg/kg for albendazole (Table 2). Logistic regression revealed no statistically significant association between body weight and cure rates between the different treatment arms which received an active drug.

Efficacy against Other Soil-Transmitted Helminths

Less than 10% of the children were infected with hookworm (n = 48; 7%) and *A. lumbricoides* (n = 31; 4%). Albendazole was highly efficacious against both parasites with cure rates of 100% against *A. lumbricoides* and 81.8% against hookworm (95% CI, 54.6–100%). Nitazoxanide showed moderate efficacy against those two nematode species with cure rates of 66.7% (95% CI, 35.4–98.0%) against hookworm and 62.5% (95% CI 19.2–100.0%) against *A. lumbricoides* (Table 2). These data, however, lacked statistical significance. Of note, placebo had an apparent cure rate against hookworm infection of 55.6%.

Adverse Events

In total, 678 of the treated children answered a standardized questionnaire pertaining to adverse events. However, not all of these children responded at each of the five follow-up time points (Table 3). Before treatment, 28 (4.1%) children reported minor symptoms (e.g., headache and abdominal pain). After treatment (both first and second round of treatment) a total of 244 children complained at least once about minor adverse events at one of the four follow-up examinations (3 and 24 hours after each treatment). Only one child had moderate adverse events, namely headache 24 hours after receiving nitazoxanide. This child was treated with paracetamol and the headache resolved within 3 hours. In total 307 adverse events were reported after starting the treatment regimen. Abdominal cramps and headache were the most frequent ones (165 times (53.7%) and 69 times (22.5%), respectively) (Table S2). Other reported adverse events were nausea (6.8%), vertigo (5.5%), diarrhea (4.6%), fever (3.6%), allergic reaction (1.6%), vomiting (1.3%), and fatigue (0.3%).

Three hours after the first treatment, minor adverse events were reported by 101 (14.9%) participants. Children who received placebo reported significantly more about minor adverse events compared to the pretreatment situation (OR, 2.97; 95% CI, 1.50–6.21) (Table 3). Children who were given single nitazoxanide had significantly more adverse events 3 hours after treatment compared to the placebo recipients at the same time point (OR, 2.02; 95% CI, 1.28–3.24). On the next day (24 hours after the first treatment) 56 (8.3%) children reported adverse events. Participants receiving nitazoxanide still had significantly higher odds of reporting adverse events (OR, 2.49; 95% CI, 1.38–4.50). Three hours after the second treatment, children treated with albendazole did not report significantly more adverse events than placebo recipients (OR, 1.47; 95% CI, 0.59–3.70). At this time point, nitazoxanide was no longer associated with significantly more adverse events than placebo recipients (OR, 2.04; 95% CI, 0.85–

Table 2. Effect of albendazole, nitazoxanide, sequentially administered albendazole-nitazoxanide combination, and placebo against soil-transmitted helminths.

Characteristic	Nitazoxanide+albendazole	Only albendazole	Only nitazoxanide	Only placebo
Trichuris trichiura				
No. of infected children	131	131	136	135
No. of children not cured after treatment	110	112	127	123
Cure rate % (95% CI)	16.0	14.5	6.6	8.9
	(9.7–22.4)	(8.4–20.6)	(2.4–10.8)	(4.0–13.8)
Geometric mean, EPG				
Before treatment	152	164	145	154
After treatment	69	89	125	127
Egg reduction rate % (95% CI*)	54.9	45.6	13.4	17.6
	(37.7–67.9)	(25.9–61.0)	(0.0–33.7)	(0.0–36.7)
Hookworm				
No. of infected children	14	11	12	9
No. of children not cured after treatment	2	2	4	4
Cure rate % (95% CI)	85.7	81.8	66.7	55.6
	(64.7–100.0)	(54.6–100.0)	(35.4–98.0)	(15.0–96.1)
Ascaris lumbricoides				
No. of infected children	5	9	8	7
No. of children not cured after treatment	0	0	3	7
Cure rate % (95% CI)	100.0	100.0	62.5	0.0
			(19.2–100.0)	
Range of actual treatment dose (mg/kg)				
Nitazoxanide mean (min, max)	38.2	-	38.9	-
	(22.7–64.9)		(23.9–54.1)	
Albendazole mean (min, max)	15.3	15.0	-	-
	(9.1–26.0)	(7.7–27.2)		

Effect of different treatments calculated with the per-protocol analysis.
*95% CI of egg reduction rates were calculated using bootstrap resampling.
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4.91) and both drugs combined (treatment group 1) showed no cumulative effect regarding adverse events (OR, 0.67; 95% CI, 0.21–2.18). Twenty-four hours after the second treatment adverse events were resolved. Logistic regression revealed ORs of 0.77 (95% CI 0.35–1.71) for albendazole, 0.76 (95% CI 0.35–1.69) for nitazoxanide, and 1.40 (95% CI 0.44–4.41) for the sequentially administered nitazoxanide-albendazole combination.

Discussion

Following up on the promising trichuricidal properties observed in *in vitro* studies [16], we carried out the first randomized, double-blind, placebo-controlled trial administering nitazoxanide as a single dose of 1,000 mg to *T. trichiura*-infected school-aged children in a highly endemic area in Pemba. In addition, since combination chemotherapy is being advocated in many therapeutic areas, as it enhances efficacy and lowers the risk of resistance development [26], one group of children was treated with a nitazoxanide-albendazole combination which was administered over two consecutive days.

A high single dose of nitazoxanide (1,000 mg) showed no therapeutic effect against *T. trichiura*. This result is in contrast to previous studies, which reported high cure rates when the drug was administered as multiple dose treatment regimen (6 times 200 mg or 500 mg) to *T. trichiura*-infected patients [17–19]. It is plausible that pharmacokinetic properties of "multiple doses a day" nitazoxanide are superior to "once a day" nitazoxanide in that it achieves a longer half life. However, since the global strategy targeting neglected tropical diseases advocates preventive chemotherapy (i.e., regular deworming with single oral doses), multiple dosing is currently not recommended, as it poses operational and financial challenges [27]. A rigorous diagnostic approach as performed in this study (two times duplicate Kato-Katz thick smears before and after treatment) can lead to lower observed cure rates, since, especially light infections, are more likely to be detected [28]. Nevertheless, two of the above mentioned studies which found high cure rates and ERRs for nitazoxanide against *T. trichiura* infections also collected several stool samples after treatment, and hence pursued a thorough diagnostic approach. Diagnosis, therefore, does not seem to be the main reason for the contradictory results.

The standard treatment albendazole revealed a very low cure rate against *T. trichiura* when given at a single dose of 400 mg, which is in agreement with the results of several previous studies [21,29,30]. It is interesting to note that the ERR following albendazole treatment was still moderate (73%) in the same setting 12 years ago, but was similarly low as the ERR obtained in this trial, in a study conducted by Knopp et al. in 2009 in neighboring Unguja, Zanzibar [29,30]. This might be an indicator of tolerance

Table 3. Assessed adverse events among the four treatment arms during a randomized, placebo-controlled trial carried out on Pemba Island, Tanzania.

Time point	Adverse events: none/mild				
	Percentage of people with adverse events				
	Overall	Nitazoxanide+albendazole	Albendazole	Nitazoxanide	Placebo
Before treatment	648/28	155/9	163/8	166/6	164/5
	4.1%	5.5%	4.7%	3.5%	3.0%
3 hours after first treatment	577/101	133/31*	155/16	139/34*	150/20
	14.9%	18.9%	9.4%	19.7%	11.8%
24 hours after first treatment	622/56[a]	145/19[a]	160/11	153/20	164/6
	8.3%	11.6%	6.4%	11.6%	3.5%
3 hours after second treatment	606/51	144/15	157/12	151/16	154/8
	7.8%	9.4%	7.1%	9.6%	4.9%
24 hours after second treatment	576/51	138/12	147/12	149/12	142/15
	8.1%	8.0%	7.5%	7.5%	9.6%

Adverse events were assessed at five different time points (before treatment, 3 and 24 hours after first treatment, and 3 and 24 hours after second treatment). Nitazoxanide was given on the first day of treatment, while albendazole was given on the second day of treatment.
*statistically significant more adverse events in nitazoxanide-treated children compared to children receiving placebo.
[a]One of these adverse events was classified as moderate.
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or resistance development to albendazole against *T. trichiura*. Of note, Levecke et al. [31] recently showed that ERRs can differ strongly between individual settings and that albendazole has higher ERRs in settings with low infection intensities. Our trial could not confirm this hypothesis since albendazole achieved only a low ERR in children characterized by low infection intensities.

The combination of albendazole and nitazoxanide had slightly, though not significantly higher cure rates and ERRs than albendazole alone. Since drug interactions have not been evaluated for this combination, drugs were administered on subsequent days. One disadvantage of spacing the drug is that synergistic effects might be missed. On the other hand, a recent study which examined the effect of a simultaneous nitazoxanide-albendazole combination against adult *T. muris in vitro* detected an antagonistic effect [32].

Only a few children were (co)-infected with hookworm and/or *A. lumbricoides*. Nonetheless, these data illustrate that the standard treatment albendazole has a high efficacy against these parasites. The good activity against these two helminths and low efficacy against *T. trichiura* is also supported by the baseline prevalences (*T. trichiura* (82.2%), hookworm (6.8%), and *A. lumbricoides* (4.6%)) observed in the current trial. The settings where the study was conducted (Wawi and Al-Sadik schools) are part of the annual deworming in Zanzibar and are therefore regularly treated with albendazole.

The placebo-controlled treatment group had a cure rate of 55.6% against hookworms. This finding might indicate that the diagnostic tool used to detect hookworm eggs in this study was not sufficiently sensitive. One reason might be that subjects with light infections may only shed very few or sometimes no eggs, resulting in a negative Kato-Katz test result [33,34]. To overcome this problem in future studies it might be advisable to use an additional diagnostic technique with higher sensitivity such as the FLOTAC technique, which allows using a larger amount of stool, or indirect diagnostic technique such as multiplex real-time PCR [33,35–38].

We observed a high frequency of adverse events, but these were mostly mild, were, at times, already reported before treatment, and also by placebo recipients [39]; and hence were not consistently treatment related. Nevertheless, 3 and 24 hours after the first day of treatment (administration of nitazoxanide or a nitazoxanide-matching placebo) children treated with nitazoxanide reported significantly more adverse events compared to those who had received placebo. This increase in adverse events was not observed after the second day of treatment (children treated with albendazole suffered from a similar number of adverse events episodes than children who had obtained placebo), suggesting that the standard treatment albendazole triggers less adverse events than nitazoxanide. Adverse events related to treatment were resolved 24 hours after the second treatment, and the highest number of adverse events was reported from placebo-treated children.

In conclusion, a single oral dose of nitazoxanide cannot be recommended for the treatment of infection with *T. trichiura* since we observed low cure rate and ERR as well as significantly more adverse events than the standard drug albendazole. Note that, nitazoxanide is also much more expensive than the benzimidazoles, pyrantel pamoate, or levamisole. Moreover, also albendazole showed a low efficacy against *T. trichiura* in our study setting which did also not significantly improve by adding nitazoxanide on the next treatment day. Therefore the discovery and development of novel anthelmintic drugs, in particular against infections with *T. trichiura*, has a high priority.

Supporting Information

Checklist S1 CONSORT Checklist.
(PDF)

Protocol S1 Trial Protocol.
(DOC)

Table S1 Effect of albendazole, nitazoxanide, sequentially administered albendazole-nitazoxanide combination, and placebo against soil-transmitted helminths calculated with available-case analysis.
(DOC)

Table S2 Number of specific adverse events assessed at different time points.
(DOC)

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Author Contributions

Conceived and designed the experiments: JK MA JU JH BS S. Ame. Performed the experiments: BS S. Ame JK S. Ali MA. Analyzed the data: BS JH. Contributed reagents/materials/analysis tools: RA. Wrote the paper: BS JK JU JH MA S. Ame S. Ali RA.

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Chapter 2b

Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide

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RESEARCH Open Access

Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide

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Abstract

Background: Pathogenic intestinal protozoa infections are common in school-aged children in the developing world and they are frequently associated with malabsorption syndromes and gastrointestinal morbidity. Since diagnosis of these parasites is difficult, prevalence data on intestinal protozoa is scarce.

Methods: We collected two stool samples from school-aged children on Pemba Island, Tanzania, as part of a randomized controlled trial before and 3 weeks after treatment with (i) single-dose albendazole (400 mg); (ii) single-dose nitazoxanide (1,000 mg); (iii) nitazoxanide-albendazole combination (1,000 mg–400 mg), with each drug given separately on two consecutive days; and (iv) placebo. Formalin-fixed stool samples were examined for the presence of intestinal protozoa using an ether-concentration method to determine the prevalence and estimate cure rates (CRs).

Results: Almost half (48.7%) of the children were diagnosed with at least one of the (potentially) pathogenic protozoa *Giardia intestinalis*, *Entamoeba histolytica/E. dispar* and *Blastocystis hominis*. Observed CRs were high for all treatment arms, including placebo. Nitazoxanide showed a significant effect compared to placebo against the non-pathogenic protozoon *Entamoeba coli*.

Conclusions: Intestinal protozoa infections might be of substantial health relevance even in settings where they are not considered as a health problem. Examination of a single stool sample with the ether-concentration method lacks sensitivity for the diagnosis of intestinal protozoa, and hence, care is indicated when interpreting prevalence estimates and treatment effects.

Keywords: Intestinal protozoa, *Blastocystis hominis*, *Entamoeba histolytica/E. dispar*, *Giardia intestinalis*, Albendazole, Nitazoxanide, Ether-concentration method

Background

Infection with pathogenic intestinal protozoa (e.g. *Entamoeba histolytica* and *Giardia intestinalis*) result in considerable gastrointestinal morbidity, malnutrition and mortality worldwide, particularly among young children in developing countries [1,2]. It has been estimated that *E. histolytica*, the causative agent of amoebiasis, kills between 40,000 and 100,000 people each year; hence, is one of the deadliest parasitic infections worldwide [2,3]. In the People's Republic of China alone, *G. intestinalis* affects an estimated 28.5 million people every year [1]. The prevalence of *G. intestinalis* has been estimated at 2–3% in the industrialized world and 20–30% in developing countries [4]. *Cryptosporidium* spp. is another major causal agent of diarrhoea, primarily affecting immunocompromised individuals such as those infected with HIV [3,5,6]. *Blastocystis hominis* is a common additional anaerobic intestinal protozoon and

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its pathogenicity is still under debate [7-9]. Lack of access to clean water, sanitation and hygiene are strong drivers for infection with intestinal protozoa [10-12].

Several drugs are currently available to treat intestinal protozoa infections. Most commonly used are 5-nitroimidazole compounds, including metronidazole, tinidazole, ornidazole and secnidazole [13]. Alternative effective agents, when given as multiple doses, include nitazoxanide and albendazole [14-16].

Information on the prevalence of intestinal protozoa infections is scarce and little data are available from sub-Saharan Africa. For example, to the best of our knowledge, the prevalence of intestinal protozoa infections on Pemba Island has been assessed only twice and these investigations date back to 1984 and 1992 [17,18]. In the 1984 study, the prevalence of *G. intestinalis* and *E. histolytica* among children and adults combined were 5.6% and 3.1%, respectively. Prevalences of 35.6%, 4.4%, 2.9%, 0.7% and 0.7% were reported for *Entamoeba coli*, *Endolimax nana*, *Chilomastix mesnili*, *Entamoeba hartmanni* and *Iodamoeba bütschlii*, respectively (Figure 1) [17]. The study conducted in 1992 reported prevalences of 25.4% for *E. histolytica* and 6.6% for *G. intestinalis* among children aged 9–17 years [18].

The study reported here was integrated in a randomized controlled trial carried out in school-aged children on Pemba Island, Tanzania in mid-2011 to asses the efficacy and safety of single-dose nitazoxanide and albendazole and a nitazoxanide-albendazole combination against *Trichuris trichiura* and other soil-transmitted helminth infections [19]. The aim of the present work was to evaluate the prevalence of intestinal protozoa infections in these children and to determine whether the study drugs had an effect on intestinal protozoa. Throughout the study, the ether-concentration method was used on formalin-fixed stool samples.

Methods

Ethics statement

Our randomized controlled trial was approved by the ethics committee of Basel (EKBB; reference no. 225/10), and the Ministry of Health and Social Welfare of Zanzibar (ZAMREC; reference no. 0001/010) and is registered at Current Controlled Trials (trial identifier: ISRCTN08336605). Participating in the trial required written informed consent from parents or legal guardians and oral assent from children. Parents and children were counseled that participation is voluntary and withdrawal was possible at any time without specification of reasons and further obligations. At the end of the trial, all children who were diagnosed with soil-transmitted helminths received albendazole (single oral dose of 400 mg).

Study area and design

Details of the study area, population and procedures have been described previously [19]. In brief, the study was carried out in June and July 2011 in children aged 7–15 years attending two schools (Wawi and Al-Sadik) located within a radius of 10 km from Chake Chake, the main city of Pemba Island. The study was a randomized controlled trial with four treatment arms: (i) single-dose albendazole (400 mg); (ii) single-dose nitazoxanide (1,000 mg); (iii) nitazoxanide-albendazole combination (1,000 mg–400 mg) with each drug given separately on two consecutive days; and (iv) placebo. Of the 657 children, who completed the trial, 550 (83.7%) had formalin-fixed stool samples before and after treatment and were therefore included in the present study (treatment arms: (i) n = 136, (ii) n = 145, (iii) n = 117, and (iv) n = 152; Wawi school: n = 404, Al-Sadik school: n = 146).

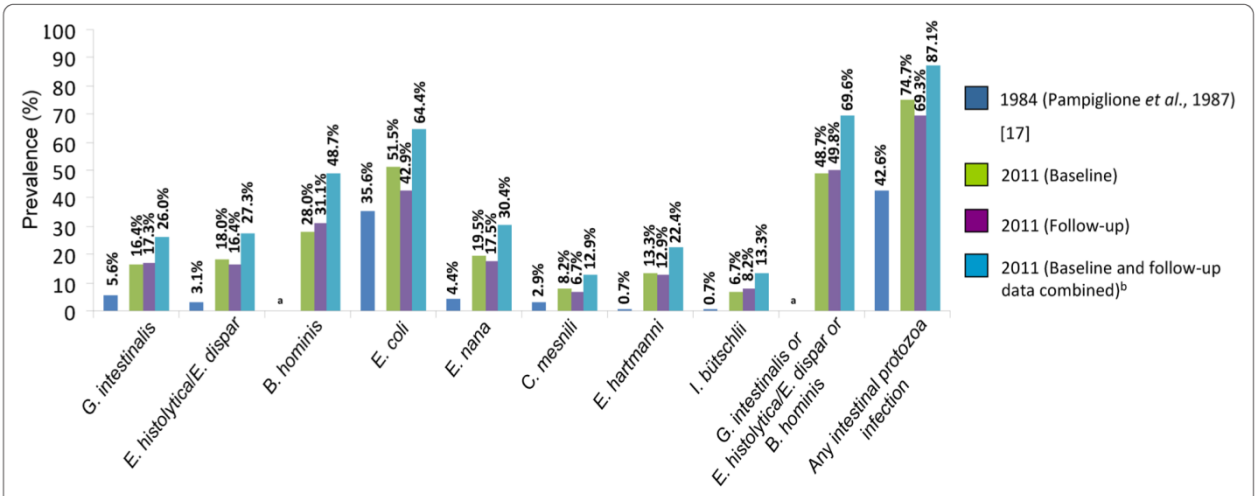

Figure 1 Comparison of prevalence of intestinal protozoa infections assessed in mid-2011 at baseline, 3 weeks post-treatment follow-up and combined results (current study) against prevalence determined in 1984 (study done by Pampiglione *et al.* [17]) on Pemba Island, Tanzania.

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Procedures for the diagnosis of intestinal protozoa

Approximately 2 g of stool were fixed in 10 ml of 5% formalin in Falcon tubes labeled with unique identifiers. Stool samples were thoroughly broken down and homogenized with a wooden spatula. The formalin-fixed samples were transferred to the Swiss Tropical and Public Health Institute (Basel, Switzerland) and examined within 10 months.

The fixed stool samples were processed with an ether-concentration method [20,21]. Briefly, the homogenized stool sample was filtered through a medical gauze into a new tube and then centrifuged for 1 min at 500 *g*. The supernatant was discarded. To the remaining pellet, 7 ml of physiological NaCl-solution and 2–3 ml of diethyl ether was added. Tubes were shaken and centrifuged again for 3 min at 500 *g*. The upper three layers were discarded. The entire sediment was examined by experienced laboratory technicians under a microscope for soil-transmitted helminths at a magnification of 100×, and intestinal protozoa at a magnification of 400× or 500× using oil immersion. The current analysis focuses on intestinal protozoa, including the pathogenic intestinal protozoa *G. intestinalis* and *E. histolytica*/*E. dispar* (of note, these two *Entamoeba* species cannot be differentiated by microscopy [22]); the potentially pathogenic protozoon *B. hominis*, and the non-pathogenic protozoa *E. coli*, *E. hartmanni*, *E. nana*, *C. mesnili* and *I. bütschlii* [7-9,23]. *Cryptosporidium* spp. was not included, since it cannot easily be detected with the formalin-ether concentration technique, and would have required staining with the modified Ziehl-Neelsen method [24]. Infection intensities were classified as follows: (i) negative (no cysts or trophozoites in the entire sediment); (ii) light (one to five cysts or trophozoites per slide); (iii) moderate (one cyst or trophozoite per observation field at the 400× or 500× magnification); and (iv) heavy (more than one cyst or trophozoite per observation field) [21,25].

Statistical analysis

Data were double-entered into an Excel file (Microsoft 2010) and cross-checked. Statistical analysis was performed using Stata version 10.1 (StataCorp; College Station, USA).

The prevalence of intestinal protozoa was determined before treatment (baseline) and at the 3-week post-treatment follow-up. Differences between prevalence at baseline and follow-up were assessed using x^2 test. In the baseline prevalence analyses, the odds of being infected with a specific intestinal protozoon species for boys compared to girls and for children from Wawi compared to Al-Sadik school was calculated using logistic regression.

Cure rates (CRs) were estimated for each intestinal protozoon species for the different treatment arms as the percentage of positive children at baseline diagnosed negative after treatment. Differences in CRs among treatment arms were examined using logistic regressions. Reduction of infection intensity was assessed as the difference in infection intensities before and after treatment among all individuals (negative individuals before treatment were included in the analysis). Mean of differences in infection intensities was calculated for each treatment arm and for each intestinal protozoon species together with 95% confidence intervals (CIs). Decrease in infection intensity was assumed as significant when 95% CI was below 0.

Results

Prevalence and intensity of intestinal protozoa infection

Based on the analysis of one formalin-fixed stool sample per child at baseline, 74.7% of the children harboured at least one intestinal protozoa species. About half of the children (48.7%) were infected with at least one of the three (potentially) pathogenic intestinal protozoa. The prevalence of *E. histolytica/E. dispar* and *G. intestinalis* was 18.0% and 16.4%, respectively before treatment (Table 1). The potentially pathogenic intestinal protozoon *B. hominis* was diagnosed in 28.0% of the children. Prevalences for the other intestinal protozoa were 51.5% for *E. coli*, 19.5% for *E. nana*, 13.3% for *E. hartmanni*, 8.2% for *C. mesnili* and 6.7% for *I. bütschlii.* For the pathogenic intestinal protozoon *G. intestinalis*, 44.4%, 38.9% and 16.7% of the infections were classified as light, moderate and heavy, respectively. The infection intensities of other intestinal protozoa species are listed in Table 1.

Examination of the follow-up stool samples revealed the following prevalences: 49.8% of children remained infected with at least one of the three (potentially) pathogenic intestinal protozoa and 69.3% of the children were found positive for at least one intestinal protozoa. The prevalence of *G. intestinalis*, *E. histolytica/E. dispar* and *B. hominis* at the 3-week post-treatment follow-up was 17.3%, 16.4% and 31.1%, respectively (Figure 1). Prevalence for the non-pathogenic intestinal protozoa species were 42.9% for *E. coli*, 17.5% for *E. nana*, 12.9% for *E. hartmanni*, 8.2% for *I. bütschlii* and 6.7% for *C. mesnili.* According to the x^2 test, only *E. coli* (p = 0.004) and all intestinal protozoa combined (p = 0.046) showed significantly lower prevalence at follow-up compared to baseline.

The odds of being infected with any intestinal protozoa at baseline was significantly higher for girls than boys (odds ratio (OR) = 1.97; 95% CI 1.32–2.93) (Table 2). There was a trend (p <0.1) that girls were at a higher odds of being infected with *E. coli* than boys (OR = 1.40; 95% CI 1.00–1.96). Children from Wawi school had significantly lower odds of being infected with *B. hominis* (OR = 0.64; 95% CI 0.43-0.97), *E. nana* (OR = 0.63; 95% CI 0.40–1.00) and *I. bütschlii* (OR = 0.50; 95% CI 0.25–1.00) considering only the baseline stool sample than children from Al-Sadik. Additionally, there was a trend (p <0.1) that children from Wawi school had a lower odds of being infected with *G. intestinalis* (OR = 0.66; 95% CI 0.41–1.07) and any of

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Table 1 Baseline characteristics of included school-aged children on Pemba Island in mid-2011 with regard to intestinal protozoa infection

Characteristic, intestinal protozoa	N (prevalence in %)	Girls (%)/boys (%)	Wawi (%)/Al-Sadik school (%)
	low (%)/moderate (%)/heavy (%)		
No. of children tested	550	271/279	404/146
G. intestinalis	90 (16.4)	37 (13.7)/53 (19.0)	59 (14.6)/31 (21.1)
	40 (44.4)/35 (38.9)/15 (16.7)		
E. histolytica/E. dispar	99 (18.0)	53 (19.6)/46 (16.5)	77 (19.1)/22 (15.1)
	57 (57.6)/33 (33.3)/9 (9.1)		
B. hominis	154 (28.0)	73 (26.9)/81 (29.0)	103 (34.9)/51 (25.5)
	125 (81.2)/25 (16.2)/4 (2.6)		
E. coli	283 (51.5)	151 (55.7)/132 (47.3)	210 (52.0)/73 (50.0)
	101 (35.7)/104 (36.7)/78 (27.6)		
E. nana	107 (19.5)	58 (21.4)/49 (17.6)	71 (17.6)/36 (24.7)
	62 (57.9)/40 (37.4)/5 (4.7)		
E. hartmanni	73 (13.3)	38 (14.0)/35 (12.0)	52 (12.9)/21 (14.4)
	57 (78.1)/14 (19.2)/2 (2.7)		
C. mesnili	45 (8.2)	27 (6.5)/18 (10.0)	31 (7.7)/14 (9.6)
	21 (46.7)/18 (40.0)/6 (13.3)		
I. bütschlii	37 (6.7)	18 (6.6)/19 (6.8)	22 (5.4)/15 (10.3)
	31 (83.8)/5 (13.5)/1 (2.7)		
G. intestinalis or *E. histolytica/E. dispar* or *B. hominis*	268 (48.7)	135 (49.8)/133 (47.8)	187 (46.3)/81 (55.5)
Any intestinal protozoa	411 (74.4)	219 (80.8)/192 (68.8)	297 (73.5)/114 (78.1)

the three (potentially) pathogenic intestinal protozoa combined (OR = 0.68; 95% CI 0.47–1.00).

Effect of antiparasitic treatment against intestinal protozoa

Observed CRs were moderate to high for all intestinal protozoa regardless of the treatments administered (Table 3). The highest CR was observed for the albendazole-nitazoxanide combination against *E. nana* (CR 91.3%; 95% CI 78.8–100.0%). The lowest CR among the antiparasitics tested was documented for single-dose albendazole against *E. coli* (CR 33.3%; 95% CI 22.4–44.3%). Note that the group of children receiving placebo had moderate to high CRs against intestinal protozoa infections. Comparing the outcomes among treatment arms using logistic regression revealed that single-dose nitazoxanide had a significant effect on *E. coli* (OR = 0.35; 95% CI 0.18–0.68). Furthermore, we observed a trend (p <0.1) with the combination of nitazoxanide plus albendazole against *E. nana* (OR = 0.18; 95% CI 0.02–1.27). All other results of the logistic regressions revealed p-values above 0.1; hence, there was no significant effect compared to placebo.

Table 2 Odds ratios (OR) of being infected with intestinal protozoa among school-aged children on Pemba Island in mid-2011, as assessed by logistic regression

Intestinal protozoa	OR girls vs. boys (95% CI)	OR Wawi vs. Al-Sadik school (95% CI)
G. intestinalis	0.67 (0.44–1.11)	0.66 (0.41–1.07)
E. histolytica/E. dispar	1.21 (0.78–1.87)	1.30 (0.77–2.19)
B. hominis	0.94 (0.64–1.37)	0.64 (0.43–0.97)*
E. coli	1.40 (1.00–1.96)	1.04 (0.71–1.53)
E. nana	1.34 (0.87–2.05)	0.63 (0.40–1.00)*
E. hartmanni	1.15 (0.70–1.89)	0.87 (0.50–1.50)
C. mesnili	1.65 (0.88–3.09)	0.74 (0.38–1.44)
I. bütschlii	1.04 (0.53–2.04)	0.50 (0.25–1.00)*
G. intestinalis or *E. histolytica/E. dispar* or *B. hominis*	1.13 (0.81–1.58)	0.68 (0.47–1.00)
Any intestinal protozoa	1.97 (1.32–2.93)*	0.72 (0.46–1.14)

*p <0.05.
CI, confidence interval.

Comparing the mean intensity of intestinal protozoa infection before and 3 weeks after treatment in the different arms revealed no significant effect for most of the assessed intestinal protozoa (results not shown). The only significant reductions of infection intensity (95% CI below 0) were observed in the albendazole-nitazoxanide combination against *E. histolytica/E. dispar* (–0.21; 95% CI -0.34 to -0.09) and *E. coli* (–0.37; 95% CI -0.58 to -0.16) and in the nitazoxanide single-dose treatment against *E. coli* infection intensity (–0.37; 95% CI -0.58 to -0.16).

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Table 3 Effect of albendazole, nitazoxanide, sequentially administered albendazole-nitazoxanide combination, and placebo against intestinal protozoa infections among school-aged children on Pemba Island in mid-2011

Characteristic	Single-dose albendazole	Single-dose nitazoxanide	Nitazoxanide-albendazole combination	Placebo
G. intestinalis				
No. of infected children	25	21	19	25
No. of children not cured after treatment	10	9	11	12
CR, % (95% CI)	60.0	57.1	42.1	52.0
	(39.4–80.6)	(34.1–80.2)	(17.7–66.6)	(31.0–73.0)
E. histolytica/E. dispar				
No. of infected children	23	23	31	22
No. of children not cured after treatment	10	9	10	10
CR, % (95% CI)	56.5	60.9	67.7	54.5
	(34.6–78.4)	(39.3–82.4)	(50.3–85.2)	(31.9–77.1)
B. hominis				
No. of infected children	35	37	33	49
No. of children not cured after treatment	15	16	10	16
CR, % (95% CI)	57.1	56.8	69.7	67.3
	(39.9–74.4)	(40.0–73.5)	(53.1–86.2)	(53.7–81.0)
E. coli				
No. of infected children	75	82	56	70
No. of children not cured after treatment	50	38	27	50
CR, % (95% CI)	33.3	53.7	51.8	28.6
	(22.4–44.3)	(42.6–64.7)	(38.3–65.3)	(17.7–39.4)
E. hartmanni				
No. of infected children	14	22	18	19
No. of children not cured after treatment	5	5	4	7
CR, % (95% CI)	64.3	77.3	77.8	63.2
	(35.6–93.0)	(58.3–96.3)	(56.5–99.1)	(39.3–87.0)
E. nana				
No. of infected children	24	25	23	35
No. of children not cured after treatment	10	9	2	15
CR, % (95% CI)	58.3	64.0	91.3	57.1
	(37.1–79.6)	(43.8–84.2)	(78.8–100.0)	(39.9–74.4)
C. mesnili				
No. of infected children	17	10	6	12
No. of children not cured after treatment	4	1	1	5
CR, % (95% CI)	76.5	90.0	83.3	58.3
	(54.0–99.0)	(67.4–100.0)	(40.5–100.0)	(25.6–91.1)
I. bütschlii				
No. of infected children	5	13	8	11
No. of children not cured after treatment	1	2	2	4
CR, % (95% CI)	80.0	84.6	75.0	63.6
	(24.5–100.0)	(61.9–100.0)	(36.3–100.0)	(29.7–97.5)

CI, confidence interval; CR, cure rate.

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Discussion

Since very little is known on the epidemiology of intestinal protozoa in sub-Saharan Africa, we analyzed formalin-fixed stool samples obtained from 550 school-aged children who participated in a randomized controlled trial on Pemba Island to assess the efficacy and safety of nitazoxanide, albendazole and a combination of both drugs against *T. trichiura* and other soil-transmitted helminths. This trial found low CRs against soil-transmitted helminth species [19]. Importantly, the study provided an opportunity to shed new light on the extent of intestinal protozoa infections in a child cohort and to determine whether the different study treatments had an effect on intestinal protozoa species.

Our results confirm that intestinal protozoa are a public health issue on Pemba Island. Indeed, almost half of the children were infected with at least one of the three (potentially) pathogenic intestinal protozoa. When considering the results from the baseline and the 3-week post-treatment follow-up, assuming that a child who was diagnosed positive at follow-up was diagnosed as a false-negative case at baseline, the prevalence of intestinal protozoa infection was even higher (Figure 1). This issue is most likely explained by the lack of sensitivity when examining only a single stool sample with the ether-concentration technique, an important limitation of our study. Hence, in future studies, multiple stool samples should be examined and subjected to the ether-concentration method or more sensitive molecular approaches employed to improve diagnostic accuracy [26,27].

A recent study reported only moderate sensitivity for the ether-concentration technique compared to the FLOTAC technique when examining the same formalin-fixed stool samples [28]. However, a study among five European reference laboratories showed that the agreement of diagnostic results was only moderate for pathogenic intestinal protozoa although the participating centres adhered to the same standard operating procedures for the ether-concentration technique [21]. In particular, *E. histolytica* is frequently misdiagnosed, even by experienced laboratory personnel [29].

We found that girls were generally at higher odds of an infection with any of the intestinal protozoa encountered than boys. Similar results were found by Mohammed Mahady and colleagues in Malaysia [30]. On the other hand, Traoré *et al.*, in a study carried out in school-aged children in Côte d'Ivoire, reported a considerably higher prevalence of intestinal protozoa among boys than girls [31], corroborating findings by Cifuentes *et al.* from Mexico, where boys were at higher odds of a *G. intestinalis* infection that girls [32]. Other studies found no gender difference at all [33]. These findings indicate that intestinal protozoa infections may be related to gender-specific behaviour within a community. In addition, we observed statistically significant differences for some of the intestinal protozoa between the two schools. Hence, even though the schools are located only a few kilometers apart and the two settings were quite similar, at least in terms of socioeconomic status, availability of safe water supply and sanitation infrastructure, different infection profiles were observed. This highlights the possibility of 'micro-geographic' variability in endemicity of intestinal protozoa infection. One explanation could be that Al-Sadik school is located close to an orphanage, where transmission of intestinal protozoa might be enhanced. Other behavioural, infrastructure or environmental factors that may account for our observation should be investigated in future studies.

Intestinal protozoa often co-occur with intestinal nematodes and it is therefore important to determine whether anthelminthic and other antiparasitic drugs have an effect on concomitant intestinal protozoa infections. Albendazole, for example, was found in a recent meta-analysis to be as effective as metronidazole against *G. intestinalis* [34]. The observed CRs against all intestinal protozoa were moderate to high in the three treatment groups. However – and contrary to what we expected – there was also a moderate treatment efficacy in the placebo group, which is difficult to explain other than a diagnostic dilemma. Our findings therefore have to be interpreted with caution. The high 'cure rate' observed within the placebo group underscores that analysis of a single stool sample with the formalin-ether concentration technique is unreliable, and hence, the CRs for the three treatment schemes investigated here are likely overestimated. Still, our results show that none of the drugs administered as single dose resulted in cure of all infected children. Only moderate CRs were observed against *G. intestinalis*, *E. histolytica/E. dispar* and the potentially pathogenic *B. hominis*. A further limitation is the relatively small number of positive children for specific intestinal protozoa. Infection intensity did not significantly decrease after treatment with the exception of *E. coli* in the nitazoxanide group. Note that intestinal protozoa multiply within the host, which can also influence infection intensity results [35]. Further, the incubation time for different intestinal protozoa is between 7 and 28 days, meaning that it is possible that within the 3-week period before and after treatment, re-infection had occured [36-40]. The clinical relevance of the moderate CRs suggests that single-dose albendazole or nitazoxanide or a combination of the two drugs do not have sufficient efficacy against pathogenic intestinal protozoa. However, multiple stool samples should be examined to strengthen the diagnostic accuracy for these infections.

Conclusion

Our study revealed that intestinal protozoa infections are highly prevalent among school-aged children on Pemba Island, a setting where these intestinal protozoa were not considered of major importance thus far. The difficulty in accurately diagnosing intestinal protozoa infections, also experienced in our study, might be one of the chief reasons why these pathogens and the diseases they cause are often neglected, even in settings where mass treatment are underway against intestinal helminth infections [21]. The prevalence of intestinal protozoa is therefore unknown in many settings and consequently the disease burden cannot be fully quantified [41]. Accurate diagnostic tools that can be applied at the point-of-care are thus urgently required. Finally, drugs and effective treatment regimens for intestinal protozoa infections to be used in public health campaigns are still far out of reach.

Competing interests

None of the authors has any conflict of interest concerning the work reported in this paper.

