Soil-transmitted helminthiasis: the efficacy of recommended drugs, new drugs and combinations

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Table of Contents

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Summary

About 1.5 billion people are infected with at least one of the soil-transmitted helminths (STH); *Ascaris lumbricoides*, hookworm or *Tichuris trichiura.* One of the groups most affected by STH are school aged children, living under poor conditions in the least developed settings. Whereas light infections are typically asymptomatic, moderate and heavy infections with these parasites cause severe morbidity. In 2015, the estimated burden of STH infections was 3.4 million disability adjusted life years. Preventive chemotherapy (PC), the administration of anthelminthic drugs to at-risk populations, is the current strategy of the World Health Organization (WHO). Only in 2015, over 1 billion tablets of benzimidazoles (albendazole and mebendazole) were distributed around the world aiming to reduce the disease burden caused by moderate and heavy infections. The success of PC is largely threatened by low efficacy of the two benzimidazoles in particular against *T. trichiura* and by potential resistance development due to immense drug pressure. Hence, new drugs with high efficacy against *T. trichiura* and new drugs to replace albendazole and mebendazole in case of drug resistance are urgently needed.

The objectives of this PhD were closely related to PC. The **first objective** was to review and meta-analyse the efficacy of the current anthelminthic drugs and to provide the first-time summary estimates of egg reduction rates (ERR), the key measurement for anthelminthic drug efficacy. From the two benzimidazoles, only albendazole has a satisfactory efficacy against hookworm, whereas mebendazole is moderately effective against *T. trichiura*. Alarmingly, a significantly reduced efficacy of albendazole for treating hookworm infections and both against *T. trichiura* was demonstrated. Moreover, the estimated ERRs were doubtful or reduced based on the current WHO reference efficacies for monitoring drug resistance. According to WHO, the helminth drug efficacy should be calculated using the ERRs based on the arithmetic mean. However, the choice

of measurement of central tendency (i.e. arithmetic or geometric) is the subject of current discussion, which was extended in this thesis by consulting helminth experts with a questionnaire including different infection and treatment scenarios. Based on the expert's opinion, the arithmetic mean does not accurately reflect the egg burden and consequently, drug efficacy. Highest agreement with the experts' opinion was reached with the Hölder and geometric mean, however, final conclusions have not yet been drawn since the work is still in progress.

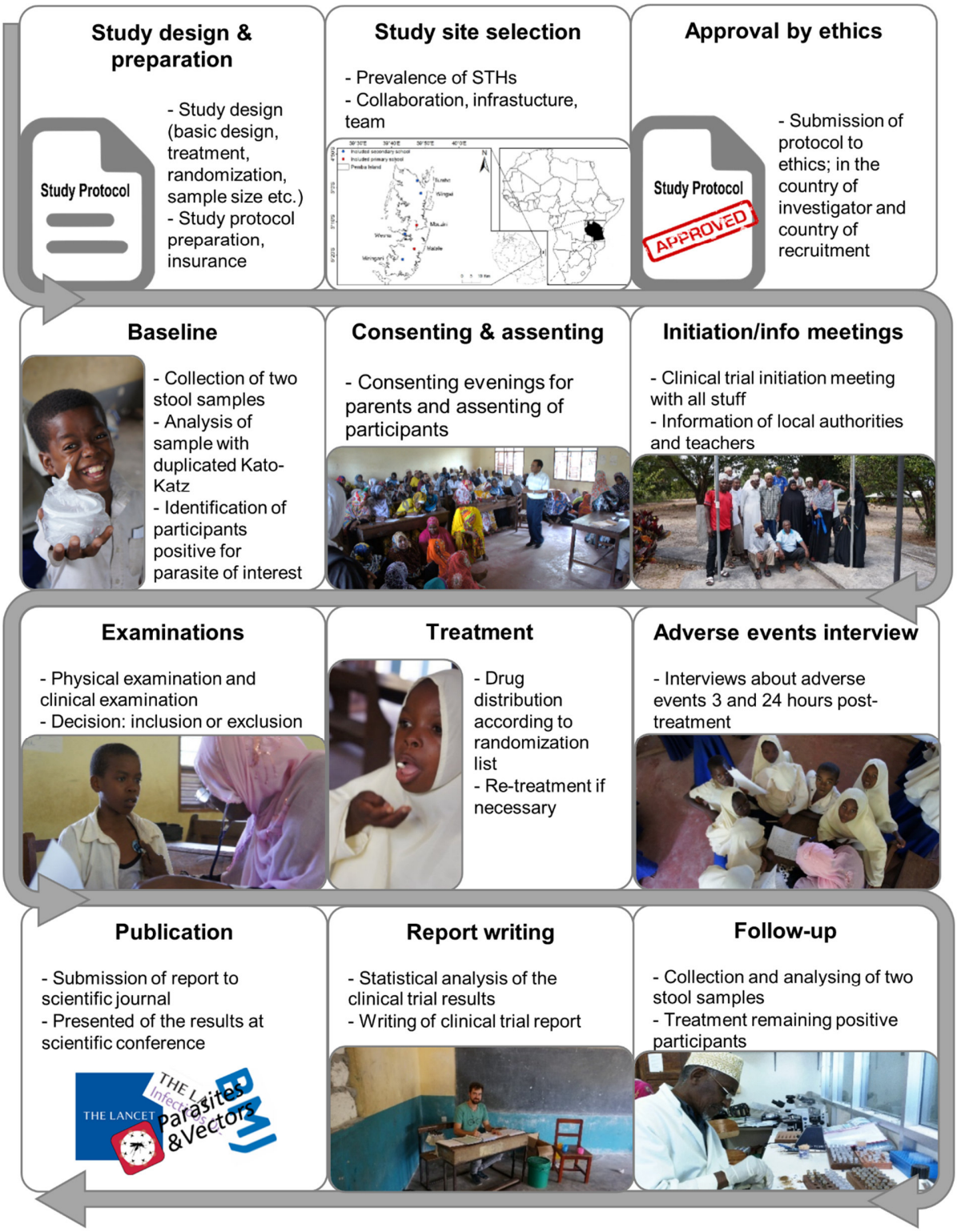
The review highlighted the compelling need of new drugs against *T. trichiura* and for the simultaneous treatment of all STH species, which was addressed with the next objectives. Oxantel pamoate has proven its high trichuricidal activity in two recent clinical trials. In order to include oxantel pamoate in large scale PC programs, a weight-independent dose is required. The **second objective** included a dose-finding trial to evaluate the optimal and a weight-independent dose (500 mg) for accelerating the delivery process once oxantel pamoate is used for PC. The **third objective** aimed to evaluate the new Chinese drug tribendimidine in different combinations and to investigate the improved efficacy of two triple drug therapies (TDT). In the second clinical trial of this PhD thesis, the co-administrations tribendimidine plus ivermectin or oxantel pamoate were assessed for non-inferiority to albendazole-oxantel pamoate against hookworm infections. Tribendimidine could complement or replace albendazole in case of drug resistance in PC programs, since it has a similar efficacy profile. In the third clinical trial, we investigated the improved efficacy of albendazole-oxantel pamoate combined with pyrantel pamoate against hookworm. A significantly increased efficacy was reached by the TDT, compared to albendazole-oxantel pamoate, which has currently highest efficacy against any STH species. On the downside, even after successful treatment, reinfection is relatively fast for *A. lumbricoides* and *T. trichiura* and somehow slower for hookworm, which was confirmed with an 18 weeks follow-up study.

Since particularly drug efficacy estimates rely on accurate diagnostic tools and the current method Kato-Katz is limited by low sensitivity for low infection intensity, new diagnostic tools are needed. Therefore, the **fourth objective** included the evaluation of FECPAK^{G2}, a new remote location online diagnostic tool, in comparison to Kato-Katz. Despite lower sensitivity and egg recovery rates, FECPAK^{G2} offers the advantage of capturing an image, which can be stored and uploaded

onto an internet cloud for later analysis of STH in human stool. An additional study under this objective was to explore the Butha-Buthe district in Lesotho as a potential new study site. A lower STH prevalence was found, compared to the national survey, indicating that PC is no longer required.

In conclusion, the review indicated the short-coming of the two widely used drugs albendazole and mebendazole particularly against *T. trichiura* and a decreased efficacy over time, resulting in doubtful or reduced efficacy, according to the current WHO references. The meta-analysed ERRs and the in-depth scrutinized measurement of central tendency call for an adaptation of the WHO guidelines, first, of the reference efficacies for monitoring anthelminthic drug resistance and second, for the assessment of anthelminthic drug efficacy in terms of ERRs. During this PhD thesis two novel drugs were evaluated, which demonstrated high efficacy against *T. trichiura* (oxantel pamoate), *A. lumbricoides* and hookworm (tribendimidine and pyrantel pamoate). We have shown an improved efficacy of different co-administration over single-drugs against any STH species. Furthermore, co-administrations could prevent anthelminthic drug resistance since the drugs act on different targets. Among the combinations tested in the scope of this PhD thesis, albendazole-oxantel pamoate proved the most outstanding efficacy, which was additionally increased by adding pyrantel pamoate. Our findings compel the evidence to support an adaption of the WHO guidelines towards the use of combination chemotherapy.

Clinical trial process flow



Abbreviation

STH	Soil-transmitted helminths
LF	Lymphatic filariasis
CR	Cure rate
ERR	Egg reduction rate
EPG	Egg per gram of stool
GM	Geometric mean
AM	Arithmetic mean
CI	Confidence interval
SD	Standard deviation
N (or n)	Sample size
OR	Odds ratio
TDT	Triple drug therapy
Lao PDR	Lao Peoples Democratic Republic
WHO	World Health Organization
PC	Preventive chemotherapy
ZAMREC	Zanzibar Medical Research and Ethics Committee, Tanzania
EKNZ	Ethical committee of Northern and Central Switzerland
CNER	Comité National d'Ethique et de la Recherche, Côte d'Ivoire
NECHR	National Ethics Committee for Health Research, Lao PDR
Swiss TPH	Swiss Tropical and Public Health Institute
PHL-IdC	Public Health Laboratory-Ivo de Carneri

Chapter 1- Introduction

1.1. Soil-transmitted helminths

The three major groups of parasitic worms (helminths) include nematodes (roundworms), trematodes (flukes) and cestodes (tapeworms). Soil-transmitted helminthiasis is caused by an infection with the nematodes *Ascaris lumbricoides,* hookworm (*Necator americanus* and *Ancyclostoma duodenale*) and *Trichuris trichiura*. The helminths are transmitted through feces-contaminated soil, hence the group's name: soil-transmitted helminths (STH). Infection with STH occurs by larval penetration of the skin (hookworm) or via oral uptake of contaminated soil in the case of *A. lumbricoides* and *T. trichiura*. STH infections are a major public health concern in the poor and most vulnerable populations of Asia, Sub-Saharan Africa and the Americas. The prevalence is highest among pre-and school-aged children living under poor conditions in the least developed settings [1,2]. Hence, STH belong to the group of neglected tropical diseases (NTD) [3].

1.1.1. Global distribution and burden

Looking back into history, already the old Romans were familiar with various intestinal worms including *A. lumbricoides* [4]. The earliest findings of parasitic worms originate from human coprolites and date back to the year 2277 BC [5]. Until recently, STH infection were a public health concern in Europe and the United States of America, where hookworm infections were responsible for the slowdown of economic growth in the early 20th century [6,7]. In the wealthier parts of Europe and USA, helminth infections were brought under control in the mid-20th century through improved sanitation and increased awareness. The most striking example was reported

from Japan: shortly after the Second World War, the prevalence of *A. lumbricoides* and *T. trichiura* exceeded 50% [7,8]. With improved socioeconomic conditions, sanitation and water supply, health education and treatment of children and adults with anthleminthic drugs, STH infection disappeared within a period of less than 20 years [7,8].