Authors' contributions

BS, ShMA, SaMA, JU, MA and JK designed the study; BS, HM, ShMA, SaMA, and JK implemented the study; BS managed the data; BS, HM, IIB, JU and JK analyzed and interpreted the data; BS wrote the first draft of the paper; HM, ShMA, SaMA, IIB, JU, MA and JK revised the manuscript. All authors read, and approved the manuscript prior to submission and assisted with the final revision of the manuscript.

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Chapter 3

Oxantel pamoate-albendazole for *Trichuris trichiura* infection

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ORIGINAL ARTICLE

Oxantel Pamoate–Albendazole for *Trichuris trichiura* Infection

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ABSTRACT

BACKGROUND

Infections with soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) are widespread and often occur concomitantly. These parasitic-worm infections are typically treated with albendazole or mebendazole, but both drugs show low efficacy against *T. trichiura*. Albendazole is the drug of choice against hookworm.

METHODS

In this double-blind trial conducted on Pemba Island, Tanzania, we randomly assigned children, 6 to 14 years of age, to receive one of four treatments: oxantel pamoate at a dose of 20 mg per kilogram of body weight, plus 400 mg of albendazole, administered on consecutive days; oxantel pamoate at a single dose of 20 mg per kilogram; albendazole at a single dose of 400 mg; or mebendazole at a single dose of 500 mg. We assessed the efficacy and safety profile of oxantel pamoate–albendazole when used in the treatment of *T. trichiura* infection (primary outcome) and concomitant soil-transmitted helminth infection (secondary outcome). Efficacy was determined by means of assessment of the cure rate and egg-reduction rate. Adverse events were assessed four times after treatment.

RESULTS

Complete data were available for 458 children, of whom 450 were infected with *T. trichiura*, 443 with hookworm, and 293 with *A. lumbricoides*. The cure rate of *T. trichiura* infection was significantly higher with oxantel pamoate–albendazole than with mebendazole (31.2% vs. 11.8%, P=0.001), as was the egg-reduction rate (96.0% [95% confidence interval {CI}, 93.5 to 97.6] vs. 75.0% [95% CI, 64.2 to 82.0]). The cure rate with albendazole (2.6%) and the egg-reduction rate with albendazole (45.0%; 95% CI, 32.0 to 56.4) were significantly lower than the rates with mebendazole (P=0.02 for the comparison of cure rates). Oxantel pamoate had low efficacy against hookworm and *A. lumbricoides*. Adverse events (mainly mild) were reported by 30.9% of all children.

CONCLUSIONS

Treatment with oxantel pamoate–albendazole resulted in higher cure and egg-reduction rates for *T. trichiura* infection than the rates with standard therapy. (Funded by the Medicor Foundation and the Swiss National Science Foundation; Current Controlled Trials number, ISRCTN54577342.)

Soil-transmitted helminthiasis is caused by chronic infection with nematode worms, *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*.[1] More than 1 billion people are infected with one or several species of soil-transmitted helminths. Infection with *T. trichiura*, a roundworm commonly known as whipworm, causes a global burden of 638,000 disability-adjusted life-years.[1-3]

The periodic administration of anthelmintic drugs (i.e., albendazole or mebendazole) to at-risk populations is the global strategy for controlling morbidity due to soil-transmitted helminth infection.[4] The goal of control programs is to eliminate childhood illness caused by soil-transmitted helminth infection — that is, to decrease the prevalence of moderate and heavy infection intensity among school-age children to less than 1%.[5]

Treatment with albendazole or mebendazole, in a single-dose regimen, results in high cure rates against infection with *A. lumbricoides*; only albendazole is associated with a satisfactory cure rate against hookworm. Both drugs are associated with a low cure rate against *T. trichiura* infection.[6-10] Although *T. trichiura* infection is not highly pathogenic unless the infection intensity is high, there is a growing recognition of the public health effects of trichuriasis.[11] Hence, there is a need to develop new, effective, and broad-spectrum anthelmintic drugs.[12]

Oxantel is the m-oxyphenol analogue of pyrantel and has been marketed as a veterinary drug since 1974. It shows high trichuricidal activity.[13-15] A number of exploratory trials showed that oxantel pamoate was effective when given as a single dose of 10 to 20 mg per kilogram of body weight.[16-20]

The aim of the present study was to assess the efficacy and safety profile of a combination of oxantel pamoate and albendazole (Zentel, GlaxoSmithKline) in children infected with *T. trichiura* (primary outcome). We also studied the effect of this combination therapy on concurrent infections with hookworm and *A. lumbricoides* (secondary outcome). Monotherapies with oxantel pamoate, albendazole, and mebendazole (Vermox, Johnson & Johnson) served as comparators.

METHODS

STUDY DESIGN AND PATIENTS

We conducted this randomized, controlled, double-blind trial from September through November 2012 in two primary schools, Mchangamdogo and Shungi, on Pemba Island, Tanzania. Children 6 to 14 years of age were invited to provide two stool samples, and children who were positive for either *T. trichiura* or hookworm were considered eligible for inclusion in the trial. Children presenting with *T. trichiura*–hookworm coinfection were enrolled with highest priority. A medical history was obtained from children who met the inclusion criteria, and each child underwent a physical examination. Children who had any systemic illness (e.g., clinical malaria or hepatosplenic schistosomiasis), as assessed by a medical doctor at the initial clinical assessment, were excluded from the trial.

STUDY OVERSIGHT

Ethical approval was obtained from the Ministry of Health and Social Welfare of Zanzibar, Tanzania, and from the ethics committee of Basel, Switzerland. Written informed consent was obtained from all the parents or guardians, and all the children provided verbal assent. All the authors take full responsibility for the study design; the collection, analysis, and interpretation of the data; and the fidelity of the report to the study protocol (available with the full text of this article at NEJM.org).

RANDOMIZATION

Children were randomly assigned, with the use of block sizes of four, to receive one of four treatments: oxantel pamoate at a dose of 20 mg per kilogram, plus 400 mg of albendazole; oxantel pamoate at a dose of 20 mg per kilogram; 400 mg of albendazole; or 500 mg of mebendazole. Children, study-site investigators, and laboratory technicians were unaware of the study-group assignments.

Each child received tablets on 2 consecutive days. On the first day, children were given either oxantel pamoate or, in the study groups that did not include therapy with oxantel pamoate, identical placebo tablets. Oxantel pamoate and identical matching placebo were given to the nearest half tablet according to the calculated dose per kilogram of body weight. On the second day, children were administered two tablets. Participants in the two treatment groups that included albendazole received albendazole and a placebo matching mebendazole, children in the mebendazole group received mebendazole plus a placebo matching albendazole, and children in the

oxantel pamoate monotherapy group received a placebo matching albendazole plus a placebo matching mebendazole.

There was no industry involvement; the drugs were purchased. Placebos exactly matching albendazole and mebendazole were purchased from Fagron. Oxantel pamoate and the matching placebo were manufactured at the University of Basel.[21] Drug quality was assured for all products used.

STUDY PROCEDURES

We explained the purpose and procedures of the study, including potential benefits and risks, to the parents or guardians of the children. At baseline, children who were willing to participate provided us with the informed-consent form signed by a parent or guardian and with two stool samples obtained over consecutive days. Stool samples were transferred to the Public Health Laboratory–Ivo de Carneri. From each sample, duplicate Kato–Katz thick smears were prepared and examined for soil-transmitted helminth eggs by one of six experienced laboratory technicians (all of whom were unaware of the treatment assignments).[22] For quality control, 10% of the slides were randomly chosen and reexamined; the agreement was more than 95%.

Before treatment, children were asked about clinical signs and symptoms, and their weight and height were measured. Adverse events were assessed and graded by means of active questioning at four time points after treatment — at 3 hours and 24 hours after the first and second treatments (for details about judging the severity of adverse events, see the study protocol).

Treatment efficacy was assessed 18 to 23 days after treatment, after children had submitted an additional two stool samples. At the end of the study, all school-going children were offered albendazole (at a dose of 400 mg) according to national guidelines.[4,23]

SAMPLE SIZE

We calculated that with a sample of 70 children infected with *T. trichiura* per treatment group the study would have 80% power to test the primary hypothesis that the oxantel pamoate–albendazole combination would result in a higher cure rate than the current drug of choice (i.e., mebendazole). Our calculations were based on an estimated cure rate against *T. trichiura* of 35% with mebendazole[6] and an estimated cure rate of 60% with oxantel pamoate–albendazole. To account for loss to follow-up, we increased the sample in each treatment group to 95 participants, resulting in a total of 380 school-age children with *T. trichiura* infection in the four treatment groups.

To provide the study with sufficient power to determine the efficacy of the combination therapy against concomitant hookworm infection (secondary outcome, with the prevalence of hookworm infection expected to be 60%), the sample was increased to 500 children. We also performed analyses to determine whether oxantel pamoate–albendazole was superior to its single components with respect to the primary and secondary outcomes. No adjustment was made for multiple testing.

STATISTICAL ANALYSIS

Data were double-entered into a database (Excel 2010, Microsoft), cross-checked, and analyzed with the use of Stata software, version 10.1 (StataCorp). The multiple imputation sensitivity analysis was performed with the use of R software, version 3.0.0 (www.r-project.org).

Potential imbalances in the baseline characteristics of the enrolled children (i.e., age, sex, school, weight, height, and log geometric-mean soil-transmitted helminth egg counts) were compared with the use of logistic and linear regression models, as appropriate. In the between-group comparisons, the mebendazole group was the reference group.

An available case analysis[24] was performed, which included all children with primary outcome data. A sensitivity analysis that used an intention-to-treat approach was performed for the primary hypothesis with the use of several different methods for imputation of missing data (i.e., best-case and worst-case scenarios and a multiple-imputation approach with the use of an iterative regression imputation, with age, sex, weight, height, treatment group, and log-transformed soil-transmitted helminth egg counts at baseline as predictors).

The cure rate, which was our primary outcome measure, was calculated as the percentage of the children who became egg-negative after treatment among those who had had eggs in their stool at baseline. The number of eggs per gram of stool was assessed by adding up the egg counts from the quadruplicate Kato–Katz thick smears and multiplying this number by six. Infection intensity was classified according to World Health

Organization (WHO) cutoffs.[25] Cure rates were further stratified according to infection intensity before treatment.

In addition, we calculated the number of children with moderate or heavy infection before treatment who had no infection or only light infection after treatment — a key goal of the WHO global program for control of soil-transmitted helminthiasis.[5] Crude logistic regressions (including all four treatment groups) and adjusted logistic regressions (with adjustment for age, sex, and school) were used to calculate differences in cure rates among treatment groups.

To test the primary hypothesis, logistic regression was used to compare the cure rates with oxantel pamoate–albendazole and with mebendazole among children with *T. trichiura* infection. Monotherapy comparators (i.e., oxantel pamoate and albendazole) were compared with mebendazole to assess which of the two drugs (oxantel pamoate or albendazole) was more efficacious. Adverse events were evaluated descriptively as the difference in the proportion of children reporting adverse events before and after treatment.

Geometric-mean soil-transmitted helminth egg counts were calculated for the treatment groups before and after treatment to assess the corresponding egg-reduction rate, an equally important variable for drug efficacy and therefore our secondary outcome; the egg-reduction rate was equal to $100 \times (1 - [\text{mean at follow-up} \div \text{mean at baseline}])$. (For arithmetic means, which have been recommended recently as a methodologic alternative,[26] see Table S1 in the Supplementary Appendix, available at NEJM.org.) A bootstrap-resampling method with 10,000 replicates was used to calculate 95% confidence intervals of the geometric means for the egg-reduction rates.[27] The differences in egg-reduction rates were determined under the assumption that nonoverlapping confidence intervals indicated statistical significance.

RESULTS

PARTICIPANTS AND BASELINE DATA

Of 900 children who were invited to participate, 798 had complete baseline data (Fig. 1). Of these, 774 children (97.0%) were positive for *T. trichiura*, 464 (58.1%) were infected with hookworm, and 461 (57.8%) had an *A. lumbricoides* infection. Triple-species infections were diagnosed in 316 children (39.6%).

Since we were interested in the efficacy of the drugs against *T. trichiura* infection (primary outcome) and concomitant soil-transmitted helminth infection (secondary outcome), we included all children with a *T. trichiura*–hookworm coinfection (456 of the 774 children with *T. trichiura* infection). In addition, to reach our overall estimated sample size, we included 16 children with single *T. trichiura* infection and 8 with single hookworm infection. Among these 480 children, 309 were coinfected with *A. lumbricoides*.

No significant between-group differences were observed with regard to any of the baseline characteristics ($P>0.05$). On average, children at the Mchangamdogo school, as compared with those from the Shungi school, had a lower baseline *T. trichiura* infection intensity, which was associated with lower cure rates (Table S2 in the Supplementary Appendix). A total of 4 children were absent during treatment and the follow-up survey. A total of 18 children were lost to follow-up after treatment because they did not provide two stool samples (14 children) or because identification materials were mislabeled (4). Hence, no primary-outcome data were available for 22 children.

Demographic and baseline laboratory characteristics of the 480 children included in the analysis are summarized in Table 1. Treatment groups were well balanced with respect to age, sex, weight, and height. Classifications of infection intensities according to WHO cutoffs are presented in Table 1.

EFFICACY AGAINST *T. TRICHIURA*

Cure rates and egg-reduction rates among 450 children with *T. trichiura* infection are shown in Table 2. Treatment with oxantel pamoate–albendazole resulted in a significantly higher cure rate among children with *T. trichiura* infection than did mebendazole (31.2% vs. 11.8%, $P=0.001$). Oxantel pamoate alone was associated with a significantly higher cure rate than mebendazole (26.3% vs. 11.8%, $P=0.01$). Albendazole monotherapy resulted in a significantly lower cure rate than mebendazole monotherapy (2.6% vs. 11.8%, $P=0.02$). Adjustment for school, sex, and weight did not influence these estimates. Stratification according to infection intensity showed that in both treatment groups that received oxantel pamoate, the cure rate among lightly infected children was approximately 39% (39.0% with oxantel pamoate–albendazole and 39.3% with oxantel pamoate monotherapy), whereas the cure rates

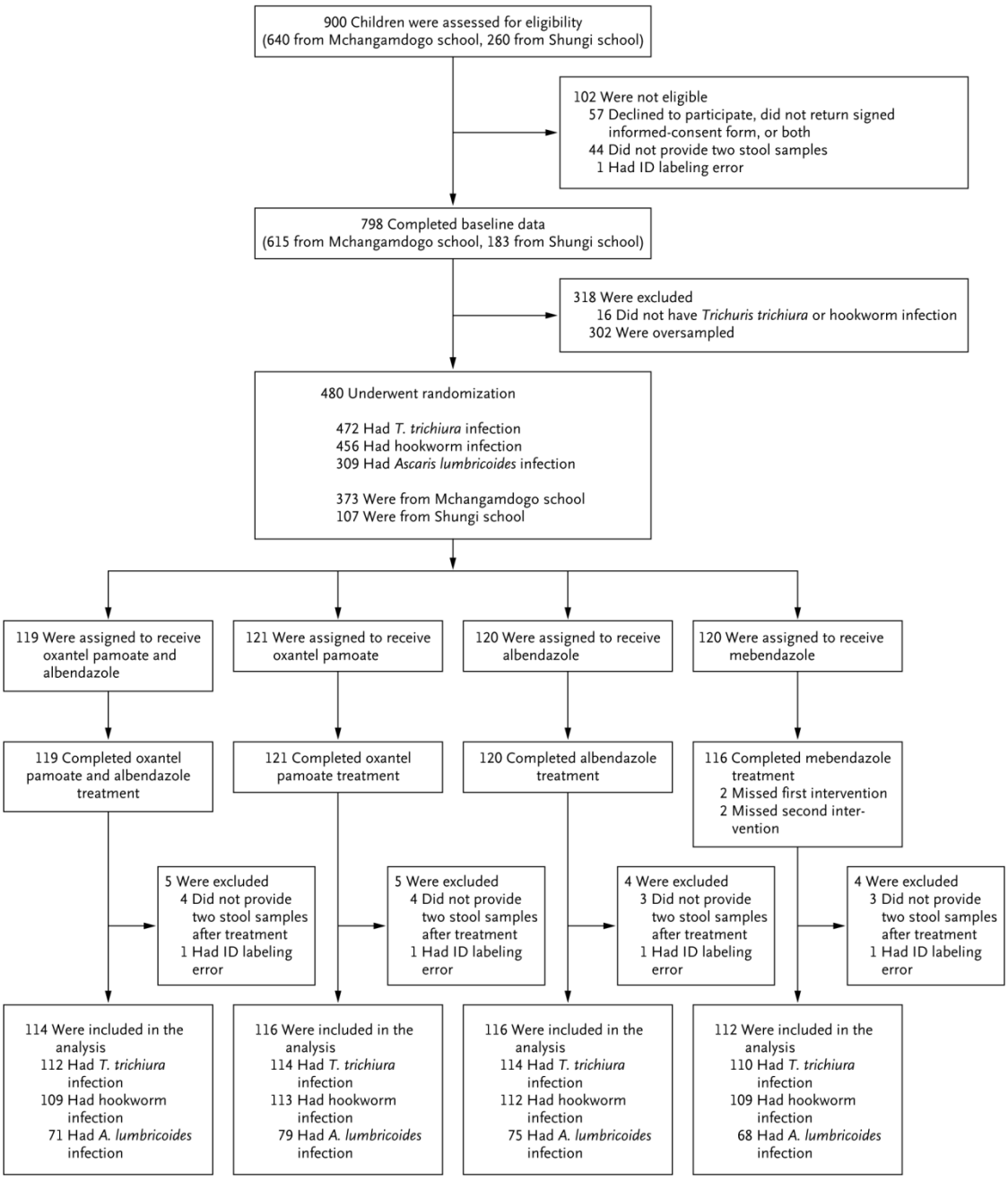

Figure 1. Study Enrollment, Randomization, and Follow-up.
ID denotes identification.

Table 1. Characteristics at Baseline.*

Characteristic	Oxantel Pamoate–Albendazole (N=119)	Oxantel Pamoate (N=121)	Albendazole (N=120)	Mebendazole (N=120)	Total (N=480)
Age — yr	9.6±1.6	9.9±1.8	9.9±1.7	9.6±1.6	9.7±1.7
Sex — no.					
Girls	58	59	55	61	233
Boys	61	62	65	59	247
School — no.					
Mchangamdogo	92	94	94	93	373
Shungi	27	27	26	27	107
Weight — kg†	25±4	26±5	25±5	25±4	25±5
Height — cm‡	128±12	128±17	129±13	128±8	128±13
Trichuris trichiura infection					
Children infected — no. (%)	117 (98.3)	119 (98.3)	118 (98.3)	118 (98.3)	472 (98.3)
Geometric mean no. of eggs/g of stool	807	885	883	847	855
Infection intensity — no. (%)§					
Light	61 (52.1)	59 (49.6)	67 (56.8)	61 (51.7)	248 (52.5)
Moderate	54 (46.2)	58 (48.7)	49 (41.5)	52 (44.1)	213 (45.1)
Heavy	2 (1.7)	2 (1.7)	2 (1.7)	5 (4.2)	11 (2.3)
Hookworm infection					
Children infected — no. (%)	113 (95.0)	118 (97.5)	116 (96.7)	117 (97.5)	464 (96.7)
Geometric mean no. of eggs/g of stool	133	122	108	112	118
Infection intensity — no. (%)¶					
Light	111 (98.2)	117 (99.2)	115 (99.1)	114 (97.4)	457 (98.5)
Moderate	0	0	1 (0.9)	2 (1.7)	3 (0.6)
Heavy	2 (1.8)	1 (0.8)	0	1 (0.9)	4 (0.9)
Ascaris lumbricoides infection					
Children infected — no. (%)	74 (62.2)	82 (67.8)	79 (65.8)	74 (61.7)	309 (64.4)
Geometric mean no. of eggs/g of stool	1920	3126	2366	2143	2368
Infection intensity — no. (%)‖					
Light	38 (51.4)	38 (46.3)	43 (54.4)	41 (55.4)	160 (51.8)
Moderate	35 (47.3)	44 (53.7)	33 (41.8)	32 (43.2)	144 (46.6)
Heavy	1 (1.4)	0	3 (3.8)	1 (1.4)	5 (1.6)

* Plus–minus values are means ±SD. There were no significant between-group differences.
† Data were missing for four children in the mebendazole group.
‡ Data were missing for two children in the albendazole group and four in the mebendazole group.
§ The intensity of *T. trichiura* infection was categorized as light (1 to 999 eggs per gram of stool), moderate (1000 to 9999 eggs per gram of stool), or heavy (≥10,000 eggs per gram of stool).
¶ The intensity of hookworm infection was categorized as light (1 to 1999 eggs per gram of stool), moderate (2000 to 3999 eggs per gram of stool), or heavy (≥4000 eggs per gram of stool).
‖ The intensity of *A. lumbricoides* infection was categorized as light (1 to 4999 eggs per gram of stool), moderate (5000 to 49,999 eggs per gram of stool), or heavy (≥50,000 eggs per gram of stool).

Table 2. Cure Rates and Egg-Reduction Rates.*

Variable	Oxantel Pamoate–Albendazole	Oxantel Pamoate	Albendazole	Mebendazole
T. trichiura				
No. of children positive for infection				
Before treatment	112	114	114	110
After treatment	77	84	111	97
Cure rate — % (95% CI)	31.2 (22.5–40.0)	26.3 (18.1–34.5)	2.6 (0.0–5.6)	11.8 (5.7–17.9)
No. of children cured/total no. with infection (%)				
From light infection	23/59 (39.0)	22/56 (39.3)	3/67 (4.5)	12/57 (21.1)
From moderate infection	12/52 (23.1)	8/57 (14.0)	0/45	1/49 (2.0)
From heavy infection	0/1	0/1	0/2	0/4
Geometric mean no. of eggs/g of stool				
Before treatment	769	874	853	813
After treatment	31	59	469	203
Egg-reduction rate — % (95% CI)	96.0 (93.5–97.6)	93.2 (90.0–95.7)	45.0 (32.0–56.4)	75.0 (64.2–82.0)
Moderately or heavily infected children with no or light infection after treatment — % (95% CI)†	84.9 (74.9–94.9)	77.6 (66.5–88.6)	46.8 (32.0–61.1)	58.5 (44.8–72.2)
Hookworm‡				
No. of children positive for infection				
Before treatment	109	113	112	109
After treatment	53	101	45	90
Cure rate — % (95% CI)	51.4 (41.8–60.9)	10.6 (4.9–16.4)	59.8 (50.6–69.0)	17.4 (10.2–24.7)
Geometric mean no. of eggs/g of stool				
Before treatment	136	127	108	109
After treatment	6	78	4	45
Egg-reduction rate — % (95% CI)	95.6 (92.8–97.3)	38.6 (19.5–55.3)	96.3 (93.9–97.6)	58.7 (42.6–71.6)
A. lumbricoides				
No. of children positive for infection				
Before treatment	71	79	75	68
After treatment	4	71	6	6
Cure rate — % (95% CI)	94.4 (88.9–99.9)	10.1 (3.3–16.9)	92.0 (85.7–98.3)	91.2 (84.3–98.1)
No. of children cured/no. with infection (%)				
From light infection	36/36 (100.0)	6/35 (17.1)	36/40 (90.0)	38/40 (95.0)
From moderate infection	31/34 (91.2)	2/44 (4.5)	31/32 (96.9)	24/28 (85.7)
From heavy infection§	0/1	—	2/3 (66.7)	—
Geometric mean no. of eggs/g of stool				
Before treatment	1967	3452	2426	1876
After treatment	<1	2472	1	1
Egg-reduction rate — % (95% CI)	99.98 (99.96–100.00)	28.4 (0.0–54.2)	99.97 (99.91–99.99)	99.94 (99.82–99.98)
Moderately or heavily infected children with no infection or light infection after treatment — % (95% CI)†	97.1 (91.3–100.0)	13.6 (3.1–24.2)	97.1 (91.3–100.0)	89.3 (77.1–100.0)

* CI denotes confidence interval.
† The goal of the World Health Organization global program for the control of soil-transmitted helminthiasis is to reduce the rate of illness from infection with soil-transmitted helminths in school-age children to below a level that would be considered a public health problem (i.e., to reduce soil-transmitted helminth infection of moderate and high intensity among school-age children to <1%).[5]
‡ Most hookworm infections (>95%) were classified as light.
§ No children in the oxantel pamoate or mebendazole monotherapy group had heavy infection.

Table 3. Adverse Events and Related Symptoms Assessed at Five Time Points.*

Time Point	Oxantel Pamoate–Albendazole	Oxantel Pamoate	Albendazole	Mebendazole	Total
	number/total number (percent)				
Before treatment	12/119 (10.1)	21/120 (17.5)	13/120 (10.8)	13/115 (11.3)	59/474 (12.4)
After first treatment					
3 hr	9/119 (7.6)	16/121 (13.2)	15/120 (12.5)	8/116 (6.9)	48/476 (10.1)
24 hr	18/119 (15.1)	20/121 (16.5)	12/120 (10.0)	21/116 (18.1)	71/476 (14.9)*
After second treatment					
3 hr	15/119 (12.6)	15/121 (12.4)	11/120 (9.2)	6/116 (5.2)	47/476 (9.9)
24 hr	15/117 (12.8)	25/118 (21.2)	15/115 (13.0)	11/110 (10.0)	66/460 (14.3)

* One child in the oxantel pamoate–albendazole group was observed with moderate episodes of diarrhea and fever 24 hours after treatment. All other adverse events were characterized as mild.

with mebendazole and albendazole were 21.1% and 4.5%, respectively, among lightly infected children. Cure rates based on different intention-to-treat approaches are shown in Table S3 in the Supplementary Appendix.

Oxantel pamoate–albendazole and oxantel pamoate monotherapy were associated with high egg-reduction rates among children with *T. trichiura* infection (96.0%; 95% confidence interval [CI], 93.5 to 97.6, and 93.2%; 95% CI, 90.0 to 95.7, respectively). A significantly lower egg-reduction rate was observed in the group that received mebendazole than in either treatment group that received oxantel pamoate (75.0%; 95% CI, 64.2 to 82.0), and the egg-reduction rate with albendazole was significantly lower than the rate with mebendazole (45.0%; 95% CI, 32.0 to 56.4).

A total of 84.9% of the children with moderate or heavy *T. trichiura* infection at baseline had either no infection or a light infection after treatment with oxantel pamoate–albendazole (Table 2). In contrast, considerably fewer children receiving mebendazole or albendazole had no infection or a light infection after treatment (58.5% and 46.8%, respectively).

EFFICACY AGAINST HOOKWORM

A total of 443 children infected with hookworm were included in the analysis. Albendazole monotherapy resulted in a significantly higher cure rate against hookworm than any other applied treatment (59.8%, vs. 17.4% with mebendazole; P<0.001). Adding oxantel pamoate did not increase the efficacy (59.8% with albendazole alone vs. 51.4% with oxantel pamoate–albendazole; P=0.21) (Table 2). High egg-reduction rates against hookworm were observed with albendazole (96.3%; 95% CI, 93.9 to 97.6) and with albendazole combined with oxantel pamoate (95.6%; 95% CI, 92.8 to 97.3). As compared with the rates after treatments that included albendazole, the egg-reduction rates were significantly lower after treatment with mebendazole (58.7%; 95% CI, 42.6 to 71.6) and after oxantel pamoate monotherapy (38.6%; 95% CI, 19.5 to 55.3).

EFFICACY AGAINST *A. LUMBRICOIDES*

Complete data were available for 293 children infected with *A. lumbricoides*. Treatment with albendazole and mebendazole resulted in high cure rates among children with *A. lumbricoides* infection (92.0% and 91.2%, respectively) (Table 2). The cure rate with oxantel pamoate–albendazole was 94.4% (95% CI, 88.9 to 99.9). All the children infected with *A. lumbricoides* who were treated with albendazole or mebendazole had egg-reduction rates close to 100%. Monotherapy with oxantel pamoate resulted in low cure and egg-reduction rates among children with *A. lumbricoides* infection.

SAFETY

Adverse events were assessed in 476 children, but not all children were available at all time points after treatment (Table 3). No serious adverse events were noted during the study. Before treatment, 59 children (12.4%) had mild symptoms. When we pooled the children in the two treatment groups that included oxantel pamoate, we found that 13.8% of the children had mild symptoms before treatment. At 3 hours and 24 hours

after the administration of oxantel pamoate, mild adverse events were observed in 10.5% and 15.8% of the children, respectively, in the pooled group. The proportions of children with mild adverse events in the groups that received the placebo matching oxantel pamoate on the first day were 11.1% before treatment and 9.7% and 14.0% at 3 hours and 24 hours after treatment, respectively. Similarly, the number of mild adverse events after the administration of albendazole and mebendazole differed only slightly from the levels observed before treatment (maximum increase, 2.7 percentage points).

The highest number of adverse events was observed 48 hours after the administration of oxantel pamoate monotherapy (24 hours after the administration of placebo), occurring in 25 children (21.2%), as compared with 21 children with adverse events (17.5%) before treatment. The largest increase in the proportion of mild adverse events was seen in the mebendazole group 24 hours after administration of the placebo matching oxantel pamoate (increase from before treatment, 6.8 percentage points). Children receiving oxantel pamoate were not absent more often during the assessment of adverse events than were those who received standard treatments.

Abdominal cramps and headache were the most frequently reported adverse events (in 15.1% and 12.4% of children, respectively) that were observed at one or more of the four time points after treatment. However, these were also the most frequently observed clinical signs and symptoms before treatment (Fig. 2, and Table S4 in the Supplementary Appendix). Overall, 147 children (30.9%) had a total of 349 adverse events, all of which were mild except for 2 moderate episodes observed in 1 child.

DISCUSSION

Given the trichuricidal properties of oxantel pamoate,[13-20] we assessed this drug in a randomized, controlled trial in a highly endemic area.[7,10] Because the geographic distribution of *T. trichiura* and other soil-transmitted helminth infections overlap,[1,2,28] we combined oxantel pamoate with albendazole to further the spectrum of activity against multiple soil-transmitted helminths. We chose albendazole because it shows the highest activity among the anthelmintic drugs currently on the market against hookworm infection.[6]

We found that oxantel pamoate (with or without albendazole) was significantly more efficacious against *T. trichiura* than was albendazole or mebendazole monotherapy. Furthermore, egg-reduction rates were more than 90% in the two treatment groups that included oxantel pamoate. As we had anticipated, oxantel pamoate showed little effect against hookworm infection.[15] In addition, little effect was observed with oxantel pamoate against *A. lumbricoides*.

As expected, albendazole showed high efficacy against hookworm and *A. lumbricoides* infections, whereas mebendazole showed only low-to-moderate activity against hookworm but high efficacy against *A. lumbricoides*.[6] The low cure and egg-reduction rates with albendazole and mebendazole among children with *T. trichiura* infection corroborate findings from prior studies.[7,10,29] Particularly low cure rates were observed among children with moderate- or high-intensity *T. trichiura* infection, regardless of whether albendazole or mebendazole was administered.

In addition, only approximately half the children presenting with moderate or heavy infection at baseline received a diagnosis of no infection or light infection after treatment with albendazole or mebendazole. These are worrying findings, because the WHO recommends the periodic administration of albendazole and mebendazole for control of morbidity due to soil-transmitted helminthiasis, but clearly, the stated goal — to reduce illness from infection with soil-transmitted helminths in school-age children to below a level that would be considered a public health problem (i.e., to reduce soil-transmitted helminth infection of moderate and high intensity among school-age children to <1%) — was not met in our study.

Adverse events, most of which were mild, were observed in approximately 30% of the children. The number of clinical symptoms observed before treatment was similar to that after treatment. The somewhat higher frequencies of symptoms in the two groups that received oxantel pamoate, as compared with the groups that received albendazole or mebendazole, had been observed even before administration of the drug. Hence, there is no indication of an increase in adverse events in the treatment groups that received oxantel pamoate, as compared with the groups that received a standard treatment. Given the limited absorption of oxantel pamoate from

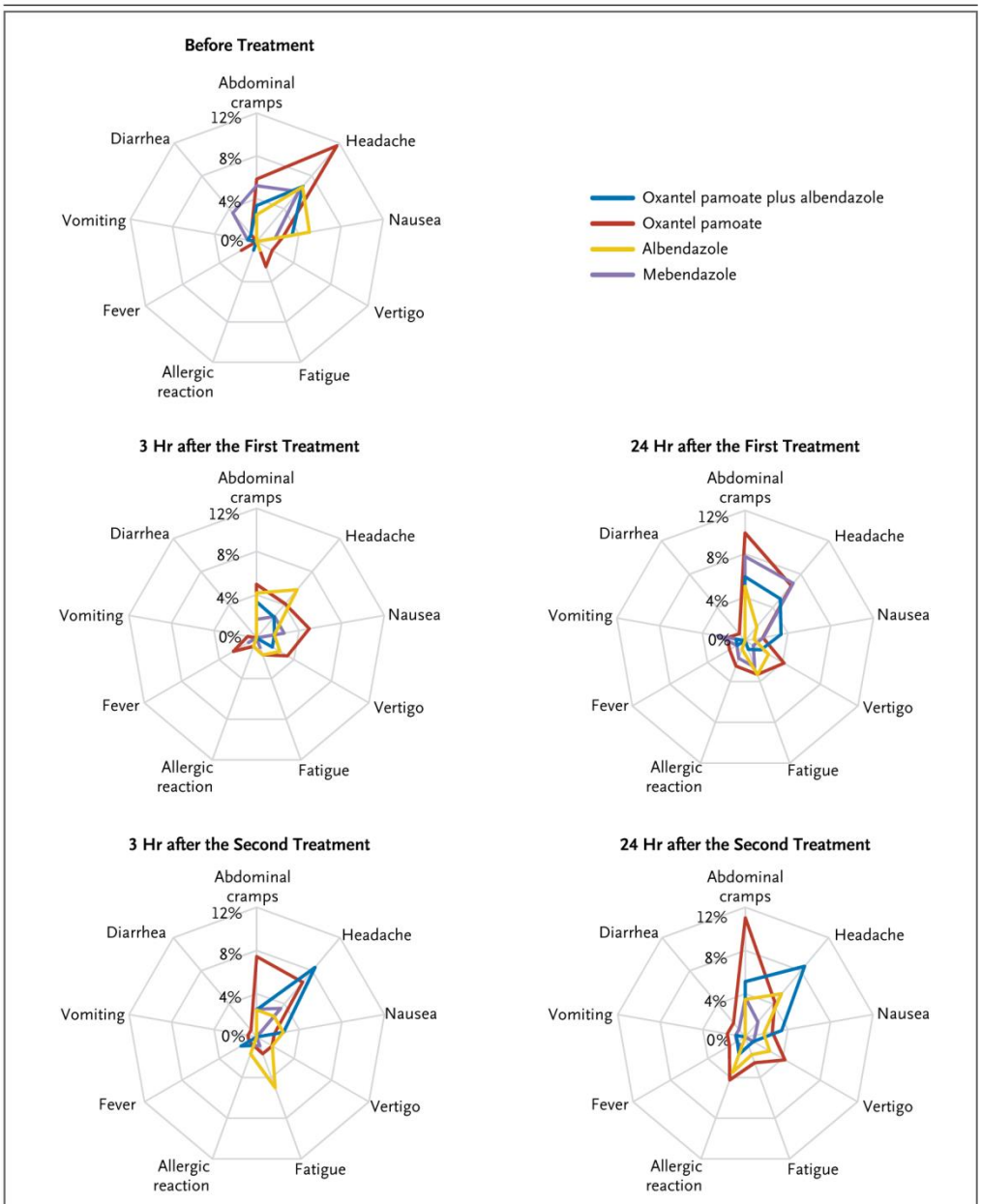

Figure 2. Clinical Symptoms and Adverse Events.

Spider plots indicate the percentage of observed clinical symptoms (before treatment) and adverse events (after treatment) in the four treatment groups. Oxantel pamoate was administered on the first day of treatment, whereas albendazole and mebendazole were administered on the second day of treatment.

the gastrointestinal tract, as reported in the veterinary health literature, our findings seem reasonable.[30] Nevertheless, the safety of oxantel pamoate warrants further scientific inquiry. Drug interaction between albendazole and oxantel pamoate should be studied in particular, because it would be operationally more convenient to administer both drugs simultaneously.

In conclusion, the combination of oxantel pamoate and albendazole had significantly higher efficacy against *T. trichiura* than did albendazole or mebendazole. Moderate and heavy *T. trichiura* infection intensities were cleared in a large proportion of the children after the administration of oxantel pamoate (with or without albendazole) — results that contrast with findings after standard treatments. Hence oxantel pamoate, particularly in combination with albendazole, could be useful in the global strategy for the control of soil-transmitted helminthiasis.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Chapter 4

Efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, albendazole plus oxantel pamoate, and mebendazole against *Trichuris trichiura* and concomitant soil-transmitted helminth infections: a four arm, randomised controlled trial

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Articles

Efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, albendazole plus oxantel pamoate, and mebendazole alone against *Trichuris trichiura* and concomitant soil-transmitted helminth infections: a four-arm, randomised controlled trial

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Summary

Background Existing anthelmintic drugs (eg, albendazole and mebendazole) have low efficacy against the intestinal nematode species *Trichuris trichiura* and the drug pipeline is exhausted. We aimed to investigate the strategy of combination chemotherapy with existing drugs to establish whether their efficacy could be enhanced and broadened.

Methods In this randomised controlled trial, we compared three drug combinations and one standard drug alone in children aged 6–14 years in two schools on Pemba Island, Tanzania infected with *T trichiura* and concomitant intestinal nematodes. We assigned children, via a randomisation list with block sizes of either four or eight, to orally receive albendazole (400 mg) plus ivermectin (200 μg/kg); albendazole (400 mg) plus mebendazole (500 mg); albendazole (400 mg) plus oxantel pamoate (20 mg/kg); or mebendazole (500 mg) alone. The primary endpoints were the proportion of children cured of *T trichiura* infection and the reduction of *T trichiura* eggs in stool based on geometric means, both analysed by available case. This study is registered with ISRCTN, number ISRCTN80245406.

Findings We randomly assigned 440 eligible children infected with *T trichiura* between Sept 2, and Oct 18, 2013, to one of the four treatment groups (110 children per group). Data for 431 children were included in the analysis for the primary endpoints. Albendazole plus oxantel pamoate (74 of 108 children cured [68·5%, 95% CI 59·6–77·4]; egg reduction 99·2%, 98·7–99·6) and albendazole plus ivermectin (30 of 109 cured [27·5%, 19·0–36·0]; egg reduction 94·5%, 91·7–96·3) were significantly more effective against *T trichiura* than mebendazole alone (nine of 107 cured [8·4%, 3·1–13·8]; egg reduction 58·5%, 45·2–70·9). Albendazole plus mebendazole had similar low efficacy (nine of 107 cured [8·4%, 3·1–13·8; egg reduction 51·6%, 35·0–65·3) to mebendazole alone. About a fifth of the children reported adverse events, which were mainly mild. Abdominal cramps and headache were the most common adverse events after treatment; abdominal cramps were reported by 13 (12·0%) children for albendazole plus ivermectin, 10 (9·3%) for albendazole plus mebendazole, 20 (18·2%) for albendazole plus oxantel pamoate, and 16 (14·5%) for mebendazole; headaches were reported by 5 (4·6%) children for albendazole plus ivermectin, 6 (5·6%) for albendazole plus mebendazole, 12 (10·9%) for albendazole plus oxantel pamoate, and 7 (6·4%) for mebendazole.

Interpretation Our head-to-head comparison of three combination chemotherapies showed the highest efficacy for albendazole plus oxantel pamoate for the treatment of infection with *T trichiura*. Further studies should investigate the combination of albendazole plus oxantel pamoate so that it can be considered for soil-transmitted helminthiasis control programmes.

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Introduction

Globally, an estimated 465 million people were infected with the intestinal nematode species *Trichuris trichiura* in 2010.[1] Additionally, an estimated 819 million people were infected with *Ascaris lumbricoides*, 439 million with hookworm,[1] and about 100 million people with *Strongyloides stercoralis*.[2] These intestinal nematodes are grouped together as soil-transmitted helminths,[3] and the global strategy for their control emphasises the large-scale use of safe, single-dose anthelmintic drugs as preventive chemotherapy.[4] In view of their straightforward administration, good safety profiles, and large-scale donations, the benzimidazoles albendazole and mebendazole are the two most widely used drugs for soil-transmitted helminth infections in preventive chemotherapy programmes.[5,6] Although one-dose treatment with both drugs shows high efficacy against *A lumbricoides*, only albendazole cures a satisfactory proportion of hookworm infections. Both drugs show low efficacy against *T trichiura*.[7–12] Hence, to accomplish the ambitious goals put forth by the London declaration on neglected tropical diseases to control soil-transmitted helminth infections by 2020, broad-spectrum anthelmintic treatments need to be developed.[13] In view of the

For the **London declaration** see http://www.who.int/neglected_diseases/London_Declaration_NTDs.pdf

absence of new products for soil-transmitted helminth infections in research and development,[14] combination chemotherapy with existing drugs offers an avenue to increase and broaden their effectiveness against these organisms and lower the risk of resistance development.

Albendazole plus ivermectin,[9,15,16] albendazole plus mebendazole,[10] and albendazole plus oxantel pamoate[12] have emerged as potential combination therapies against soil-transmitted helminth infections in a series of randomised controlled trials. These drug combinations were well tolerated and improved the proportions of patients cured of *T trichiura*, and concurrent *A lumbricoides* and hookworm infections. However, because drug efficacy is affected by myriad factors (eg, baseline infection intensity, parasite strain, host factors, and the diagnostic approach used), the findings are difficult to compare and interpret.[17,18]

We therefore undertook a randomised controlled trial to compare the efficacy and safety of albendazole plus ivermectin, albendazole plus mebendazole, and albendazole plus oxantel pamoate, with a standard treatment (one-dose mebendazole), to identify the intervention with the greatest potential against *T trichiura* and concomitant soil-transmitted helminths.

Methods

Study design and participants

We did this randomised controlled trial on Pemba Island, Tanzania. We selected two schools (Mchangamdogo and Shungi) on the basis of high prevalence of soil-transmitted helminths shown in a previous study.[12] Children in these schools receive treatment twice a year as part of mass drug-administration programmes. Before the onset of the study, we asked the school headteachers for permission to do the trial at their schools. The parents or guardians of the children were invited to the schools to be told about the aim and procedures of the study, including potential benefits and risks. Parents and teachers were encouraged to ask questions in an open discussion forum and sufficient time was given to decide whether or not to allow children to participate.

We screened children aged 6–14 years for eligibility. We obtained written informed consent from their parents or guardians; the children themselves gave assent orally. Children who tested positive for *T trichiura* were deemed eligible for the trial. Oral medical history and physical examinations were obtained from each child meeting the inclusion criteria. Children presenting with any abnormal medical disorder, as judged by the study physician, or who had a history of acute or severe chronic

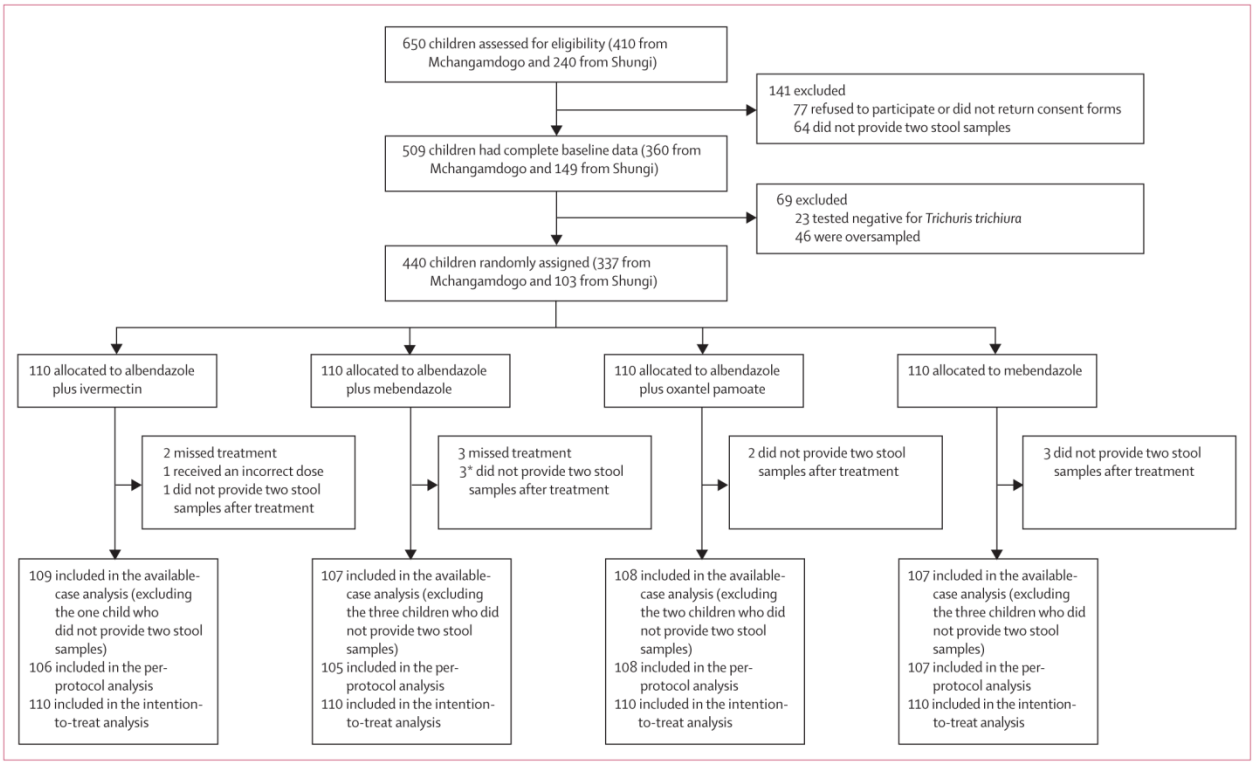

Figure 1: **Trial profile**
*One child was also absent on the day of treatment.

disease, were excluded from the trial. We allocated eligible children in the two schools to one of four oral treatment groups: albendazole (400 mg) plus ivermectin (200 µg/kg), albendazole (400 mg) plus mebendazole (500 mg), albendazole (400 mg) plus oxantel pamoate (20 mg/kg), or mebendazole alone (500 mg).

Ethical clearance was obtained from the Ministry of Health and Social Welfare in Zanzibar, Tanzania (Zamrec, 0001/June/13) and from the cantonal ethics commission of Basel, Switzerland (EKBB 123/13). Written informed consent was obtained for all children.

Randomisation and masking

We used a randomisation list (with varying random block sizes of either four or eight), provided by an independent statistician, to randomly assign children to one of the four treatment groups.

Sealed and labelled plastic bags, which included the tablets assigned by the randomisation list, were prepared by two independent pharmacists. Group assignments were concealed from the participants, investigators, and laboratory technicians doing the diagnostic tests; however, the investigator giving the treatment could recognise the drugs because of their different forms.

Procedures

We recorded the name, sex, age, and school grade of all eligible children. We gave them a consent form and an empty stool container, both labelled with unique identification numbers. Children who returned the signed consent form together with a fresh sample of their own morning stool were given a second container and asked to provide a second sample on the consecutive day. Stool samples were transferred to the Public Health Laboratory–Ivo de Carneri. Duplicate Kato-Katz thick smears were prepared from each stool sample by use of 41·7 mg templates.[19] Kato-Katz thick smears were examined by skilled laboratory technicians for eggs of *T trichiura*, hookworm, and *A lumbricoides*. All slides were read within 60 min after preparation to avoid overclearing of hookworm eggs.[20] To assure a high quality of the microscopic diagnosis, 10% of the Kato-Katz thick smears were randomly chosen and re-examined. In case of discordant results, slides were read for a third time and the results discussed until a consensus was reached; the agreement between initial readings of the Kato-Katz thick smears and the quality control was more than 95%. All stool samples were further analysed for *S stercoralis* by use of the Koga-agar plate and the Baermann techniques, in accordance with standard protocols.[21,22] Koga-agar plates containing 1–2 g of stool were cultivated for 48 h. For the Baermann technique, 20–30 g of stool was placed in a funnel for 2 h. After centrifugation, the sediment from both techniques was examined for *S stercoralis* larvae.

Children were asked what clinical symptoms they had before treatment. Their height was measured to the nearest cm and weight to the nearest 0·1 kg. Doses of ivermectin were rounded up to the nearest 3 mg tablet, and oxantel pamoate was given rounded up to the nearest half-400 mg tablet on the basis of the calculated dose per kg of bodyweight. Albendazole (400 mg) and mebendazole (500 mg) were each given as one tablet. Children received treatment just once. Albendazole, ivermectin, and mebendazole were produced by GlaxoSmithKline, Merck, and Johnson & Johnson, respectively, and oxantel pamoate was manufactured at the University of Basel.[23] We actively assessed adverse events 3 h and 24 h after treatment and provided symptomatic relief if necessary. Children gave another two stool samples 18–23 days after treatment for assessment of treatment efficacy.[24] At the end of the study, all children attending the schools were offered anthelmintic treatment as part of preventive chemotherapy programmes done by the Public Health Laboratory–Ivo de Carneri.

Outcomes

The primary endpoints were the proportion of children cured and the reduction in the number of eggs of *T trichiura* analysed by available case. Secondary outcomes were the proportion of children cured and the

	Albendazole plus ivermectin (n=110)	Albendazole plus mebendazole (n=110)	Albendazole plus oxantel pamoate (n=110)	Mebendazole (n=110)
Age* (years)	9·0 (0·1)	8·9 (0·1)	8·9 (0·1)	8·8 (0·1)
Girls	54 (49%)	53 (48%)	52 (47%)	55 (50%)
Mchangamdogo	84 (76%)	85 (77%)	84 (76%)	84 (76%)
Shungi	26 (24%)	25 (23%)	26 (24%)	26 (24%)
Weight* (kg)	22·8 (0·3)	22·6 (0·4)	22·5 (0·3)	22·3 (0·3)
Height* (cm)	125 (0·6)	124 (0·7)	124 (0·6)	124 (0·7)
Infected with *Trichuris trichiura*	110 (100%)	110 (100%)	110 (100%)	110 (100%)
Geometric mean EPG	482 (3·7)	396 (4·5)	502 (4·3)	469 (3·9)
Infection intensity				
Light (1–999 EPG)	75 (68%)	78 (71%)	72 (66%)	82 (75%)
Moderate (1000–9999 EPG)	35 (32%)	31 (28%)	37 (34%)	28 (26%)
Heavy (≥10 000 EPG)	0 (0%)	1 (1%)	1 (1%)	0 (0%)
Infected with hookworm	43 (39%)	48 (44%)	55 (50%)	43 (39%)
Geometric mean EPG	108 (4·4)	134 (4·0)	88 (4·0)	79 (3·6)
Infection intensity				
Light (1–1999 EPG)	41 (95%)	46 (96%)	54 (98%)	43 (100%)
Moderate (2000–3999 EPG)	2 (5%)	1 (2%)	1 (2%)	0 (0%)
Heavy (≥4000 EPG)	0 (0%)	1 (2%)	0 (0%)	0 (0%)
Infected with *Ascaris lumbricoides*	50 (46%)	40 (36%)	48 (44%)	47 (43%)
Geometric mean EPG	2023 (14·0)	1074 (22·2)	1230 (14·1)	922 (15·3)
Infection intensity				
Light (1–4999 EPG)	27 (54%)	20 (50%)	29 (60%)	30 (64%)
Moderate (5000–49 999 EPG)	20 (40%)	17 (43%)	18 (38%)	16 (34%)
Heavy (≥50 000 EPG)	3 (6%)	3 (8%)	1 (2%)	1 (2%)
Infected with *Strongyloides stercoralis*†	6 (6%)	10 (9%)	7 (6%)	8 (7%)

Data are mean (SD) or n (%) unless otherwise stated. EPG=eggs per gram of stool. *434 records. †Diagnosed with a different approach to the other infections, and infection intensities could not be calculated.

Table 1: **Baseline characteristics**

	Albendazole plus ivermectin	Albendazole plus mebendazole	Albendazole plus oxantel pamoate	Mebendazole
Trichuris trichiura				
Children positive before treatment (n)	109	107	108	107
Children cured after treatment (95% CI)	30 (27·5%; 19·0–36·0, p=0·001)	9 (8·4%; 3·1–13·8, p=1·000	74 (68·5%; 59·6–77·4, p<0·0001)	9 (8·4%; 3·1–13·8)
Geometric mean EPG				
Before treatment	489 (3·7)	409 (4·5)	491 (4·4)	479 (3·8)
After treatment	27 (10·2)	198 (8·5)	4 (13·2)	199 (8·4)
Egg reduction (95% CI)	94·5% (91·7–96·3)	51·6% (35·0–65·3)	99·2% (98·7–99·6)	58·5% (45·2–70·9)
Hookworm				
Children positive before treatment (n)	42	46	55	41
Children cured after treatment (95% CI)	21 (50·0%; 34·2–65·8, p=0·018)	22 (47·8% 32·8–62·8, p=0·026)	25 (45·5%; 31·9–59·0, p=0·036)	10 (24·4%; 10·7–38·1)
Geometric mean EPG				
Before treatment	108 (4·5)	137 (4·1)	88 (4·0)	79 (3·7)
After treatment	5 (7·1)	10 (11·8)	8 (9·1)	32 (9·6)
Egg reduction (95% CI)	95·4% (90·8–98·3)	92·7% (85·7–96·6)	90·9% (84·8–95·5)	59·5% (34·2–78·8)
Ascaris lumbricoides				
Children positive before treatment (n)	50	40	47	44
Children cured after treatment (95% CI)	49 (98·0%; 94·0–100·0, p=0·495)	39 (97·5%; 92·4–100·0, p=0·619)	46 (97·9%; 93·6–100·0, p=0·528)	42 (95·5%; 89·0–100·0)
Geometric mean EPG				
Before treatment	2023 (14·0)	1074 (22·2)	1378 (12·8)	1035 (14·3)
After treatment	0·04 (1·3)	0·29 (5·1)	0·22 (3·9)	0·36 (4·4)
Egg reduction (95% CI)	100·0% (99·9–100·0)	99·9% (99·8–100·0)	99·9% (99·9–100·0)	100·0% (99·8–100·0)
****Strongyloides stercoralis***				
Children positive before treatment (n)	6	10	6	7
Children cured after treatment (95% CI)	6 (100%; 54·1–100·0)	10 (100%; 69·2–100·0)	6 (100%; 54·1–100·0)	6 (85·7%; 50·8–100·0)

Data are n; n (%, 95% CI); % (95% CI); or mean (SD) unless otherwise stated. EPG=eggs per gram of stool. *Diagnosed with a different approach to the other infections, and infection intensities could not be calculated.

***Table 2*: Efficacy outcomes**

reduction in the number of eggs of concomitant nematode infections and drug safety (assessed at two timepoints) analysed by intention to treat, per protocol, and available case.

Statistical analysis

To test the primary hypothesis that a drug combination has greater efficacy than one dose of mebendazole against infection with *T trichiura*, we needed at least 92 children per group to detect a difference between the proportion of children cured of 10% and 30% (as showed in our previous study comparing mebendazole with albendazole plus oxantel pamoate),[12] with a power of 90% and a two-sided significance level of 0·05. The detection of significant differences in the proportion of children cured between the drug-combination treatments would need sample sizes that would be operationally unrealistic. However, computer simulations showed us that the expected sample size (ie, 92 children per treatment group) did allow detection of a significant difference in the egg reduction rate (ERR) of 99% versus 95% with a power of 80%. We increased the final sample size to 110 per group to account for loss to follow-up. We did not make any adjustments for multiple comparisons, because superiority of a drug combination would require each of the null hypotheses to be rejected (ie, higher proportion of children cured compared with mebendazole and larger reductions in the number of eggs compared with the two competitor combinations).

Data were double entered into a spreadsheet (Excel 2010; Microsoft (Redmond, Washington) and crosschecked. Statistical analysis was done with Stata version 10.1 (StataCorp; College Station, TX). We mainly did an available-case analysis with all children who reached the primary endpoint. Secondary analyses included a per-protocol analysis of all children who did not miss treatment, did not receive an overdose, and did not miss giving either of the two stool samples at follow-up, and an intention-to-treat analysis. For the intention-to-treat analysis, different approaches were done to impute missing data—ie, best-case scenario (all individuals with no endpoint data were assumed to be free of infection) and worst-case scenario (all individuals with no endpoint data were assumed to have the same egg counts as before treatment). The proportion of children cured was calculated as the number (percentage) of infected children at baseline who were free from eggs at follow-up. The egg counts from the quadruplicate Kato-Katz thick smears were added and multiplied by a factor of six to obtain the number of eggs per gram of stool (EPG). Cutoff measures from WHO were

used to classify infection intensities.[25] Differences between the proportions of children cured were analysed with crude logistic regressions and logistic regressions adjusted for age, sex, school, and height. Adverse events were analysed descriptively as the change in proportion of reported adverse events before and after treatment.

Geometric mean egg counts were calculated for each treatment group before and after treatment to assess the corresponding ERR (ERR=(1–[mean at follow-up/mean at baseline])×100). CIs for ERR were calculated using bootstrap resampling methods with 10 000 replicates.[26] Differences in ERR were established with the assumption that non-overlapping CIs show statistical significance.

The trial is registered at Current Controlled Trials (ISRCTN80245406).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

We randomly assigned 440 eligible children infected with *T trichiura* between Sept 2, and Oct 18, 2013, to one of the four treatment groups (110 children per group (figure 1). Nine children were excluded from the available-case analysis because they did not provide two stool samples after treatment, and 14 were excluded from the per-protocol analysis because they either missed treatment or did not provide primary endpoint data (figure 1). Treatment groups were well balanced at baseline in terms of age, sex, weight, and height (table 1). About 70% of *T trichiura* infections were classified as light; only two children had a heavy infection (>10 000 EPG; table 1). Most hookworm infections (97%) were classified as light. For *A lumbricoides*, 57% were diagnosed with a light infection, 38% with a moderate infection, and 4% with a heavy infection (table 1).

Albendazole plus oxantel pamoate cured the highest proportion of children infected with *T trichiura* of the four treatment groups, and the difference was significant compared with mebendazole (table 2). Albendazole plus ivermectin also cured significantly more children infected with *T trichiura* than did mebendazole. On the basis of the proportion of children cured of *T trichiura*, albendazole plus mebendazole was no more effective than mebendazole alone. Adjustment for school, sex, and height did not change these results (data not shown). Albendazole plus oxantel pamoate cured a significantly greater proportion of children infected with *T trichiura* than did albendazole plus ivermectin (74 [69%] of 108 *vs* 30 [28%] of 109, p<0·0001), although this comparison was not prespecified in the study protocol.

The greatest reduction in *T trichiura* eggs in the stool was achieved with albendazole plus oxantel pamoate (table 2). The reduction with albendazole plus oxantel pamoate was significantly greater than with albendazole plus ivermectin, albendazole plus mebendazole, and mebendazole alone. As with the proportion of children cured of *T trichiura*, the reduction of *T trichiura* eggs after treatment with albendazole plus mebendazole did not differ from that achieved with mebendazole alone. Egg reductions based on arithmetic means[27] are in the appendix. Secondary analyses are also presented in the appendix.

See Online for appendix

The adjusted logistic regression analysis suggested that the proportion of children cured were significantly affected by school setting (odds ratio [OR] 2·02, 95% CI 1·03–3·75). Shungi had higher infection intensities before treatment and lower proportions of children cured than Mchangamdogo, but the numbers of eggs were similar (appendix).

The proportions of children cured of hookworm infection were significantly higher for albendazole plus ivermectin and albendazole plus oxantel pamoate than for mebendazole alone (table 2). Hookworm eggs in stool were reduced by more than 90% for all three combinations; all significantly higher than for mebendazole alone (table 2). We noted no significant differences between the three drug combinations with respect to reductions in hookworm eggs. All four treatments cured more than 95% of *A lumbricoides* infections and reduced *A lumbricoides*

	Albendazole plus ivermectin (n=108)*	Albendazole plus mebendazole (n=107)	Albendazole plus oxantel pamoate (n=110)	Mebendazole (n=110)
Symptoms present before treatment	21 (19%)	11 (10%)	13 (12%)	17 (15%)
Moderate	4 (4%)	0 (0%)	1 (1%)	2 (2%)
Mild	16 (15%)	11 (10%)	12 (11%)	14 (13%)
None	88 (81%)	96 (90%)	97 (88%)	94 (85%)
Abdominal cramps	7 (6%)	4 (4%)	4 (4%)	6 (5%)
Headache	7 (6%)	6 (6%)	5 (5%)	4 (4%)
Symptoms present 3 h after treatment	10 (9%)	9 (8%)	12 (13%)	7 (6%)
Moderate	0 (0%)	1 (1%)	2 (2%)	0 (0%)
Mild	10 (9%)	8 (7%)	12 (11%)	7 (6%)
None	98 (91%)	98 (92%)	96 (87%)	103 (94%)
Abdominal cramps	4 (4%)	7 (6%)	11 (10%)	4 (4%)
Headache	4 (4%)	3 (3%)	8 (7%)	2 (2%)
Symptoms present 24 h after treatment	15 (16%)	12 (11%)	19 (17%)	18 (16%)
Moderate	0 (0%)	1 (1%)	5 (5%)	2 (2%)
Mild	17 (16%)	11 (10%)	14 (12%)	15 (14%)
None	91 (84%)	95 (89%)	91 (83%)	93 (84%)
Abdominal cramps	9 (8%)	3 (3%)	14 (13%)	12 (11%)
Headache	1 (1%)	4 (4%)	6 (5%)	7 (6%)

Data are n (%). *One child allocated to albendazole plus ivermectin received additional mebendazole because of an identification error and was therefore overdosed; this child did not report any adverse events 3 h or 24 h after treatment. There was no sign of adverse events that could be attributed to the drugs alone.

Table 3: **Adverse events**

eggs in stool by nearly 100%; we noted no significant differences between the combination treatments and mebendazole alone with respect to the number of children cured of *A lumbricoides* infection, nor any significant differences in egg reduction between the four treatment groups. With the exception of a single child, all *S stercoralis* infections were cured irrespective of treatment regimen (table 2; note that *S stercoralis* infections are diagnosed with a different approach, and infection intensities could not be calculated).

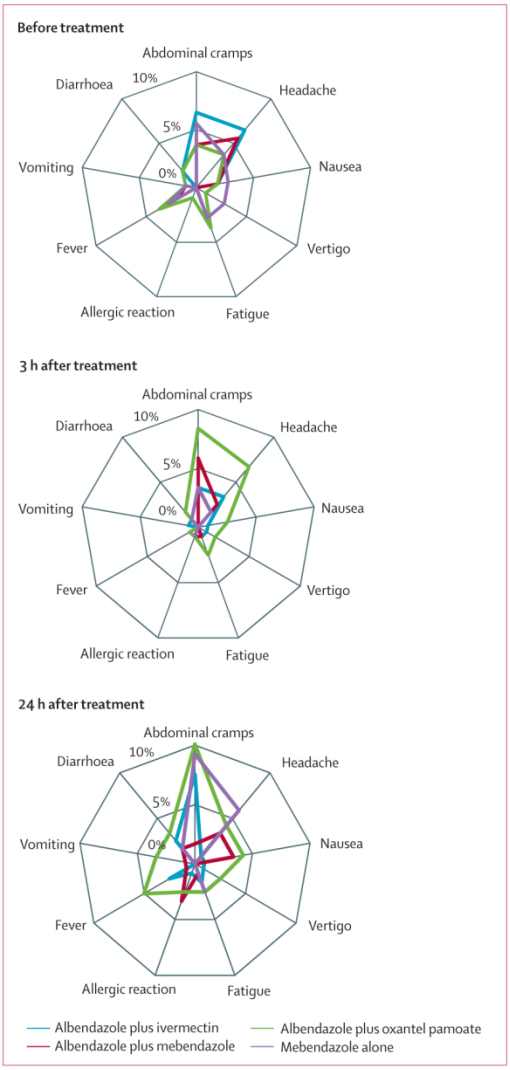

***Figure 2*: Mild clinical symptoms (before treatment) and mild adverse events (after treatment)**

Number of moderate events: Before treatment with albendazole plus ivermectin—three diarrhoea, one allergic reaction. 3 h after treatment with albendazole plus ivermectin—none. 24 h after treatment with albendazole plus ivermectin—one abdominal cramp, one fever; Before treatment with albendazole plus mebendazole—none. 3 h after treatment with albendazole plus mebendazole—one vomiting. 24 h after treatment with albendazole plus mebendazole—one abdominal cramp; Before treatment with albendazole plus oxantel pamoate—one headache. 3 h after treatment with albendazole plus oxantel pamoate—two vomiting, one abdominal cramp, one allergic reaction. 24 h after treatment with albendazole plus oxantel pamoate—three vomiting, one diarrhoea, two abdominal cramps, one fatigue; Before treatment with mebendazole alone—two diarrhoea. 3 h after treatment with mebendazole alone—none. 24 h after treatment with mebendazole alone—one abdominal cramp, one allergic reaction.

Adverse events that occurred 3 h and 24 h after treatment were assessed in all 435 treated children (table 3). Before treatment, 60 children (14%) reported clinical symptoms including seven moderate episodes (five episodes of diarrhoea, one child with headache, and one child with an allergy). 3 h after treatment, the investigators assessed and reported adverse events in 40 children (9%). 24 h after treatment, the overall proportion of children reporting symptoms increased slightly to 65 (15%), with slight differences between the four treatment groups (differences from before to after treatment ranging from –3% for albendazole plus ivermectin to 6% for albendazole plus oxantel pamoate). Eight children reported moderate adverse events 24 h after treatment; five of these eight children were in the albendazole plus oxantel pamoate group.

Abdominal cramps and headache were the most common adverse events after treatment and were reported at least once after treatment by 13 (12%) and 5 (5%) children for albendazole plus ivermectin, 10 (9%) and 6 (6%) for albendazole plus mebendazole, 20 (18%) and 12 (11%) for albendazole plus oxantel pamoate, and 16 (15%) and 7 (6%) for mebendazole. These symptoms were already the most common before treatment (figure 2, appendix). Overall at the two timepoints after treatment, 88 children (20%) had a total of 171 adverse events, of which 15 episodes (reported by ten children) were moderate. No serious adverse events were reported.

Discussion

Albendazole plus ivermectin, and albendazole plus oxantel pamoate had greater efficacy against *T trichiura* compared with the established standard treatment of one-dose mebendazole. Albendazole plus oxantel pamoate showed the greatest efficacy in this study, confirming its promising anthelmintic potential shown in our predecessor trial (see panel). New drugs are needed to improve control and potentially achieve elimination of soil-transmitted helminthiasis and other neglected tropical diseases, yet a systematic assessment has emphasised the dry pipeline in drug and vaccine development for most neglected tropical diseases.[14] The use of drug combinations might help to bridge this gap in drug discovery and development, and lower the risk of resistance development.[28] Previous studies of drug combinations documented high efficacy against *T trichiura*.[9,10,12,15,16] However, direct comparison between individual studies is not possible because of differences between the settings and methods used. To our knowledge, this is the first comparative appraisal of three broad-spectrum anthelmintic drug combinations with a

randomised controlled design in a highly endemic area.[11,12,29] For our study, only albendazole-based combinations were selected because of the high efficacy of albendazole against *A lumbricoides* and hookworm infections, which often co-occur in settings where *T trichiura* is endemic.[1]

On the basis of WHO recommendations and assessments of efficacy based on arithmetic means[27] (appendix) rather than geometric mean egg counts (presented in this paper), a somewhat higher reduction in the number of eggs was shown for albendazole plus ivermectin compared with albendazole plus oxantel pamoate, mainly caused by one extreme patient value. Recalculating the reduction in the number of eggs using the 10% trimmed arithmetic mean after treatment (see appendix)—which reduces the effects of outliers—resulted in reductions of 87% for albendazole plus ivermectin and 93% for albendazole plus oxantel pamoate (data not shown). The large reduction in the number of eggs noted for albendazole plus ivermectin accords with results from a clinical trial that assessed this combination.[9] We could not substantiate results by Namwanje and colleagues,[10] (46% children cured [95% CI 35·7–56·4], 93% egg reduction) because the efficacy for the albendazole plus mebendazole combination was similar to that of mebendazole alone. The different susceptibility of parasite strains at the two study sites might account for this contradictory finding.[30,31]

As expected, mebendazole had very poor efficacy in this study. The proportion of children cured of *T trichiura* by mebendazole was even slightly lower in this study than in a previous trial (8% *vs* 12%),[12] despite lower intensities of baseline infection. According to WHO, patients with reductions in the number of eggs (calculated from arithmetic means) that are less than 50% for *T trichiura* and hookworm are of concern and should undergo further investigations.[32] The reductions in the number of eggs established for mebendazole in this trial (eg, 14% for *T trichiura* and 12% for hookworm; see appendix) are in this category; however, although they are lower than in previous studies,[33] they are not untypical for the setting of Pemba Island[11,12] and could denote tolerance of, or resistance to, the benzimidazoles. The poor efficacy of the standard drugs clearly shows that new treatment options are needed.

Compared with our work with albendazole plus oxantel pamoate done in 2012 at the same schools on Pemba Island,[12] in the present study this combination therapy cured a higher proportion of children of *T trichiura*. Comparison of the proportions of children cured between studies can be problematic[18] and the underlying factors for varying drug efficacy are often difficult to identify, perhaps because different infection intensities at baseline probably affect the proportion of children cured.[12] Another possibility for the increased proportion of children cured with albendazole plus oxantel pamoate in the present study might be the presence of synergistic effects from the simultaneous use of albendazole and oxantel pamoate. In our previous trial, we spaced out the administration of albendazole and oxantel pamoate by a day, because any drug interactions had not been studied at that time.[12]

As projected, all combinations tested had high efficacy against co-infections with *A lumbricoides* and hookworm, but mebendazole showed high efficacy only against *A lumbricoides* co-infections. We documented a low baseline prevalence of 7% of *S stercoralis* infections. Although ivermectin, albendazole, and mebendazole have potential in the treatment of *S stercoralis* infection,[34–37] the proportions of children cured were surprisingly high. This might be attributable to the insensitive diagnostic techniques used[38] and that infections were light.

About 20% of children had adverse events after treatment during at least one assessed timepoint (3 h or 24 h after treatment). Eight children had moderate adverse events 24 h after treatment; five of these children had been treated with albendazole plus oxantel pamoate. This finding might be a random outcome because before treatment, moderate symptoms were clustered in one treatment group (ie, albendazole plus ivermectin). However, future studies with larger sample sizes are warranted to examine the safety of oxantel pamoate, and to detect any possible differences in adverse events with this particular combination therapy compared with the standard treatments.

In conclusion, we identified two combination therapies against *T trichiura* that have significantly higher efficacy than the standard treatment of mebendazole alone: albendazole plus oxantel pamoate and albendazole plus ivermectin. On one hand, the combination including oxantel pamoate cured a larger proportion of patients than the combination with ivermectin. On the other hand, ivermectin has the advantage of also being the standard treatment against *S stercoralis*, the most

***Panel:* Research in context**

Systematic review

We searched PubMed for any article published before Aug 31, 2014, with the search terms "*Trichuris trichiura*", "treatment", and "efficacy". We identified strong evidence, including a systematic review and meta-analysis[7] and some clinical trials,[8–12] for the low efficacy of the existing drugs albendazole and mebendazole against *T trichiura*. We identified three albendazole-based drug combinations (albendazole [400 mg] plus ivermectin [200 μg/kg],[9,15,16] albendazole [400 mg] plus mebendazole [500 mg],[10] and albendazole [400 mg] plus oxantel pamoate [20 mg/kg])[12] that showed high efficacy against *T trichiura*. However, the efficacy and safety of these three drug combinations had not been assessed within a head-to-head clinical trial.

Interpretation

Our study provides the first evidence that a combination of albendazole plus oxantel pamoate is the most effective treatment for infections with *T trichiura*. By contrast with previous work, our study directly compared three combined treatments. Our results suggest that mebendazole alone or combined with albendazole cannot be recommended as treatment for these infections. The frequency and severity of adverse events noted after combination chemotherapy were similar to those reported for mebendazole alone.

neglected of the intestinal nematode species.[2,38] Safety and dose-finding studies with oxantel pamoate should be done because mass drug-administration programmes would benefit from the availability of a fixed-dose that is independent from patient's weight.

Contributors

BS, IIB, MA, JHa, JU, and JK designed the study. RA and JHu produced the oxantel pamoate tablets. BS, SMAl, SMAm, IIB, and JK implemented the study. BS, JHa, and JK analysed and interpreted the data. BS and JK wrote the first draft of the report, and IIB, MA, JHa, and JU reviewed it. All authors read and approved the final version.

Declaration of interests

We declare no competing interests.

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Chapter 5

Comparison of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections in the framework of a randomised controlled trial

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ARTICLE

Comparison of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections in the framework of a randomised controlled trial

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Abstract Soil-transmitted helminth infections are a major public health problem. An accurate diagnosis is important in order to identify individuals and communities in need of intervention, and for monitoring drug efficacy and potential emergence of resistance. We compared the accuracy of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections within a randomised controlled trial. Quadruplicate Kato-Katz thick smears (duplicate Kato-Katz from two stool samples each) were examined before (baseline) and 3 weeks after treatment (follow-up). Additionally, at baseline and follow-up, the first stool sample was subjected to an ether-concentration method. We determined the prevalence, sensitivity, negative predictive value, diagnostic agreement and cure rates for single and duplicate Kato-Katz thick smears from the first stool sample, quadruplicate Kato-Katz thick smears produced from two stool samples and single ether-concentration as compared to our 'gold' standard (i.e. quadruplicate Kato-Katz plus ether-concentration). Quadruplicate Kato-Katz revealed a higher sensitivity than single ether-concentration for *Trichuris trichiura* at baseline (94.3 % vs. 88.5 %, $p=0.002$) and follow-up (93.8 % vs. 83.5 %, $p<0.001$). In contrary, at follow-up, ether-concentration showed a higher sensitivity than quadruplicate Kato-Katz for *Ascaris lumbricoides* diagnosis (86.7 % vs. 46.7 %, $p=0.012$). The ether-concentration method showed similar or slightly higher sensitivity than the Kato-Katz technique based on a single stool sample for all soil-transmitted helminth infections. The estimated cure rates were heavily dependent on the diagnostic technique and sampling effort. In conclusion, data on the prevalence of soil-transmitted helminth infections and the efficacy of anthelminthics are greatly influenced by the diagnostic method and sampling effort. The ether-concentration technique is a valuable alternative to the Kato-Katz method for helminth diagnosis.

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Introduction

Chronic infections with one or several of the common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) cause an estimated global burden of 5.2 million disability-adjusted life years (DALYs) [1]. Preventive chemotherapy, that is, the large-scale administration of anthelminthic drugs (mainly the two benzimidazoles albendazole and mebendazole) to at-risk populations to avert morbidity, is the mainstay of control [2–4]. Accurate diagnostic tools are of pivotal importance for the identification of infected individuals, assessing endemicity in a given epidemiological setting and monitoring of drug efficacy and response to interventions, including resistance development [5, 6]. The most widely used technique to diagnose intestinal helminth infections (i.e. soil-transmitted helminths, *Schistosoma*

japonicum, *S. mansoni* and *S. mekongi*) is the Kato-Katz method [7–11]. A single Kato-Katz thick smear examines, on average, 41.7 mg of stool on a microscopic slide for the detection and quantification of helminth eggs. The diagnostic sensitivity of the Kato-Katz method can be improved by examining multiple thick smears from a single stool sample or by examining multiple stool samples [8, 12–17]. Nevertheless, the Kato-Katz technique has limitations, in particular, when the prevalence of soil-transmitted helminth infections is lower than 20 % or when infection intensities are low [5, 18].