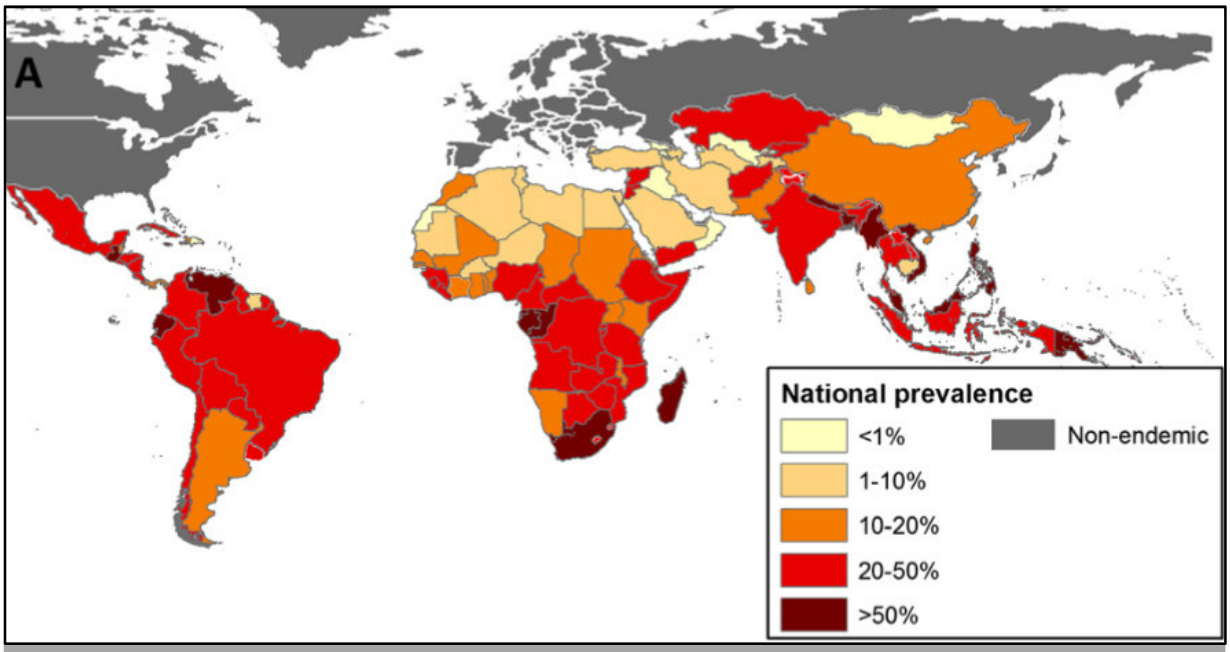


Fig 1. The global prevalence of soil-transmitted helminths. (adapted from Pullan et al., 2014.)

About 5.3 billion people lived in areas of STH transmission, whereas 1.45 billion people were infected with at least one of the three STH species (Figure 1) [9]. Considering the world's population of the same year (6.9 billion), more than one out of five people (21%) worldwide were infected with one or several STHs (Figure 2). In more detail, the highest infection numbers were reported for *A. lumbricoides* (819.0 million people[1]), followed by *T. trichiura* (464.6 million) and hookworm (438.9 million). Despite the highest reduction of STH prevalence over the past two decades, Asia continues to have the highest prevalence of STH infections, followed by Sub-Sahara Africa and South America [2]. Likewise, the STH prevalence in Sub-Sahara Africa decreased from the year 2000 onwards, which might be associated with socioeconomic development and intensified control programs [10].

[1]https://esa.un.org/unpd/wpp/index.htm

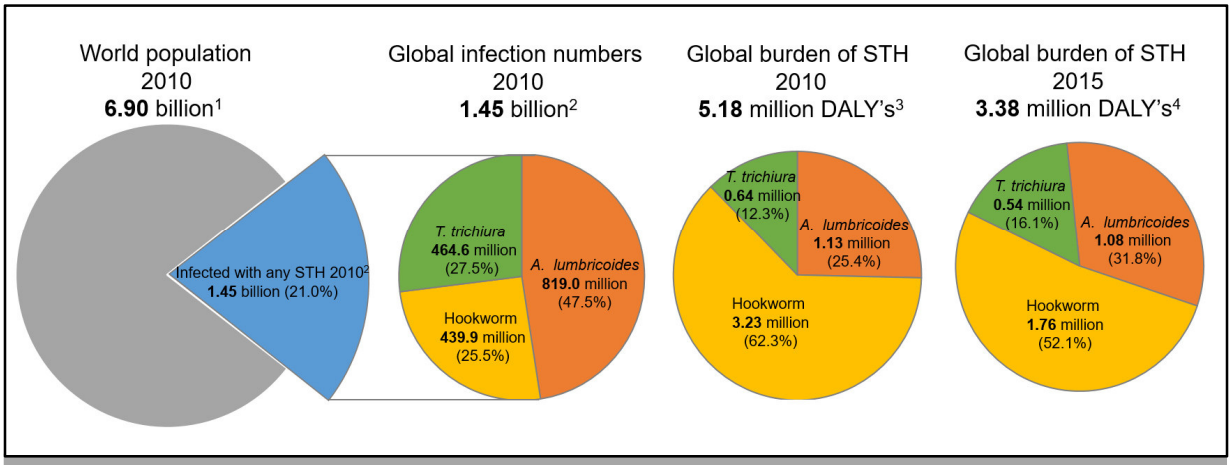


Figure 2. Global numbers of soil-transmitted helminth infections and disability adjusted life years (DALY).
References: 1 (https://esa.un.org/unpd/wpp/index.htm), 2 (Pullan et al., 2014), 3 (Murray et al., 2012), 4 (GBD 2015 DALYs and HALE Collaborators, 2016)

In Eastern Europe, STH are still endemic in the native population of Albania, Armenia, Slovakia and Turkey, where helminth infections are associated with extreme poverty and poor sanitation [11–13]. Because of political instabilities and numerous violent conflicts, in 2015, the number of displaced people reached their highest numbers since the Second World War. About 65 million people were forcedly displaced, in particular coming from the Middle East and Africa [14]. Europe is facing the largest refugee movements since the WWII, which are mainly caused by illegal wars in the Middle-East initiated by the United States and its military allies (i.e. NATO-partners and Saudi Arabia) [14–16]. With the large movement of immigrants from STH endemic countries, STH infections attract the attention of the European health authorities. A study from Germany reported STH infections in unaccompanied minor refugees and showed the necessity for screening and treating new arrivals, which are most vulnerable [17,18].

Non-specific symptoms are associated with an STH infection, which complicates the estimation of the global disease burden and the number of deaths attributed to STH infections. Furthermore, soil-transmitted helminthiasis are often co-endemic with malaria, an often fatal disease of major public health importance [19]. Therefore, the number of deaths specifically caused by soil-transmitted helminthiasis are consequently highly varying across publications and range from 10‘000 to 135‘000 deaths per year [20]. Most recent estimates from 2015 reported a number of 2’700 deaths attributed to *A. lumbricoides* [21], while no estimates for hookworm and *T. trichiura* were given. In the same report, an estimated 54‘200 deaths are

attributed to iron-deficiency anaemia (IDA), which is also associated with malaria and hookworm [22].

For the year 2010, the global burden of STH infections was 5.2 million disability adjusted life years (DALY) [23]. Based on the latest estimates from 2015, a lower burden of 3.4 million DALYs was estimated [24]. In more detail, hookworm caused the main burden of 1.8 million DALYs, followed by *A. lumbricoides* with 1.1 million DALYs and *T. trichiura* with 0.5 million DALYs. Despite twice the infection rates for *A. lumbricoides*, the hookworm burden is higher due to IDA [22,25]. Generally speaking, DALY estimations are variable and influenced by the estimation of a range of factors, i.e. the disability weight and likelihood for hookworm caused anaemia. Consequentially, the DALYs caused by hookworm range from 1.8 million [24], over 3.2 million [23] or up to 4.1 million [25] depending on the publication.

1.1.2. Life cycle

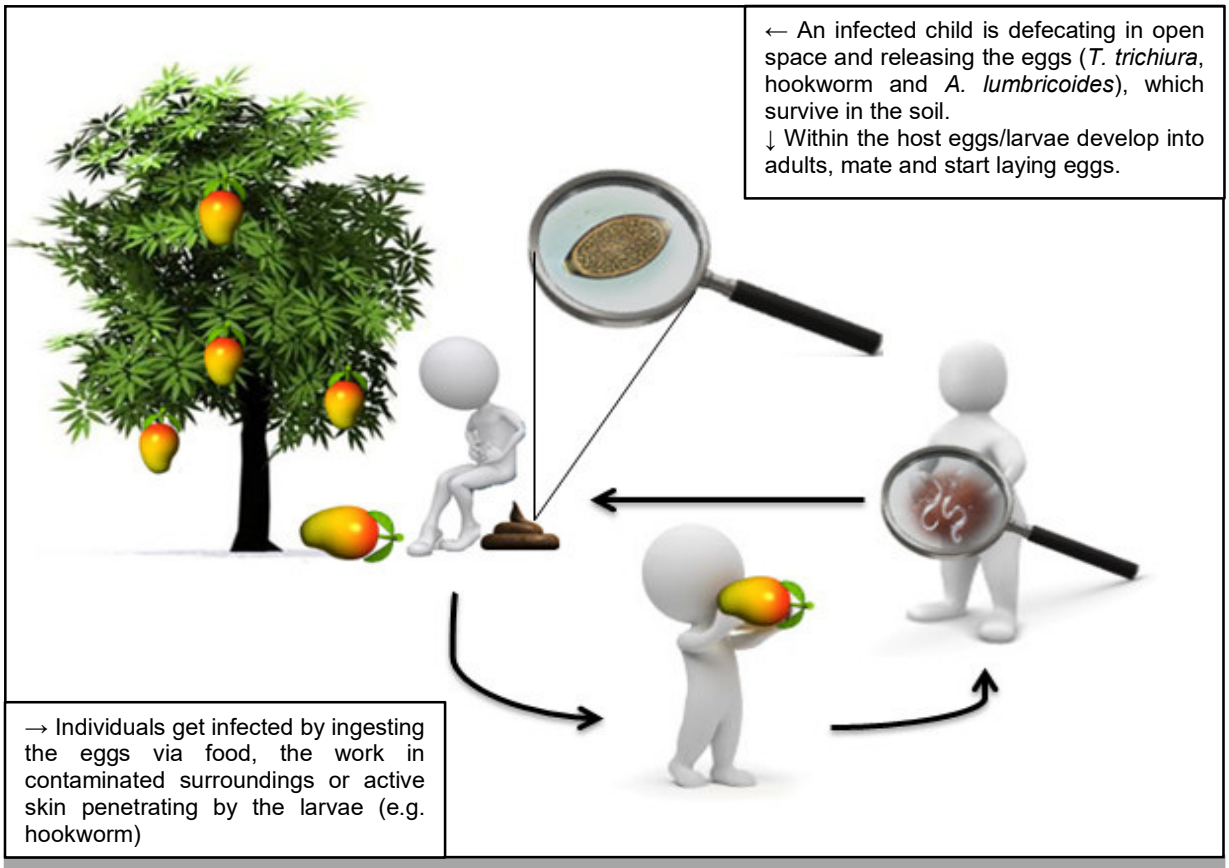


Figure 3. Life cycle of soil-transmitted helminths.

Large numbers of STH eggs are released by infected humans and reach the soil via open defecation (Figure 3). The eggs of *A. lumbricoides* and *T. trichiura* and the larvae of the two hookworm species (*N. americanus* and *A. duodenale*) develop in the soil to the infective stage. Humans are infected by accidentally ingesting the eggs of *A. lumbricoides* and *T. trichiura.* Once *A. lumbricoides* eggs developed into larvae, they migrate out of the intestine to the liver before entering the lungs. Next, they penetrate the alveolar space and end up in the pharynx. After the larvae get swallowed, they reach the small intestines and develop into adults, which are able to produce eggs after 9-11 weeks [1,26,27]. *T. trichiura* eggs moult within the host before the larvae travel to the colon and develop into adult worms [1].

The two hookworm species, *N. americanus* and *A. duodenal* moult twice in the soil until they develop into the infective larval stage (L3), which is able to penetrate human skin (*N. americanus* and *A. duodenale*) or gets orally ingested by humans in case of *A. duodenale* [1,28]. Post infection, the larvae migrate to the lungs, pass over the epiglottis and continue the migration to the upper smaller intestines. 4-7 weeks after entering the host, hookworm adults are able to produce eggs [1,28–30].