The ether-concentration method is a diagnostic approach which can detect soil-transmitted helminth eggs within a large amount of a fixed stool sample [19]. For example, a recent study conducted in the People's Republic of China revealed that the ether-concentration method based on one single stool sample is nearly as sensitive as the Kato-Katz method based on two to three stool samples for the diagnosis of soil-transmitted helminth infections [14].

The aim of the present study was to assess the sensitivity of single and multiple Kato-Katz thick smears in comparison to the ether-concentration method for the diagnosis of soil-transmitted helminths before and after a treatment intervention. Stool samples were collected in the frame of a randomised controlled trial, carried out among school-aged children on Pemba Island, Tanzania, which evaluated the efficacy and safety of nitazoxanide, albendazole and nitazoxanide–albendazole against *T. trichiura* infections [20]. We assessed the influence of the diagnostic technique and sampling effort on the prevalence of soil-transmitted helminth infections before and after treatment, as well as on observed cure rates (CRs).

Materials and methods

Ethics statement

The data presented here stem from a randomised controlled trial carried out among school-aged children on Pemba Island, Tanzania. The ethics statement has been presented previously [20]. In short, ethical clearance was obtained from the Ministry of Health and Social Welfare in Zanzibar (ZAMREC, reference no. 0001/010) and from the ethics committee of Basel, Switzerland (EKBB, reference no. 225/10). The study is registered at Current Controlled Trials (ISRCTN08336605). Written informed consent was acquired from the children's parents or legal guardians to participate in this trial. Children provided assent. At the end of the study, all children remaining positive for soil-transmitted helminths were offered standard treatment (i.e. 400 mg albendazole).

Collection and processing of stool samples

Stool samples were collected from school-aged children participating in the trial, which assessed the safety and efficacy of the following four treatments: (i) nitazoxanide–albendazole combination (1,000 mg–400 mg), with each drug given separately on two consecutive days; (ii) single albendazole (400 mg); (iii) single nitazoxanide (1,000 mg); and (iv) placebo. The clinical trial took place from June to July 2011. The study setting and trial procedures have been described elsewhere [20].

Two stool samples collected before (baseline) and 3 weeks after treatment (follow-up) were available from 657 children. From each stool sample, duplicate Kato-Katz thick smears were prepared according to guidelines put forward by the World Health Organization (WHO) [18]. Within 30 min, Kato-Katz thick smears were examined quantitatively for hookworm eggs. Subsequently, the slides were re-read for *A. lumbricoides* and *T. trichiura* eggs that were counted and recorded separately. Additionally, from 550 children, approximately 2 g of stool from the first specimen before and after treatment were fixed in 10 ml of 5 % formalin in 15-ml Falcon tubes. Formalin-fixed samples were transferred to the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland, and semi-quantitatively analysed for soil-transmitted helminth eggs and intestinal protozoan cysts within 10 months, using an ether-concentration method [21, 22]. Results pertaining to intestinal protozoa infections have been described elsewhere [23]. Complete datasets were available from 528 individuals (i.e. quadruplicate Kato-Katz thick smears before and after treatment, as well as a single formalin-fixed stool sample subjected to an ether-concentration method before and after treatment) and considered for the present work.

Statistical analysis

All data were double-entered into an Excel file (Microsoft 2010) and cross-checked. For statistical analysis, Stata version 10.1 was used (StataCorp., College Station, TX, USA).

The prevalence at baseline and treatment follow-up was calculated as the percentage of individuals diagnosed positive for a specific soil-transmitted helminth species, considering the following diagnostic approaches: (i) the first Kato-Katz thick smear from the first stool sample; (ii) duplicate Kato-Katz thick smears from the first stool sample; (iii) quadruplicate Kato-Katz thick smears (two stool samples, each subjected to duplicate Kato-Katz); and (iv) single ether-concentration test from the first stool sample. As the diagnostic 'gold' standard, we considered the combined results from quadruplicate Kato-Katz thick smears plus single ether-concentration. Each sample found positive with either method was considered as "true positive". The sensitivity and negative predictive value (NPV) were calculated for the different diagnostic

approaches and sampling efforts before and after treatment, assuming that the 'gold' standard had a sensitivity of 100 %. Differences in prevalence were determined under the assumption that non-overlapping 95 % confidence intervals (CIs) indicate statistical significance. Cohen's kappa measure was used to assess agreement between the two methods and between different sampling efforts for the Kato-Katz technique, as follows: $\kappa<0$, no agreement; $\kappa=0$–0.20, poor agreement; $\kappa=0.21$–0.40, fair agreement; $\kappa=0.41$–0.60, moderate agreement; $\kappa=0.61$–0.80, substantial agreement; and $\kappa=0.81$–1.00, nearly perfect agreement [24, 25]. The McNemar test was used to examine differences in sensitivity between diagnostic methods. Therefore, only individuals who were positive according to the 'gold' standard were included. For a sample size of 20 or fewer individuals in the discordant pair, the exact McNemar value was taken (p*), otherwise, we used the McNemar χ^2 value (p) [26].

The CR was calculated as the percentage of individuals who were diagnosed negative at the 3-week post-treatment follow-up, but had a positive diagnostic test before drug administration. CRs were calculated for each diagnostic approach and for each treatment arm individually. Differences between CRs among the two diagnostic methods and varying sampling efforts were compared with a two-sample test of proportion.

Results

Baseline data

The overall prevalence in school-aged children in the study setting before treatment based on our 'gold' standard was 87.1 % (95 % CI, 84.3–90.0 %) for *T. trichiura*, 11.2 % (95 % CI, 8.5–13.9 %) for hookworm and 6.8 % (95 % CI, 4.7–9.0 %) for *A. lumbricoides* (Table 1). For all three soil-transmitted helminth species, statistically significantly lower prevalences were calculated when relying on the Kato-Katz method from a single stool sample compared to the 'gold' standard, regardless of whether stool samples were subjected to single or duplicate Kato-Katz thick smears.

Duplicate Kato-Katz thick smears identified a larger number of children with *T. trichiura* and *A. lumbricoides* infection compared to the ether-concentration method, while the ether-concentration method detected slightly more hookworm cases. However, these differences lacked statistical significance, as indicated by overlapping 95 % CIs.

Our study revealed a high sensitivity for all diagnostic methods for the detection of *T. trichiura* eggs (Table 1). On the contrary, the sensitivity for hookworm and *A. lumbricoides* was considerably lower for all diagnostic methods. Quadruplicate Kato-Katz thick smears showed the highest sensitivity for *T. trichiura* and *A. lumbricoides* diagnosis compared to less intensive sampling and the ether-concentration method. The McNemar test revealed a statistically significantly higher sensitivity at baseline using quadruplicate Kato-Katz thick smears compared to the ether-concentration method for diagnosing *T. trichiura* (94.3 % vs. 88.5 %; $p=0.002$; Table 2). The ether-concentration method had the highest sensitivity for hookworm. We observed a significant difference for hookworm diagnosis with the ether-concentration method compared to a single Kato-Katz (69.5 % vs. 39.0 %; $p=0.004$).

NPVs were high for both methods and different sampling efforts of the Kato-Katz technique for hookworm and *A. lumbricoides* diagnosis (92.9–98.2 %), while lower NPVs were observed for *T. trichiura*, ranging from 49.6 % (single Kato-Katz) to 72.3 % (quadruplicate Kato-Katz).

The agreement between the ether-concentration method and quadruplicate Kato-Katz thick smears was moderate for all helminth species before treatment (*T. trichiura*, $\kappa=0.54$; *A. lumbricoides*, $\kappa=0.48$; hookworm, $\kappa=0.47$) (Table 2).

Treatment follow-up

The overall prevalence 3 weeks after treatment according to our 'gold' standard was 84.9 % (95 % CI, 81.8−87.9 %) for *T. trichiura*, 7.2 % (95 % CI, 5.0−9.4 %) for hookworm and 5.7 % (95 % CI, 3.7−7.7 %) for *A. lumbricoides* (Table 3). Single stool examination with the Kato-Katz and ether-concentration methods resulted in a significantly lower prevalence for *T. trichiura* compared to the 'gold' standard. Duplicate Kato-Katz thick smears revealed a significantly higher number of *T. trichiura* compared to the ether-concentration method. Duplicate Kato-Katz showed a better diagnostic performance than the ether-concentration method with regard to detecting hookworm eggs. On the other hand, the ether-concentration method detected more *A. lumbricoides* cases compared to quadruplicate Kato-Katz thick smears. However, these differences lacked statistical significance.

The McNemar test revealed that quadruplicate Kato-Katz thick smears were more sensitive for diagnosing *T. trichiura* than the ether-concentration method (93.8 % vs. 83.5 %; $p<0.001$; Table 2). On the other hand, the ether-concentration method showed a significantly higher sensitivity for *A. lumbricoides* compared to the Kato-Katz method based on one stool sample (86.7 vs. 40.0; $p=0.003$), but also compared to quadruplicate Kato-Katz thick smears (86.7 vs. 46.7; $p=0.012$).

The NPVs calculated at the 3-week post-treatment follow-up for *T. trichiura* ranged from 55.2 % (single Kato-Katz) to 74.1 % (quadruplicate Kato-Katz), while the NPVs for hookworm and *A. lumbricoides* diagnosis were above 95 % for both techniques, regardless of the sampling effort. The agreement between quadruplicate Kato-Katz thick smears and the ether-concentration method was moderate at follow-up

Table 1 Prevalence of soil-transmitted helminth infections on Pemba Island, Tanzania, in mid-2011 according to different diagnostic methods and their corresponding sensitivities and negative predictive values at baseline

Parasite	Diagnostic technique	Number of infected children			Sensitivity in % (95 % CI)	NPV (95 % CI)
		n	%	95 % CI		
T. trichiura	'Gold' standard	460	87.1	84.3–90.0	100.0	100.0
	Single Kato-Katz	391	74.1	70.3–77.8	85.0 (81.4–88.1)	49.6 (41.0–58.3)
	Duplicate Kato-Katz	406	76.9	74.4–80.5	88.3 (85.0–91.1)	55.7 (46.5–64.6)
	Quadruplicate Kato-Katz	434	82.2	78.9–85.5	94.3 (91.8–96.3)	72.3 (62.0–80.8)
	Ether-concentration	407	77.1	73.5–80.7	88.5 (85.2–91.2)	56.2 (46.9–65.1)
Hookworm	'Gold' standard	59	11.2	8.5–13.9	100.0	100.0
	Single Kato-Katz	23	4.4	2.6–6.1	39.0 (26.5–52.6)	92.9 (90.2–94.9)
	Duplicate Kato-Katz	24	4.6	2.8–6.3	40.7 (28.1–54.3)	93.1 (90.4–95.0)
	Quadruplicate Kato-Katz	38	7.2	5.0–9.4	64.4 (50.9–76.4)	95.7 (93.4–97.3)
	Ether-concentration	41	7.8	5.5–10.1	69.5 (56.1–80.8)	96.3 (94.1–97.7)
A. lumbricoides	'Gold' standard	36	6.8	4.7–9.0	100.0	100.0
	Single Kato-Katz	14	2.7	1.3–4.0	38.9 (23.1–56.5)	95.7 (93.5–97.2)
	Duplicate Kato-Katz	16	3.0	1.6–4.5	44.4 (27.9–61.9)	96.1 (93.9–97.5)
	Quadruplicate Kato-Katz	27	5.1	3.2–7.0	75.0 (57.8–87.9)	98.2 (96.5–99.1)
	Ether-concentration	21	4.0	2.3–5.7	58.3 (40.8–74.5)	97.0 (95.1–98.3)

(*T. trichiura*, κ=0.49; *A. lumbricoides*, κ=0.48; hookworm, κ=0.42) (Table 2).

Estimated CRs

CRs calculated for each diagnostic approach for the three soil-transmitted helminths, stratified by treatment arm, are presented in Table 4. According to our 'gold' standard, CRs for *T. trichiura* were 11.7 % (95 % CI, 5.3–18.0 %) for the nitazoxanide–albendazole combination, 9.1 % (95 % CI, 3.6–14.5 %) for single albendazole and 1.6 % (95 % CI, 0.0–3.9 %) for single nitazoxanide. Overall CRs (including the placebo treatment arm), according to our 'gold' standard, were significantly lower compared to all diagnostic tests relying on a single stool sample ('gold' 7.2 %; single Kato-Katz, 16.4 %; duplicate Kato-Katz, 15.8 %; ether-concentration, 17.2 %; 'gold' vs. single Kato Katz $p<0.001$; 'gold' vs. duplicate Kato-Katz, $p<0.001$; 'gold' vs. ether-concentration $p<0.001$). Borderline significance was observed for quadruplicate Kato-Katz thick smears (CR: 10.8 %) compared to the 'gold' standard ($p=0.056$). CRs based on quadruplicate Kato-Katz thick smears were significantly lower compared to diagnostic approaches based on a single stool sample (quadruplicate Kato-Katz vs. single Kato-Katz, $p=0.012$; duplicate Kato-Katz vs. single Kato-Katz, $p=0.035$; quadruplicate Kato-Katz vs. ether-concentration, $p=0.008$).

The overall CR according to the 'gold' standard against hookworm was 59.3 % (95 % CI, 46.4–72.2 %). The CR for the individual diagnostic approaches were higher (Table 4); however, the differences lacked statistical significance. For *A. lumbricoides*, we determined an overall CR of 63.9 % (95 % CI, 47.4–80.4 %) using the 'gold' standard. Using single or duplicate Kato-Katz from a single stool sample and the ether-concentration technique resulted in lower overall CRs than the 'gold' standard, while higher CRs were recorded when analysing quadruplicate Kato-Katz (all $p>0.05$).

Discussion

Accurate diagnostic methods are mandatory for assessing soil-transmitted helminth infections, drug efficacies and the possible development of drug resistance [3, 5, 6, 27]. The most widely used technique for diagnosing soil-transmitted helminth infections is the Kato-Katz method [18]. Because of the low sensitivity of a single Kato-Katz thick smear, it is recommended to collect consecutive stool samples that are subjected to multiple Kato-Katz thick smears to enhance the sensitivity [12, 14–16]. We assessed the diagnostic performance of single, duplicate (from a single stool sample) and quadruplicate Kato-Katz thick smears (from two samples) in the frame of a randomised controlled trial and compared the results with an ether-concentration technique using formalin-fixed stool samples. The latter method is rarely used in settings where soil-transmitted helminths are endemic, although it is often used in reference laboratories in Europe [22].

Our results demonstrate that the ether-concentration method has a similar or even higher sensitivity for the diagnosis of all three soil-transmitted helminths than single or duplicate Kato-Katz prepared from a single stool sample. Hence, we

Table 2 Agreement between ether-concentration and Kato-Katz (different sampling efforts) for the diagnosis of soil-transmitted helminths among school-aged children on Pemba Island, Tanzania, in mid-2011

Before treatment		Ether-concentration		Total	Kappa	McNemar	After treatment		Ether-concentration		Total	Kappa	McNemar
		−	+						−	+			
T. trichiura													
Single Kato-Katz	−	83	54	137	0.53	$p=0.095$	Single Kato-Katz	−	101	44	145	0.55	$p=0.361$
	+	38	353	391				+	53	330	383		
	Total	121	407	528				Total	154	374	528		
Duplicate Kato-Katz	−	74	48	122	0.49	$p=0.918$	Duplicate Kato-Katz	−	99	43	142	0.54	$p=0.225$
	+	47	359	406				+	55	331	386		
	Total	121	407	528				Total	154	374	528		
Quadruplicate Kato-Katz	−	68	26	94	0.54	$p=0.002$	Quadruplicate Kato-Katz	−	80	28	108	0.49	$p<0.001$
	+	53	381	434				+	74	346	420		
	Total	121	407	528				Total	154	374	528		
Hookworm													
Single Kato-Katz	−	476	29	505	0.34	$p=0.004$	Single Kato-Katz	−	495	14	509	0.37	$p=0.549$
	+	11	12	23				+	11	8	19		
	Total	487	41	528				Total	506	22	528		
Duplicate Kato-Katz	−	476	28	504	0.36	$p=0.007$	Duplicate Kato-Katz	−	495	14	509	0.31	$p=0.549$
	+	11	13	24				+	11	8	19		
	Total	487	41	528				Total	506	22	528		
Quadruplicate Kato-Katz	−	469	21	490	0.47	$p=0.631$	Quadruplicate Kato-Katz	−	490	11	501	0.43	$p=0.336$
	+	18	20	38				+	16	11	27		
	Total	487	41	528				Total	506	22	528		
A. lumbricoides													
Single Kato-Katz	−	504	10	514	0.62	$p=0.092$*	Single Kato-Katz	−	499	17	516	0.46	$p=0.003$*
	+	3	11	14				+	3	9	12		
	Total	507	21	528				Total	502	26	528		
Duplicate Kato-Katz	−	502	10	512	0.58	$p=0.302$*	Duplicate Kato-Katz	−	499	17	516	0.46	$p=0.003$*
	+	5	11	16				+	3	9	12		
	Total	507	21	528				Total	502	26	528		
Quadruplicate Kato-Katz	−	492	9	501	0.48	$p=0.221$	Quadruplicate Kato-Katz	−	498	16	514	0.48	$p=0.012$*
	+	15	12	27				+	4	10	14		
	Total	507	21	528				Total	502	26	528		

*Instead of the McNemar χ^2 value, the exact McNemar value was taken because a sample size of 20 individuals or less was in the discordant pair

speculate that a diagnostic examination based on two stool samples using the ether-concentration technique reveals a higher sensitivity than quadruplicate Kato-Katz thick smears. For diagnosing *A. lumbricoides*, the ether-concentration method showed a significantly higher sensitivity compared to quadruplicate Kato-Katz thick smears at the 3-week post-treatment follow-up. This might be explained by the large amount of stool (~2 g) examined with the ether-concentration method, which should allow the detection of low infection intensities. The ether-concentration method offers the advantage of preservation. Stool samples are maintained in low-concentration formalin or within sodium acetate-acetic acid formalin (SAF), and, hence, can be kept for several weeks or months prior to microscopic examination for soil-transmitted helminth eggs as well as for intestinal protozoan cysts at a later time point [21, 28]. This diagnostic technique is, therefore, a useful alternative to the Kato-Katz method, particularly in remote resource-constrained settings [29]. It is interesting to note that mobile phone microscopy might be an alternative promising diagnostic method in underserviced rural areas. The proof-of-concept of a 'first-generation' mobile phone light microscope was recently demonstrated in our study setting on Pemba Island [30].

The diagnostic accuracy was the same if either single or duplicate Kato-Katz thick smears were examined (single Kato-Katz vs. duplicate Kato-Katz). This result is in contrast to a recent study, which reported that multiple Kato-Katz thick smears from a single stool sample enhances the overall sensitivity for hookworm and *T. trichiura* [16]. Setting-specific differences in prevalence and infection intensities might

Table 3 Prevalence of soil-transmitted helminth infections on Pemba Island, Tanzania, in mid-2011, according to different diagnostic methods and their corresponding sensitivities and negative predictive values 3 weeks post-treatment

Parasite	Diagnostic technique	Number of infected school children			Sensitivity in % (95 % CI)	NPV (95 % CI)
		n	%	95 % CI		
T. trichiura	'Gold' standard	448	84.9	(81.8–87.9)	100	100
	Single Kato-Katz	383	72.5	(68.7–76.4)	85.5 (81.9–88.6)	55.2 (46.7–63.4)
	Duplicate Kato-Katz	386	73.1	(69.3–76.9)	86.2 (82.6–89.2)	56.3 (47.8–64.6)
	Quadruplicate Kato-Katz	420	79.6	(76.1–83.0)	93.8 (91.1–95.8)	74.1 (64.6–81.8)
	Ether-concentration	374	70.8	(66.9–74.7)	83.5 (79.7–86.8)	51.9 (43.8–60.0)
Hookworm	'Gold' standard	38	7.2	(5.0-9.4)	100	100
	Single Kato-Katz	19	3.6	(2.0–5.2)	50.0 (33.4–66.6)	96.3 (94.1–97.7)
	Duplicate Kato-Katz	19	3.6	(2.0–5.2)	50.0 (33.4–66.6)	96.3 (94.1–97.7)
	Quadruplicate Kato-Katz	27	5.1	(3.2–7.0)	71.1 (54.1–84.6)	97.8 (96.0–98.8)
	Ether-concentration	22	4.2	(2.5–5.9)	57.9 (40.8–73.7)	96.8 (94.8–98.1)
A. lumbricoides	'Gold' standard	30	5.7	(3.7–7.7)	100	100
	Single Kato-Katz	12	2.3	(1.0–3.6)	40.0 (22.7–59.4)	96.5 (94.4–97.9)
	Duplicate Kato-Katz	12	2.3	(1.0–3.6)	40.0 (22.7–59.4)	96.5 (94.4–97.9)
	Quadruplicate Kato-Katz	14	2.7	(1.3–4.0)	46.7 (28.3–65.7)	96.9 (94.9–98.1)
	Ether-concentration	26	4.9	(3.1–6.8)	86.7 (69.3–96.2)	99.2 (97.8–99.7)

explain this observation [8, 31–33]. On the other hand, collecting multiple stool samples had a significant effect on improving the diagnostic sensitivity, and this observation corroborates previous findings [12, 14–16]. Hence, collecting multiple stool samples is often the method of choice for diagnosing soil-transmitted helminths in intervention trials.

Table 4 Cure rates (CR) determined following treatment with nitazoxanide–albendazole, albendazole, nitazoxanide and placebo against soil-transmitted helminths among school-aged children on Pemba Island, Tanzania, according to different diagnostic techniques

	Cure rate				
	Overall	Nitazoxanide–albendazole	Albendazole	Nitazoxanide	Placebo
T. trichiura					
Single Kato-Katz (95 % CI)	16.4 (12.7–20.1)	19.8 (11.2–28.4)	20.4 (12.3–28.5)	12.9 (6.2–19.5)	13.2 (6.7–19.8)
Duplicate Kato-Katz (95 % CI)	15.8 (12.2–19.3)	19.3 (10.9–27.7)	19.8 (11.9–27.7)	11.2 (5.1–17.3)	13.6 (7.1–20.2)
Quadruplicate Kato-Katz (95 % CI)	10.8 (7.9–13.8)	14.6 (7.4–21.8)	15.2 (8.2–22.2)	5.3 (1.1–9.4)	9.2 (4.0–14.5)
Ether-concentration (95 % CI)	17.2 (13.5–20.9)	22.0 (13.3–30.6)	17.3 (9.7–25.0)	19.4 (11.9–27.0)	10.9 (5.0–16.8)
'Gold' standard	7.2 (4.8–9.5)	11.7 (5.3–18.0)	9.1 (3.6–14.5)	1.6 (0.0–3.9)	7.2 (2.6–11.8)
Hookworm					
Single Kato-Katz (95 % CI)	69.6 (49.2–89.9)	80.0 (24.5–100.0)	83.3 (40.5–100.0)	83.3 (40.5–100.0)	33.3 (0.0–87.5)
Duplicate Kato-Katz (95 % CI)	66.7 (46.3–87.0)	80.0 (24.5–100.0)	83.3 (40.5–100.0)	71.4 (26.3–100.0)	33.3 (0.0–87.5)
Quadruplicate Kato-Katz (95 % CI)	71.1 (55.9–86.2)	88.9 (63.3–100.0)	80.0 (49.8–100.0)	66.7 (28.2–100.0)	50.0 (12.3–87.7)
Ether-concentration (95 % CI)	73.2 (59.0–87.3)	83.3 (58.6–100.0)	100.0 (–)	33.3 (0.0–71.8)	75.0 (46.3–100.0)
'Gold' standard	59.3 (46.4–72.2)	75.0 (51.2–98.8)	75.0 (46.3–100.0)	38.5 (7.9–69.1)	50.0 (24.4–75.6)
A. lumbricoides					
Single Kato-Katz (95 % CI)	57.1 (27.5–86.8)	100.0 (–)	100.0 (–)	50.0 (0.0–100.0)	0.0 (–)
Duplicate Kato-Katz (95 % CI)	62.5 (35.9–89.1)	100.0 (–)	100.0 (–)	50.0 (0.0–100.0)	0.0 (–)
Quadruplicate Kato-Katz (95 % CI)	70.4 (52.0–88.8)	100.0 (–)	100.0 (–)	70.0 (35.4–100.0)	16.7 (0.0–59.5)
Ether-concentration (95 % CI)	52.4 (29.1–75.7)	100.0 (–)	100.0 (–)	50.0 (12.3–87.7)	0.0 (–)
'Gold' standard	63.9 (47.4–80.4)	100.0 (–)	87.5 (57.9–100.0)	60.0 (31.9–88.1)	14.3 (0.0–49.2)

We observed significantly lower CRs for *T. trichiura*, preparing duplicate Kato-Katz thick smears from two stool samples compared to all diagnostic methods relying on only one stool sample, which re-emphasises the importance of an accurate diagnostic test in clinical trials. Further, our 'gold' standard had lower CRs compared to quadruplicate Kato-Katz thick smears for *T. trichiura*. Even though this result showed borderline significance, it demonstrates that sensitive tools for diagnosing soil-transmitted helminth infections are warranted. On the other hand, it is interesting to note that duplicate Kato-Katz revealed higher CRs for *A. lumbricoides*. However, no statistical significance was observed, but this might be explained by the relatively small sample size. Several studies showed that the FLOTAC technique revealed higher sensitivities compared to multiple stool samples examined with the Kato-Katz method [15, 16, 32, 34–36] and also compared to the ether-concentration technique [16]. However, the FLOTAC technique, as well as the ether-concentration method, requires additional laboratory equipment, such as a centrifuge, and is also more expensive than the Kato-Katz method [10].

Our study has the following limitations. First and foremost, for the ether-concentration method, helminth eggs were only counted in a semi-quantitative manner. Therefore, it was only possible to calculate the prevalence and CR, but no data could be provided on infection intensities and egg reduction rates, an important parameter for the evaluation of anthelminthic drug efficacy [37]. Second, all positive results obtained by the different techniques were recorded as "true positive". Though this is a common standard in helminth diagnosis [33, 38], it is worth highlighting that false-positive results might be reported even by well-trained laboratory technicians due to artefacts in stool that are misinterpreted as helminth eggs or due to writing errors on the entry forms. To our knowledge, the effect of false-positive results for the diagnosis of soil-transmitted helminths has not yet been studied. Third, all diagnostic approaches revealed high CRs against hookworm in the placebo group (as high as 75 % for the ether-concentration technique), indicating a diagnostic problem for hookworm in general. We have previously attributed this finding to the low prevalence and intensity of hookworm infections [20]. Finally, as mentioned before, the sample size for hookworm as well as for *A. lumbricoides* was relatively small due to the low prevalence of these infections in our study setting (11.2 % and 6.8 % at baseline, respectively). Therefore, even though we did not find significant differences between the diagnostic techniques for these two helminth species, we should not assume that they do not exist. Note that, also, the sensitivities and NPVs are strongly influenced by prevalence [5]. Thus, these parameters should only be compared among different diagnostic methods and not among helminth species. A negative diagnostic result is more likely to be true (high NPV) when the prevalence is low, as was the case in our study for hookworm and *A. lumbricoides*.

To conclude, our study confirmed that a sensitive diagnostic method is crucial in order to reliably assess the prevalence of soil-transmitted helminth infections, as well as determine CRs in clinical trials, and that using insensitive diagnostic methods overestimates drug efficacy when CR is employed as the outcome measure. Our trial re-emphasises that collecting multiple stool samples is useful to enhance the sensitivity, especially in settings where infection intensities are low. The diagnostic accuracy of the ether-concentration method from a single formalin-fixed stool sample revealed moderate diagnostic agreement with quadruplicate Kato-Katz thick smears and a similar or even higher sensitivity for the diagnosis of all three soil-transmitted helminths than single or duplicate Kato-Katz thick smears prepared from a single stool sample. Hence, the ether-concentration method provides an alternative in settings where fresh stool samples cannot be directly examined.

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Conflict of interest The authors declare that they have no conflict of interest.

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Chapter 6

The effect of sanitation facilities and water treatment on infections with intestinal parasites

Chapter 6a

Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis

Chapter 6b

Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis

Chapter 6a

Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis

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Effect of Sanitation on Soil-Transmitted Helminth Infection: Systematic Review and Meta-Analysis

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Abstract

Background: In countries of high endemicity of the soil-transmitted helminth parasites *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm, preventive chemotherapy (i.e., repeated administration of anthelmintic drugs to at-risk populations) is the main strategy to control morbidity. However, rapid reinfection of humans occurs after successful deworming, and therefore effective preventive measures are required to achieve public health goals with optimal efficiency and sustainability.

Methods and Findings: We conducted a systematic review and meta-analysis to assess the effect of sanitation (i.e., access and use of facilities for the safe disposal of human urine and feces) on infection with soil-transmitted helminths. PubMed, Embase, ISI Web of Science, and the World Health Organization Library Database were searched without language restrictions and year of publication (search performed until December 31, 2010). Bibliographies of identified articles were hand-searched. All types of studies reporting data on sanitation availability (i.e., having access at own household or living in close proximity to sanitation facility), or usage, and soil-transmitted helminth infections at the individual level were considered. Reported odds ratios (ORs) of the protective effect of sanitation on soil-transmitted helminth infections were extracted from the papers or calculated from reported numbers. The quality of published studies was assessed with a panel of criteria developed by the authors. Random effects meta-analyses were used to account for observed heterogeneity. Thirty-six publications, consisting of 39 datasets, met our inclusion criteria. Availability of sanitation facilities was associated with significant protection against infection with soil-transmitted helminths (OR = 0.46 to 0.58). Regarding the use of sanitation, ORs of 0.54 (95% confidence interval [CI] 0.28–1.02), 0.63 (95% CI 0.37–1.05), and 0.78 (95% CI 0.60–1.00) were determined for *T. trichiura*, hookworm, and *A. lumbricoides*, respectively. The overall ORs, combining sanitation availability and use, were 0.51 (95% CI 0.44–0.61) for the three soil-transmitted helminths combined, 0.54 (95% CI 0.43–0.69) for *A. lumbricoides*, 0.58 (95% CI 0.45–0.75) for *T. trichiura*, and 0.60 (95% CI 0.48–0.75) for hookworm.

Conclusions: Despite a number of limitations (e.g., most studies used a cross-sectional design and were of low quality, with potential biases and considerable heterogeneity), our results reveal that sanitation is associated with a reduced risk of transmission of helminthiases to humans. Access to improved sanitation should be prioritized alongside preventive chemotherapy and health education to achieve a durable reduction of the burden of helminthiases.

Please see later in the article for the Editors' Summary.

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Abbreviations: CI, confidence interval; OR, odds ratio

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Introduction

An estimated 4.5 billion people are at risk of infection with one of the three common soil-transmitted helminths, namely, the roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1,2]. Infection with soil-transmitted helminths is intimately connected with poverty, with the highest prevalence rates observed in low- and middle-income countries where hygiene is poor, access to safe, clean water is lacking, and sanitation is absent or inadequate [3–7]. More than 1 billion people are infected with one or multiple species of soil-transmitted helminths, and the global burden of disease owing to soil-transmitted helminthiases is estimated at 39 million disability-adjusted life years [2,8–10]. Anemia and other morbidities (e.g., reduced physical and cognitive development) are the main reasons for this large global burden [4,11,12]. People are infected after ingesting eggs from contaminated soil or food (*A. lumbricoides* and *T. trichiura*), or through active penetration of the skin by infective larval stages present in contaminated soil (hookworm) [3]. Soil-transmitted helminths do not reproduce in the human host, and hence, each established helminth in the human body is a result of an infection event.

In 2001, the World Health Organization endorsed preventive chemotherapy as the global strategy to control morbidity due to soil-transmitted helminthiasis and schistosomiasis [9]. The key component of this strategy is to regularly administer safe and efficacious anthelmintic drugs to at-risk populations, with a target of reaching at least 75%, and up to 100%, of school-aged children [9,13,14]. While this strategy has a direct impact on morbidity, it does not prevent reinfection [15,16], and it is recognized that complementary interventions are necessary to reduce the frequency of reinfection [16–19]. A large body of historic evidence [20–22] and recent experiences from China [23] suggest that integrated control approaches are essential for the interruption of transmission and local elimination of helminthiases. Improved access to sanitation is a key factor of integrated control programs [15–19,24,25].

We were interested in the evidence regarding sanitation (i.e., access to, and use of, facilities for the safe disposal of human urine and feces) and its effects on infection of humans with soil-transmitted helminths. A systematic review and meta-analysis were carried out to determine whether the availability and/or use of sanitation facilities was associated with a reduced risk of infection with soil-transmitted helminths from single or multiple species.

Methods

Search Strategy and Inclusion Criteria

We performed a systematic review and meta-analysis adhering to the MOOSE guidelines for reporting meta-analyses of observational studies (see Text S1) [26]. Our protocol is available in Text S2. In brief, we systematically searched PubMed, Embase, and ISI Web of Science, which are readily available and widely used electronic databases for systematic reviews in the health sciences. Additionally, the World Health Organization Library Database and the authors' own collections of articles were examined. Preliminary searches using the Cochrane Library and the CAB Abstracts revealed no additional studies, and hence these databases were not considered further. No restrictions on language or year of publication were made. Our search was performed until December 31, 2010. We employed a broad search using the following keywords: "sanitation," "sanitary engineering," "water supply," and "waste management," in combination with one of the following soil-transmitted helminth-related terms: "helminth," "soil-transmitted helminth," "geohelminth," "ascaris," "lumbricoides," "trichuris," "trichiura," "hookworm," "ancylostoma," "duodenale," "necator," and "americanus."

Additionally, two previous general reviews pertaining to water and sanitation and parasitic worm infections were examined for relevant references [27,28]. The bibliographies of publications identified and deemed relevant were hand-searched for potential additional important articles. If an article was considered relevant, but data were not available in the format needed for our meta-analysis, the corresponding authors were contacted by E-mail and asked for supplementary information. All study types were eligible if they reported the prevalence (i.e., number of people infected among the examined population) of *A. lumbricoides*, *T. trichiura*, hookworm, or all three soil-transmitted helminths combined, stratified by the presence or absence of sanitation facilities or by the use or non-use of sanitation facilities. Since insufficient data were available to distinguish between different types of sanitation facilities, all types of latrines (e.g., pit latrines, ventilated improved pit latrines, and flush toilets) were pooled. Hence, studies reporting only the presence or absence of latrines without further specificity regarding the type of latrines were eligible for inclusion. Open defecation was defined as no sanitation. Studies that only compared the effect of different toilet types (e.g., flush toilet *versus* pit latrine) were excluded. Regarding the use of sanitation, we also applied a broad set of inclusion criteria. For instance, studies that employed a questionnaire and asked one of the following questions "do you use a sanitary facility?" or "where do you defecate?" were included.

However, most intervention studies were excluded, because of specific aspects of the design, setting, and the complexity of interventions (e.g., multiple control measures) where the studies were implemented. Indeed, it is difficult to compare intervention studies carried out over different time frames and to distinguish studies that used single or multiple interventions (sanitation plus water supply, preventive chemotherapy, and health education) [29].

Data Extraction and Quality Assessment

In the first step, studies identified in our computer-aided search that failed to meet at least one inclusion criterion after scrutinizing the title and, if available, the abstract, were excluded. In the second step, two reviewers (K. Z. and B. S.) independently examined the full text of potentially relevant articles using a standard protocol developed by the authors (see Text S2). In case of disagreement, a third reviewer (J. K. or J. U.) independently examined such articles, and the assessors' findings were discussed until consensus was reached.

Relevant data, including a brief description of the study (e.g., study design, setting, year, and sample size), the primary research question pursued by the study, details of the study population (e.g., all age groups, school-aged children only, or other special groups) and the selection of study population (e.g., random selection), specificities on sanitation facilities (i.e., availability or use), and the helminth species investigated were extracted from all eligible studies by K. Z. using a standard protocol and independently cross-checked by B. S.

The reported odds ratios (ORs) served as effect measures. For studies that did not report ORs, these were calculated from 2×2 contingency tables of sanitation facility (availability or use) and infection status with soil-transmitted helminths, compared to the infection status of those who do not have access to, or use, sanitation facilities. Whenever possible, reported ORs were used; if

both adjusted and unadjusted ORs were reported, we considered unadjusted ORs. Studies reporting effect measures for more than one helminth species were considered, and relevant results were fed into the respective meta-analyses.

Inspired by the GRADE methodology [30], we developed a panel of criteria to assess the quality of identified studies. Our criteria focused on parasitological/diagnostic features, sanitation, and overall strengths and limitations of the studies. With regard to parasitological/diagnostic features, a study was given one point if the diagnostic approach (clinical assay) was clearly spelled out. Studies that employed a rigorous diagnostic approach (i.e., multiple stool samples examined and/or concurrent use of several diagnostic tests) received one additional point. Finally, studies that detailed an approach for quality control (e.g., 10% of stool examinations checked by a senior laboratory technician) were further given one additional point. Of note, no qualitative ranking of the different diagnostic tests was performed, as the sensitivity and specificity of a particular test depends on the overall endemicity (prevalence and intensity) of soil-transmitted helminthiasis. Conversely, studies that did not mention clinical/diagnostic assays were given zero points. With regard to sanitation-related quality assessment, a study was given one point if the toilet status (e.g., cleanliness and condition of superstructure) was investigated by the research team. Repeated spot checks of random sub-samples of sanitation availability and use were deemed sufficient to obtain a point. However, no point was assigned if the toilet status was assessed using a questionnaire, as questionnaires were not considered sufficient to be awarded a quality point. Finally, studies were scrutinized for other strengths (+1 point) and limitations (−1 point) (e.g., no random population sample, but instead high-risk group only). Two assessors (K. Z. and B. S.) performed the quality assessment independently and documented the results in separate tables. Results were discussed; in case of discrepancies, a third reviewer (J. K. or J. U.) examined the respective articles, and the ratings were discussed until consensus was reached among the assessors. Overall, a study could obtain an overall score ranging between −1 and +6 points. Since these ratings are mainly to inform the reader about the overall quality of individual studies, no studies were excluded because of low quality.

All studies were pooled in the meta-analyses and stratified by soil-transmitted helminth species (overall OR). Furthermore, we carried out separate meta-analyses for *A. lumbricoides*, *T. trichiura*, hookworm, and soil-transmitted helminths combined, stratified by (i) availability or use of sanitation facility; (ii) data for children, adults, or all age groups; and (iii) geographical area (Africa, Asia, South and Central America, and the United States).

Statistical Analysis

ORs were calculated for specific soil-transmitted helminths by comparing prevalence rates among those individuals having access to, or using, sanitation and those without, or not using, facilities employing the "metan" code of Stata version 10 (StataCorp). StatsDirect version 2.4.5 (StatsDirect) was used for meta-analyses, performed for *A. lumbricoides*, *T. trichiura*, hookworm, and soil-transmitted helminths combined. Egger's test was utilized to investigate whether there was a publication bias (a small study bias is evident if $p<0.1$) [31]. Heterogeneity between studies was determined using Moran's I^2 and Cochran's Q-tests. Factors specified a priori as potential explanations for observed heterogeneity were age and type of toilet. Since there was some evidence for heterogeneity ($I^2>50\%$), random effects models [32] were used throughout, and pooled ORs for the effect of sanitation on the prevalence of helminth infections were employed. Studies with an OR less than 1.0 indicate a decrease in the odds of being infected with soil-transmitted helminths among those individuals having access or using sanitation facilities.

Results

Inclusion, Exclusion, and Yielded Studies

Our computer-aided search yielded 2,537 publications (Figure 1A), with the majority retrieved by Embase (1,841 hits) and PubMed (882 hits) (Figure 1B). From the titles and, when available, the abstracts of these articles, 146 publications were deemed relevant, hence, were fully screened by two of us (K. Z. and B. S.). The majority of relevant articles were obtained from Embase and PubMed (Figure 1C). Bibliographies of these 146 articles revealed an additional 16 studies that were also investigated by the first two authors. We noted missing data to address our research question in 34 publications, and, hence, the corresponding authors were contacted by E-mail. We received the requested data from ten authors pertaining to 12 studies, which were included in our analyses. Table S1 provides a summary of the 162 fully screened publications, including the reasons why studies were excluded. Thirty-six studies met our inclusion criteria—consisting of 39 datasets that were finally included in our meta-analyses—investigating the relationship between sanitation facilities and prevalence of soil-transmitted helminth infections.

Twenty-five publications investigated the effect of sanitation availability on infection with soil-transmitted helminths, whereas the remaining 11 articles focused on the use of sanitation and infection with soil-transmitted helminths. From the 36 publications, 16 focused on Asia [33–48], 11 on Africa [49–59], four on Central America [60–63], four on South America [64–67], and one on the United States [68]. The study conducted in the United States was the oldest one identified (published in 1970). With the exception of one article published in Spanish [66] and one in Chinese [38], articles were published in English. There were only two studies that reported results on intensity of soil-transmitted helminth infection, as determined by the number of helminth eggs per gram of stool [37,69].

Of note, multiple studies dating back to the early decades of the last century from the southern part of the United States, Panama, and elsewhere also reported an impact of sanitation (often in combination with chemotherapy and other control measures) on soil-transmitted helminth infections [20,22,70–72]. However, these studies did not report data in the format needed for the current meta-analysis, and it was not possible to contact the authors by E-mail; hence, these studies were not considered further (see Table S1).

Study Characteristics and Data Quality

Most of the publications identified were descriptive cross-sectional surveys, assessing single or multiple risk factors for infection with soil-transmitted helminths (Table 1). Only one intervention study was included in our meta-analysis, and this study was included because complete baseline data were available [49]. In 16 publications it was possible to obtain relevant data in a 2×2 contingency table format directly from the respective articles. The ten authors who kindly supplied the requested supplementary data for 12 studies upon E-mail inquiry did this in the form of 2×2 contingency tables as per our request. In five studies, the ORs provided in the articles were retrieved and used for subsequent meta-analysis. In three surveys, data were reanalyzed to obtain the respective contingency table information for meta-analyses. Study participants were chosen at random, either at individual or at household level in more than half of the relevant studies. In 14 studies, all individuals of a particular community, village, or

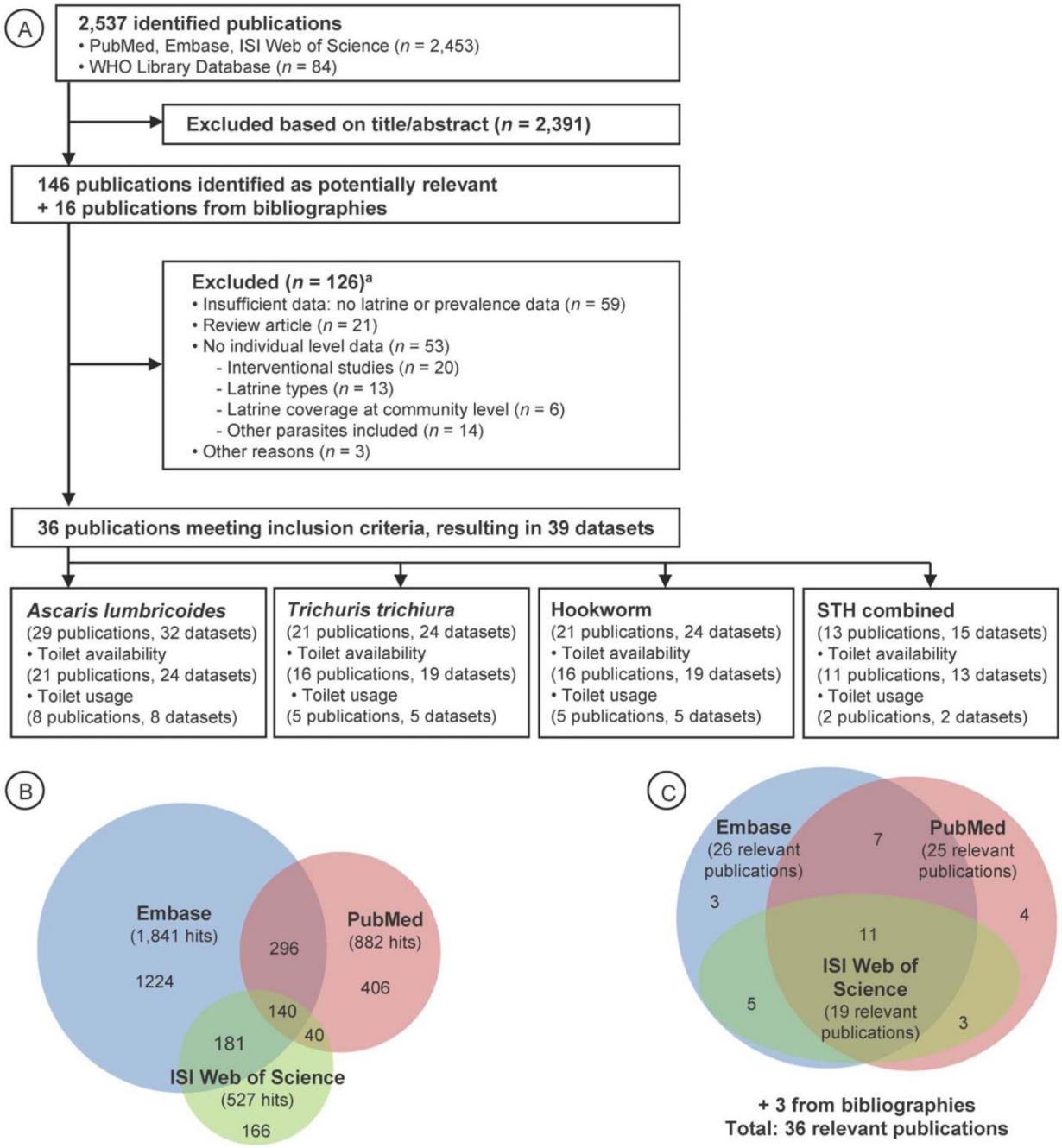

Figure 1. Flowchart visualizing the procedure for identifying relevant publications. Overall, 36 publications were identified, containing 39 datasets (A). Number of hits (B) and ultimate identification of relevant publications (C) are also shown, for three different electronic databases. STH, soil-transmitted helminths. [a]Multiple exclusion criteria possible.
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special population group were enrolled, whereas no selection criteria for study participation were specified in four studies.

The diagnostic technique utilized for assessing soil-transmitted helminth infection status was mentioned in all the studies meeting our inclusion criteria. The Kato-Katz technique [73] was the most widely used diagnostic approach (n = 20). Three studies mentioned that quality control for microscopic examination of stool samples was performed. Only one study explicitly stated that repeated spot checks for sanitation facilities were done per protocol by the researchers [41].

Table 1 also summarizes the overall quality of the included studies. On our scale from −1 (worst quality) to +6 (best quality),

Table 1. Characteristics of studies examining the association of sanitation availability or sanitation use with soil-transmitted helminth infections, including quality assessment.

Reference	Study Design and Setting	Year	Study Population (Selection)	Availability (A) or Use (U) of Sanitation	Soil-Transmitted Helminth Species	Data Obtained	Diagnostic Approach (D)		Sanitation (S)		Other Strengths and Limitations (O)	Points	
							Method	Quality Control	Toilet Status Assessment Method	Spot Checks		D/S/O	Total
Al-Mekhlafi et al. [33]	Descriptive study in one school in Malaysia	2006	Sc (random)	A	A.l.	OR (MVA)	K-K (Hw: H-M)	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Asaolu et al. [52]	Descriptive study in two communities in Nigeria	1998	PSc (all)	A	A.l.	2×2 table	Mod. K-K	n.s.	Questionnaire	No	—	+1/0/0	+1
Basualdo et al. [64]	Descriptive study in one town of Argentina	2002	All age groups (n.s.)	A	A.l.; T.t.	2×2 table[a]	Mod. T-L (five samples)	n.s.	n.s.	n.s.	—	+2/0/0	+2
Belo et al. [53]	Descriptive study in three schools in Sao Tomé	2000	Sc (random)	U	A.l.; T.t.	OR (UVA)	K-K; T-L	n.s.	Questionnaire	n.s.	—	+2/0/0	+2
Chongsuvivatwong et al. [41]	Descriptive study in four villages in Thailand	n.s.	All >6 y (random HH)	U	Hw	2×2 table	K-K	n.s.	Questionnaire	Yes	Spent 11 mo prior to study to establish good relationship	+1/+1/+1	+3
Corrales et al. [61]	Case-control study in eight communities in El Salvador	n.s.	All age groups (random HH; all solar latrine owners)	A	A.l.; T.t.; Hw	OR (MVA)	Mod. RFEC	n.s.	n.s.	n.s.	—	+1/0/0	+1
de Souza et al. [65]	Descriptive study in two villages in Brazil	2004	All age groups (all)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	SS	No	Questionnaire	No	—	+1/0/0	+1
Ensink et al. [34]	Descriptive study in four communities in Pakistan	2002	Adult men and children (only textile laborers, wastewater farmers, farmers)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	FES	n.s.	n.s.	n.s.	Only high-risk groups	+1/0/−1	0
Erlanger et al. [46]	Descriptive study in 17 villages in Lao People's Democratic Republic	2001 /2002	All age groups (random)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	FES	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Gloor et al. [68]	Descriptive study in eight schools in the US	1968	Sc (all)	A	STH; Hw	2×2 table	ZSF	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Gunawardena et al. [35]	Descriptive study in one village in Sri Lanka	2000	All >2 y (random HH, participants)	A	A.l.; T.t.[a]	2×2 table[a]	K-K	n.s.	Questionnaire	n.s.	—	+1/0/0	+1

Table 1. Cont.

Reference	Study Design and Setting	Year	Study Population (Selection)	Availability (A) or Use (U) of Sanitation	Soil-Transmitted Helminth Species	Data Obtained	Diagnostic Approach (D)		Sanitation (S)		Other Strengths and Limitations (O)	Points	
							Method	Quality Control	Toilet Status Assessment Method	Spot Checks		D/S/O	Total
Gunawardena et al. [36]	Descriptive study in two plantations in Sri Lanka	2000	All age groups (n.s.)	A	Hw	2×2 table[a]	K-K	n.s.	Questionnaire	No	—	+1/0/0	+1
Hagel et al. [67]	Descriptive study in an urban slum in Venezuela	1993	Children (representing overall socio-economic structure)	A	A.l.; T.t.	2×2 table	Stoll	n.s.	Prior door-to-door interviews	n.s.	—	+1/0/0	+1
Holland et al. [60]	Descriptive study in one health center in Panama	1983	PSc (random)	A	A.l.; T.t.; Hw	2×2 table	n.s.	n.s.	Questionnaire (with mother or caregiver of child)	n.s.	—	+1/0/0	+1
Ilechukwu et al. [59]	Descriptive study in three nurseries and three schools in Nigeria	2003	PSc, Sc (random)	U	STH; A.l.; T.t.; Hw	2×2 table	K-K	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Jombo et al. [54]	Descriptive study in three communities in Nigeria	2004	All age groups (random)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	Mod. DS	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Kightlinger et al. [50]	Descriptive study in southeast Madagascar	n.s.	Children (n.s.)	U	A.l.	2×2 table	FES	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Knopp et al. [58]	Descriptive study in two communities in Zanzibar	2008	All age groups (all adults; first 100 children)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	K-K, BM, KAP	10% of stool samples	Questionnaire	n.s.	—	+3/0/0	+3
Matthys et al. [55]	Descriptive study in six communities in Côte d'Ivoire	2004	All age groups (all farmers; non-farmers: random)	U	Hw	2×2 table[a]	FEC, K-K (two slides)	10% of stool samples	Questionnaire	n.s.	—	+3/0/0	+3
Morales-Espinoza et al. [63]	Descriptive study in 32 communities in Mexico	1998	Children (systematic)	A	A.l.	OR (UVA)	Faust (three samples)	n.s.	Questionnaire	n.s.	—	+2/0/0	+2
Nguyen et al. [47]	Descriptive study among women of reproductive age in Viet Nam	1995	Women (random cluster sampling)	U	A.l.; T.t.; Hw	2×2 table	K-K	n.s.	Questionnaire	n.s.	Only data from closed latrine vs. "bush," since open latrine not clearly defined	+1/0/0	+1
Nishiura et al. [37]	Descriptive study in five schools in Pakistan	2000	Sc (random)	U	A.l.	2×2 table	K-K (one stool)	n.s.	Questionnaire	n.s.	—	+1/0/0	+1

Table 1. Cont.

Reference	Study Design and Setting	Year	Study Population (Selection)	Availability (A) or Use (U) of Sanitation	Soil-Transmitted Helminth Species	Data Obtained	Diagnostic Approach (D)		Sanitation (S)		Other Strengths and Limitations (O)	Points	
							Method	Quality Control	Toilet Status Assessment Method	Spot Checks		D/S/O	Total
Olsen et al. [51]	Descriptive study in three villages in Kenya	1994	All age groups (all >4 y)	A	A.l.; Hw	2×2 table	K-K (two stools, two slides)	n.s.	Questionnaire	n.s.	—	+2/0/0	+2
Raja'a et al. [43]	Descriptive study in one town in Yemen	n.s.	Sc (random)	U	STH	2×2 table	Mod. K-K	n.s.	Questionnaire	No	—	+1/0/0	+1
Steinmann et al. [48]	Descriptive study in 51 schools in Kyrgyzstan	2009	Sc (random)	U	A.l.	Cal.	K-K (two slides)	n.s.	Questionnaire	n.s.	Toilet use during the night	+1/0/−1	0
Stephenson et al. [49]	Intervention study in two villages in Kenya	1975–1980	Sc, PSc (all)	A	A.l.	Cal.	Mod. T-L	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Stothard et al. [56]	Descriptive study in ten villages in Zanzibar	2006	Mothers and children (n.s.)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	K-K (one slide)	10% of stool samples	Questionnaire	n.s.	—	+2/0/0	+2
Sun et al. [38]	Descriptive study in three counties in China	2003	All age groups (random)	A	STH	2×2 table	Mod. K-K	n.s.	n.s.	n.s.	—	+1/0/0	+1
Toma et al. [39]	Descriptive study in four villages in Indonesia	1994	All age groups (random HH)	A	A.l.; T.t.; Hw	2×2 table	Mod. K-K (Hw: mod. H-M)	n.s.	Questionnaire	No	—	+1/0/0	+1
Torres et al. [66]	Descriptive study in six schools in Chile	1993	Sc (all)	A	A.l.; T.t.	2×2 table	PAFS	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Trang et al. [45]	Descriptive study in two communities in Viet Nam	2003	Adults (random HH; exclusion of farmers)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	DS	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Trang et al. [44]	Descriptive study in a peri-urban area in Viet Nam	2002	All age groups (random HH)	A	STH; A.l.; T.t.; Hw	2×2 table[a]	DS (one stool)	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Traub et al. [40]	Descriptive study in three communities in India	2000	All age groups (random HH)	U	A.l.; T.t.; Hw	Cal.	SS (one stool)	n.s.	Questionnaire	n.s.	Always the same interviewer	+1/0/+1	+2
Ugbomoiko et al. [57]	Descriptive study in one village in Nigeria	2005	Children (random HH)	A	A.l.	2×2 table	K-K (one sample)	n.s.	Questionnaire	n.s.	—	+1/0/0	+1
Wördemann et al. [62]	Descriptive study in two municipalities (19 schools) in Cuba	2003	Sc (all)	U	A.l.; T.t.; Hw	OR (UVA)	DS, K-K (two slides)	n.s.	Questionnaire	n.s.	—	+2/0/0	+2

Table 1. Cont.

Reference	Study Design and Setting	Year	Study Population (Selection)	Availability (A) or Use (U) of Sanitation	Soil-Transmitted Helminth Species	Data Obtained	Diagnostic Approach (D)		Sanitation (S)		Other Strengths and Limitations (O)	Points	
							Method	Quality Control	Toilet Status Assessment Method	Spot Checks		D/S/O	Total
Yajima et al. [42]	Descriptive study in one community in Viet Nam	2007	All age groups (random participant)	A	A.l.; T.t.; Hw	2×2 table	K-K (two slides)	n.s.	Questionnaire	n.s.	Small sample size (only three with no latrine)	+1/0/−1	0

[a]Data provided by author.

A.l., *Ascaris lumbricoides*; BM, Baermann (technique); Cal., calculated; DS, direct smear; FEC, formalin-ether concentration (technique); FES, formalin-ether sedimentation (technique); HH, household; H-M, Harada-Mori (technique); Hw, hookworm; KAP, Koga agar plate (technique); K-K, Kato-Katz (technique); Mod., modified; MVA, multivariate analysis; PAFS, polyvinyl alcohol fixative solution; PSc, pre-school children; n.s., not stated; RFEC, Ritchie's formalin-ether concentration (technique); Sc, schoolchildren; SS, stool sedimentation (technique); STH, soil-transmitted helminths; T-L, Teleman-Lima (technique); T.t., *Trichuris trichiura*; UVA, univariate analysis; ZSF, zinc sulfate flotation (technique).

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most studies had a score of +1 ($n = 23$) and, hence, were of relatively low quality. Quality of three studies was even lower (zero points), whereas the remaining ten studies had a score of +2 ($n = 7$) or +3 ($n = 3$). Two of the studies with the highest score pursued a rigorous diagnostic approach for detecting infections with soil-transmitted helminths (i.e., multiple stool samples, different techniques employed, and quality control) [55,58]. One study had such a small sample size (i.e., only three persons without latrine), that one quality point was subtracted [42].

Effect of Sanitation Availability and Use on Infections with Soil-Transmitted Helminths

Figures 2–5 present the effect estimates of sanitation availability and use for *A. lumbricoides* (Figure 2), *T. trichiura* (Figure 3), hookworm (Figure 4), and soil-transmitted helminths combined (Figure 5). The observed heterogeneity for the different sub-group meta-analyses, I^2, ranged from 0% (e.g., soil-transmitted helminths combined for studies conducted in Asia, and *T. trichiura* for studies carried out in Africa) to 90.5% (*A. lumbricoides*, sanitation use for studies carried out in Africa), justifying the use of random effects models for all meta-analyses (Table 2).

The 36 publications identified included 32 datasets on the effect of sanitation on infection with *A. lumbricoides*, 24 on infection with *T. trichiura*, 24 on infection with hookworm, and 15 on infection with all three soil-transmitted helminths combined. The estimated pooled random effects ORs of either having or using sanitation facilities compared to those individuals who neither have nor use a latrine were 0.54 (95% confidence interval [CI] 0.43–0.69) for infection with *A. lumbricoides*, 0.58 (95% CI 0.45–0.75) for *T. trichiura*, 0.60 (95% CI 0.48–0.75) for hookworm, and 0.51 (95% CI 0.43–0.61) for infection with soil-transmitted helminths combined.

Twenty-eight datasets were identified that specifically examined the relationship between availability of sanitation facilities and the prevalence of infection with soil-transmitted helminths. Among these, 24 reported data on *A. lumbricoides*, 19 on *T. trichiura*, 19 on hookworm, and 13 on soil-transmitted helminths combined. Although we observed wide ranges in effectiveness estimates, most studies showed that having access to a sanitation facility reduces the odds of being infected with soil-transmitted helminths, regardless of the species. The highest protective effect was observed for *A. lumbricoides* and soil-transmitted helminths combined, with respective summary estimates of 0.46 (95% CI 0.33–0.64; Figure 2) and 0.49 (95% CI 0.40–0.60; Figure 5). For infection with *T. trichiura* or hookworm, ORs of 0.56 (95% CI 0.46–0.70) and 0.58 (95% CI 0.45–0.76), respectively, were found (Figures 3 and 4). Evidence for publication bias was found for infection with soil-transmitted helminths combined pertaining to usage and availability of sanitation ($p = 0.017$). We found a borderline significance for publication bias for sanitation availability alone ($p = 0.054$). All other meta-analyses revealed no evidence of publication bias (Egger's test, $p>0.1$).

Use of sanitation facilities was reported in 11 publications. Stratified by soil-transmitted helminth species, meta-analyses included eight studies for *A. lumbricoides* (Figure 2), five for *T. trichiura* (Figure 3), and five for hookworm (Figure 4). Only two publications reported the relationship between use of sanitation facilities and infection with soil-transmitted helminths combined (OR = 0.56; 95% CI 0.34–0.92). In the comparison of individuals who use a latrine with those who do not, the odds of being infected with *A. lumbricoides*, *T. trichiura*, and hookworm were 0.78 (95% CI 0.60–1.00), 0.54 (95% CI 0.28–1.02), and 0.63 (95% CI 0.37–1.05), respectively.

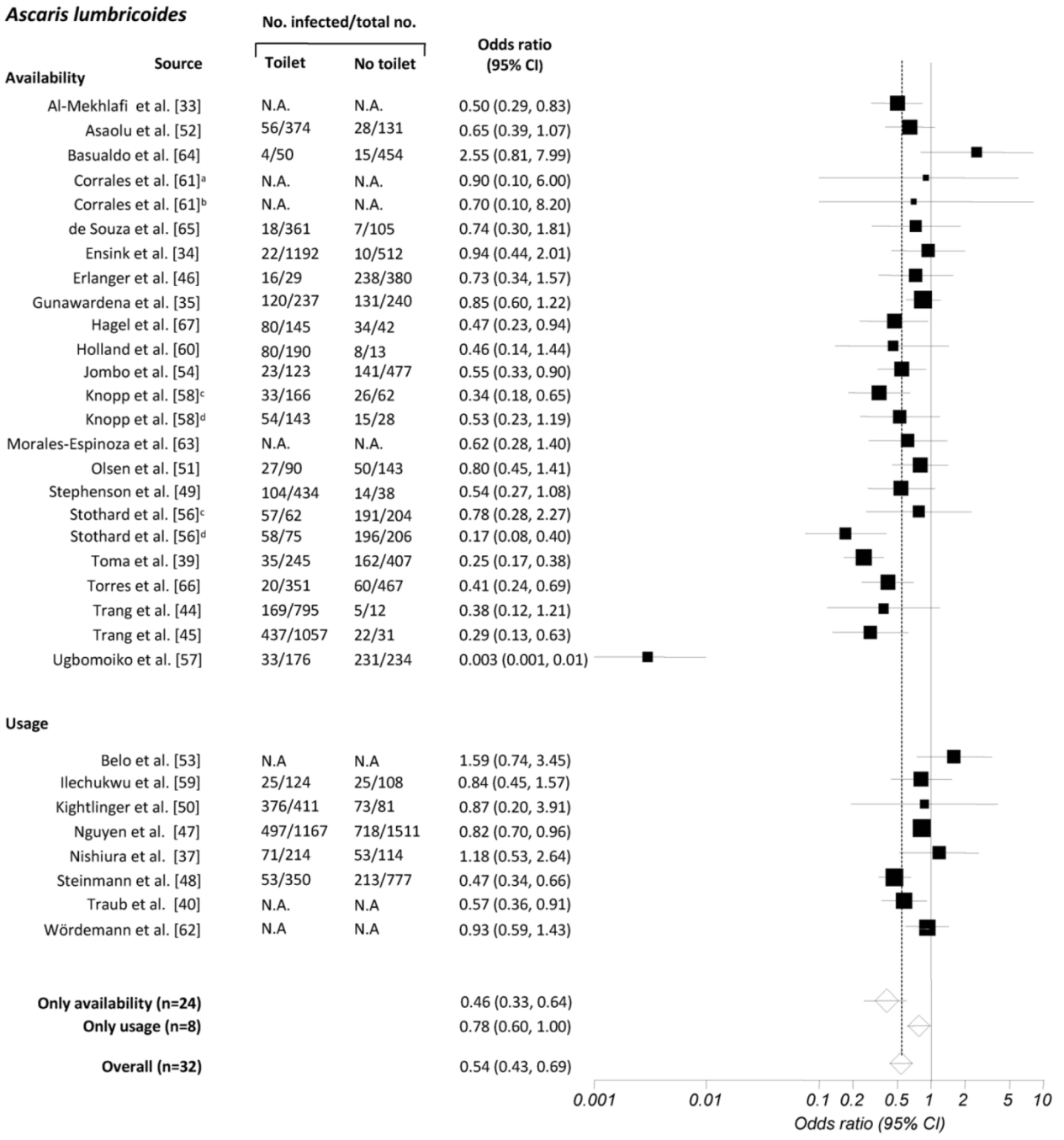

Figure 2. Meta-analysis examining the association of sanitation facilities with *A. lumbricoides* infection. Data are presented separately for availability and use of sanitation. Rectangles indicate ORs, and sizes of the rectangles represent the weight given to each study in the meta-analysis; open diamonds and vertical dashed lines indicate combined ORs; and horizontal lines indicate 95% CIs. Data are presented separately for [a]only pit latrine, [b]only solar urine-diverting desiccating latrine, [c]only adults, [d]only children. N.A., not assessed.
doi:10.1371/journal.pmed.1001162.g002

Results from different sub-group analyses are summarized in Table 2. The pooled OR of datasets examining only children (including pre-school and school-aged children [aged below 16 y]) ranged from 0.35 (95% CI 0.21–0.57) for infection with hookworm to 0.47 (95% CI 0.37–0.60) for infection with *T. trichiura*, suggesting a stronger association of sanitation with helminth infection in children than in the whole population. However, 95% CIs are strongly overlapping. Analyses of studies conducted in different geographical areas (Africa, Asia, South and Central America, and the United States) revealed no difference in associations between availability or use of sanitation facilities and infection with any of the common soil-transmitted helminth species.

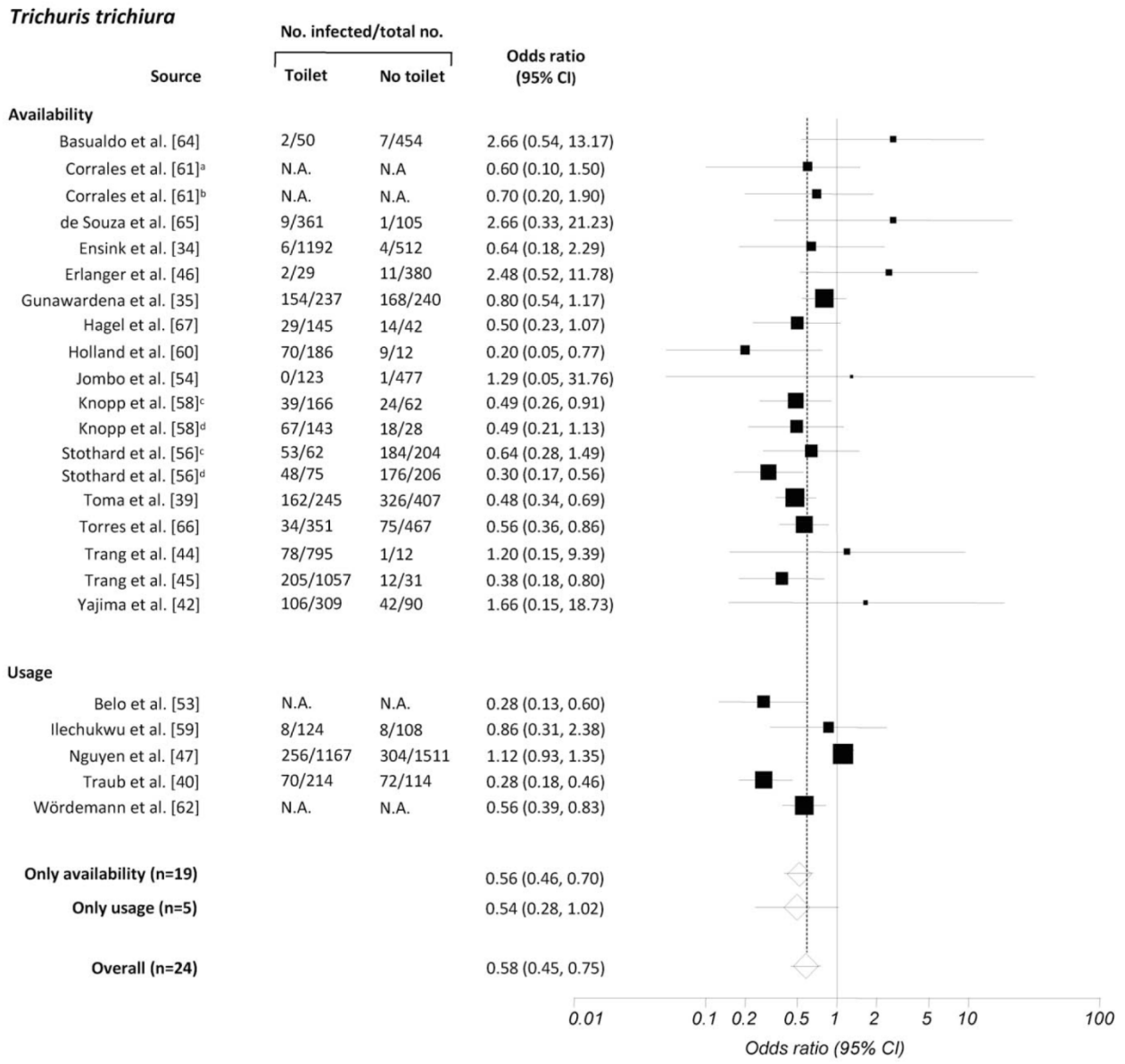

Figure 3. Meta-analysis examining the association of sanitation facilities with *T. trichiura* infection. Data are presented separately for availability and use of sanitation. Rectangles indicate ORs, and sizes of the rectangles represent the weight given to each study in the meta-analysis; open diamonds and vertical dashed lines indicate combined ORs; and horizontal lines indicate 95% CIs. Data are presented separately for [a]only pit latrine, [b]only solar urine-diverting desiccating latrine, [c]only adults, [d]only children. N.A., not assessed.
doi:10.1371/journal.pmed.1001162.g003

Discussion

Since the International Drinking Water Supply and Sanitation Decade (1980–1990), adequate sanitation, safe drinking water, and appropriate hygiene have been forgotten pillars of health, until recently [18,19,74,75]. Fortunately, though, interest in access to safe, clean drinking water and adequate sanitation and improved hygiene has been renewed, and a road map of what needs to be done has been established [75]. Indeed, the United Nation's Millennium Development Goal 7c aims at halving the proportion of the population without sustainable access to safe drinking water and basic sanitation by 2015 [76], and the United Nation's General Assembly recently adopted access to water and sanitation as a basic human right [77]. Progress toward Millennium Development Goal 7c and recognizing water and sanitation as a basic human right will undoubtedly result in major health gains and improved well-being, such as lower incidence of diarrheal episodes and infant mortality, and enhanced human dignity, apart from other benefits [18,75].

In our meta-analysis we found that the availability and use of sanitation facilities were associated with a reduction in the prevalence of infection with soil-transmitted helminths. Considering all of the studies that met our inclusion criteria, summary ORs ranging between 0.54 and 0.60 for the three common soil-transmitted

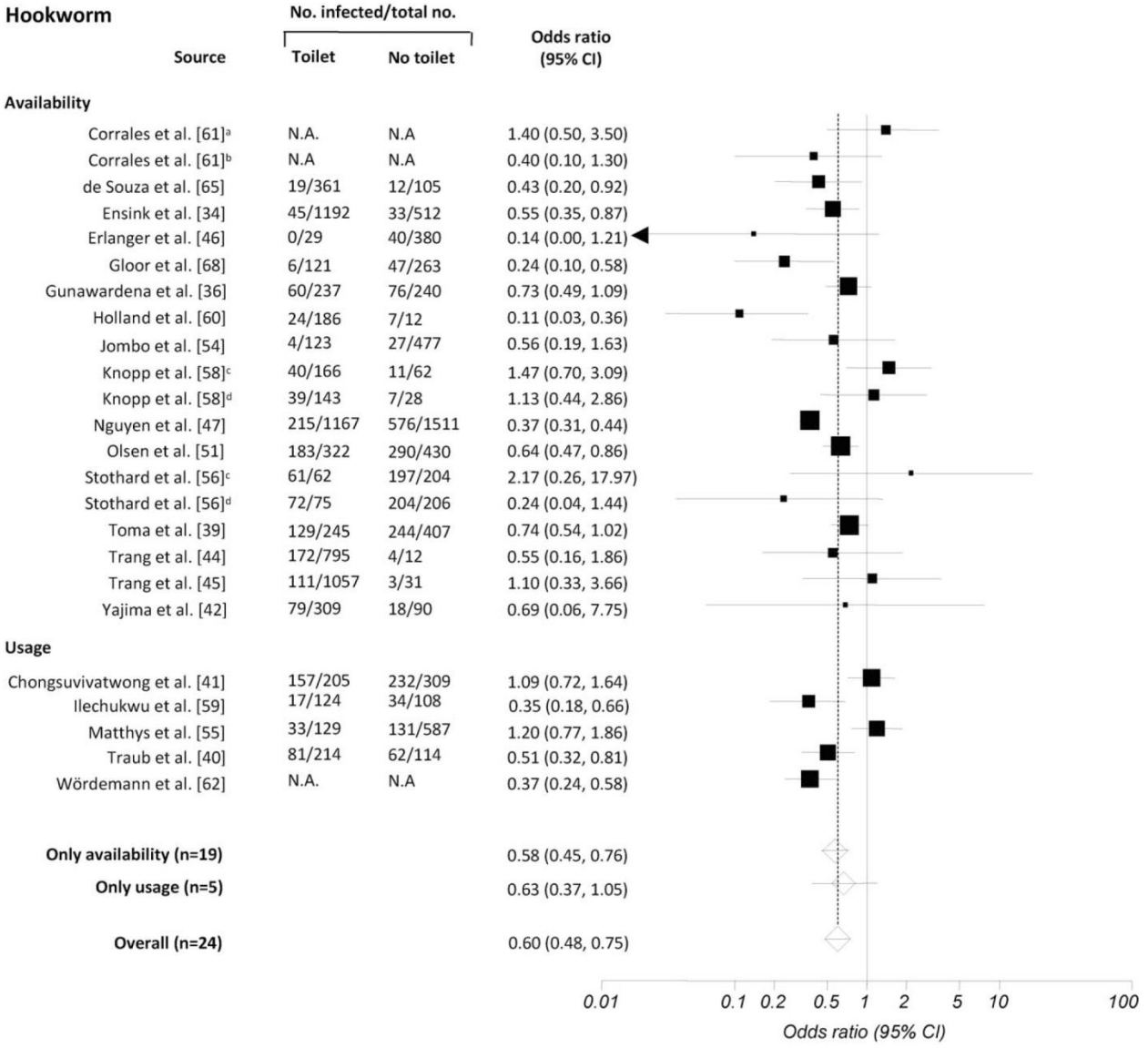

Figure 4. Meta-analysis examining the association of sanitation facilities with hookworm infection. Data are presented separately for availability and use of sanitation. Rectangles indicate ORs, and sizes of the rectangles represent the weight given to each study in the meta-analysis; open diamonds and vertical dashed lines indicate combined ORs; and horizontal lines indicate 95% CIs. Data are presented separately for [a]only pit latrine, [b]only solar urine-diverting desiccating latrine, [c]only adults, [d]only children. N.A., not assessed.
doi:10.1371/journal.pmed.1001162.g004

helminth species were found. Similar estimates were obtained when studies were stratified by availability (ORs between 0.46 and 0.58) *versus* use of sanitation facilities (ORs between 0.54 and 0.78). Subgroup analysis, with stratification according to geographical area or children *versus* all age groups, showed no differences.