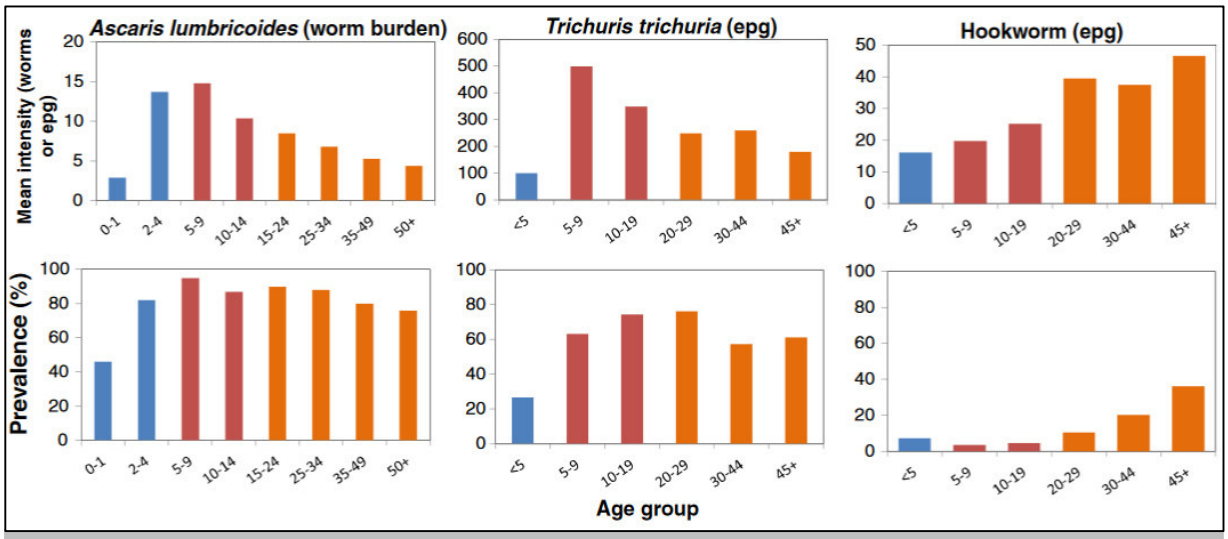


Figure 4. The mean intensity and prevalence of soil-transmitted helminths (*Ascaris lumbricoides, Trichuris trichiura* and hookworm) according to the age class. (Figure from Truscott et al. 2014 [33])

Local STH transmission is determined by the micro-climate and the soil properties. Because of the strong outer shell of *A. lumbricoides* and *T. trichiura* eggs, they are more resistant to

environmental influences and survive several months in the soil, in contrast to only a few weeks for the hookworm larvae [19,31]. Factors including temperature, pH, light etc., have an influence on the larval development. For example, *N. americanus* hatch only between 15°C and 35°C [32]. *A. lumbricoides* and *T. trichiura* are most prevalent in school-aged children, while the age specific epidemiology of hookworm differs. Likewise, high prevalences are found in school-aged children and highest infections are commonly in adults (Figure 4) [33].

1.1.3. Clinical symptoms

Based on the egg counts of the duplicated Kato-Katz thick smear, the World Health Organization (WHO) classifies STH infections into three categories; light, moderate and heavy [34]. The morbidity is strongly associated with the number of worms infecting a host. It is unknown if light infections are associated with morbidity [2], while moderate and heavy infections cause severe morbidity [35]. The prevalence is not necessarily associated with morbidity, however moderate and heavy infections are more predominant with higher prevalence. The major morbidity caused by adult hookworms is blood loss, induced by the adult worms feeding on blood in the gut. *A. duodenale* feeds more blood than *N. americanus*, which results in ten times more blood loss [36] and a greater amount of IDA [37]. The ingestion of *A. duodenale* can provoke the so-called Waka syndrome, which is characterized by nausea, vomiting and pharyngeal irritation [1,30]. The migration of *A. lumbricoides* larvae through the liver and lungs induce different symptoms including pneumonitis. Pneumonitis could result in asthma, cough, substernal pain, fever and eosinophilia [38] or even deathly [39]. Moderate *T. trichiura* infections lead to diarrhoea, vomiting, headache, weight loss, whereas heavy infections cause bloody diarrhoea, severe anaemia, chronic dysentery, rectal prolapse and colitis [1,40].

1.1.4. Helminth therapy

The “hygiene hypothesis” was developed in 1989 and claims that with improved living standards and hygiene conditions, the risk of developing an allergic reaction is increasing [41]. With more

careful hygiene practices and the sterilization of the environment, humans are less exposed to helminths, bacteria, viruses or fungi. With a lack of exposure to external pathogens, especially early in life, it is hypothesized that the human immune system is rendered hyper-sensitized to non-pathogenic substances and host endogenous molecules and cells. Subsequent epidemiological studies supported this concept by demonstrating that proper immune system activation in early human development plays an important role in shaping the immune system and the suppression of allergic reactions [42]. For example, a childhood infection with *T. trichiura* was shown to reduce the prevalence of allergen skin test reactivity and eczema later in life [43]. A systematic review and meta-analysis revealed a reduced risk for asthma for individuals with a hookworm infection, which was positively association with infection intensity [44]. Knowing this, therapies with helminths for autoimmune and other inflammatory disorders have been evaluated. While helminth therapy indicated promising results in animal models, studies in humans were less confirmatory. In clinical trials, two helminth species have been evaluated as therapy: *T. suis* and *N. americanus* [45]. *T. suis* is a common parasite of pigs and rarely infects humans, which remains only for a few weeks and is self-limiting. In one study, the ingestion of *T. suis* eggs by Crohn's disease patients, where the eggs were repeatedly ingested for several weeks, a high response rate and eventually remission were demonstrated, which was hypothesized to be due to the inhibition of intestinal inflammatory mechanisms [46]. However, further studies with *T. suis* as therapy for Crohn's disease and ulcerative colitis showed contradictory results. A Phase 2 trial with *T. suis* against Crohn's disease was even stopped at an early stage because of the lack of efficacy [45]. The treatment approach with *N. americanus* showed even less promising results [45,47]. The discrepancy between the animal and human studies is likely due to dosing; in most of the cases, significantly higher doses of helminths were used in animals, which would not be safe for humans [45].

1.2. Control programmes – preventive chemotherapy

Preventive chemotherapy (PC), the administration of anthelmintic drugs to at-risk populations without prior diagnosis, was endorsed in 2001 by the World Health Assembly resolution WHA54.19 [48]. Member states for which STH is a public health problem, were urged to control morbidity with PC programs (Table 1).

Year	Org.	Resolution	Content	Aims
International resolutions				
2001	World Health Assembly	WHA54.19 [48]	WHO founds Department for NTD	- Endemic countries start preventive chemotherapy - 75% of school-aged children are treated until 2010
2012	WHO, WB, B&MG, etc.	London Declaration on Neglected Tropical Diseases [51]	Increase in funding of research and drug donation by the pharmaceutical industry	- Eliminate or control 10 NTDs by 2020 - Control morbidity of STH by 2020 - Sustain or expand drug access programs (e.g. preventive chemotherapy) - progress reports using scorecards are provided annually on www.unitingtocombatntds.org
World Health Organization – STH recommendations				
2006	WHO	Preventive chemotherapy for human helminthiasis [52]	Manual for health professionals and program manager	Annual treatment of school-aged children for countries with STH prevalence 20%-50% and bi-annual for ≥50%
2008	WHO	Monitoring anthelminthic efficacy for STH [53]	Manual for assessing anthelminthic drug resistance	Monitoring anthelmintic drug resistance using the arithmetic or geometric FECRT
2012	WHO	Accelerating work to overcome the global impact of neglected tropical diseases [49]	A roadmap for implementation of STH control programs	By 2015; 50% of preschool and school-aged children receive PC and 100% of affected countries have a plan of action By 2020; 75% of preschool-aged and school-aged children receive PC and 100% of the effected countries reach 75% coverage
2012	WHO	Eliminating soil-transmitted helminthiases as a public health problem in children [50]	Progress report 2001−2010 and strategic plan 2011−2020	Goal: Reduce the prevalence of STH infections of moderate and high intensity among school-aged children below 1% by 2020 Objectives: 100% countries start deworming by 2015 and 100% of countries reach a 75% coverage by 2020
2013	WHO	Assessing the efficacy of anthelminthic drugs against schistosomiasis and STH [34]	Updated guidelines	Detailed guidelines to harmonize including indicators of efficacy, sample size, follow-up, diagnostic method, resistance monitoring, statistical analysis and interpretation of data
2017	WHO	Guideline: Preventive chemotherapy to control STH infections in at-risk population groups [54]	Global, evidence-informed recommendations for preventive chemotherapy	Recommendation: PC with annual or biannual albendazole or mebendazole for young children, pre-and school-aged children, adolescent, non-pregnant women and pregnant women (after first trimester)
Annual	WHO	Weekly epidemiological record – soil-transmitted helminthiasis progress reports	Report about the number of children treated per year in PC programs	Progress report including the number of distributed albendazole and mebendazole Tablets and the achieved coverage of PC

Table 1. International resolution with an impact for soil-transmitted helminth (STH) control programmes and recommendations by the World Health Organization.

In 2012, WHO released the roadmap to guide the implementation of NTD control and elimination programs including recommendations for STHs [49]. In the same year WHO released a progress report and strategic plan for STH control (and schistosomiasis), after failing the initial goals of the WHA54.19 resolution. The corrected goals included the expansion of PC to 50% of pre-and school-aged children in need for regular treatment by 2015 and to 75% by 2020 for eliminating STH as a public health problem [50].

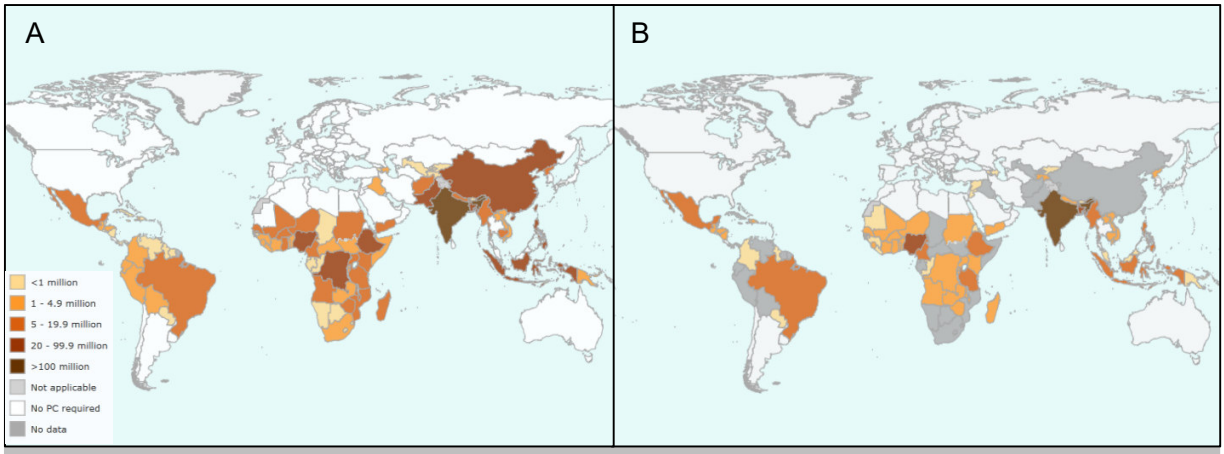


Figure 5. Population requiring (A) and receiving (B) PC against soil-transmitted helminths in 2015. (adapted from http://apps.who.int/gho/cabinet/pc.jsp)

Inspired by the WHO roadmap, a collaborative control program (London Declaration on Neglected Tropical Diseases) was launched in 2012. More than 70 pharmaceutical companies, governments and global health organisations endorsed their commitment to combat NTDs [51]. The pharmaceutical companies pledged to continue the donations of albendazole and mebendazole. In 2014, about 900'000 doses albendazole and 135'000 doses of mebendazole were donated to treating STH and LF [55]. In 2015, the overall coverage with PC reached 48.6% preschool-aged children and 64.7% for school-aged children in need for treatment (Figure 4) [56].

1.3. Limitation of preventive chemotherapy

The success of PC to control soil-transmitted helminthiasis is threatened by three major limitations. First, commonly school-aged children are treated in PC programs against STH [57]. However, most recent WHO guidelines from 2017 include young children, pre-and school-aged children, adolescent girls, non-pregnant women of reproductive age and pregnant women after the first trimester in hookworm or *T. trichiura* endemic areas with anaemia as a severe public health problem [54]. Whereas older recommendations also included adults with a high occupational risk [50]. To date, most countries only treat school-aged children on a regular base. Considering hookworm prevalence increases with age [33], the expansion of PC to adolescent and adults could be a strategy for hookworm endemic settings.

The second limitation of PC is the low efficacy of the two main benzimidazoles, albendazole and mebendazole. Both drugs reveal high efficacy in terms of cure rates (CRs) and egg reduction rates (ERR) against *A. lumbricoides.* A systematic review and meta-analysis from 2008 [58], indicated the moderate efficacy of albendazole against hookworm (CR: 72%) and low efficacy of pyrantel pamoate (31%) and mebendazole (15%) [58]. The main challenge of PC, however, is the low efficacy of albendazole (CR: 28%) and mebendazole (CR: 36%) against *T. trichiura*. Since the last review about anthelminthic drug efficacy, almost a decade has passed and new clinical trials were conducted, whereof one was hinting even lower CRs against *T. trichiura* for albendazole (CR: 3%) and mebendazole (CR: 12%) [59]. Hence, the first objective of this PhD thesis was to update the systematic review by using a new network meta-analysing approach and to provide summary estimates for ERRs, the key parameter for anthelminthic drug efficacy (page 24, objective 1). For measuring anthelminthic drug efficacy and to monitor drug resistance, which is detected by a decrease in efficacy compared to a reference efficacy for albendazole or mebendazole, the arithmetic ERR are recommended by the WHO (Table 1) [34]. However, the choice of measure of central tendency (i.e. arithmetic or geometric mean) for calculating ERRs is a longstanding discussion among human and animal parasitologists [60–62]. A side project of this PhD thesis was to further investigate the measurement of central

tendency to recommend one of the mean, however, the work is still in progress and part of it will be discussed.

Even after successful treatment, the protection is only transient and reinfections in high endemic settings occur quickly. Six to 12 months after successful treatment with albendazole or mebendazole, pre-treatment levels are reached for *A. lumbricoides* and *T. trichiura*, while reinfection with hookworm tends to be slower [63–65]. Most epidemiological reinfection studies can only provide inaccurate estimation of *T. trichiura* reinfection data since an effective treatment against *T. trichiura* is lacking. In the framework of this PhD, a 18 weeks follow-up study for estimating the reinfection was conducted after the successful treatment with different drug combinations by Speich and colleagues (page 24, objective 3) [66].

Third, there are no effective new drugs to replace the current anthleminthic in case of resistance. In veterinary medicine, resistance against anthelminthic drugs has been shown relatively quickly after the initial approval of several drugs [67,68]. For example, only nine years after the approval of levamisole for sheep, resistance was reported in 1979 [67]. Despite the increased use of anthelminthic drugs in PC against STH, no resistance in human medicine has been found to date. In veterinary nematodes, the frequent use of benzimidazole led to resistance, which was caused by a single nucleotide polymorphism in the parasite's β-tubulin at position 200. Diawara and colleagues showed the same substitution in *T. trichiura* recovered from humans in Kenya and Panama, which might explain the low efficacy of the two benzimidazole against *T. trichiura* [69].

1.3.1. Promising drugs

Given the limitations of current treatments, new drugs and drugs with higher efficacy particularly against *T. trichiura* are warranted. In the framework of this PhD thesis, the efficacy of the two novel drug candidates tribendimidine and oxantel pamoate were assessed in three clinical trials (see page 24), and hence I will briefly introduce these drugs.

1.3.1.1. Tribendimidine

During the mid-1980s, tribendimidine was developed as a wide-ranging anthelminthic drug in China by the National Institute of Parasitic Diseases in Shanghai. The first data about tribendimidine were published exactly 3 decades ago, in 1987 [70]. After a series of pre-clinical and clinical trials, tribendimidine was approved as a human anthelmintic by the Chinese Food and Drug Administration in 2004 [71]. Today, tribendimidine is the only new developed chemotherapeutical antiparasitic drug in the past 30 years, however it does not yet have market approval outside of China [72].

The mode of action was explored in *Caenorhabditis elegans,* where tribendimidine was classified as an L-type nicotinic acetylcholine receptor agonist (nAChR), similar to levamisole and pyrantel pamoate (Table 2) [73]. These findings were later revised with experiments in *Oesophagostomum dentatum* and *A. suum*, where tribendimidine caused depolarization antagonized by the nicotinic mecamylamine in the body muscles of the helminths. This indicated that tribendimidine is rather an agonist of muscle B-subtype nAChR [74]. Hence, the mode of action differs from levamisole and pyrantel pamoate.

Tribendimidine has demonstrated an excellent safety profile in clinical trials from China [75]. The drug was well tolerated and patients showed no abnormalities in routine blood and urine tests, hepatic and renal functions, and ECG examinations [71]. Moreover, all studies until 2013 reported only low numbers of adverse events for the recommended single dose treatment (200 mg for children and 400 mg for adults) and adverse events were even lower for children than adults [75]. Post-approval, further studies confirmed the large anthelminthic spectrum of tribendimidine, including activities against more than 20 nematode (e.g. *Enterobius vermicularis* [76,77]), trematode (e.g. *Taenia spp.* [78]) or cestode (e.g. *Opistorchis viverrini* [79,80] and *Clonorchis sinensis* [81]) species. Against STH, tribendimidine has a similar activity spectrum compared to albendazole, i.e. excellent activity against hookworm and *A. lumbricoides*. In more details, a weight-independent dose of 400 mg for 3 consecutive days resulted in a CR of 89.5% against hookworm [82]. Two phase IV clinical trials examined the efficacy of tribendimidine against hookworm in children and adults with a dose regimen of 200 mg and 400 mg,

respectively. A similar high efficacy was found in children (CRs: 72.7 – 84.5% [83]) and adults (CRs of 72.2 – 93.1% [84]). Since tribendimidine revealed high CRs against hookworm and *A. lumbricoides* [75] and a somehow lower CR against *T. trichiura,* further studies should focus on the combination of tribendimidine with a partner drug against *T. trichiura.* To combine tribendimidine with a trichuricidal drug (i.e. ivermectin or oxantel pamoate) and to evaluate tribendimidine as alternative drug to albendazole in case of drug resistance was the goal of one clinical trial conducted during this PhD thesis (page 24, objective 3).

1.3.1.2. Oxantel pamoate

In 1974, oxantel pamoate was introduced on the market [85]. The combination of oxantel pamoate combined with pyrantel pamoate (Quantrel; Pfizer) is approved for human use in Colombia, Peru and the Philippines, however, Quantrel is no longer produced (Kopp and Keiser 2017, unpublished). Both, oxantel pamoate and pyrantel pamoate belong to the tetrahydropyrimidines. While pyrantel pamoate has a high activity against *A. lumbricoides* and hookworm, oxantel pamoate is only active against *T. trichiura*. They are fast acting anthelminthics, which selectively bind to the gated (N-subtype) acetylcholine receptor ion-channels of nerves and muscles (Table 2) [86] and cause spastic paralysis of nematodes until the worms are expelled. Oxantel pamoate is very poorly absorbed and hence, the concentration in the caecum and colon could be attained without the risk of systematic reactions [87]. Extensive biochemical, hematologic and urine examination did not reveal any drug related changes [88]. All studies from the past century and two recent clinical trials described predominantly low adverse events [59,66,88–91].

Oxantel pamoate is of particularly high interest due to its unprecedented high activity against *T. trichiura*. An early study from 1976, reported CRs of 93% and 100% after treating the patients with a weight-dependent dose of 20 mg/kg. With only half of the dose regimen (10 mg/kg), the CRs dropped to 56.6% and 77%, respectively [88,96]. Several decades later, Speich and colleagues resumed the work with oxantel pamoate for treating human trichuriasis. They reported a lower CR of 26.3%, with an administered dose of 20 mg/kg and in combination with

albendazole increased CRs of 31.2% and 68.5%, respectively [59,66]. Considering that the goal of the WHO to reduce moderate and heavy infections, the ERR is the key parameter of anthelmintic drug efficacy [49,34]. In contrast to the moderate CRs, the ERRs reported by Speich and his team were as high as 93.2% [66].

Class	Drug	Mode of action	Cause to parasite	Ref
Benzimidazole	Albendazole, Mebendazole	Inhibition of microtubule polymerization by selective binding to the β-tubulin	Destruction of cell structure, death of parasite	[92]
Avermectin	Ivermectin	Agonist of glutamate-gated chloride channels in nerve and muscle cells	Paralysis and death	[93]
Imidazothiazoles	Levamisole	L-subtype nAChR agonist. Binding to the nAChR on body wall muscles	Spastic paralysis of worm, expulsion of parasite	[94]
Tetrahydrophyrimidines	Pyrantel pamoate	L-subtype nAChR agonist.	Spastic paralysis of worm, expulsion of parasite	[95]
	Oxantel pamoate	N-subtype nAChR agonist.	Spastic paralysis of worm, expulsion of parasite	[86]
Tribendimidine	Tribendimidine	B-subtype nAChR agonist.	Spastic paralysis of worm, expulsion of parasite	[74]

Table 2. Mode of action of the currently used and from two potential anthelminthic drugs.

Oxantel pamoate administered in combination with albendazole reached ERRs of 99.2% and 96%, respectively [59,66]. In light of these results, oxantel pamoate is currently the most efficacious drug candidate against *T. trichiura*. Because the effect of oxantel pamoate is limited to *T. trichiura*, a combination with a partner drug (i.e. albendazole) could reach high efficacy against any STH. To continue the work on oxantel pamoate and further explore its advantages was the major goal of this PhD thesis. Hence, three randomized clinical trials (page 24, objective 2 and 3) including oxantel pamoate were conducted in the past three years; a dose-finding trial and two exploratory trial including oxantel pamoate co-administrations (i.e. with tribendimidine, albendazole, pyrantel pamoate and mebendazole).

1.3.2. Drug combinations

Two different strategies could lead to effective treatments against all three STH; the development of new drugs and the combination of new and old drugs. Most anthelminthic drugs against human STH were developed and approved for veterinary medicine. Apart from the currently used anthelminthics, an array of veterinary drugs have been discussed for their potential to treat human soil-transmitted helminthiasis [97]. Because only very few drugs are currently in the anthelminthic drug pipeline, the combination of old and new drugs might be a step forward. The advantage of drug combinations are i) the increased efficacy compared to single drugs and ii) the increased protection against drug resistance due to the combined drugs acting on different targets [98]. Only a few new drugs have been evaluated in combination with the drugs from the WHO List of Essential Medicines against STH (Table 3) [99].

Drug combination	Reference		*A. lumbricoides*	Hookworm	*T. trichiura*
ALB-MEB	Namwanje et al. 2011 [100]	CR			46.1
		ERR			93.2
	Speich et al. 2015 [66]		97.5	47.8	8.4
			99.9	92.7	51.6
ALB-IVR	Speich et al. 2015 [66]	CR	98.0	50.0	27.5
		ERR	>99.9	95.4	94.5
	Knopp et al. 2010 [101]	CR	92.9	66.7	37.9
		ERR	>99.9	95.9	91.1
	Ndyomugyen et al. 2008 [102]	CR	-	92.6	70.6
		ERR	-	63.9	-
	Belizario et al. 2003 [103]	CR	78.1	-	65.1
		ERR	99.5	-	97.5
	Beach et al. 1999 [104]	CR	100.0	100.0	79.6
		ERR	100.0	100.0	68.0
MEB-IVR	Knopp et al. 2010 [101]	CR	100.0	25.7	55.1
		ERR	100.0	50.0	96.7
ALB-DIE	Belizario et al. 2003 [103]	CR	77.5	-	19.2
		ERR	96.6	-	79.4
ALB-OXP	Speich et al. 2014 [59]	CR	94.4	51.4	31.2
		ERR	>99.9	95.6	96.0
	Speich et al. 2015 [66]	CR	97.9	45.5	66.3
		ERR	99.9	90.9	99.5
ALB-NIT	Speich et al. 2012 [105]	CR	-	85.7	16.0
		ERR	-	-	54.9
ALB-OXP-PYP	Shichun et al. 1989 [106]	CR	95.0	92.6	92.9
		ERR	100.0	99.3	97.3
MEB-OXP-PYP	Sinniah et al. 1980 [91]	CR	100.0	66.7	81.0
		ERR	100.0	85.7	93.5

Table 3. The efficacy of different combination with albendazole (ALB), mebendazole (MEB), ivermectin (IVR), diethylcarbamazine (DIE), oxantel pamoate (OXP), nitazoxanide (NIT), pyrantel pamoate (PYP).

The combinations albendazole with diethylcarbamazine [103] and nitazoxanide [105] showed no improved efficacy against *T. trichiura* and hence, no further clinical trials were conducted. Contradicting results were reported for the combination of the two most widely used drugs albendazole and mebendazole; compared to mebendazole monotherapy one study showed improved [100], while another study did not show improved efficacy against *T. trichiura* [66]. A study from Knopp and colleagues indicated the potential of combining ivermectin with albendazole or mebendazole [101]. The combination with mebendazole reached a high efficacy against *T. trichiura*.

Further studies with albendazole plus ivermectin indicated similar results against all three STH [66,102–104]. However, the most promising results were derived from two clinical trials that tested the combination of albendazole and oxantel pamoate [59,66]. This combination has currently the highest efficacy against any STH, which might even be improved by adding pyrantel pamoate [91], which was the goal of one clinical trial (page 24, objective 3) realized during this PhD thesis.

1.4. Integrated control approach

PC might be able to reduce moderate and heavy infections, however given its limitations the elimination of STH might not be possible. With an integrated approach, i.e. with extended PC, improved water, sanitation, hygiene (WASH) and health education, the interruption of STH transmission might be feasible. Japan implemented a similar approach and was able to eliminate the initially high STH prevalence within 20 years [7,8]. Because improved WASH is integral to reducing poverty, promoting equality and supporting socioeconomic development for poor and vulnerable populations, drinking water and sanitation were included in the ‘Millennium Development Goals’ [107] and the successive ‘Sustainable Development Goals’ [108].

WASH promotes the prevention of open defecation with the installation of ventilated-improved pith latrines and a faecal disposal management, which decreases the contamination of soil, food and hands with STH eggs from infected people. Improved hygiene practices, i.e. hand-washing

with soap before eating, after using toilets and the wearing of shoes, could lead to reduced transmission [109]. Indeed, two systematic reviews reported lower odds of STH infections with increased access and use of sanitation facilities [110,111]. Although not all studies including WASH interventions have shown an immediate reduction in STH prevalence [112], WASH has an important impact on many different pathogens, including enteric bacteria, intestinal protozoa [113] and several NTDs [109].