Our findings revealed a somewhat stronger negative association of lack of sanitation with infection with soil-transmitted helminths than previous general reviews in which the introduction of water supply and/or sanitation interventions was associated with a reduction in the prevalences of *A. lumbricoides* and hookworm of only 29% and 4%, respectively [27,28]. These previous reviews included only one and four intervention studies for *A. lumbricoides*, and both identified only one relevant study for hookworm [78]. Interestingly, these earlier general reviews did not identify estimates for the association of sanitation with infection with *T. trichiura* and soil-transmitted helminth infections combined.

Strengths and Limitations

We adhered to the MOOSE guidelines for reporting meta-analysis of observational studies (see Text S2) and performed electronic searches on three readily available and widely used databases (i.e., PubMed, Embase, and ISI Web of Science), supplemented with hand-searches of bibliographies of relevant

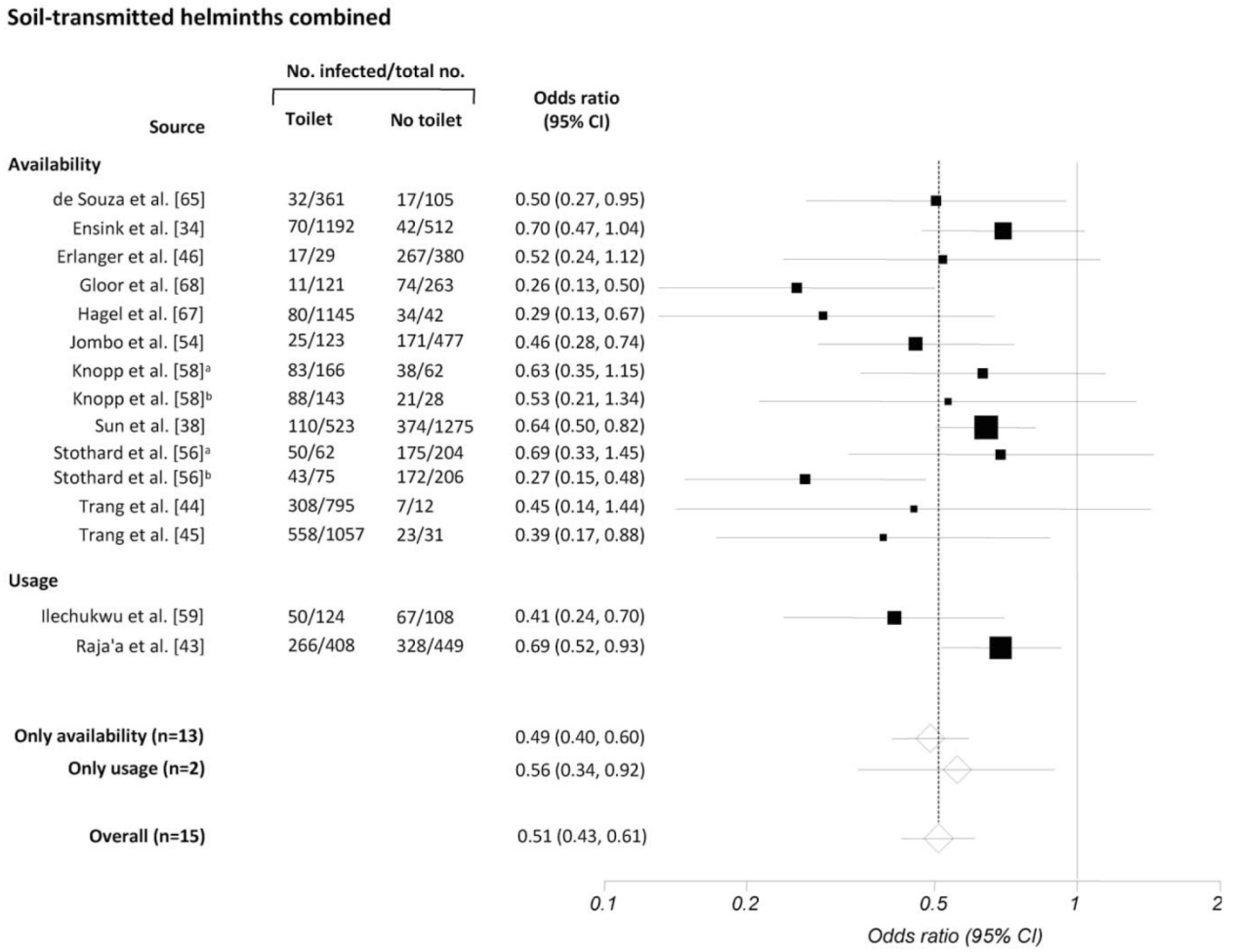

Figure 5. Meta-analysis examining the association of sanitation facilities with infection with the three common soil-transmitted helminths combined. Data are presented separately for availability and use of sanitation. Rectangles indicate ORs, and sizes of the rectangles represent the weight given to each study in the meta-analysis; open diamonds and vertical dashed lines indicate combined ORs; and horizontal lines indicate 95% CIs. Data are presented separately for [a]only adults and [b]only children.
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articles and other sources, until December 31, 2010. We assessed and graded the quality of included studies (see Table 1). However, a number of shortcomings must be highlighted. First, the majority of studies identified reported only on prevalence of infections with soil-transmitted helminths rather than intensity, although the latter measure is of key relevance for morbidity. Indeed, only two of the identified studies assessed the effect of sanitation on infection intensity of soil-transmitted helminths, and hence, no meta-analysis could be performed. Second, we focused on individual-level data. We were therefore not able to address how intervention coverage and use in a community would modify the effect on the individual. It is conceivable that the health effect of changes in intervention coverage in a community from, say, 10% to 70% is distinctively different for the individual living in that community than if coverage increased from 70% to 100%. Unfortunately, this kind of data could not be extracted from the final set of studies included in our meta-analysis. The change in coverage and use of sanitation facilities between the time of baseline and follow-up is a potentially important determinant of impact and a potential explanation of heterogeneity. Third, we noted a publication bias regarding the results of all three soil-transmitted helminth species combined. However, Egger's tests on the individual helminth species did not indicate any publication bias, and hence, the reported ORs for the soil-transmitted helminths combined seem to be justified. Fourth, we did not include "grey literature" or expert consultations. Although this might have yielded important additional studies, we felt that standardization would have been too complicated and, hence, might have introduced additional biases.

Another aspect worth mentioning is that availability, access, ownership, and use of sanitation facilities are not one and the same. Indeed, availability of sanitation facilities does not automatically mean that people use them [43]. Therefore, we stratified results into availability and use of sanitation facilities in our meta-analysis. Our results do not suggest that use of sanitation facilities is more strongly associated than availability with infection by soil-

Table 2. Summary results of sub-group analysis examining the association of sanitation with soil-transmitted helminth infections.

Characteristics	*A. lumbricoides*			*T. trichiura*			Hookworm			Soil-Transmitted Helminths Combined		
	n	Random Effects Pooled OR (95% CI)	I^2 (%)	*n*	Random Effects Pooled OR (95% CI)	I^2 (%)	*n*	Random Effects Pooled OR (95% CI)	I^2 (%)	*n*	Random Effects Pooled OR (95% CI)	I^2 (%)
Overall	32	0.54 (0.43, 0.69)	80.7	24	0.58 (0.45, 0.75)	69.4	24	0.60 (0.48, 0.75)	71.0	15	0.51 (0.44, 0.61)	35.5
Only availability	24	0.46 (0.33, 0.64)	81.2	19	0.56 (0.46, 0.70)	20.5	19	0.58 (0.45, 0.76)	65.8	13	0.49 (0.40, 0.60)	33.3
Only use	8	0.78 (0.60, 1.00)	56.1	5	0.54 (0.28, 1.02)	90.5	5	0.63 (0.37, 1.05)	79.1	2	0.56 (0.34, 0.92)	N.A.
All age groups	16	0.61 (0.43, 0.80)	68.2	16	0.69 (0.49, 0.98)	71.5	18	0.70 (0.54, 0.90)	71.8	9	0.60 (0.51, 0.70)	0.0
Only children	16	0.46 (0.30, 0.71)	86.0	8	0.47 (0.37, 0.60)	14.3	6	0.35 (0.21, 0.57)	51.5	6	0.39 (0.26, 0.59)	66.7
Africa	12	0.41 (0.22, 0.77)	89.0	7	0.44 (0.32, 0.59)	0	8	0.77 (0.51, 1.17)	60.0	6	0.46 (0.35, 0.60)	14.7
Asia	11	0.57 (0.43, 0.77)	77.3	9	0.66 (0.41, 1.05)	82.7	10	0.62 (0.45, 0.86)	74.4	6	0.64 (0.55, 0.75)	0
Central and South America	9	0.67 (0.48, 0.96)	34.3	8	0.58 (0.43, 0.79)	14.3	5	0.42 (0.22, 0.78)	62.3	2	0.41 (0.24, 0.69)	N.A.
US	0			0			1	0.24 (0.10, 0.58)	N.A.	1	0.26 (0.13, 0.50)	N.A.

N.A., not assessed.

doi:10.1371/journal.pmed.1001162.t002

transmitted helminths. This finding is not surprising, since one of the methodological shortcomings of our analysis is that studies reporting on the availability and use of sanitation facilities were both included. Availability and use of sanitation facilities was primarily assessed by questionnaires rather than verified by random spot checks or direct observations. It is conceivable that the question "Where do you defecate?" is prone to reporting bias, as people might be ashamed to state that they practice open defecation [79]. Moreover, farmers, fishermen, street vendors, and traders might have sanitation facilities at home and use them, but may be forced to practice open defecation or defecate in unimproved latrines (open pits) with highly contaminated surroundings during extended periods away from home. In view of this, one study focusing on school-aged children was excluded because the authors examined the availability of sanitation facilities only at school, and not at home [80].

Finally, in most of the included studies the type of sanitation facilities available or used was not mentioned, but such information is important, as the types of sanitation might be differentially associated with the prevalence of infection with different soil-transmitted helminth species [81]. If the type of sanitation facilities was mentioned, a wide variety of terms was used (e.g., flush toilet, water closet, ventilated improved pit latrine, pit latrine, and open latrine). Hence, there is a need for a more unified classification of latrine types. The "sanitation ladder" proposed by the World Health Organization/United Nations Children's Fund Joint Monitoring Programme for Water Supply and Sanitation is a first step in this direction [82]. In the current study, however, stratified analysis according to toilet type was not possible because of the lack of data. Other determinants that were not investigated in our meta-analysis were coverage levels of toilet availability and toilet use in a community, and the maintenance of sanitation facilities. Proper maintenance of toilets is crucial, as otherwise sanitation facilities can turn into "hookworm-traps" [83,84]. Coverage plays an important role; only a few individuals defecating openly can maintain the transmission of helminths [85]. In addition, a recent study carried out in Viet Nam found high prevalence of soil-transmitted helminth infections despite the fact that 98.1% of the households owned a latrine. This was explained by the use of "night soil" (human excreta) as fertilizer, which is a common agricultural practice in many Asian countries [42].

There were no randomized controlled trials evaluating the impact of sanitation facilities on the prevalence of infection with soil-transmitted helminths identified in our systematic review. Although randomized controlled trials provide the most robust evidence [86], this experimental design is not always feasible, as seen in the current review and in other environmental interventions that have been tested to reduce the burden of infectious diseases [28,87–91]. Intervention studies have the disadvantage that in addition to sanitation, more complex interventions were implemented, including health education, improvement of water supplies, and preventive chemotherapy. Obviously, it is then the package of interventions and not just one component that is associated with the outcomes [89,91]. Furthermore, most studies have only short evaluation periods, and it is difficult to draw inferences regarding sustainability [92,93]. It is interesting to note that only a few such complex integrated interventions were identified for sanitation and prevalence of helminth infections, and all except one were excluded. In cross-sectional observation studies, which make up most of our included studies, sanitation facilities had been in place for several years, and hence, the long-term effect on soil-transmitted helminth infections could be assessed. However, observational studies bear the risk of confounding, since people owning sanitation facilities may be different from those without. For example, community members owning and using sanitation facilities may be wealthier, their educational level might be higher, or they might be more health conscious [94].

Policy Implications

The results of our meta-analysis reveal that sanitation is associated with a reduction in the prevalence of soil-transmitted helminth infections. Our findings, therefore, underscore what the Rockefeller Sanitary Commission stated more than 70 years ago: "Cure alone is almost useless in stamping out hookworm disease, because the patient can go out and immediately pick up more hookworms. The cure should be accompanied by a sanitation campaign for the prevention of soil pollution" [6]. Implementation

of sanitation facilities and integrated control approaches go far beyond the prevention and control of intestinal helminths; they impact other neglected tropical diseases, such as schistosomiasis, trachoma, and diarrhea [23–25,95], and can even help promote social and educational advances for women and girls [96]. For a durable impact, the process of implementing improved sanitation requires community involvement and setting-specific information, education, and communication strategies as key factors to ultimately change human behaviors. Now that the elimination of neglected tropical diseases is coming to the forefront of global attention, integrated control approaches—using a combination of preventive chemotherapy; information, education, and communication campaigns; and improvements to basic sanitation and access to safe, clean water—cannot be emphasized enough.

Supporting Information

Table S1 Details of all the publications that were fully screened by the first two authors ($n = 162$). Reasons why studies have been excluded are given ($n = 126$). Studies included in our meta-analysis are shaded grey ($n = 36$).
(DOC)

Text S1 PRISMA checklist.
(DOC)

Text S2 Study protocol for systematic review and meta-analysis to determine the effect of sanitation on soil-transmitted helminth infection.
(DOC)

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Author Contributions

Conceived and designed the experiments: KZ BS JK JU. Performed the experiments: KZ BS. Analyzed the data: KZ BS JK JU. ICMJE criteria for authorship read and met: KZ BS DM RB JK JU. Agree with the manuscript's results and conclusions: KZ BS DM RB JK JU. Wrote the first draft of the paper: KZ BS. Contributed to the writing of the paper: DM RB JK JU. Interpreted the data: KZ BS DM JK JU.

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Chapter 6b

Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis

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Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis

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Summary

Background **Pathogenic intestinal protozoa infections are responsible for substantial mortality and morbidity, particularly in settings where people lack improved sanitation and safe drinking water. We assessed the relation between access to, and use of, sanitation facilities and water treatment and infection with intestinal protozoa.**

Methods **We did a systematic review and searched PubMed, ISI Web of Science, and Embase from inception to June 30, 2014, without restrictions on language. All publications were examined by two independent reviewers and were included if they presented data at the individual level about access or use of sanitation facilities or water treatment, in combination with individual-level data on human intestinal protozoa infections. Meta-analyses using random effects models were used to calculate overall estimates.**

Findings **54 studies were included and odds ratios (ORs) extracted or calculated from 2×2 contingency tables. The availability or use of sanitation facilities was associated with significantly lower odds of infection with *Entamoeba histolytica* or *Entamoeba dispar* (OR 0·56, 95% CI 0·42–0·74) and *Giardia intestinalis* (0·64, 0·51–0·81), but not for *Blastocystis hominis* (1·03, 0·87–1·23), and *Cryptosporidium* spp (0·68, 0·17–2·68). Water treatment was associated with significantly lower odds of *B hominis* (0·52, 0·34–0·78), *E histolytica* or *E dispar* (0·61, 0·38–0·99), *G intestinalis* (0·63, 0·50–0·80), and *Cryptosporidium* spp infections (0·83, 0·70–0·98).**

Interpretation **Availability and use of sanitation facilities and water treatment is associated with lower odds of intestinal protozoa infections. Interventions that focus on water and sanitation, coupled with hygiene behaviour, should be emphasised to sustain the control of intestinal protozoa infections.**

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Introduction

Infections with intestinal protozoa (eg, *Cryptosporidium* spp, *Entamoeba histolytica*, and *Giardia intestinalis*) cause substantial gastrointestinal morbidity, malnutrition, and mortality worldwide.[1–4] For example, the annual mortality due to amoebiasis caused by *E histolytica* has been estimated at 100 000 cases and hence, amoebiasis is one of the deadliest parasitic infections.[1,3] *G intestinalis* has an estimated prevalence of 2–3% in high-income countries and 20–30% in developing countries.[5] *Cryptosporidium* spp is another important diarrhoeal agent. Immunocompromised individuals such as people infected with HIV are at highest risk for severe disease caused by *Cryptosporidium* spp.[3,6,7] The pathogenicity of the intestinal protozoon *Blastocystis hominis* is still under debate.[8–10] The Global Burden of Disease Study 2010[4] estimated that cryptosporidiosis and amoebiasis were, respectively, responsible for 8·4 million and 2·2 million disability-adjusted life years (DALYs) in 2010, but could not provide any estimates for giardiasis or blastocystiasis. In general, data on the burden of disease caused by intestinal protozoa is scarce, probably also because of the challenge of an accurate diagnosis.[11]

Children in low-income and middle-income countries who lack access to clean water and improved sanitation are at particular risk of acquiring intestinal protozoa infections.[1,2,12,13] Because intestinal protozoa are transmitted by the faecal–oral route and through drinking contaminated water,[5,6] infections are present where access to clean water and improved sanitation is inadequate. An estimated 780 million and 2·5 billion people have no access to improved drinking water sources and improved sanitation, respectively.[14] The UN Millennium Development Goal 7c aims to halve the proportion of people without sustainable access to safe drinking water and basic sanitation by 2015.[15] Although the goal for access to drinking water will probably be reached by the end of 2015,[14] it is unlikely that the target pertaining to sanitation will be met. However, substantial geographical heterogeneity exists in the use of improved water supply and sanitation, particularly in developing regions.[16]

Interest is growing in high-level evidence on the association of water, sanitation, and hygiene (WASH) on neglected tropical diseases.[17–21] For example, systematic reviews and meta-analyses showed that people without sanitation are at higher odds of being infected with soil-transmitted helminths compared with those with sanitation.[17,20] However, no evidence on this relation is available for intestinal protozoa. Against this background, it was our aim to quantify the possible protective effect of sanitation facilities (access to, and use of, facilities for the

safe disposal of faeces) and water treatment (eg, boiling, filtering, and UV treatment) against intestinal protozoa infections. We did a systematic review and summarised all quantitative data in a series of meta-analyses.

Methods

Search strategy and inclusion criteria

See Online for appendix

The study protocol for this systematic review and meta-analysis is in the appendix. We adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement[22] (appendix) and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[23] We did a systematic search of PubMed, ISI Web of Science, and Embase. The databases were searched from inception to June 30, 2014, without language restriction. We used the MeSH terms and keywords "sanitation", "water", "risk factor", and "toilet facilities". These terms were combined with "*Giardia intestinalis*", "*Entamoeba histolytica*", "*Entamoeba dispar*", "*Blastocystis hominis*", and "*Cryptosporidium*". Of note, stool microscopy does not allow to differentiate between *E histolytica* and *E dispar*, and hence these intestinal protozoa were grouped together.

Irrespective of the study type, publications were eligible for inclusion if they reported individual-level data on any of the aforementioned intestinal protozoa species, stratified by one of the following criteria: access or no access to sanitation facilities (with or without specification of toilet type); use or non-use of sanitation facilities; and application or non-application of water treatment at the place of consumption (with or without details on water treatment reported). If data were not available in the format needed, the corresponding author of the article was contacted by e-mail and asked to provide the data in the form of 2×2 contingency tables. Publications reporting on water treatment prepared by the municipal water company were excluded. To avoid potential publication bias (ie, selective outcome reporting bias), the corresponding authors of articles presenting the results of significant outcomes only (ie, omitting data from other non-significant outcomes) were also contacted by email and the respective publications were included only if complete data were provided. If authors did not respond to our email request, one reminder was sent about 4 weeks after the initial correspondence. Additionally, publications that examined risk factors after a single outbreak were excluded. Studies reporting only chlorination as a water treatment were excluded because chlorination is not deemed an efficacious treatment against intestinal protozoa.[24,25]

All identified titles and abstracts were carefully examined by two independent reviewers (BS and DC). The full text of articles considered as potentially relevant based on title and abstract were independently examined by the same two reviewers. The study protocol (appendix) was used to assess the eligibility of each article. If there was disagreement between the two reviewers, a third reviewer (TF) was consulted and the respective article discussed until agreement was reached.

Data extraction and quality assessment

The following information was extracted from all included publications: study design, study site (country), year of the study, selection of the study population (eg, randomly at school, from community), age group, whether availability or use of sanitation facilities was reported, whether the type of water treatment was reported, and how the data were presented in the publication (eg, 2×2 contingency table, odds ratio [OR]).

OR was the main outcome measure. In some publications, ORs were reported directly. For studies that presented data in 2×2 contingency tables or in text format, we calculated the ORs ourselves. If both adjusted and unadjusted ORs were presented, we consistently used unadjusted ORs to allow for cross-study comparison.

The quality of the included publications was assessed based on recently developed criteria.[17,20] These criteria were created based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) method[26] and included the diagnostic approach to identify intestinal protozoa infections; the method to assess the sanitation status and use of water treatment; and careful considerations of strengths and limitations. A scoring approach was used for grading and up to six points assigned to each study. One point was assigned to a study if the diagnostic approach was clearly described. Since a single examination often results in low sensitivity for intestinal protozoa,[27,28] publications implementing a rigorous diagnostic approach (ie, examining several consecutive stool samples from the same individual or subjecting stool samples to many diagnostic tests) received an additional point. A third point was assigned if quality control for the diagnostics was explicitly mentioned in the report (eg, 10% of all microscope slides re-examined by a senior laboratory technician). Publications that did not report on the diagnostic approaches received no point. Regarding the availability of sanitation facilities and use of water treatment, publications received two points when their availabilities were checked on the spot. One point was assigned if the access and use of sanitation facility and water treatment were assessed by questionnaire and at least a sub-sample was checked on the spot. No points were given if only a questionnaire was administered. For further study strengths (eg, pre-testing of questionnaires) and limitations (eg, no random sampling) an additional point was given or subtracted, respectively. Publications with a total score of four to six points were considered to be of high quality, two or three points were moderate quality, whereas lower scores indicated low quality. No publications were excluded based on the result of the quality assessment.

Statistical analysis

ORs from 2×2 contingency tables were calculated and forest plots generated using StatsDirect version 2.7.9

(StatsDirect, Cheshire, UK) for each intestinal protozoon species separately. Overall estimates were assumed to be free of publication bias if Egger's test was above 0·1.[29] Higgin's I^2 test was used to determine heterogeneity of the studies.[30] Since evidence for heterogeneity was found (I^2>50%), we used random effect models throughout. ORs less than 1 indicate lower odds of being infected for individuals using or having access to sanitation facilities, or for people using water treatment, respectively. Subgroup analyses were done with regard to availability (ie, having access to any sort of toilet) and use (ie, using any sort of toilet) of sanitation facility; economic capacity of the countries where the study was done (according to World Bank classification[31]); and methods of water treatment in which different techniques were not merged and could be assessed separately.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

After removing duplicates, we retrieved 4426 publications from PubMed, ISI Web of Science, and Embase (figure 1). Of these initial hits, 3887 were excluded based on title or abstract. The remaining 539 articles had full-text screening. Of these, 377 articles were excluded after consulting the full text (the main reasons for exclusion are listed in the appendix). 123 publications did not present the data in the required format or presented only data of significant outcomes. Hence, efforts were made to contact corresponding authors from these articles by e-mail. However, we could not obtain a valid e-mail address from 14 corresponding authors. From the 109 authors contacted, we received 15 datasets that allowed us to extract the relevant information for subsequent meta-analyses. The other 94 studies were excluded because we were unable to obtain the data in the required format. Finally, a total of 54 publications were included for our meta-analyses (figure 1).

Characteristics of the included publications are listed in table 1 (for more details, see appendix). In brief, 23 publications described the effect of sanitation availability, 11 articles investigated the use of sanitation, whereas two studies did not clearly differentiate between use and availability of sanitation facilities. The effect of water treatment at the place of consumption was described by 29 publications. The identified studies were conducted worldwide (figure 2). 29 datasets investigated the effect of sanitation facilities on infections with *G intestinalis*, whereas only five datasets examined the effect of sanitation facilities on

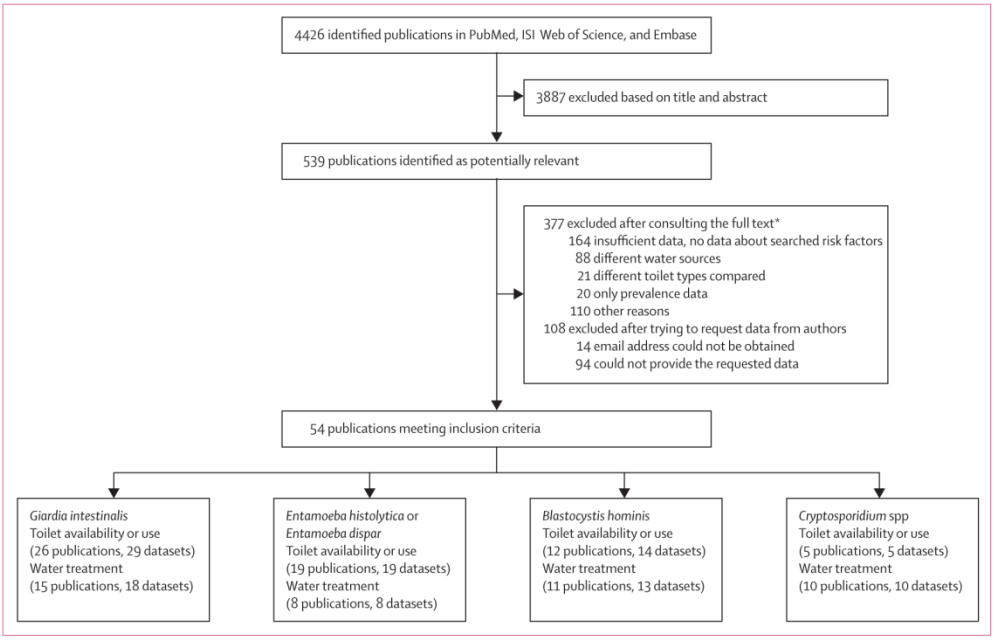

Figure 1: **Study selection**
*Multiple exclusion criteria possible; a detailed list of excluded publications and reasons for exclusion based on full text is provided in the appendix.

Articles

	General information								Information about rating			
	Study design	Country	Study year	Age group	Availability (A) or use (U) of toilet	Water treatment method	Intestinal protozoa species	Data obtained	Diagnostic	Toilet status or water treatment assessment method	Other strengths and limitations	Total points
Abdulsalam et al (2012)[32]	C-S	Malaysia	2010	Chi	A	NA	Bh	OR	1·5	0	0	1·5
Abdulsalam et al (2013)[33]	C-S	Libya	2010	All	NA	B/F/C	Bh	OR	1·5	0	0	1·5
Al-Shamiri et al (2010)[34]	C-C	Yemen	2006–07	Chi	NA	NS	Cry	2×2 T	2	0	0	2
Alyousefi et al (2011)[35]	C-S	Yemen	NS	All	NA	NS	Gi, Eh/Ed	OR	2	0	0	2
Ankarklev et al (2012)[36]	C-S	Uganda	2007	Chi	U	NA	Gi	2×2 T	2	0	0	2
Anuar et al (2012)[37]	C-S	Malaysia	2011	All	U	NS	Gi	2×2 T	2	0	0	2
Anuar et al (2012)[38]	C-S	Malaysia	2011	All	U	NS	Eh	2×2 T	2	0	0	2
Anuar et al (2013)[39]	C-S	Malaysia	2011	All	NS	NS	Bh	2×2 T	2	0	0	2
Benetton et al (2005)[40]	C-S and C-C	Brazil	2001–02	All	A	B/F/C	Eh/Ed	2×2 T	3	0	0	3
Blessmann et al (2002)[41]	C-S	Vietnam	1999	Adu	A	NA	Eh	2×2 T	2	0	0	2
Boontanom et al (2011)[42]	C-S	Thailand	2007	Chi	NA	F/B	Gi	2×2 T	3	0	0	3
Carrero and Helena (2013)[43]	C-S	Colombia	NS	Chi	NA	B	Gi, Eh/Ed, Bh, Cry	2×2 T*	1	0	0	1
Cifuentes et al (2000)[44]	C-S	Mexico	1990	Chi	A	NA	Gi	2×2 T	0	0	0	0
Crump et al (2007)[45]	RCT	Guatemala	2001–02	Chi	NA	5 water treatments	Gi, Cry	2×2 T	2	2	1	5
El Kettani et al (2006)[46]	C-C	Morocco	NS	All	A	NA	Gi	2×2 T	2	0	0	2
Esrey et al (1989)[47]	Int	Lesotho	1984	Chi	A	NA	Gi	OR	1	0	0	1
Fuentes et al (2011)[48]	C-S	Lesotho	2007	Chi	A	C/B	Gi, Eh/Ed, Bh	2×2 T*	1	0	0	1
Gamboa et al (2009)[49]	C-S	Argentina	NS	All	A	NA	Gi, Bh	2×2 T*	2	0	0	2
Hellard et al (2001)[50]	RCT	Australia	1997–99	All	NA	F/UV	Gi, Cry	2×2 T	0	2	0	2
Jeppsen et al (2013)[51]	C-S*	China	2012*	Chi	U	B	Gi, Cry	2×2 T*	1*	1*	0*	2*
Júlio et al (2012)[52]	C-S	Portugal	2002–08	Chi	A	NA	Gi	2×2 T	2	0	0	2
Katsumata et al (1998)[53]	C-C	Indonesia	1992–93	All	NA	NS	Cry	OR	2	0	0	2
Khalakdina et al (2003)[54]	C-C	USA	1999–01	All	NA	B	Cry	OR	0	0	0	0
Lander et al (2012)[55]	C-S	Brazil	2010	Chi	A	NA	Gi, Eh/Ed, Bh, Cry	2×2 T*	3	0	0	3
Leelayoova et al (2008)[56]	C-S	Thailand	2005	Chi	NA	F/B	Bh	2×2 T	2	0	0	2
Li et al (2007)[57]	C-S	China	NS	All	NA	B	Bh	OR	1	0	0	1
Licea et al (2003)[58]	C-S	Mexico	2000	Adu	U	B/P	Bh	2×2 T	2	0	0	2
Mahdy et al (2008)[59]	C-S	Malaysia	2006	All	U	NA	Gi	2×2 T	2	0	0	2
Mahmud et al (1995)[60]	Coh	Egypt	1987–89	Chi	A	NA	Gi	2×2 T	1	0	0	1
Mahmud et al (2014)[61]	C-S	Ethiopia	NS	Adu	A	NA	Gi, Eh/Ed	OR	2	0	1	3
Mathur and Kaur (1972)[62]	C-S	India	NS	All	U	NA	Eh/Ed	2×2 T	3	2	0	5
Matthys et al (2007)[63]	C-S	Côte d'Ivoire	2004–05	All	A	NA	Gi, Eh/Ed, Bh	2×2 T*	2	0	1	3
McElligott et al (2013)[64]	C-S	Uganda	2011	All	A	NA	Gi, Eh/Ed	2×2 T*	1	0	0	1
Molloy et al (2011)[65]	C-S	Nigeria	2007	Chi	U	B/F	Cry	2×2 T*	2	0	0	2
Morales-Espinoza et al (2003)[66]	C-S	Mexico	1998	Chi	A	NA	Gi, Eh/Ed	OR	2	0	0	2
Núñez et al (2003)[67]	Coh	Cuba	NS	Chi	NA	B	Gi	2×2 T	2	0	1	3
Nxasana et al (2013)[68]	C-S	South Africa	2009	Chi	A	NA	Gi, Eh/Ed, Bh	2×2 T*	2	0	0	2
Pham Duc et al (2011)[69]	C-C	Vietnam	2008	All	A	NA	Eh/Ed	2×2 T	2	0	1	3
Prado et al (2003)[70]	C-S	Brazil	NS	Chi	A	B/F	Gi	2×2 T	1	1	1	3
Quihui et al (2006)[71]	C-S	Mexico	1997–98	Chi	U	NA	Gi, Eh/Ed	2×2 T*	2	0	0	2
Rao et al (1971)[72]	C-S	NS	1970	All	U	NA	Gi, Eh/Ed	Cal	2	1	–1	2
Rinne et al (2005)[73]	C-S	Ecuador	2003	Chi	A	NA	Gi, Eh/Ed	2×2 T	1	0	0	1
Rondón et al (2003)[74]	C-C	Peru	1999	All	A	B	Bh	OR	1	0	0	1
Roy et al (2004)[75]	C-C	USA	1999–01	All	NA	F	Cry	2×2 T	2	0	1	3
Santos et al (2012)[76]	C-S	Brazil	2007–08	Chi	NA	B/F	Gi	2×2 T	3	0	1	4

(Table 1 continues on next page)

	General information								Information about rating			
	Study design	Country	Study year	Age group	Availability (A) or use (U) of toilet	Water treatment method	Intestinal protozoa species	Data obtained	Diagnostic	Toilet status or water treatment assessment method	Other strengths and limitations	Total points
(Continued from previous page)												
Sayasone et al (2011)[77]	C-S	Lao PDR	NS	All	A	NA	Gi, Bh	2×2T*	2	0	1	3
Schmidlin et al (2013)[78]	C-S	Côte d'Ivoire	2011	All	A	NA	Gi, Eh/Ed, Bh	2×2T*	2	0	1	3
Stuart et al (2003)[79]	C-C	UK	1998–99	All	NA	F	Gi	2×2T	0	0	1	1
Taamasri et al (2000)[80]	C-S	Thailand	1998	All	NA	B/F	Bh	2×2T	3	0	0	1
Taye et al (2014)[81]	C-S	Ethiopia	2007	All	A	NA	Gi, Eh/Ed, Cry	2×2T*	2	0	0	2
Tian et al (2012)[82]	C-S	China	NS	Adu	NA	B	Bh	2×2T*	1	0	0	1
Torres et al (1992)[83]	C-S	Chile	1987	All	U	NA	Gi, Eh/Ed, Bh	2×2T	1	0	0	1
Wanyiri et al (2013)[84]	C-S	Kenya	2009–10	Adu	NS	B	Gi, Eh/Ed, Cry	2×2T*	2	0	0	2
Wördemann et al (2006)[85]	C-S	Cuba	2003–04	Chi	NA	B	Gi, Eh/Ed	OR	1	0	0	1

C-S=cross-sectional study. Chi=children and infants. NA=not applicable because the respective reference does not report this parameter. Bh=*Blastocystis hominis*. OR=odds ratio directly from the publication. All=all age groups (adults and children). B=boiling. F=filtering. C=chlorination. C-C=case-control study. NS=not stated. Cry=*Cryptosporidium* spp. 2×2T=odds ratio calculated from a 2×2 table. Eh/Ed=*Entamoeba histolytica* or *E dispar*. Adu=adults. Gi=*Giardia intestinalis*. RCT=randomised controlled trial. Int=intervention study. UV=treatment with ultraviolet light. P=purifying. Coh=cohort study. Cal=2×2 table calculated from numbers within the text. *Data provided by authors.

Table 1: **Characteristics of the included studies**

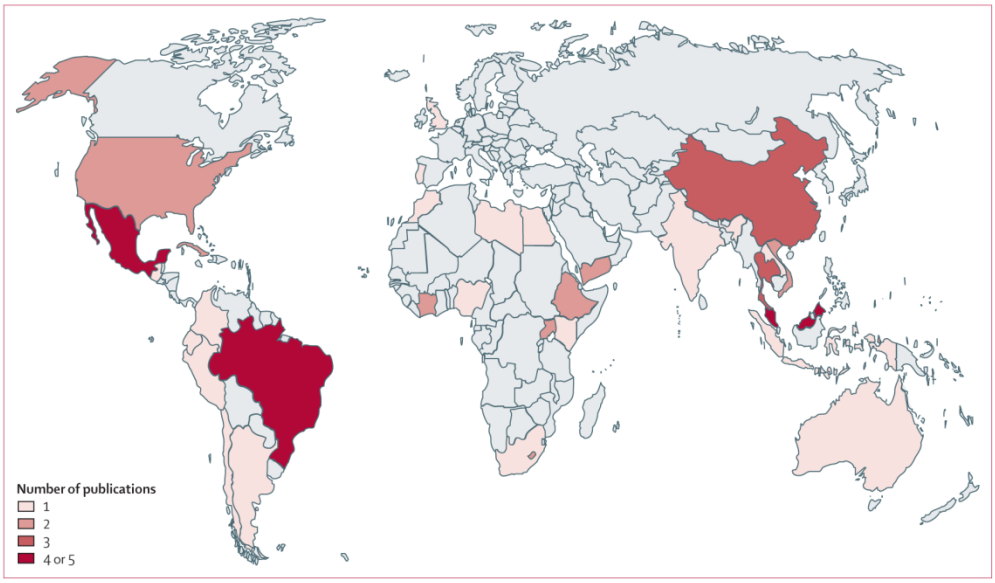

Figure 2: **Geographical distribution of the included studies**
One of the 54 publications did not state the country.