1.5. Diagnostics

Today's STH research is highly dependent on an accurate diagnostic method for estimating prevalence, evaluating infection intensities, assessing helminth drug efficacies in clinical trials or for monitoring drug resistance [50,114,115]. WHO is currently recommending the duplicate Kato-Katz method, which is the basis for the infection intensity classifications into light, moderate and heavy infection according to the egg counts [116]. The Kato-Katz method was developed almost half a decade ago [34,117] and is not without limitations. The main limitations are the low sensitivity for low infection intensities [118], which leads to bias towards false negative results [118], followed by the disintegration of hookworm eggs after one hour [119] and the short sample storage time [120]. With the addition of multiple samplings, the sensitivity of Kato-Katz thick smears can be slightly increased [121–124].

Figure 6. Association between the infection intensity in an endemic area and the required sensitivity of a diagnostic method. High infection intensities are already detected with a low sensitive method, while micro-infections require highly sensitive methods. (adapted from Bergquist et al., 2009a)

In the successive steps from morbidity reduction to transmission control to the elimination of STH, different characteristics for the diagnostic method are of importance [114]. For morbidity control, the major importance is simplicity and low costs. Kato-Katz is a good tool for these settings and the low sensitivity for light infections is an acceptable trade-off (Figure 6). Kato-

Katz requires only little preparation time and equipment, which still allows for the quantification of eggs in stool. The preparation of a single Kato-Katz thick smear requires about 20 minutes and costs around 1.7$ [123]. Apart from Kato-Katz, several other techniques with comparable sampling and time effort have been developed (ether concentration, McMaster and Mini-FLOTAC) or are still under development (FECPAK^{G2}) [123,125–127]. The last objective of this PhD thesis (page 24), was to evaluate the new diagnostic tool FECPAK^{G2} in the framework of a clinical trial. FECPAK^{G2} is an online, remote location diagnostic tool, which is used in veterinary medicine and currently under development for human use (Figure 7). The FECPAK^{G2} method comprise sample preparation (1), the capturing of an image from a cassette filled with the sample (2), storage of the image in an internet cloud and marked-up of the image using a software (3).

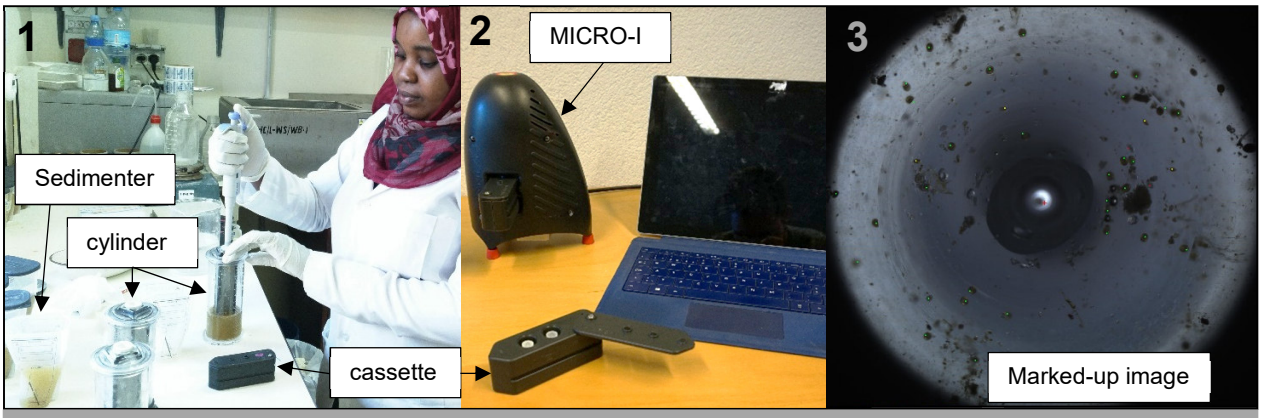


Figure 7. FECPAK^{G2} system.

Moving forward to transmission control in low infection intensity settings, diagnostic tools with increased sensitivity are necessary (Figure 6) [114]. A potential candidate is the FLOTAC system. A review and meta-analysis of the currently literature revealed the highest sensitivity for FLOTAC (92.7%), whereas for Kato-Katz, the sensitivity depended on the sampling effort and ranged from 74-95% [118]. The more steps a country has taken towards elimination, the higher the importance of predicting an infection correctly (positive predictive value). The focus is currently on different molecular methods, which are developed towards the detection of micro-infection, such as real time polymerase chain reaction [128]. However, these methods are time consuming, require costly laboratory equipment and highly skilled laboratory technicians.

1.6. Study sites

1.6.1. Tanzania

Two clinical trials of this PhD were conducted on Pemba Island, Tanzania. The Zanzibar archipelago is situated in the Indian Ocean close to the cost of Tanzania and includes apart from smaller, the two major islands Unguja and Pemba (Figure 7). In Zanzibar, large-scale administration of anthelminthic drugs (i.e. albendazole or mebendazole) started in the mid-1990s, because the STH prevalence exceeded 90%. Annually, albendazole or mebendazole was distributed among school-aged children [129]. Despite regular administration of anthelmintic drugs since decades, the STH prevalence remains high. In two recent clinical trials of Speich and colleagues the *T. trichiura* prevalence in school-aged children exceeded 90% [59,66].

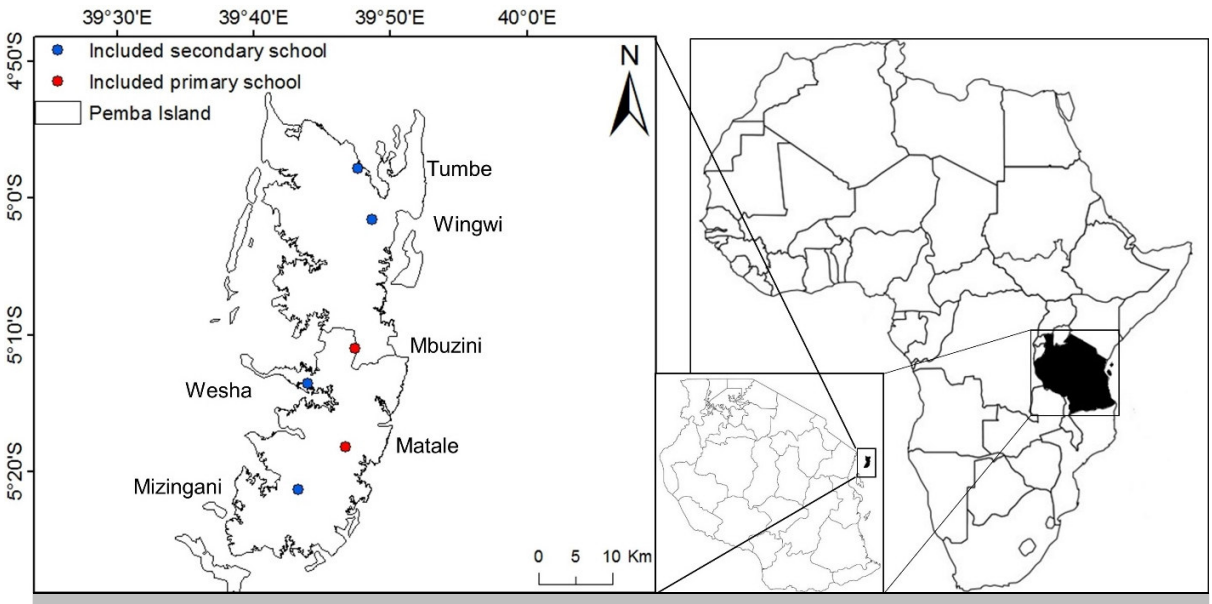


Figure 7: Included primary and secondary school on Pemba Island, Tanzania.

The remaining high prevalence, good infrastructure and skilled team of the Public Health Laboratory – Ivo de Carneri, present an ideal setting for epidemiological studies and clinical trials. The two clinical trials conducted during this PhD thesis, profited from the local leaders, field and laboratory workers with long-term experience. For the first study in 2014, two primary schools were determined by the local collaborators; Mbuzini and Matale (Figure 7). Two years

later, the four secondary schools were chosen as clinical trial site; Tumbe, Wingwi, Wesha and Mizingani.

1.6.2. Côte d'Ivoire

One part of a clinical trial of this PhD thesis was located in Tanzania and Côte d'Ivoire. In Côte d'Ivoire, the southern district Agboville and with the two villages Azaguié and Rubino were chosen as study site (Figure 8).

In 2014, the population of the Agboville district reached almost 300'000 inhabitants, whereas around 22'000 people lived in Azaguié and 36'000 people in Rubino [130]. Azaguié is co-endemic for STH and schistosomiasis (*Schistosoma mansoni* and *S. haematobium*) and was already the study setting of multiple epidemiological studies and clinical trials [131–133].

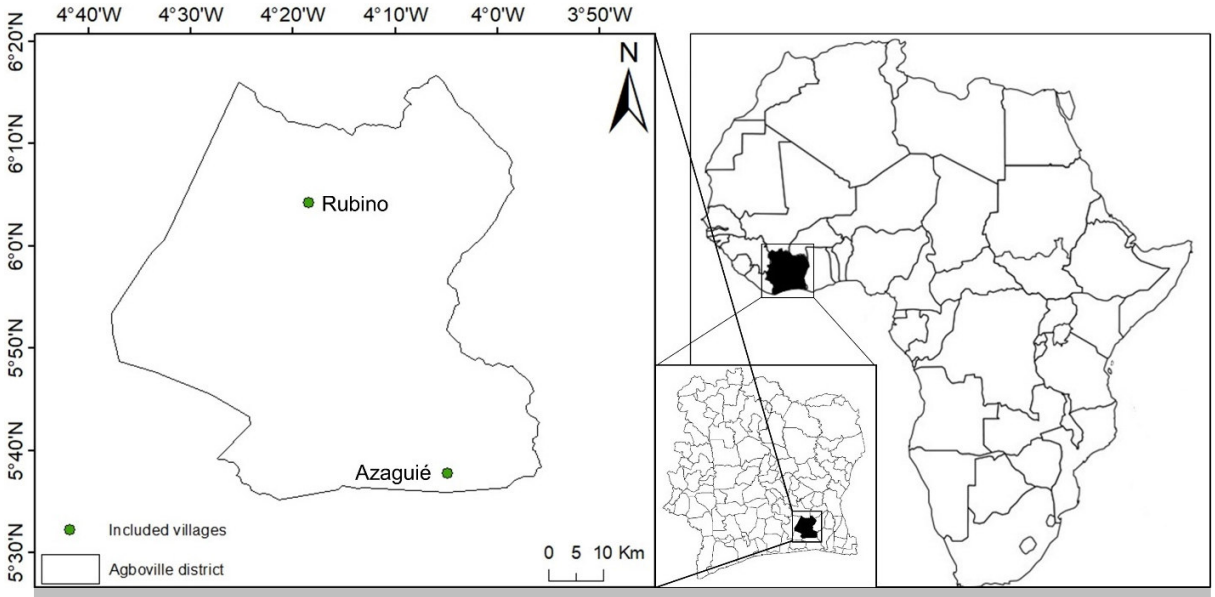


Figure 8. Included villages of the Abgoville district in Côte d'Ivoire.

1.6.3. Lao People's Democratic Republic

NTDs are still a major public health concern in Lao People's Democratic Republic (PDR). Highest prevalences are found for *Opisthorchis viverrini*, followed by STH, lymphatic filariasis and schistosomiasis [134]. The last clinical trial, which was conducted during this PhD was situated in the Luang Prabang district in Lao PDR. Two secondary (Xomphou and Namthouam)

and two primary and secondary schools combined (Nayang and Nakhone) were selected. A previous, country wide survey, indicated high hookworm prevalence (43%) for the Luang Prabang district [135]. Additionally, children from the primary school of Namthouam (Phonmany) were screened for hookworm. Due to a misunderstanding with the teachers, all children received anthelminthic treatment during the screening process and the school had to be excluded from the study.

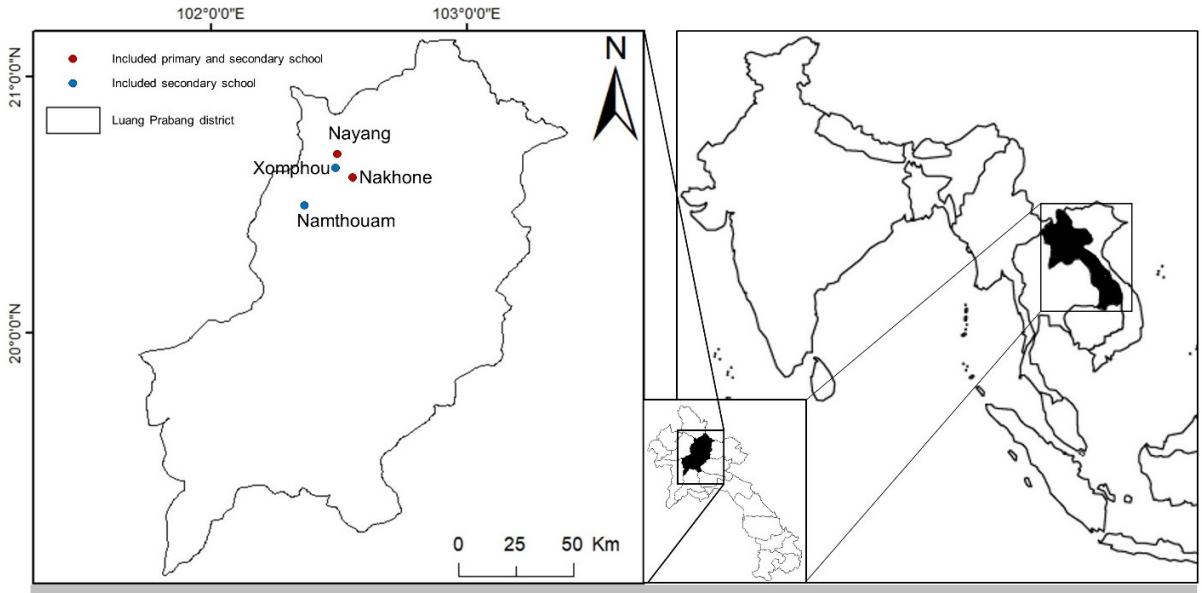


Figure 9. The primary and secondary schools included in Lao People's Democratic Republic.

1.6.4. Lesotho

In 2015 an unpublished NTD survey from the Ministry of Health in Lesotho reported high *T. trichiura* prevalence in the Butha-Buthe district of Lesotho. For re-assessing STH prevalences and for evaluating the Butha-Buthe district as a potential study site for clinical trials, a small epidemiological study was conducted during this PhD thesis (page 24, objective 4). The prevalence was assessed in six primary schools (Marakabei, St Charles, Lebesa, Lekopa, Khukhune and Damaseka) (Figure 10).

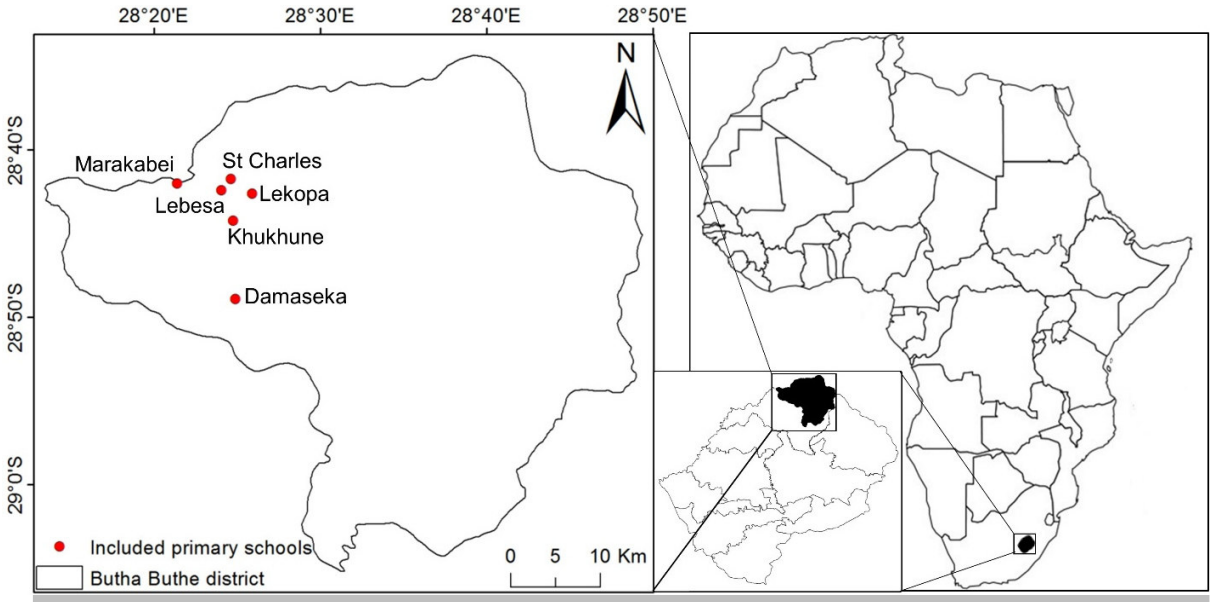


Figure 10. The primary schools included in the cross-sectional study in the Butha-Buthe district in Lesotho.

1.7. Aim and objectives

The current standard approach against STH is PC with anthelminthic drugs. The goal of this PhD thesis was closely related to the limitations of PC. The major aim was to review and meta-analyse the efficacy of the current anthelminthic drugs and to further evaluate the promising drug oxantel pamoate and tribendimidine. Additional objectives comprised the evaluation of a new diagnostic tool and to evaluate Lesotho as a potential clinical study site. In more detail, the following objectives were specified to accomplish these goals:

1. Updating the evidence about the current anthelminthic drug efficacies: To review and meta-analyse the efficacy of the most commonly used anthelminthic drugs in PC programs (chapter 2).

2. Assessing the efficacy of different doses of oxantel pamoate against *T. trichiura* to be able to calculate a weight independent dose: To assess the efficacy and safety of different oxantel pamoate doses in school-aged children infected with *T. trichiura* on Pemba Island, Tanzania (chapter 3).

3. Assessing different drug combinations against STH and reinfection: To assess the efficacy and safety of i) tribendimidine-ivermectin and tribendimidine-oxantel pamoate (chapter 4a) and i) pyrantel pamoate-oxantel pamoate and albendazole-pyrantel pamoate-oxantel pamoate (chapter 4b) against hookworm and concomitant helminth infections. Moreover, to assess the reinfection rate after treatment with different drug combinations (chapter 4c).

4. Exploring novel diagnostic tools and potential study sites: To compare the novel diagnostic tool FECPAK^{G2} to Kato-Katz (chapter 5a) and to evaluate the potential of a new study sites (chapter 5b).

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Chapter 2

Efficacy of the current drugs against soil-transmitted helminths: systematic review and network meta-analysis

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RESEARCH

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Efficacy of recommended drugs against soil transmitted helminths: systematic review and network meta-analysis

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ABSTRACT

OBJECTIVE
To evaluate efficacies of anthelmintic drugs against soil transmitted helminths in terms of cure rates and egg reduction rates.

DESIGN
Systematic review and network meta-analysis.

DATA SOURCES
PubMed, ISI Web of Science, Embase, ScienceDirect, the Cochrane Central Register of Clinical Trials, and the World Health Organization library database from 1960 until 31 December 2016.

STUDY SELECTION
Randomised controlled trials evaluating the efficacy of a single dose regimen of albendazole, mebendazole, levamisole, and pyrantel pamoate against *Ascaris lumbricoides*, hookworm (*Necator americanus* and *Ancylostoma duodenale*) and *Trichuris trichiura*. The primary outcomes included cure rates analysed by network meta-analysis with mixed logistic regression models and egg reduction rates with mixed linear models.

RESULTS
55 and 46 randomised controlled trials were included in the analysis of cure rates and egg reduction rates, respectively. All drugs were highly efficacious against *A lumbricoides*. Albendazole showed the highest efficacy against hookworm infections with a cure rate of 79.5% (95% confidence interval 71.5% to 85.6%) and an egg reduction rate of 89.6% (81.9% to 97.3%). All drugs had low efficacy against *T trichiura*, with mebendazole showing the highest cure rate of 42.1% (25.9% to 60.2%) and egg reduction rate of 66.0% (54.6% to 77.3%). Estimates for the years 1995 and 2015 showed significant reductions in efficacy of albendazole against *T trichiura*: by 2015 the egg reduction rates fell from 72.6% (53.7% to 91.5%) to 43.4% (23.5% to 63.3%; P=0.049) and the cure rates fell from 38.6% (26.2% to 52.7%) to 16.4 (7.7% to 31.3%; P=0.027).

CONCLUSIONS
All four currently recommended drugs show limitations in their efficacy profile. While only albendazole showed good efficacy against hookworm infection, all drugs had low efficacy against *T trichiura*. The decrease in efficacy of albendazole against *T trichiura* over the past two decades is of concern. The findings indicate the need for strengthening efforts to develop new drug treatments, with a particular focus on drugs against *T trichiura*.

WHAT IS ALREADY KNOWN ON THIS TOPIC
The current strategy against soil transmitted helminths is preventive chemotherapy, mainly with albendazole, mebendazole, and, to a lesser extent, levamisole and pyrantel pamoate
A previous meta-analysis presented summary estimates of cure rates of these drugs based on a small number of randomised controlled trials

WHAT THIS STUDY ADDS
The study provides up to date estimates of cure rates and egg reduction rates with network meta-analysis
The two most commonly used drugs have shortcoming in their efficacy profile: mebendazole has low efficacy against hookworm and albendazole and mebendazole show low performance against *T trichiura*
Efficacy albendazole and mebendazole against *T trichiura* has decreased over the past decades

Introduction
Soil transmitted helminthiasis is caused by infections with the nematode worm *Ascaris lumbricoides*, the hookworms *Necator americanus* and *Ancylostoma duodenale*, and *Trichuris trichiura*. An estimated 5.3 billion of people are at risk, while 1.5 billion are infected with at least one of the soil transmitted helminths.[1] Despite a global decline in infections, prevalence remains high in Asia, followed by sub-Saharan Africa and Latin America.[1] *A lumbricoides* and *T trichiura* infections particularly affect preschool and school aged children, while hookworm infections are more prevalent in adults. Infected people predominantly live in poor conditions in the least developed countries, where households lack adequate facilities and clean water. Morbidity correlates with the number of worms harboured by infected individuals. While light infections commonly remain asymptomatic, moderate and heavy infections cause severe morbidity,[2] including growth stunting, intellectual impairment, cognitive and educational deficits, malnutrition, and iron deficiency anaemia.[3] In 2015, the global burden of infections with soil transmitted helminths was estimated at 3.4 million disability adjusted life years (DALYs).[4]

The goal of the World Health Organization (WHO) is to reduce the prevalence of moderate and heavy infections with soil transmitted helminths in preschool and school aged children to below 1% by 2020.[5-7] To achieve this goal, school aged children in endemic areas are regularly treated in so called preventive chemotherapy programmes.[5 6 8] In 2015, about 573 million children received preventive chemotherapy against soil transmitted helminths, corresponding to a global coverage of 59.5%.[9] The ultimate target is to cover at least 75% of school aged children in need of treatment.[6] Albendazole, mebendazole, levamisole, and pyrantel pamoate are currently on the WHO list of essential medicines for the treatment of such infections,[6 7] while the two

benzimidazoles—albendazole and mebendazole—are the most widely used drugs in preventive chemotherapy programmes.[8]

The efficacy of albendazole, mebendazole, and pyrantel pamoate has been assessed in a systematic review for different dose regimens[10] and by means of meta-analysis of randomised controlled trials for single doses.[11] Albendazole, mebendazole, and pyrantel pamoate had high efficacy against *A lumbricoides* in terms of cure rates. Only albendazole was found to be efficacious in single dose regimen against hookworm (cure rate 72%). Both albendazole and mebendazole had unsatisfactory results against *T trichiura* at single doses with cure rates of 28% and 36%, respectively.[11] Of concern, recent results from randomised trials on Pemba Island (Tanzania) showed even lower cure rates for albendazole (2.6%) and mebendazole (11.8%) against *T trichiura*.[12]

We updated the findings from the two systematic reviews,[10 11] including new evidence and applying network meta-analysis methods. The comparison of intervention effects among randomised controlled trials with conventional meta-analysis is limited by the constraint that only drugs tested in the same study can be compared.[11] In contrast, network meta-analysis draws strength from direct and indirect comparisons through common comparators (such as placebo). Furthermore, multiple drugs can be compared and ranked.[13-17] In addition, for the first time we meta-analysed egg reduction rates, the standard key parameter for drug efficacy.[18] Our analysis provides current evidence on anthelmintic drug efficacy, which is of considerable relevance to policy makers as they call for an adaptation of current treatment guidelines.

Methods

Search strategy and selection criteria

This review and meta-analysis is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) extension statement for network meta-analysis.[19] The study protocol is provided in appendix 1. We conducted an electronic literature search on PubMed, ISI Web of Science, Embase, ScienceDirect, the Cochrane Central Register of Clinical trials, and the WHO library database. All studies from 1960 until 31 December 2016 were considered. The search was not restricted to any language, and, in case of non-English articles, native speakers were consulted for full text translations. The triple MeSH search terms included "albendazole", "mebendazole", "levamisole", and "pyrantel pamoate" combined with either "trial", "study", or "case report" and "*Ascaris lumbricoides*", "ascariasis", "hookworm", "*Ancylostoma duodenale*", "*Necator americanus*", "*Trichuris trichiura*", "trichuriasis", or "soil-transmitted helminths" (table A, appendix 1).