Cryptosporidium spp. In terms of epidemiological design, 40 of the included publications were cross-sectional[32,33,35–39,41–44,48,49,51,52,55–59,61–66,68,70–73,76–78,80–85] and eight were case-control studies.[34,46,53,54,69,74,75,79] Two studies were randomised controlled trials,[45,50] two were cohort studies,[60,67] one was a combined cross-sectional and case-control study,[40] and one was an intervention study.[44] Detailed information about study design and interventions are given in the appendix. Overall, 42 publications provided the required data in a 2×2 contingency table, 11 publications presented ORs directly, and one study allowed the reconstruction of 2×2 contingency table from data presented in the article. 46 papers were written in English, six were in Spanish,[43,48,49,67,74,83] one in French,[46] and one in Chinese.[82] The oldest study was done in 1970.[72] In 11 publications, the year when the study was done could not be retrieved. 35 studies used a rigorous diagnostic approach with

Articles

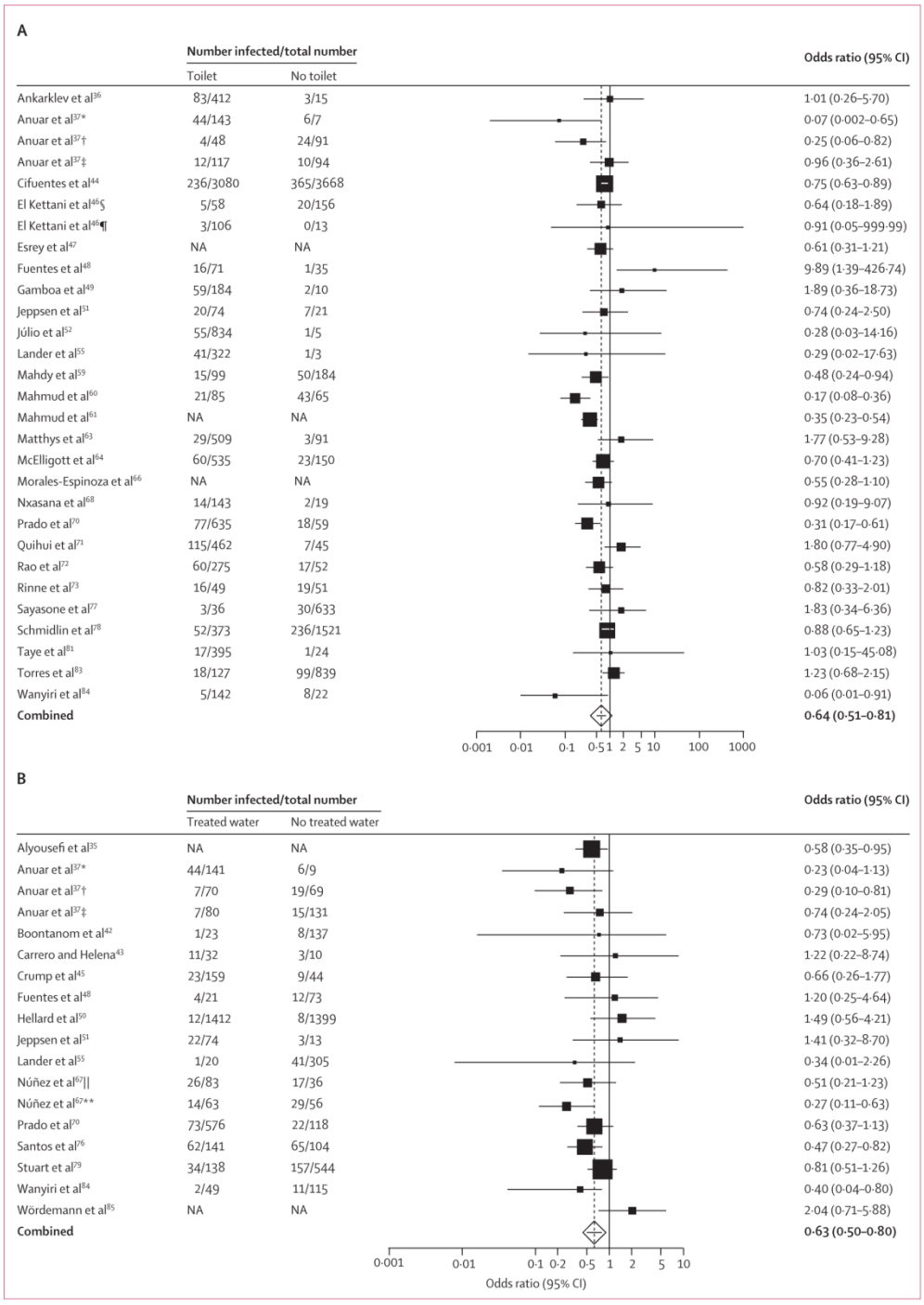

Figure 3: **Meta-analysis of the association of sanitation facilities (A) and water treatment (B) with *Giardia intestinalis* infections**
NA=not assessed. *Proto-Malay tribe. †Neggrito tribe. ‡Senoi tribe. §Village using raw sewage in agriculture. ¶Village using no sewage water in agriculture. ||Group used magnesium salt to verify boiling of water. **Group did not verify boiling of water.

more than one stool sample examined or several diagnostic approaches done on the same stool sample. Ten publications explicitly stated quality control for the diagnosis of intestinal protozoa infections.

According to our criteria, three publications were of high quality (4 or 5 points).[45,62,76] 34 publications had quality scores of 2 and 3 points indicating moderate quality, whereas the remaining 17 publications were of low quality.

Forest plots on the effect of sanitation and water treatment on *G intestinalis*, *E histolytica* or *E dispar*, *B hominis*, and *Cryptosporidium* spp are presented in figures 3–6. Egger's test showed publication bias (p<0·1) for two of the eight meta-analyses (ie, association between sanitation facility and *E histolytica* or *E dispar*, and between water treatment and *B hominis*).

The estimated pooled random effects ORs of either having or using a sanitation facility compared with not having or using a sanitation facility were 0·56 (95% CI 0·42–0·74) for infection with *E histolytica* or *E dispar*, 0·64 (0·51–0·81) for infection with *G intestinalis*, 0·68 (0·17–2·68) for infection with *Cryptosporidium* spp, and 1·03 (0·87–1·23) for infection with *B hominis*. Treatment of water before consumption was associated with a lower odds of infection with *G intestinalis* (0·63, 0·50–0·80), *E histolytica* or *E dispar* (0·61, 0·38–0·99), *B hominis* (0·52, 0·34–0·78), and *Cryptosporidium* spp (0·83, 0·70–0·98).

All subgroup analyses, including those on availability versus use of sanitation facilities, economic classification of the countries where the studies were done, and

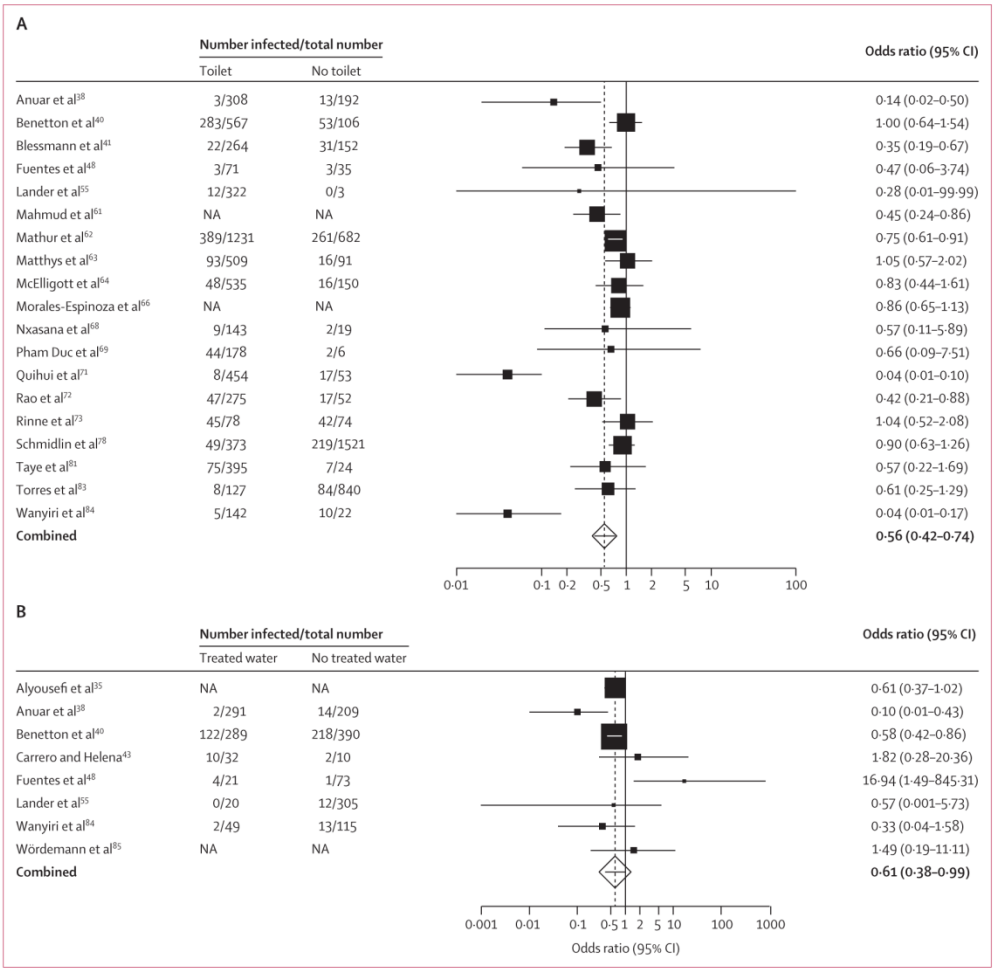

Figure 4: **Meta-analysis of the association of sanitation facilities (A) and water treatment (B) with *Entamoeba histolytica* or *Entamoeba dispar* infections**
NA=not assessed.

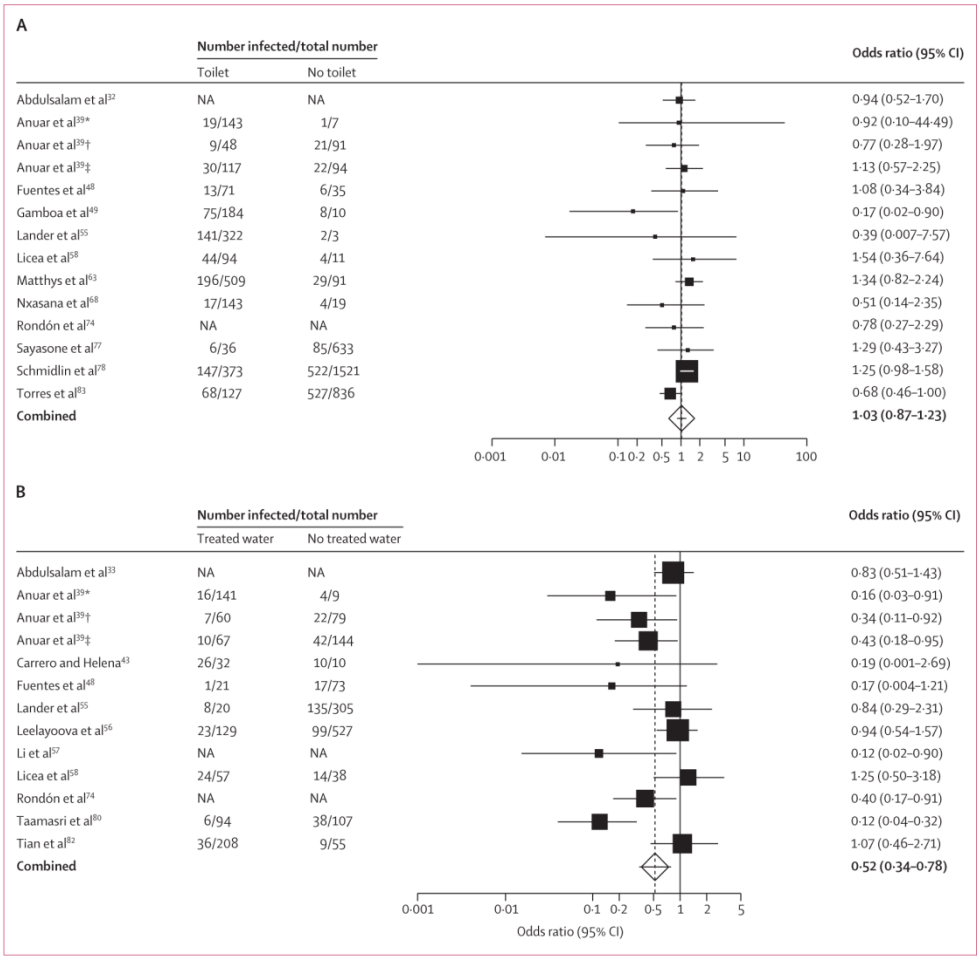

Figure 5: **Meta-analysis of the association of sanitation facilities (A) and water treatment (B) with *Blastocystis hominis* infections**
NA=not assessed. *Proto-Malay tribe. †Neggrito tribe. ‡Senoi tribe.

methods of water treatment, did not reveal any significant differences between the respective groups (as shown by overlapping 95% CIs). The only exception was either having or using sanitation facilities and *B hominis* infections in lower-middle-income countries (OR 1·26, 95% CI 1·02–1·55) and high-income countries (0·68, 0·46–1·00). The results of all subgroup analyses are summarised in table 2.

Discussion

Diarrhoeal diseases caused an estimated 282 million DALYs in 2010 and were responsible for 1·2 million deaths, primarily among children younger than 5 years.[3,4,86] For example, *Cryptosporidium* spp was identified by the Global Enteric Multicenter Study as one of the leading causes of moderate-to-severe diarrhoea.[87]

Sanitation, defined as clean water and sewage disposal, recently voted by readers of the *BMJ* as the single greatest medical milestone since 1840, could resolve the bulk of this morbidity and mortality.[88] The current systematic review and meta-analyses were done to quantify the effects of sanitation—defined as access to, and use of, facilities for the safe disposal of excreta and water treatment at the place of water consumption—against intestinal protozoa infections. Hence, it focused on two (ie, water and sanitation) of the three aspects of WASH.

Our findings imply that sanitation is an effective intervention to prevent intestinal protozoa infections. This finding is in accordance with the results of previous

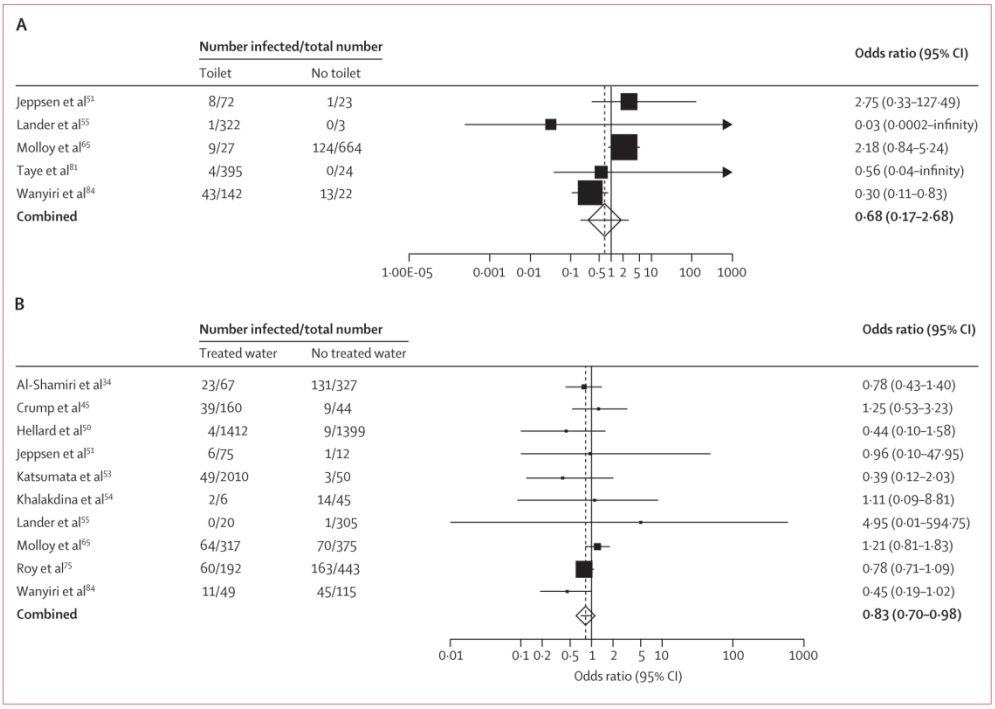

Figure 6: **Meta-analysis of the association of sanitation facilities (A) and water treatment (B) with *Cryptosporidium* spp infections**

	Giardia intestinalis			*Entamoeba histolytica* or *Entamoeba dispar*			*Blastocystis hominis*			*Cryptosporidium* spp		
	n	Random effects pooled OR (95% CI)	I^2	n	Random effects pooled OR (95% CI)	I^2	n	Random effects pooled OR (95% CI)	I^2	n	Random effects pooled OR (95% CI)	I^2
Sanitation facilities												
Overall	29	0·64 (0·51–0·81)	52·6	19	0·56 (0·42–0·74)	71·1	14	1·03 (0·87–1·23)	3·1	5	0·68 (0·17–2·68)	72·8
Only availability*	18	0·61 (0·46–0·81)	57·3	12	0·80 (0·66–0·96)	16·4	9	1·12 (0·96–1·40)	0·0	1	0·03 (<0·01–infinity)	..
Only use*	10	0·76 (0·51–1·13)	35·1	6	0·34 (0·16–0·71)	83·1	2	0·72 (0·46–1·13)	..	3	2·06 (0·98–4·32)	0
Low-income country†	5	0·48 (0·25–0·90)	50·5	4	0·36 (0·14–0·93)	79·6	..	..	..	2	0·32 (0·13–0·77)	..
Lower-middle-income country†	9	0·71 (0·48–1·05)	63·2	6	0·74 (0·56–0·97)	37·3	4	1·26 (1·02–1·55)	0	1	2·18 (0·84–5·24)	..
Upper-middle-income country†	13	0·64 (0·47–0·87)	35·1	7	0·46 (0·22–0·93)	81·6	9	0·87 (0·62–1·24)	0	2	0·36 (<0·01–35·89)	..
High-income country†	2	1·18 (0·66–2·08)	..	1	0·61 (0·25–1·29)	..	1	0·68 (0·46–1·00)	..	..	..	..
Water treatment												
Overall	18	0·63 (0·50–0·80)	15·1	8	0·61 (0·38–0·99)	30·0	13	0·52 (0·34–0·78)	54·5	10	0·83 (0·70–0·98)	0
Low-income country	1	0·42 (0·04–0·80)	..	1	0·33 (0·04–1·58)	..	..	..	..	1	0·45 (0·19–1·02)	..
Lower-middle-income country	3	0·63 (0·41–0·96)	0	2	2·20 (0·09–51·92)	..	1	0·17 (0·01–1·21)	..	4	1·00 (0·71–1·42)	12·7
Upper-middle-income country	12	0·56 (0·40–0·78)	21·3	5	0·58 (0·27–1·25)	24·3	12	0·50 (0·32–0·80)	57·0	2	1·42 (0·10–20·98)	..
High-income country	2	0·92 (0·56–1·52)	..	..	..	..	..	..	..	3	0·77 (0·63–0·95)	0
Only boiling	8	0·70 (0·39–1·28)	38·8	4	1·25 (0·38–4·11)	18·0	6	0·54 (0·29–1·01)	23·4	4	0·60 (0·31–1·16)	0
Only filtering	2	0·68 (0·44–1·07)	..	..	..	..	..	..	..	2	0·90 (0·61–1·34)	..

*Two studies, including four datasets, did not clearly state if availability or usage of toilet was observed and were therefore included in the overall meta-analysis, but not in the respective subgroup meta-analysis.
†One paper, including two datasets regarding *G intestinalis* (sanitation facilities) and *E histolytica* or *E dispar* (sanitation facilities), did not state in which country the study was done and was therefore included in the overall meta-analysis, but not in the respective subgroup meta-analysis.

Table 2: **Results of the subgroup analyses examining the association of sanitation facilities or water treatment with intestinal protozoa infections**

meta-analyses on WASH and infections with soil-transmitted helminths and diarrhoea in general.[17,20,89–91] People having access to, or using sanitation facilities have significantly lower odds of being infected with *G intestinalis* (OR 0·64) and *E histolytica* or *E dispar* (0·56), compared with their counterparts who lack access to, or do not use sanitation facilities. However, for *B hominis* and *Cryptosporidium* spp no significant associations were found, with the outcome for *Cryptosporidium* spp possibly affected by the small number of included studies.

Regarding water treatment, all four meta-analyses indicated lower odds among people who treat water before consumption, with ORs ranging between 0·52 (*B hominis*) and 0·83 (*Cryptosporidium* spp). Two of the four meta-analyses (*E histolytica* or *E dispar*, and *Cryptosporidium* spp) showed only borderline significance.

Subgroup analyses comparing the availability versus the use of sanitation facilities revealed non-significant differences. The only significant difference in the subgroup analysis was found for *B hominis* infection in lower-middle-income countries versus high-income countries. However, only one study examined the effect of sanitation facilities on *B hominis* in high-income countries, hence no meta-analysis could be done and no firm conclusion should be drawn. Most studies were done in lower-income and upper-middle-income countries. Our analyses further showed that studies are geographically clustered (figure 2) with few studies in Europe, Africa, and parts of Asia.

A potential explanation for this pattern might be that virtually all people in high-income countries have access to, and use sanitation facilities and safe water. The opposite (having no access to sanitation facilities and safe water) might also explain the few studies in low-income countries. Subgroup analysis on specific water treatments (ie, boiling, filtering) did not detect any significantly different ORs compared with all combined water treatments. In general, all subgroup analyses on water treatment revealed no significant differences. However, it is worth mentioning that several of the subgroup analyses were based on only a few studies, have low heterogeneity, and might therefore be underpowered.

Our study has several limitations. First, though we identified a substantial body of potentially relevant literature through our systematic review, we were unable to access the underlying data. Hence, even though most of these studies might not contain relevant data, there is a certain risk that we missed some evidence.

Second, according to our scoring system, most studies were only of moderate or even relatively low quality. This finding is mainly due to the epidemiological design of the studies; most were cross-sectional in nature, applying questionnaires. However, questionnaires are prone to reporting bias, as people might be ashamed to answer truthfully to questions such as "Where do you defecate?".[92] This issue has been emphasised, for example, in a study done among food vendors by Licea and colleagues[58] who assumed that "some vendors answered untruthfully thinking that it was the right answer". Consequently, the protective effect of sanitation facilities would be underestimated because persons state they use sanitation facilities and water treatment, although, in reality, they do not. Cluster randomised controlled trials would provide stronger evidence,[18,20,93] but their implementation is more challenging. A laudable exception is the randomised controlled trial of Hellard and colleagues,[50] because study participants were masked with regard to their allocation to water treatment by using placebo devices. A way forward to generate high-quality data might be using a time-series approach as a study design (ie, assessing many communities repeatedly but introducing sanitations interventions only within one community at a time).[94]

Third, observational studies might be confounded, since people who own a toilet or treat their drinking water might differ (eg, wealthier, more health conscious, and higher educational attainment) from their counterparts. Because only very few data on adjusted ORs were available (four studies), we did not include adjusted ORs in our calculations to compare like with like and be fully transparent and consistent. Furthermore, we did not have enough data on adjusted ORs to do a subgroup meta-analysis including three or more studies. However, two recent meta-analyses on the effect of sanitation facilities on soil-transmitted helminth infection revealed similar ORs using either unadjusted[17] or adjusted ORs.[20]

Fourth, the presence and sporadic use of sanitation facilities does not necessarily exclude open defecation (eg, no sanitation facility available during work). Likewise, routinely treating drinking water at home does not necessarily prevent drinking of untreated water, for example during a visit at a neighbour's house. However, this kind of study group contamination is difficult to avoid, even in randomised controlled trials.[95]

Fifth, all data were collected at individual level and from settings in which people might only partly use preventive interventions such as sanitation facilities or water treatment. However, not using sanitation facilities and water treatment by one individual might increase the general environmental contamination and thereby increases the risk of infection for all other persons living in the same setting. Such spillover effects of an individual's behaviour for the community as well as transmission via animal faeces (eg, *Cryptosporidium* spp),[96] could not be considered in our meta-analyses, and hence, could result in substantial underestimation of the positive effects of sanitation facilities and water treatment.

Finally, most studies did not explicitly state the kind of treatment applied or toilet type used, or else, merged results from different practices. As previously stated,[17] a wide range of terms was used for sanitation (eg, pit latrine, open latrine, toilet, and flush toilet). This suggests that there is a need for a unified classification of latrine types such as the sanitation ladder proposed by the Joint Monitoring Programme of the WHO and the United

Nations Children's Fund for water supply and sanitation.[97] Additionally, insufficient data about further relevant factors on sanitation facilities (eg, maintenance, condition, number of users per toilet) were available for subanalysis. Furthermore, regarding water treatment it was not always clear whether water had been previously treated by others (eg, water providers such as municipal or private water companies) and masked the beneficial effects of water treatment directly at the place of consumption. Therefore, we cannot draw any conclusion about the most effective water treatment and toilet type to prevent intestinal protozoa infections. Most of the studies examined only children or whole communities (including children and adults), whereas three out of five publications on adults only focused on individuals infected with HIV. Hence, no subgroup analysis stratified by age could be done.

In conclusion, our study shows that sanitation facilities and water treatment are associated with lower odds of infection with intestinal protozoa. Sanitation facilities and safe water can also prevent diarrhoeal diseases in general, intestinal nematode infections, lymphatic filariasis, trachoma, schistosomiasis, and malnutrition.[17,21,47,98] However, even though improved sanitation and safe water might be effective against a series of infectious diseases and cost effective in the long-run, short-term budget constraints and organisational hurdles might hamper their promotion and implementation.[18] Furthermore, changing human behaviour is a difficult long-term process and needs strong community involvement and education.[78,99] Consequently, the effect of improved sanitation facilities and safe water in combating a large number of infectious diseases cannot be emphasised enough, and should assist policy makers and practitioners to develop and implement strategies for access and use of improved sanitation facilities and water treatment.

Contributors

BS, TF, JU, and JK conceived and designed the study. BS and DC conducted the study. BS, DC, TF, JU, and JK analysed the data. BS wrote the first draft of the paper. DC, TF, JU, and JK contributed to the writing of the paper.

Declaration of interests

We declare no competing interests.

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Chapter 7

General Discussion and Conclusion

Novel tools for the control of soil-transmitted helminthiasis: Drug combinations, diagnostics, and meta-analyses on the effect of sanitation facilities

General Discussion

Rationales, objectives and aim of the present thesis

Approximately 1.5 billion people are infected with soil-transmitted helminths, causing substantial morbidity [1]. Since decades, the control strategy has been mass drug administration of albendazole and mebendazole without prior diagnosis (termed "preventive chemotherapy) to people at risk, mostly school-aged children [2,3]. For example in 2012 a total of 212 million anthelmintic tablets were donated to school-age children [4]. One of the shortcomings of the current preventive chemotherapy strategy is the low efficacy of the standard drugs against *T. trichiura* reported in several studies [5–8].

The chief objectives of this PhD thesis focused on finding a better treatment against *T. trichiura* and concomitant soil-transmitted helminths. Therefore, in a first step, the two promising compounds, nitazoxanide and oxantel pamoate, were tested within two separate clinical trials (chapter 2 and chapter 3). Both drugs were given alone as well as in combination with albendazole to broaden their spectrum of efficacy against concomitant soil-transmitted helminths (i.e. hookworm and *A. lumbricoides*). In a second step, a follow-up trial was conducted in order to compare the efficacy of the combination that achieved good results (i.e. albendazole plus oxantel pamoate; chapter 4) to two other promising albendazole-based drug combinations which had previously shown high efficacy against *T. trichiura,* in clinical trials (i.e. albendazole plus ivermectin and albendazole plus mebendazole) [6,9,10]. The aim of this trial, was to specify which drug combinations were worth taking one step further for additional studies. As the planning and implementation of a clinical trial uses a large amount of resources, we followed the

strategy of adding additional smaller and related research questions. An additional research question was regarding protozoa; explicitly, to assess the prevalence of intestinal protozoa on Pemba Island and to evaluate the efficacy of nitazoxanide and albendazole (chapter 2b). Another objective focused on the diagnosis of soil-transmitted helminths. As the diagnostic technique is important for monitoring prevalence but also for determining drug efficacies, we compared single and multiple Kato-Katz thick smears to the ether-concentration method (chapter 5). Further, various studies on novel diagnostic techniques were added to our clinical trials.

Albendazole and mebendazole are the currently most widely used drugs within preventive chemotherapy programmes. Both are highly active against *A. lumbricoides* with cure rates and egg reduction rates close to 100% (chapter 2-4, [5]). Even though efficacy is high, prevalence usually returns relatively quickly to previous levels [11], indicating that interventions other than preventive chemotherapy are also necessary to sustainably control soil-transmitted helminths. We suspected that the lack of evidence on the effect of sanitation facilities might be a reason why water, sanitation and hygiene (WASH) are not implemented within soil-transmitted helminth control programs [12]. To overcome this knowledge gap, an objective of this PhD thesis was to quantify the protective effect of sanitation facilities against infections with soil-transmitted helminths. Therefore, a systematic review and meta-analysis was conducted (chapter 6a). As intestinal protozoa were included as a side objective within the first clinical trial (chapter 2b), and because sanitation facilities and water treatment are also potential interventions against intestinal protozoa, we conducted a second systematic review and meta-analysis on the protective effect of sanitation facilities and water treatment against infections with intestinal protozoa (chapter 6b). Quantifying the effect of WASH on

single diseases is precious as it might trigger cost-effectiveness studies on WASH, which could result into promotion of an implementation of WASH into control programmes.

The results of the individual studies have already been discussed within the separate manuscripts. In this part of the thesis, I would like to discuss specific issues and to put them into wider context. Based on my gained experience during my PhD thesis and the ongoing questions within the soil-transmitted helminth community, I have chosen the following topics for an in-depth discussion

1) Efficacy of the current standard drugs albendazole and mebendazole, and the challenges in analysing cure rates and egg reduction rates
2) Efficacies of the tested drug combinations
3) Future steps for an albendazole-oxantel pamoate combination
4) Diagnostics: novel approaches
5) Generating evidence on the protective effect of sanitation facilities and water treatment.

1 Efficacy of the current standard drugs albendazole and mebendazole, and the challenges in analysing cure rates and egg reduction rates

The World Health Organization (WHO) states that the current common drugs are effective in controlling soil-transmitted helminth infections [13]. A meta-analysis conducted by Keiser and Utzinger in 2008 revealed that cure rates against *T. trichiura* are low, regardless of whether albendazole or mebendazole is administered [5]. Instead of recognising this lack of efficacy and implementing strategies to overcome this inefficacious treatment against *T. trichiura*, it was argued that cure rates are not a valid indicator for assessing drug efficacy [14]. It is true that it is difficult to compare cure rates between different studies and settings as cure rates are influenced by infection intensities at baseline and by the sensitivity of the diagnostic approach (chapter 5, [14–16]). However, cure rates of different treatments can be compared within a clinical trial without the above-stated bias. Even though the main goal of the WHO is to reduce morbidity due to soil-transmitted helminths by eliminating moderate and heavy infection intensities [3], the cure of individuals should not be neglected. The U.S. Food and Drug Administration also gave a clear sign that cure rates should be prioritised by insisting that cure rates shall be the primary outcome in a clinical trial that is currently being implemented by Jansen Research & Development on a chewable mebendazole formulation. In general, only few randomised controlled trials on soil-transmitted hemlminths are conducted. Therefore, our three clinical trials provide not only important data on new promising compounds, but also on the current anthelminthic treatments. The efficacy of albendazole and mebendazole was tested in two out of the three randomised controlled trials (i.e. albendazole in 2011 and 2012, mebendazole in 2012 and 2013). Against *T. trichiura,* both drugs revealed substantially lower cure rates

than described within the before- mentioned meta-analysis (Table 1, [5]). Our trial in 2012, where both drugs were used simultaneously, confirmed that mebendazole has significantly higher efficacy than albendazole in the treatment of *T. trichiura.* Further, egg reduction rates of both drugs were only low to moderate against *T. trichiura*. Especially worth mentioning is our analysis from the clinical trial in 2012. As the stated goal of the WHO is to "reduce soil-transmitted helminth infections of moderate and high intensity among school-age children to <1%" [3] we calculated how many of the moderate or heavy infected children had no or only light infections after treatment. The analysis showed that with albendazole or mebendazole, moderate or heavy infections could only be reduced by approximately 50%. In comparison, oxantel pamoate and the albendazole-oxantel pamoate combination reduced moderate or heavy infections by 78% and 85%, respectively (chapter 3). Certainly, one could argue that preventive chemotherapy is applied regularly and hence the reduction of prevalence and infection intensities is achieved after a number of treatments [14]. This hypothesis is confirmed by a study conducted on hookworm by Casey and colleagues which is mentioned as an example in the literature [14,17]. As a counter argument, one also has to keep the following two points in mind: (i) participants in our clinical trial were school-aged children who had profited already from one or several rounds of preventive chemotherapy. Nevertheless prevalence and infection intensities were still high in the setting we worked, indicating that the goal of eliminating heavy and moderate infection intensities cannot be achieved with this regimen. (ii) Re-infection after treatment happens rather quickly, especially for *A. lumbricoides* and *T. trichiura* [11]. Therefore, many of the children who did not suffer any more from moderate or heavy infections after treatment might acquire heavy or moderate infections within a few months. Given the fact that re-infection with hookworms happens slower [11], it is not surprising that

the example for a successful decrease in prevalence, in the frame of ongoing preventive chemotherapy, was given for hookworm [14,17]. Our three clinical trials further confirmed the high efficacy of albendazole against hookworm infections while mebendazole revealed insufficient cure rates and egg reduction rates against hookworm (Table 1). Additionally, our studies underlined the high efficacy of the current drugs against *A. lumbricoides* with cure rates above 90% and egg reduction rates close to 100% (Table 1). In the context of these results, the high prevalence found at two schools (chapter 3 and chapter 4) might indicate intensive transmission of soil-transmitted helminths. This finding indicates once more that even when an effective drug is available, other interventions such as the implementation of sanitation facilities, safe drinking water, and health education are needed for sustainable control of soil-transmitted helminth infections [12,18].

Table 1: Efficacies of novel drug regimens against soil-transmitted helminth infections. Cure rates and egg reduction rates based on geometric means (GM) and arithmetic means (AM) were assessed in three randomised controlled trials conducted in the frame of this PhD thesis from 2011-2013, on Pemba Island, Tanzania.

Year of study	Efficacy	Albendazole + Nitazoxanide	Nitazoxanide	Albendazole + Oxantel pamoate	Oxantel pamoate	Albendazole + Ivermectin	Albendazole + Mebendazole	Albendazole	Mebendazole	Placebo
	Trichuris trichiura									
2011	Cure rate %	16.0	6.6					14.5		8.9
(Chapter 2a)	Egg reduction rate (GM) %	54.9	13.4					45.6		17.6
2012	Cure rate %			31.2	26.3			2.6	11.8	
(Chapter 3)	Egg reduction rate (GM) %			96.0	93.2			45.0	75.0	
	Egg reduction rate (AM) %			68.1	66.2			31.9	41.2	
2013	Cure rate %			68.5		27.5	8.4		8.4	
(Chapter 4)	Egg reduction rate (GM) %			99.2		94.5	51.6		58.5	
	Egg reduction rate (AM) %			72.5		85.6	31.4		14.0	
	Hookworm									
2011	Cure rate %	85.7	66.7					81.8		55.6
(Chapter 2a)										
2012	Cure rate %			51.4	10.6			59.8	17.4	
(Chapter 3)	Egg reduction rate (GM) %			95.6	38.6			96.3	58.7	
	Egg reduction rate (AM) %			87.1	14.7			75.8	44.7	
2013	Cure rate %			45.5		50.0	47.8		24.4	
(Chapter 4)	Egg reduction rate (GM) %			90.9		95.4	92.7		59.5	
	Egg reduction rate (AM) %			70.3		89.6	72.7		11.6	
	Ascaris lumbricoides									
2011	Cure rate %	100.0	62.5					100.0		0
(Chapter 2a)										
2012	Cure rate %			94.4	10.1			92.0	91.2	
(Chapter 3)	Egg reduction rate (GM) %			99.98	28.4			99.97	99.94	
	Egg reduction rate (AM) %			97.8	-18.5			94.9	88.1	
2013	Cure rate %			97.9		98.0	97.5		95.5	
(Chapter 4)	Egg reduction rate (GM) %			99.98		100.00	99.97		99.97	
	Egg reduction rate (AM) %			97.0		100.0	94.3		98.8	

In general we acknowledge the importance of egg reduction rates as they are a direct indicator for reduction of morbidity and because they can be easier compared between different studies than cure rates. Nevertheless, in analysing the results from the clinical trials, we realised that egg reduction rates should also be interpreted with caution. The WHO working group on soil-transmitted helminths stated in 2008 that "the arithmetic mean does not assure homogeneity of the variance between groups compared; therefore parametric tests cannot be used for the statistical interpretation of the results" [19]. Thus, most of the clinical trials on soil-transmitted helminths used geometric means. It was shown that the geometric mean is also not ideal for calculating egg reduction rates because it overestimates egg reduction rates in relatively homogenous populations [20], as is, for example, the case in clinical trials where only positive individuals are studied. Recently it was then argued that preferably arithmetic means should be used instead of geometric [21]. As already mentioned above, working with arithmetic means has the disadvantage that strong outliers can greatly influence the overall analysis. Hence, both analyses are imperfect with certain limitations [19,22,23]. Regarding our clinical trials, we presented geometric means within the publications in order to compare our results with older studies that have used the same drug regimens. However, we would suggest to present always both (geometric and arithmetic means) as we did it for the clinical trials conducted in 2012 and 2013 (chapter 3 and chapter 4; supplementary appendix, available online at the journals website where the corresponding chapter is/will be published). Especially for the randomised controlled trial conducted in 2013 (chapter 4), we had a closer look at the analysis of egg reduction rates because results based on geometric and arithmetic means were quite different. In detail, based on geometric means, the albendazole-oxantel pamoate combination revealed higher egg reduction rates against *T. trichiura* (ERR, 99.2%) than the albendazole-ivermectin combination

(ERR, 94.5%). Based on arithmetic means, the albendazole-ivermectin had higher egg reduction rates (ERR, 85.6%) than the albendazole-oxantel pamoate combination (ERR, 72.5%). After analysing egg counts of single individuals, we realised that the relatively low egg reduction rates based on arithmetic means for the albendazole-oxantel pamoate combination were mainly caused by an extreme value of one patient who had an egg count after treatment of 20,766 eggs per gram of stool. This was by far the highest egg count within this study, even compared to baseline data. When using 10% trimmed arithmetic means which reduces the impact of strong outliners, the egg reduction rates of the albendazole-oxantel pamoate combination (ERR, 93.2%) were higher than for the albendazole-ivermectin combination (ERR, 87.1%). Based on this findings and also on the examination of the general distribution of *Trichuris trichiura* egg counts at baseline and at follow-up, we concluded that in this specific case geometric means represent the "real" situation better than arithmetic means. In addition, one should keep in mind that it is difficult to control the origin of the collected stool samples; stool samples brought by an individual might possibly originate from a different person. Similarly, the possibility that stool samples are mixed up by accident should be considered. Consequently, one should be careful when analysing egg reduction rates based on arithmetic means, especially if strong outliners are present. For better understanding the dynamics of egg reduction rates based on arithmetic and geometric means, we provided our data sets, including egg counts before and after treatment, to collaborators who requested those data. For example, Diawara and colleagues concluded after analysing multiple data sets that neither arithmetic nor geometric means give satisfactory egg reduction rates and that alternative approaches are required (Diawara et al., manuscript in preparation). The fact that geometric means can overestimate the egg reduction rates is also important in context of the efficacy of the current standard

drugs albendazole and mebendazole. In our study conducted in 2012 (chapter 3) the egg reduction rates for albendazole and mebendazole against *T. trichiura* based on arithmetic means were 31.9% and 41.2%, respectively, compared to egg reduction rates based on geometric means of 45.0% and 75.0%, respectively. The differences in egg reduction rates based on arithmetic mean versus geometric mean was even larger in the clinical trial conducted in 2013 (chapter 4). The treatment regime using mebendazole had an egg reduction rate for *T. trichiura* of only 14.0% based on arithmetic mean, while egg reduction rates based on geometric mean reached 58.5%. These data and the high prevalence observed on Pemba Island are worrisome and underlines once again that the current drugs lack efficacy in this setting. Based on arithmetic means, we also observed hookworm egg reduction rates below 90% and *A. lumbridocides* egg reduction rates below 95%, when treated with albendazole. Apparently, these values should be already viewed with concern regarding potential drug resistance [24]. Hence, alternative treatments, mostly (but not only), against *T. trichiura* are urgently needed.