To be eligible for inclusion, studies had to be level 1 randomised controlled trials (https://www.elsevier.com/__data/promis_misc/Levels_of_Evidence.pdf) that reported the efficacy against *A lumbricoides*, hookworm, and *T trichiura* in terms of cure rates, egg reduction rates, or both. For this review we selected randomised controlled trials that included at least one treatment arm of the currently recommended[7 10 20] single dose regimens of albendazole (400 mg), mebendazole (500 mg), levamisole (80 mg or 2.5 mg/kg), or pyrantel pamoate (10 mg/kg). There were no age restrictions. Studies were excluded if they were not randomised controlled trials, used different drug regimens (such as multiple doses or different drug regimens), or combined different drugs or if the follow-up was shorter than one or longer than six weeks.

Patient involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. We did not evaluate whether the studies included in the review had any patient involvement.

Data extraction and assessment of risk bias

From each eligible randomised controlled trial we extracted number of infected participants at baseline, number of cured participants at follow-up, mean number of eggs at baseline, mean number of eggs at follow-up, percentage of egg reduction, measure of central tendency (arithmetic, geometric, or not described), information on the number of treatment arms, number of eligible treatment arms, year of publication, country, diagnostic method, age range, and time between treatment and follow-up.

Two independent reviewers (WM and JK) screened titles and abstracts for potential studies. When articles met the inclusion criteria, the entire manuscripts were scrutinised, and, for eligible trials, the data were extracted independently by the same reviewers. All included trials were assessed for quality by two different methods: that described by Jadad and colleagues,[21] with scores ranging between 1 (lowest level) and 5 (highest level), and according to the Cochrane Collaboration Handbook (table A in appendix 2).[22] The latter criteria assess studies for risk of bias in six different domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, and selective reporting. Each domain is categorised into low, high, or unclear risk of bias. In case of discrepancies over extraction of data or scoring of the study quality, a third person (CS) was involved and the results discussed until consensus was reached.

Data synthesis and statistical analysis

The advantage of a network meta-analysis is the simultaneous combination of direct and indirect estimates of the treatment effect in one analysis. In data from clinical trials with direct estimates for drug A *v* B and other trials comparing A *v* C, we can estimate the relative treatment effect for B *v* C and

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all three drugs can be ranked.[13 23] To illustrate the network geometry, we have provided a separate plot for cure rates and egg reduction rates (fig 1).[23] For the network meta-analysis of the cure rates, we used a method proposed by Kessels and colleagues.[24] The method consists of rebuilding the original datasets based on sample sizes and case numbers retrieved from the publications. All datasets from studies with one, two, or more treatment arms were then pooled, and mixed logistic regression models were applied to the final pooled dataset. With this method even studies with only one eligible treatment arm can be included. The models included treatment as a fixed factor and random effects for studies and for treatment arms within studies. To mimic meta-regression analysis, we additionally included the respective regressor variable and its interactions with the treatments.

We recorded all egg reduction rates directly from the articles and used mixed linear models for the meta-analysis of these rates. These models included the fixed factors treatment, infection intensity (dichotomised as above versus below median of baseline egg counts), measure of central tendency (arithmetic mean, geometric mean, or not described), and random effects for studies. We considered baseline infection intensity to increase precision and to achieve approximate normality of regression residuals.

Table 1 shows the average cure rates and egg reduction rates per treatment derived from the underlying regression models as marginal estimates. We presented one sided 95% confidence intervals if the limits of the respective two sided interval exceeded 0 or 100%. We carried out one to one comparisons of cure rates and egg reduction rates by looking at the differences of the respective regression coefficients (fig 2). In the case of cure rates we used exponentiation to convert these differences into odds ratios. We also conducted a simple, pairwise meta-analysis of cure rates for the one to one comparison using the command metan in Stata. Table C in appendix 2 and fig B in

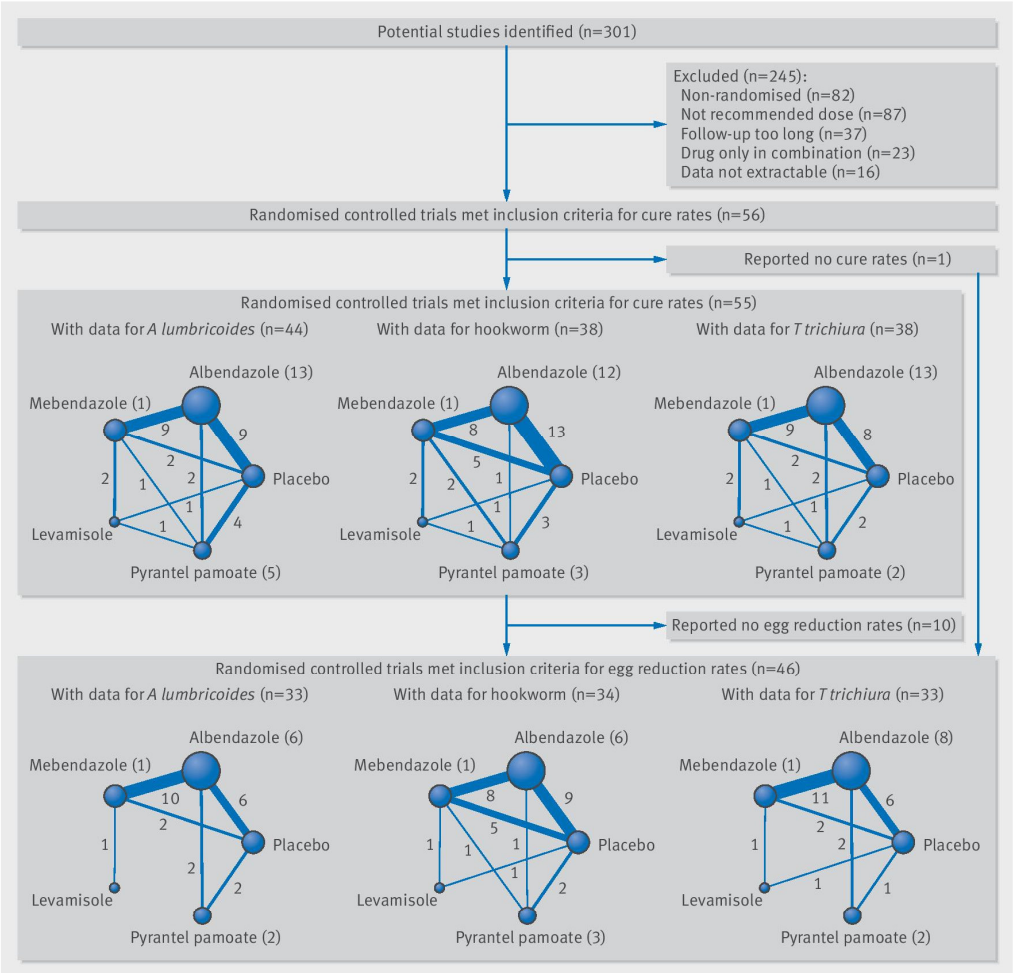

Fig 1 | Flowchart and network showing procedure for identification of relevant publications. Circular nodes show each treatment with circle size indicating amount of respective evidence and numbers in brackets indicating number of pooled studies with only one eligible treatment arm. Weight of line and number on line indicate number of direct treatment comparisons within same study

Table 1 | Average cure rates (%) and egg reduction rates (%) of albendazole, mebendazole, levamisole, and pyrantel pamoate against *A lumbricoides*, hookworm, and *T trichiura* based on network meta-analysis

	Cure rates			Egg reduction rates		
Treatment	No of included studies	No of included participants	Rate (95% CI)	No of included studies	No of included participants	Rate (95% CI)
A lumbricoides						
Placebo	14	842	12.7 (6.7 to 22.7)	9	525	20.7 (14.7 to 26.7)
Albendazole	34	3360	95.7*** (93.2 to 97.3)	26	2854	98.5*** (94.9 to 100.0)
Mebendazole	13	1548	96.2*** (92.3 to 98.1)	13	1529	98.0*** (94.0 to 100.0)
Levamisole	2	149	97.3*** (84.2 to 99.6)	1	125	96.4*** (82.3 to 100.0)
Pyrantel pamoate	11	1374	92.6*** (85.6 to 96.3)	6	284	94.3*** (88.3 to 100.0)
Total	44	7273	—	33	5137	—
Hookworm						
Placebo	18	1309	15.2 (9.3 to 23.9)	14	1046	16.2 (5.3 to 27.1)
Albendazole	30	3104	79.5*** (71.5 to 85.6)	26	2839	89.6*** (81.9 to 97.3)
Mebendazole	14	2305	32.5* (20.8 to 46.9)	14	2263	61.0*** (52.0 to 69.9)
Levamisole	2	230	10.3 (2.4 to 35.2)	1	202	61.8* (30.3 to 93.3)
Pyrantel pamoate	7	230	49.8** (29.5 to 70.1)	5	144	71.9*** (54.7 to 89.0)
Total	38	7178	—	34	6494	—
T trichiura						
Placebo	11	1417	8.6 (4.1 to 17.1)	28	1049	19.2 (6.9 to 31.4)
Albendazole	33	4432	30.7*** (21.0 to 42.5)	29	3407	49.9*** (39.0 to 60.6)
Mebendazole	13	2514	42.1*** (25.9 to 60.2)	14	2507	66.0*** (54.6 to 77.3)
Levamisole	2	203	29.5 (6.1 to 72.9)	1	197	28.3 (6.7 to 49.8)
Pyrantel pamoate	6	275	20.2 (7.3 to 44.7)	4	158	47.5** (25.5 to 69.6)
Total	38	8841	—	33	7318	—

* P<0.05, **P<0.01, ***P<0.001 for comparison with placebo.

appendix 3 show the respective summary odd ratios, I^2, and τ^2 statistics, where they are compared with the corresponding odd ratio estimates from network meta-analysis.

In a second stage, we stratified analysis for cure rates and egg reduction rates according to continent, place of the study, sensitivity of diagnostic method, quality of the study, length of follow-up, intensity of infection at baseline, and year of publication (see tables D-J in appendix 2). By letting the treatment interact in the mixed regression model separately with study size and year, we estimated cure rates and egg reduction rates

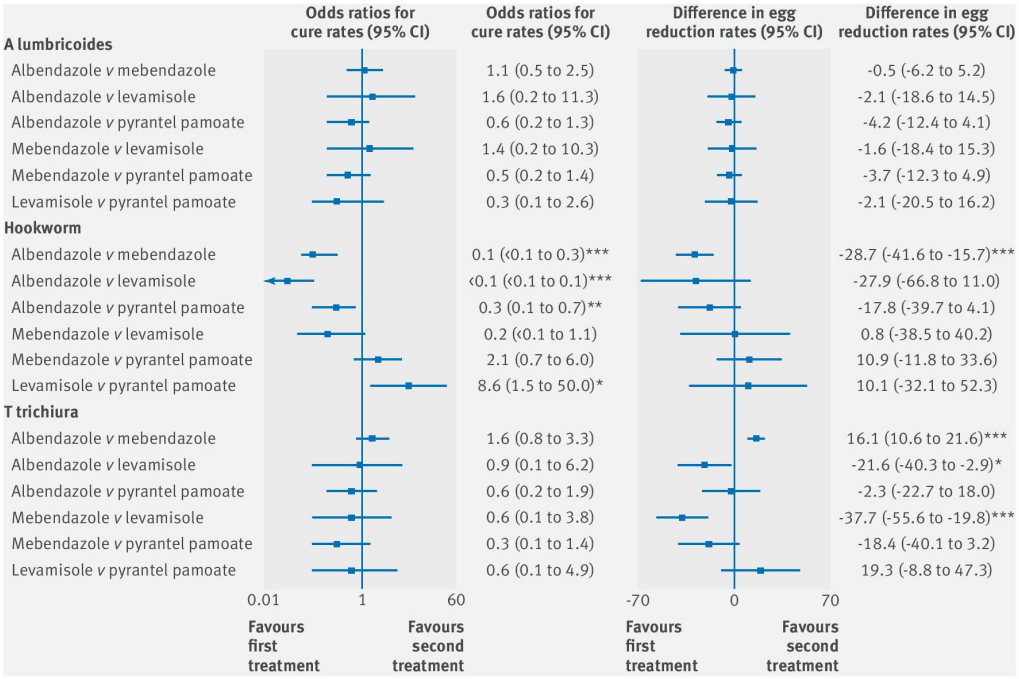

Fig 2 | Drug comparison based on network meta-analysis. Odds ratios for one to one comparisons of cure rates and difference for one to one comparisons of egg reduction rates are based on network meta-analysis for each drug and infection (*P<0.05, **P<0.01, ***P<0.001)

for small (n=30) and large (n=300; table K in appendix 2) studies and for years 1995 and 2015 (table L in appendix 2), to examine publication bias and evaluate the potential trends of efficacy over time.