2 Efficacies of the tested drug

In the frame of this PhD thesis, four promising albendazole-based drug combinations were tested in randomised controlled trials against *T. trichiura* and concomitant soil-transmitted helminths. The results shall briefly be summed up and discussed here. A number of clinical trials reported that the antiprotozoal drug nitazoxanide has high efficacy against *T. trichiura* when given in multiple doses (200 mg for children aged below 12 years and 500 mg for patients aged 12 years and above, twice daily for 3 days) [25–27]. In a small-scale study, it was also shown that a single dose was effective against *T. trichiura*, concomitant soil-transmitted helminths and intestinal protozoa infections

[28]. In our clinical trial conducted in 2011, nitazoxanide (1,000 mg) alone but also in combination with albendazole (administered on two consecutive days) was given to *T. trichiura* infected children. Nitazoxanide revealed lower cure rates and egg reduction rates than placebo, hence the potential trichuricidal properties for nitazoxanide could not be confirmed (Table 1). Of note, the efficacy of nitazoxanide against intestinal protozoa was also only moderate and comparable to single albendazole (chapter 2b). The diagnostic technique used to detect intestinal protozoa was relatively weak (one ether-concentration before and after treatment), thus cure rates for intestinal protozoa are most likely still overestimated. It is considerable that using multiple doses of nitazoxanide might have led to a longer half-life, which could eventually explain the high efficacy against *T. trichiura* observed in other studies. On the other hand, it is surprising that we found no effect at all against *T. trichiura* even though we used a relatively high dose of nitazoxanide (1,000 mg). Of note, the nitazoxanide used in our trial was sourced from Romark (Alinia®), the same product used in the two previous clinical studies, resulting in very high cure rates against *T. trichiura* [26,27]. The only study which assessed efficacy of single treatments unfortunately did not report the dosage used [28]. It is necessary to mention that three out of four studies that assessed the efficacy of nitazoxanide were financed by the manufacturer of the tablets [25–27] while the fourth study had a sample size of only 16 *T. trichiura* infected children [28]. Hence, our randomised controlled trial was probably the first independent conducted trial with a sufficient sample size for assessing the efficacy of nitazoxanide against *T. trichiura*. An *in vivo* study using nitazoxanide against *T. muris* found no effect as well [29] which is congruent with the results from our clinical trial. Hereafter, we concluded that nitazoxanide and an albendazole-nitazoxanide combination should not be considered as

an alternative treatment against *T. trichiura* and concomitant soil-transmitted helminths.

Oxantel pamoate is a veterinary drug with trichuricidal activity that showed high efficacy against *T. trichiura* in a number of exploratory trials conducted in the 1970's [30–34]. *In vivo* results in our laboratory confirmed the trichuricidal activity of oxantel pamoate [35]. As oxantel pamoate was soon combined with pyrantel pamoate [36–40], we could not find a manufacturer that could provide us with tablets that contained only oxantel pamoate. Henceforth, colleagues from the Department of Pharmaceutical Sciences at the University of Basel developed a formulation for the oxantel pamoate tablets for paediatric clinical studies [41]. Using these tablets, a randomised controlled trial evaluating the efficacy of single oxantel pamoate (20 mg/kg) and an albendazole-oxantel pamoate combination compared to the standard drugs albendazole and mebendazole was conducted (chapter 3). As the possibility of interactions between albendazole and oxantel pamoate was unknown, the drug combination was given over two consecutive days. Analysing the different treatment regimens, the treatment arms containing oxantel pamoate had significantly higher efficacies against *T. trichiura* compared to albendazole and mebendazole (Table 1). As oxantel pamoate had no or only very low efficacy against hookworm and *A. lumbricoides*, it is necessary to combine it with a drug that is effective against these soil-transmitted helminths. Our trial confirmed our choice of albendazole which revealed high efficacy against *A. lumbricoides* and hookworm, while mebendazole showed only high efficacy against *A. lumbricoides*. We concluded that the albendazole-oxantel pamoate is a promising drug combination against *T. trichiura* and concomitant soil-transmitted helminths and decided to further evaluate this combination.

Within a third randomised controlled trial we compared the efficacy of albendazole-oxantel pamoate against an albendazole-ivermectin and an albendazole-mebendazole combination. The latter two drug combinations have also proven to be highly effective against *T. trichiura* within previous clinical trials [6,7,9,10]. As it is difficult to compare efficacies of drugs among studies [16,42], it was important to compare these promising compounds in a single randomised controlled trial. In the meantime, between the second and third clinical trial, it was shown that albendazole and oxantel pamoate can be safely administered together (N. Cowan, unpublished data). Therefore, drug combinations were given together at a single day of treatment. Considering the results from our clinical trial, we could not confirm the high efficacy of the albendazole-mebendazole combination against *T. trichiura* found by Namwanje and colleagues [7]. An explanation for this contradictory finding might be the different susceptibility of parasite strains present at the two study sites [43,44]. After years of treating people in Zanzibar with albendazole and mebendazole, *T. trichiura* might have developed a high tolerability for these drugs. As both drugs have the same mechanism of action [45], it might therefore not be surprising that this combination achieved low efficacy in our setting on Pemba Island. The other two tested drug combinations revealed both significantly higher efficacies against *T. trichiura* than mebendazole (Table 1). The albendazole-oxantel pamoate combinations achieved not only a very high egg reduction rate but also a high cure rate of 69%. This cure rate was surprisingly high when compared to the cure rate from our clinical trial conducted in 2012, and might again confirm that comparing cure rates between studies is difficult [16]. We have two possible explanations for this much higher cure rate: (i) Infection intensities in the clinical trial in 2012 were higher and therefore it was probably more difficult to achieve a cure. However, considering that the cure rate of single mebendazole did not increase in

the study with lower infection intensity in 2013, the second explanation could be more likely: (ii) the simultaneous administration of albendazole and oxantel pamoate might have led to synergistic effects against *T. trichiura*. This might have resulted in higher cure rates compared to the clinical trial in 2012 where the drug combination was spaced by one day. Previous laboratory examinations found a synergistic effect for albendazole-oxantel pamoate against *T. muris in vitro*, but an antagonistic effect *in vivo* [35]. In general, *in vivo* studies are more reliable than *in vitro* experiments. However, given the high cure rates for albendazole-oxantel pamoate found in our randomised controlled trial, antagonistic effects are rather unlikely. A clear conclusion cannot be drawn, as treatment arms including single albendazole and single oxantel pamoate were not included in the clinical trial conducted in 2013. To generate clear evidence of antagonistic or synergistic effects, a follow-up study, should be conducted including the single components (i.e. albendazole, oxantel pamoate), as well as the simultaneously administered drug combination. The albendazole-ivermectin combination which had significantly higher efficacy against *T. trichiura* than mebendazole was less efficacious than albendazole-oxantel pamoate (different interpretations of egg reduction rates are discussed above). Nevertheless, in settings where *T. trichiura* coexists with *S. stercoralis* or lymphatic filariasis, albendazole plus ivermectin might be preferably used, as ivermectin is the standard treatment against these parasites and this combination is already commonly used in preventive chemotherapy programmes [6,46–48]. Compared to the albendazole-ivermectin combination, there remain some uncertainties about the albendazole-oxantel pamoate combination which would need to be investigated in future studies. Even though oxantel pamoate is only partially absorbed, the pharmacokinetics of oxantel pamoate and the albendazole-oxantel pamoate combination should be studied [49]. Additionally, within our latest clinical trial we

detected a few moderate adverse events in the albendazole-oxantel pamoate treatment arm (chapter 4). Our sample size calculation was based on efficacies. Safety studies per se would require larger sample sizes [50]; therefore, the safety of the albendazole-oxantel pamoate combination needs to be closely monitored and evaluated in future studies. A tribendimidine-oxantel pamoate combination might also be a possible alternative drug combination against *T. trichiura* and other soil-transmitted helminth infections. Tribendimidine has shown to be highly effective against *A. lumbricoides* and hookworm in a number of studies. [51–55]. As the benzimidazoles are overused alternating between albendazole and tribendimidine might be a way to delay drug resistance against albendazole.

3 Future steps for an albendazole-oxantel pamoate combination

The albendazole-oxantel pamoate combination was carefully examined within two randomised controlled trials. The highly promising results call for further action to gain additional knowledge and to promote this combination. Therefore, I list concrete future studies which should be conducted on albendazole-oxantel pamoate so that this combination could ultimately become a real alternative for preventive chemotherapy programmes.

First, in the two previous studies, oxantel pamoate was dosed weight-dependently (20 mg/kg). Dose finding studies should be conducted with the ultimate goal to find a fixed dose independent from the patient's weight. Even though praziquantel (40 mg/kg) and ivermectin (200 µg/kg) are given according to the patient's weight, a fixed dose is preferred as it would reduce logistic challenges during mass drug administration [56,57]. In a first step, different weight-dependent dosages could be tested within a

larger study. If a large spectrum of dosages remains efficacious against *T. trichiura*, a fixed dose should be chosen that is still effective at lower doses in heavy children but would not exceed a certain dose which was assessed as safe (probably 20 mg/kg) for small and light children. This considered dosage should then additionally be tested using a smaller sample size.

Second, it is known that oxantel pamoate is only poorly absorbed [30,33], however the pharmacokinetics of single oxantel pamoate and the albendazole-oxantel pamoate combination should be assessed. Preferably dry blood spots for blood sampling, transportation of blood samples and analysing pharmacokinetics could be used [58]. Dry blood spots would have the advantage that they can be easily stored and transported, and that they only require blood from a finger prick. Obtaining a finger prick blood sample can be easily and safely done within a school setting. A challenge of this technique would be that the analytical method for oxantel-pamoate and albendazole would first need to be developed within the Helminth Drug Development Group – as it has already been done for other compounds in previous studies. Such a study on the pharmacokinetics could also be attached to the second phase of a dose finding study. Further, food uptake can strongly influence pharmacokinetics [59,60]; therefore, it would be interesting to divide the participants in a group which receives a meal before treatment and a group which did not eat before treatment.

Third, further safety data on albendazole-oxantel pamoate is required and; fourth, additional data about the potential antagonistic or synergistic properties of this combination is needed. Again, data on both these questions could be collected during dose finding studies. To assess the safety, results from all studies could be merged to evaluate if oxantel pamoate and the albendazole-oxantel pamoate combination cause more adverse events than the standard treatments, albendazole and mebendazole. Fifth,

it would be important to confirm the high efficacy of the albendazole-oxantel pamoate combination in a different setting than the Zanzibar archipelago. This however, could be easily done in a last small scale study and of course only if all the previous studies confirmed the positive properties of the drug combination. Wendelin Moser, a new PhD-student in the Helminth Drug Development Group from Jennifer Keiser, will tackle these research needs within the next years of his PhD. For ultimately including oxantel pamoate within preventive chemotherapy programmes, a manufacturer needs to be found, which would produce oxantel-pamoate and donate or sell it at low costs.

4 Diagnostics: Novel approaches

The ether concentration technique for the diagnosis of soil-transmitted helminths was tested and compared to the Kato-Katz method. Further a number of novel techniques were tested in the frame of our clinical trials, where I could assist to some extent and was associated as a co-author [61,62] (Bogoch et al., accepted for publication, *Am J Trop Med Hyg*.; Barda et al., submitted to *PLoS Negl Trop Dis.*). Here, advantages and limitations of these diagnostic techniques are discussed (Figure 1). Further, I elaborate on how the diagnostic techniques can influence results from epidemiological studies and which diagnostic techniques are needed and shall be used. Currently the Kato-Katz method, which examines a relatively small amount of stool (41.7 mg) and which is also recommended by the WHO, is the most commonly used diagnosis for soil-transmitted helminth infections [13,63]. The Kato-Katz technique is rather simple, requires little equipment and allows for the quantification of eggs [3,64]. Egg counts from the Kato-Katz technique are important as the WHO classifications of infection intensities (i.e. light, moderate, and heavy) is based on Kato-Katz egg counts [3,13]. A limitation of the

Kato-Katz technique is the low sensitivity in settings characterised by light infection intensities [42,65]. Sensitivity can be increased by examining numerous Kato-Katz thick smears which are ideally produced from multiple stool samples [42,65–68].

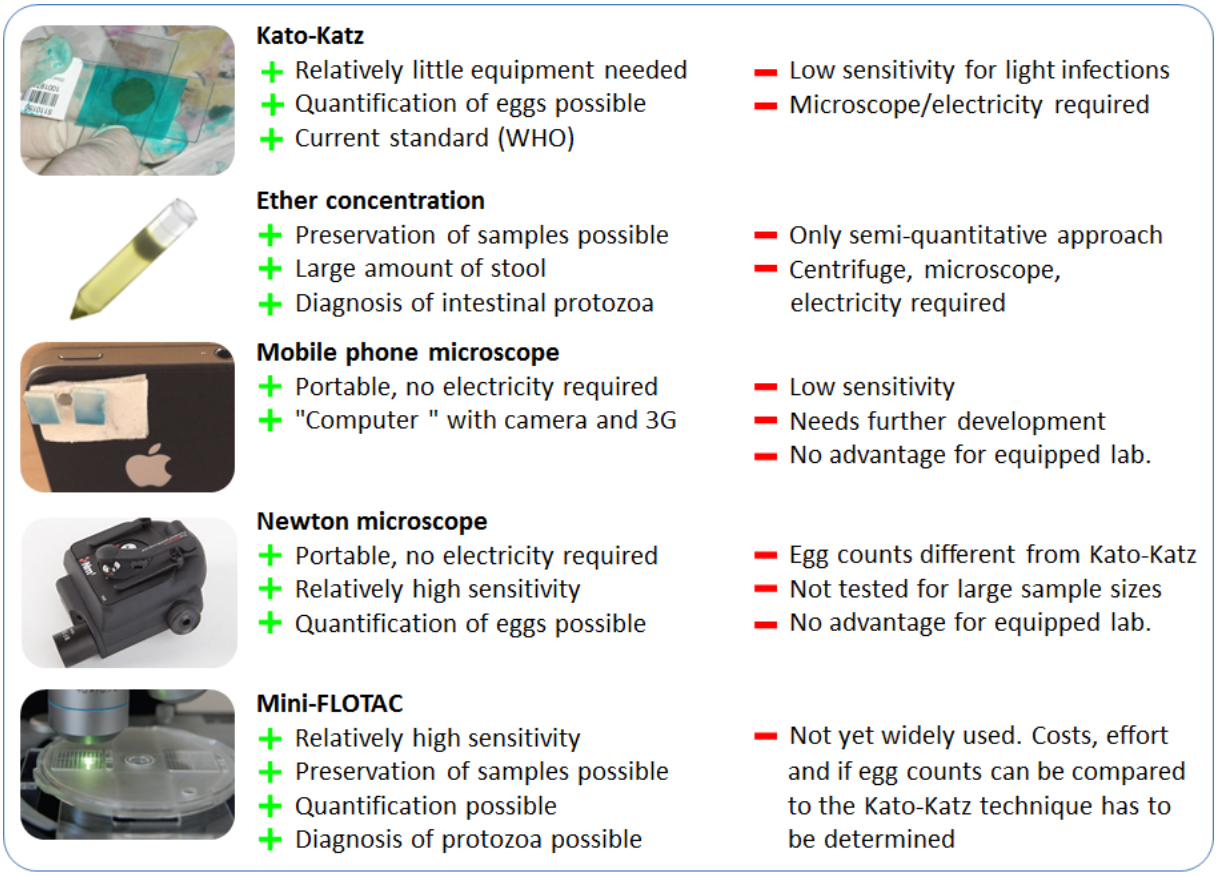

Figure 1: Advantages and disadvantages of diagnostic techniques for detecting soil-transmitted helminth infections. These five techniques were tested in the frame of the three clinical trials that were conducted for this PhD thesis (chapter 5, [61,62], Bogoch et al., accepted for publication, *Am J Trop Med Hyg*., Barda et al., submitted to *PLoS Negl Trop Dis.*).

The ether-concentration technique was primarily used within this PhD thesis for the diagnosis of intestinal protozoa (chapter 2b). However, the ether-concentration method revealed promising results for the diagnosis of soil-transmitted helminths as well. In detail, analysing formalin fixed stool samples after several months using the ether-concentration resulted in comparable sensitivity as duplicate, and in some cases even to quadruplicate, Kato-Katz thick smears performed on fresh stool samples (chapter 5). This would make the ether-concentration technique an interesting diagnostic

alternative especially in settings where samples cannot be analysed on spot. Nevertheless, the biggest limitation of this technique is that it is only-semi quantitative [69,70]. Therefore, it is not possible to assess egg counts, nor egg reduction rates. Hence, the ether-concentration technique is not recommended for most epidemiological studies, as egg counts are an important indicator. However, in studies focusing on intestinal protozoa, prevalence of soil-transmitted helminths can be an important and reliable (due to the relatively high sensitivity) secondary outcome. Another diagnostic approach which can process fixed stool samples is the FLOTAC technique. This technique has a high sensitivity, but required several working steps and was therefore quite time consuming [64,65,67]. Further, egg counts for the FLOTAC method were significantly lower compared to the Kato-Katz technique [15]. A novel approach which was tested by Barda and colleagues within the frame of our clinical trial in 2013, was the so called mini-FLOTAC (submitted to: *PLoS Negl Trop Dis.*). The mini-FLOTAC is a simplified version of the original FLOTAC technique, requiring less working steps [71–73]. In the study conducted on Pemba Island in 2013, Barda and colleagues could show that the mini-FLOTAC can analyse formalin-preserved stool samples for at least 30 days after preservation. Sensitivity as well as egg counts were similar when comparing the mini-FLOTAC to the Kato-Katz technique. Previous studies, on the other hand, reported higher sensitivity for the mini-FLOTAC compared to the Kato-Katz technique [73]. If a single mini-FLOTAC has higher or at least similar sensitivity as multiple Kato-Katz thick smears should be examined in future studies. The mini-FLOTAC could become a good diagnostic alternative if; (i) it has similarly high sensitivity as the original FLOTAC, and; (ii) if it could be confirmed that egg counts are comparable to the Kato-Katz method.

Two mobile devices for the diagnosis of soil-transmitted helminths were further tested within the frame of our clinical trials. The first study conducted by Bogoch and

colleagues was a proof of concept study that a mobile phone can be transformed into a microscope for examining Kato-Katz thick smears [61]. It could be shown that the mobile phone microscope could detect soil-transmitted helminths, but the sensitivity was still relatively low [61,62], meaning that the microscope adapter should be further improved for mobile phones. In case this technique can be improved, physicians could diagnose patients with their mobile phones for soil-transmitted helminths, which would be helpful in highly remote areas. Another mobile diagnostic device is the Newton microscope. This device showed high sensitivity for soil-transmitted helminths as well as for *Schistosoma haematobium* and *Schistosoma mansoni* [62]. In a further step, Bogoch and colleagues discovered in a study conducted on Pemba Island that the Newton microscope can also quantitatively count eggs (Bogoch et al., accepted for publication: *Am J Trop Med Hyg.*). Egg counts were significantly different compared to the Kato-Katz method, and could thus only be compared after using a correction factor. It remains to be discovered if this correction factor is stable so that it could be used for assessing infection intensities according to WHO guidelines [13]. Similar to the mobile phone microscope, the portable Newton microscope also has its strength in highly remote areas as it does not require a constant electricity source. If it could be confirmed that quantification of eggs can be performed reliably, the Newton microscope would also be an alternative for smaller epidemiological studies in highly remote areas. Nevertheless, if the infrastructure with conventional microscopes is available, the traditional Kato-Katz technique is preferred due to the simpler and more convenient handling.

The microscopic examination of Kato-Katz thick smears is rather time consuming especially in settings with heavy infection intensities. In other fields, software using algorithms were developed which can automatically detect parasites (e.g. malaria) [74,75]. Given that computers can already reliably differentiate between faces [76], it

should be possible to develop a system that differentiates and counts the different soil-transmitted helminth eggs. Such systems would simplify epidemiological studies and monitoring programmes for soil-transmitted helminths. However, in case such a system is implemented, there could also be the danger that the knowledge of the traditional diagnostic would get lost. Automatic detection systems could further be applied if mobile phone microscopes become more reliable. It is possible that an application on the mobile phone could then recognise the shape of different soil-transmitted helminth eggs. A further advantage of a mobile phone microscope is the ability to connect to the internet. Therefore, pictures of eggs could be easily transmitted and discussed with experts. Additionally, mobile phones have the advantage that they can transmit their location. Linking mobile phones with mapping software could become a powerful tool for monitoring programmes to create real time maps of the prevalence. However, as all the above mentioned diagnostic methods are not more sensitive than the traditional Kato-Katz thick smear (with the exception of the mini-FLOTAC), these methods might only become niche products for the diagnosis of soil-transmitted helminths. Even though people receive regular administration of preventive chemotherapy, the prevalence and infection intensities of soil-transmitted helminths remained high in several settings. Thus, it has to be assumed that the Kato-Katz method, using multiple thick smears, will be the diagnostic of choice in these settings within the near future as it is simple, well established and has adequate sensitivity as long as infection intensities are not very low. Multiple stool sampling often involves great efforts in logistics and can lead to high loss of follow-up rates. Consequently, having a tool that is as sensitive as multiple Kato-Katz thick smears with comparable egg counts would be a great advantage for epidemiological studies and monitoring programmes. It needs to be evaluated if the mini-FLOTAC could potentially fill this gap. As soon as the general

strategy would shift from morbidity control to transmission control, more sensitive tools (e.g. PCR) would be needed [77]. Hence, these highly sensitive tools should also be further developed and evaluated.

5 Sanitation, safe water and hygiene

Water, sanitation and hygiene (WASH) is a potential but poorly investigated tool for preventing soil-transmitted helminth infections [12]. In 2012 approximately 2.5 billion and 780 million people lacked access to adequate sanitation and drinking water, respectively [78]. Possible reasons for why WASH is neglected might be that several sectors are included in preventing diarrheal diseases, and therefore they are difficult to promote and organise [79]. Another reason is that evidence on the effectiveness and cost-effectiveness of preventive interventions such as sanitation facilities against soil-transmitted helminthiasis was missing [79,80]. We conducted a systematic review and meta-analysis to overcome this knowledge gap (chapter 6a). The first finding was that no randomised controlled trials were found which evaluated the impact of sanitation facilities on the prevalence of infections with soil-transmitted helminths. As randomised controlled trials provide the strongest evidence [81], well designed studies evaluating the impact of sanitation facilities on soil-transmitted helminth infections are warranted. For certain interventions, randomised controlled trials are difficult or impossible to conduct [82]; alternatively, studies could use an interrupted time series design [83]. This design would allow for the allocation of multiple communities to a certain intervention (e.g. implementation of sanitation facilities) at different time points. The impact of the intervention would then be assessed repeatedly, generating stronger evidence than cross-sectional or case control studies.

The first conducted meta-analysis revealed that individuals owning or using sanitation facilities have an approximate odds ratio of 0.5 of being infected with soil-transmitted helminths compared to people who do not own or use sanitation facilities (chapter 6a). Meta-analyses have certain limitations such as publication bias, or that results can be confounded when they are based on descriptive studies [84]. On the other hand, so called spill-over effects (i.e. that single individuals performing open defecation increase the risk of infection for all others) which were not considered could also result in a considerable underestimation of the positive effects of sanitation facilities. Therefore, the impact of different coverage levels has to be carefully evaluated.

Within a second systematic review and meta-analysis, we summarised the effect of sanitation facilities and water treatment on the prevalence of intestinal protozoa. For two out of three evaluated intestinal protozoa, the conducted meta-analysis revealed a significant protective effect of owning or using sanitation facilities. Water treatment before consumption was also associated with lower odds of being infected with intestinal protozoa, detecting odds ratios ranging from 0.51 (*Blastocystis hominis*) to 0.83 (*Cryptosporidium* spp.). For the literature search on intestinal protozoa, we excluded studies that only presented risk factors with significant results to avoid publication bias. Our results indicate that sanitation facilities can not only prevent soil-transmitted helminth infections but also other diarrheal diseases such as intestinal protozoa. This evidence should be generated for all diarrheal diseases so that cost-effectiveness studies based on hard evidence can be conducted. This could then again underline the importance and positive effects of sanitation facilities, and more essentially, motivate decision makers to implement and add programmes on WASH to their current control strategies. Furthermore, well designed intervention studies on WASH should be conducted, generating evidence on the effect of WASH, but also

assessing which coverage levels are needed to effectively reduce transmission of soil-transmitted helminthiasis and other diarrheal diseases.

Conclusion

In the frame of my PhD thesis, we conducted three randomised controlled trials on Pemba Island, Tanzania, assessing the efficacy of different treatment regimens against soil-transmitted helminth infections. In detail, we confirmed that the current standard treatments, albendazole and mebendazole, reveal worryingly low efficacy against *T. trichiura*. On the other hand, albendazole was highly active against *A. lumbricoides* and hookworm. The reported trichuricidal properties of nitazoxanide and an albendazole-mebendazole combination were not confirmed. Two other promising albendazole based drug combinations were identified (i.e. albendazole-oxante pamoate and albendazole-ivermectin) achieving significantly higher cure rates and egg reduction rates against *T. trichiura* compared to the standard treatments. The albendazole-oxantel pamoate combination achieved the highest efficacy against *T. trichiura* and concomitant soil-transmitted helminths. Therefore, this combination should be further evaluated (i.e. dose finding, safety, pharmacokinetics) so that it could become an alternative in settings having similar difficulties in treating *T. trichiura* as in the Zanzibar archipelago. In the meantime the albendazole-ivermectin combination could be already used, as this combination is well studied and already implemented in several preventive chemotherapy control programmes. Our diagnostic analysis confirmed that in general, a sensitive diagnostic approach should be used in clinical trials (multiple Kato-Katz thick smears from multiple stool samples) so that drug efficacy is not overestimated.

Conducting meta-analyses on the effect of sanitation, we could generate evidence that interventions other than preventive chemotherapy also have a significant effect on the prevalence of soil-transmitted helminths and further intestinal parasites. Consequently,

an ideal and sustainable control strategy for soil-transmitted helminths should include new and effective drugs together with the implementation of sanitation facilities.

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73. Barda BD, Rinaldi L, Ianniello D, Zepherine H, Salvo F, et al. (2013) Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. PLoS Negl Trop Dis 7: e2344.

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Curriculum Vitae

Benjamin Johannes Speich

Curriculum Vitae

Benjamin Johannes Speich

Epidemiologist

Name:	Benjamin Johannes Speich
Date of birth:	3rd September, 1985
Place of origin:	Münchenstein (BL)
Nationality:	Swiss

PhD Study

04/2011 – today	PhD in Epidemiology Swiss Tropical and Public Health Institute (University of Basel)
Thesis title	Novel tools for the control of soil-transmitted helminthiasis: Drug combinations, diagnostics, and meta-analyses on the effect of sanitation facilities
Supervision	Prof. Dr. Jennifer Keiserr
Collaborations	Public Health Laboratory - Ivo de Carneri (Pemba Island, Tanzania) Ivo de Carneri Foundation (Milan, Italy) Divisions of Internal Medicine and Infectious Diseases, Toronto General Hospital (Toronto, Canada)

Presentations/Meetings

April 2014	Joint EPH-MPI Student Seminar, Basel, Switzerland "Publishing from a students point of few" (oral presentation)
April 2014	British Society for Parasitology (BSP): 52nd Annual Spring meeting esearch Seminar, Cambridge, England (oral presentation)
March 2014	Research Seminar, Swiss Tropical and Public Health Institute, Basel, Switzerland: "Drug combinations against *Trichuris trichiura* and concomitant soil-transmitted helminths" (oral presentation)
March 2014	1st European Meeting for Young researchers on soil-transmitted Helminths, Vevey-Montreux, Switzerland (oral presentation)

March 2014	Deutschen Gesellschaft für Tropenmedizin und Internationale Gesundheit (DTG) Jahrestagung, Düsseldor, Germany (oral presentation)
December 2013	Mebendazole Investigator Meeting organised by Jansen Research & Development, London, England
December 2012	Swiss Society of Tropical Medicine and Parasitology (SSTMP) Student Meeting, Bern, Switzerland (oral presentation)
December 2011	Swiss Society of Tropical Medicine and Parasitology (SSTMP) Student Meeting, Basel, Switzerland (oral presentation)

Lectures

Autumn semester 2012	Master Program in Infection Biology and Epidemiology **Drug development** (1 lecture)
Autumn semester 2011	Master Program in Infection Biology and Epidemiology **Epidemiological methods** (1 lecture)

Education

09/2008 – 01/2010	Master of Science in Infection Biology and Epidemiology Swiss Tropical and Public Health Institute (Swiss TPH), University of Basel, Switzerland Major in Epidemiology Supervision: Prof. Dr. Jürg Utzinger Field stay: 3 months on Unguja Island, Tanzania
2005 – 2008	Bachelor of Science in Biology University of Basel, Switzerland Major in Organismic Biology
1999 – 2004	High school diploma (Matura) at Gymnasium Bäumlihof Basel-Stadt (specialisation in Biology and Chemistry, Thesis: "Quality of life in Kleinbasel").

Work Experience

09/2010 – 01/2011	Civilian service at the Swiss TPH Data management within the SAPALDIA (Swiss study on Air Pollution and Lung Disease in adults) study
03/2009 – 09/2009	Internship: Departement of Epidemiology and Public Health within the Ecosystem Health Sciences Unit at the Swiss TPH (supervision: Prof. Dr. Jürg Utzinger)

Additional Skills

Languages	German	(native speaker)
	English	(fluent)
	French	(basic)
Trainings	December 2009:	Good Clinical Practice (1 week)
	November 2011:	Laboratory animal course I (1 week)
	August 2013	Good Clinical Practice (1 day)

List of publications

1. **Speich B**, Croll D, Fürst T, Utzinger J, Keiser J.
Effect of sanitation and water treatment on intestinal protozoa infection: a systematic review and meta-analysis
Lancet Infect Dis. 2015 Sep 21. pii: S1473-3099(15)00349-7. doi: 10.1016/S1473-3099(15)00349-7. [Epub ahead of print]

2. Moser W, Ali SM, Ame SM, **Speich B**, Puchkov M, Huwyler J, Albonico M, Hattendorf J, Keiser J.
Efficacy and safety of oxantel pamoate in school-aged children infected with Trichuris trichiura on Pemba Island, Tanzania: a parallel, randomised, controlled, dose-ranging study.
Lancet Infect Dis. 2015 Sep 17. pii: S1473-3099(15)00271-6. doi: 10.1016/S1473-3099(15)00271-6. [Epub ahead of print]

3. Barda B, Albonico M, Ianniello D, Ame SM, Keiser J, **Speich B**, Rinaldi L, Cringoli G, Burioni R, Montresor A, Utzinger J.
How Long Can Stool Samples Be Fixed for an Accurate Diagnosis of Soil-Transmitted Helminth Infection Using Mini-FLOTAC?
PLoS Negl Trop Dis. 2015 Apr 7;9(4):e0003698.

4. **Speich B**, Ali SM, Ame SM, Albonico M, Utzinger J, Keiser J.
Quality control in the diagnosis of Trichuris trichiura and Ascaris lumbricoides using the Kato-Katz technique: experience from three randomised controlled trials
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5. **Speich B**, Ali SM, Ame SM, Bogoch II, Alles R, Huwyler J, Albonico M, Hattendorf J, Utzinger J, Keiser J.
Efficacy and safety of albendazole-ivermectin, albendazole-mebendazole, albendazole-oxantel pamoate, and mebendazole against Trichuris trichiura and concomitant soil-transmitted helminth infections: a randomised controlled trial.
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6. Bogoch II, Andrews JR, **Speich B**, Ame SM, Ali SM, Stothard JR, Utzinger J, Keiser J.
Quantitative Evaluation of a Handheld Light Microscope for Field Diagnosis of Soil-Transmitted Helminth Infection.
Am J Trop Med Hyg. 2014 Sep 22.

7. Panic G, Duthaler U, **Speich B**, and Keiser J.
Repurposing drugs for the treatment and control of helminth infections
Int J Parasitol Drugs Drug Resist 2014 Dec 4:185-200

8. Bogoch II, Coulibaly JT, Andrews JR, **Speich B**, Keiser J, Stothard JR, N'goran EK, and Utzinger J.
Evaluation of portable microscopic devices for the diagnosis of Schistosoma and soil-transmitted helminth infection.
Parasitology 2014 Apr 28:1-8

9. **Speich B**, Ame SM, Ali SM, Alles R, Huwyler J, Hattendorf J, Utzinger J, Albonico M, and Keiser J.
Oxantel pamoate-albendazole for Trichuris trichiura infection.
N. Engl. J. Med. 2014 370, 610–620
Correspondence: N Engl J Med. 2014 370: 1953–1954: Keiser J, **Speich B**, Utzinger J.

10. **Speich B**, Utzinger J, Marti H, Ame SM, Ali SM, Albonico M, and Keiser J.
Comparison of the Kato-Katz method and ether-concentration technique for the diagnosis of soil-transmitted helminth infections in the framework of a randomised controlled trial.
Eur J Clin Microbiol Infect Dis. 2014 33: 815–822.

11. Alles R, Puchkov M, Jablonski C, **Speich B**, Keiser J, Huwyler J.
Development of oxantel tablets for pediatric clinical studies: a technical note.
J Drug Deliv Sci Technol. 2013 23: 623–5.

12. Keiser J, Tritten L, Silbereisen A, **Speich B**, Adelfio R, and Vargas M.
Activity of oxantel pamoate monotherapy and combination chemotherapy against Trichuris muris and hookworms: revival of an old drug.
PLoS Negl Trop Dis. 2013 7(3): e2119.

13. Bogoch II, Andrews JR, **Speich B**, Utzinger J, Ame SM, Ali SM, and Keiser J.
Mobile phone microscopy for the diagnosis of soil-transmitted helminth infections: a proof-of-concept study.
Am J Trop Med Hyg. 2013 88: 626–629.

14. **Speich B**, Marti H, Ame SM, Ali SM, Bogoch II, Utzinger J, Albonico M, and Keiser J.
Prevalence of intestinal protozoa infection among school-aged children on Pemba Island, Tanzania, and effect of single-dose albendazole, nitazoxanide and albendazole-nitazoxanide.
Parasit Vectors. 2013 6: 3.

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Efficacy and safety of nitazoxanide, albendazole, and nitazoxanide-albendazole against Trichuris trichiura infection: a randomized controlled trial.
PLoS Negl Trop Dis. 2012 6(6): e1685.

16. Ziegelbauer K*, **Speich B***, Mäusezahl D, Bos R., Keiser J, and Utzinger J.
Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis.
PLoS Med. 2012 9(1): e1001162.
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17. Knopp S, **Speich B**, Hattendorf J, Rinaldi L, Mohammed KA, Khamis IS, Mohammed AS, Albonico M, Rollinson D, Marti H, Cringoli G, Utzinger J.
Diagnostic accuracy of Kato-Katz and FLOTAC for assessing anthelmintic drug efficacy.
PLoS Negl Trop Dis. 2011 5(4): e1036.

18. Becker SL, Lohourignon LK, **Speich B**, Rinaldi L, Knopp S, N'goran EK, Cringoli G, and Utzinger J.
Comparison of the Flotac-400 dual technique and the formalin-ether concentration technique for diagnosis of human intestinal protozoon infection.
J Clin Microbiol. 2011 49(6): 2183–2190.

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Albendazole and mebendazole administered alone or in combination with ivermectin against Trichuris trichiura: a randomized controlled trial.
Clin Infect Dis. 2010 51(12): 1420–1428.

20. **Speich B**, Knopp S, Mohammed KA, Khamis IS, Rinaldi L, Cringoli G, Rollinson D, and Utzinger J.
Comparative cost assessment of the Kato-Katz and FLOTAC techniques for soil-transmitted helminth diagnosis in epidemiological surveys.
Parasit Vectors. 2010 3: 71.