We assessed the consistency between estimated odd ratios from direct and indirect comparisons by adding indicator variables for the two respective parallel treatments to the mixed logistic regression models. The difference of their regression coefficients was exponentiated to obtain the ratio between the odds ratio from direct and the odds ratio from indirect comparison (referred to as ratio of odds ratios). The two variables were obtained as the product of the respective treatment indicator variable and the indicator variable for studies that compared both treatments directly. The ratio of odds ratios (and 95% confidence intervals) measuring inconsistency between direct and indirect estimates are shown in appendix 3 (fig C). All analyses were done with STATA version 14.0 (StataCorp, College Station, TX, USA).

Results

Characteristics of included studies and bias assessment

We identified 301 potential studies of albendazole, mebendazole, levamisole, and pyrantel pamoate for treating soil transmitted helminth infections (fig 1). From these, we excluded 245: 82 were not randomised, 87 used a different dose regimen, 37 had follow-up longer than six weeks, 23 used only drug combinations, and data were not extractable from 16 (table B in appendix 2). From the 56 remaining studies, one included only egg reduction rates, while 10 did not report egg reduction rates. A total of 44 studies had data on cure rates against *A lumbricoides*, and 38 presented data on hookworm and *T trichiura*. For the analysis of egg reduction rates we included 34 studies for hookworm and 33 for *A lumbricoides* and *T trichiura*. Studies including treatments consisting of placebo and albendazole and albendazole and mebendazole were most common. The inconsistency plot showed considerable differences between odd ratios of cure from direct and indirect comparisons for some of the drug pairs (fig C in appendix 3), but none of these differences reached significance.

The percentages of studies in the lowest categories for risk of bias were 41.1% for random sequence generation, 30.4% for allocation concealment, and 51.8% for incomplete outcome data. The percentage of studies in the highest category for risk of bias was largest for blinding of participants and personnel (25.6%). The category of unclear risk was largest in all criteria other than "incomplete outcome data." This was especially pronounced among studies published before the year 2000 (table M in appendix 2).

Drug efficacy against *A lumbricoides*

We evaluated 44 studies with an average Jadad score of 2.5 and a total of 7273 participants positive for *A lumbricoides* (table N in appendix 2 gives detailed numbers) to evaluate the effect of the four anthelmintic drugs against *A lumbricoides* (fig 1). Pooled estimates were based on 19 studies with only one treatment,[25-43] 22 studies with two treatments,[12 43-63] and three studies with three eligible treatments.[64-66]

The four anthelmintic drugs investigated showed highly significant superiority (all P<0.001) over placebo (the average cure rate with placebo was 12.7% (95% confidence interval 6.7% to 22.7%; table 1). Estimated average cure rates were 95.7% (93.2% to 97.3%) for albendazole, 96.2% (92.3% to 98.1%) for mebendazole, 97.3% (84.2% to 99.6%) for levamisole, and 92.6% (85.6% to 96.3%) for pyrantel pamoate. There were no significant differences among the four treatments in the one to one comparison (fig 2).

Thirty three studies reported egg reduction rates[12 25 26 29 31 32 34-38 43-49 51 52 54-62 64 66 67] (fig 3). All treatment arms showed significantly higher rates (P<0.001) than placebo (20.7%, 95% confidence interval 14.7% to 26.7%; table 1), while there were no significant differences between the rates with the four treatments (fig 2). The highest estimated egg reduction rate (98.5%, 94.9% to 100.0%) was for albendazole, followed by 98.0% (94.0 to 100.0) for mebendazole, 96.4% (82.3 to 100.0) for levamisole, and 94.3% (88.3 to 100.0) for pyrantel pamoate.

Drug efficacy against hookworm

For estimating the drug efficacy against hookworm, we looked at data from 7178 individuals from 38 studies (table 1; table N in appendix 2) with an average Jadad score of 2.8. Pooled estimates included 12 studies with one treatment,[25 26 30-32 34 36 38 42 43 68] 21 studies with two treatments,[12 43 44 46-58 62 63 68-70] and five studies with three treatments.[64-66 71 72]

The cure rate was 15.2% (95% confidence interval 9.3% to 23.9%) for placebo (table 1). The rate with levamisole (10.3%, 2.4% to 35.2%) did not differ significantly from the placebo rate, but was significantly higher with albendazole (79.5%, 71.5% to 85.6%; P<0.001), mebendazole (32.5%, 20.8% to 46.9%; P=0.011), and pyrantel pamoate (49.8%, 29.5% to 70.1%; P=0.001). The one to one comparison of cure rates showed a strongly increased odds of cure after the administration of albendazole compared with mebendazole (P<0.001), levamisole (P<0.001), and pyrantel pamoate (P=0.005, fig 2). The odds for levamisole were significantly lower than the odds for pyrantel pamoate (P=0.016).

We used data from 34 studies[12 25 26 30-32 34 36 38 43 44 46-49 51 52 54-58 62 64 66-72] (fig 3) to determine an egg reduction rate of 16.2% (95% confidence interval 5.3% to 27.1%) for placebo, which was significantly lower than the rates for all active treatments (table 1). Albendazole had the highest average rate of 89.6% (81.9% to 97.3%), followed by pyrantel pamoate (71.9%, 54.7% to 89.0%), levamisole (61.8%, 30.3% to 93.3%), and mebendazole (61.0%, 52.0% to 69.9%). The one to one comparison showed a significant difference between albendazole and mebendazole (P<0.001, fig 2).

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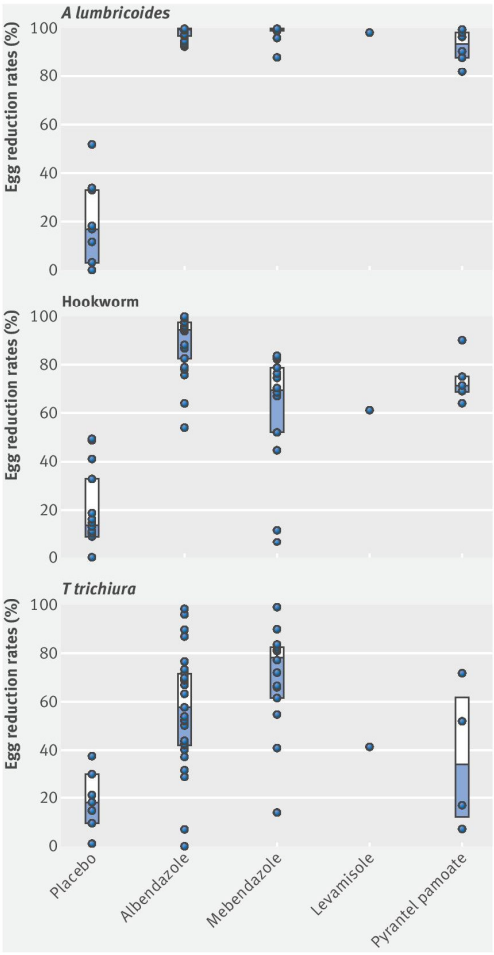

Fig 3 | Egg reduction rates for albendazole, mebendazole, levamisole, and pyrantel pamoate. Median, third quarter (white bar), second quarter (shaded bar), and individual study specific rates (solid circles) for each drug against *A lumbricoides*, hookworm, and *T trichiura*. Negative values of rates were set to zero in this figure

Drug efficacy against *T trichiura*

We used data from 38 studies (average Jadad score of 2.6), including 8841 participants positive for *T trichiura* (table 1; table N in appendix 2) for analysis of cure rates of the four drugs against *T trichiura*. Pooled estimates were based on 16 trials with one treatment,[25 26 29-33 35-38 40 42 43 73 74] 19 studies including two treatments,[12 43 44 46-51 53-59 62 63 75] and three studies with three treatments.[64-66]

The cure rate was 8.6% (95% confidence interval 4.1% to 17.1%) with placebo, which was not significantly different from the rates with levamisole (29.5%, 6.1% to 72.9%) and pyrantel pamoate (20.2%, 7.3% to 44.7%; table 1). Albendazole and mebendazole showed significantly higher efficacy than placebo, with estimated cure rates of 30.7% (21.0% to 42.5%; P<0.001) and 42.1% (25.9% to 60.2%; P<0.001), respectively. We found no significant differences among the rates of the four treatments comparing them one to one (fig 2).

We used 33 studies for analysis of the egg reduction rates[12 25 26 29 31 35-38 43 44 46-49 51 54 55-58 62-64 66 67 73-76] (fig 3). The average rate was 19.2% (95% confidence interval 6.9% to 31.4%) for placebo, which was significantly lower than the rates for albendazole (P<0.002), mebendazole (P<0.001), and pyrantel pamoate (P=0.008) but comparable with the rate for levamisole (28.3%, 6.7% to 49.8%; table 1). The highest rate of 66.0% (54.6% to 77.3%) was estimated for mebendazole, which was significantly higher than the rate for albendazole (49.9%, 39.0% to 60.6%; P<0.001) and levamisole in the one to one comparison (fig 2). For pyrantel pamoate the rate was 47.5% (25.5% to 69.6%).

Stratification by publication year (before *v* after 2000), resulted in a significantly reduced cure rate for albendazole (44.9% (95% confidence interval 29.4% to 61.5%) *v* 23.7% (14.2% to 36.7%); P=0.039; table J in appendix 2). The interaction analysis, with estimates for 1995 and 2015, showed a significant decrease in cure rates for albendazole from 38.6% (26.2% to 52.7%) to 16.4% (7.7% to 31.3%; P=0.027) and in egg reduction rates for albendazole from 72.6% (53.7% to 91.5%) to 43.4% (23.5% to 63.3%; P=0.049) and mebendazole from 91.4% (72.9% to 100.0%) to 54.7% (34.6% to 74.8%; P=0.014; table L in appendix 2).

Discussion

Summary of key findings

Albendazole, the most widely used anthelmintic drug against *A lumbricoides* and hookworm, is highly effective, both in terms of cure rates and egg reduction rates. With about 134 million doses distributed in 2015, mebendazole is the second most widely used drug for infections with soil transmitted helminths.[77] It has high efficacy against *A lumbricoides* and low activity against hookworm. Levamisole and pyrantel pamoate have high efficacy against *A lumbricoides*, and pyrantel pamoate has moderate efficacy against hookworm. The weakness of the currently available drugs is their low efficacy against *T trichiura*, for which mebendazole showed the best performance. This finding emphasises the urgent need for new drugs with higher efficacy against *T trichiura* for preventive chemotherapy programmes.[10 11]

Our review provides up to date evidence on the efficacy of the four recommended anthelmintic drugs—albendazole, mebendazole, and the less widely used levamisole and pyrantel pamoate—based on a thorough review of the literature. For the first time a network-meta analysis was applied, and we meta-analysed summary estimates on egg reduction rates, a key parameter for efficacy of anthelmintic drugs.[18]

Strength and limitations

The main strength of our study was the innovative data analysis including the two measures of efficacy of anthelmintic drugs: cure rates and egg reduction

rates. By applying a network meta-analysis, we could increase the evidence by including the efficacy results of a higher number of randomised controlled trials than in a previous meta-analysis.[11] Furthermore, the model from Kessels and colleagues[24] allowed the inclusion of studies with only one eligible treatment arm. To assess consistency of estimates, we compared odd ratios of cure from direct and indirect comparisons of the treatments with a plot (fig C in appendix 3). Although some of the differences were quite large, potentially challenging the validity of the indirect comparisons, none of the differences reached significance.

The reviewed randomised controlled trials cover the past 50 years of research. This inevitably leads to huge qualitative differences among the studies, which reflects the main challenge and limitation of our analyses. There were major disparities among the included studies, which affect drug efficacy—for instance, diagnostic method, infection intensity at baseline, statistical analyses, and sample size.

The diagnostic methods used in the reviewed studies ranged from lowest sensitivity methods, such as the direct smear, up to multiple Kato-Katz thick smears, which have a reasonable sensitivity. The sensitivity of diagnostic methods is associated with the infection intensity at baseline—for example, Kato-Katz has a reduced sensitivity for low egg counts.[78] Both the diagnostic method and infection intensity at baseline directly influence cure rates and egg reduction rates.[79-81] While the results stratified by infection intensities did not show a clear tendency for efficacy in this review, the sensitivity of the diagnostic method had an impact. Stratification of efficacy by low and moderate sensitivity of the diagnostic method significantly decreased cure rates of albendazole against *A lumbricoides* (P=0.044) and hookworm (P=0.023). We cannot, however, explain the increase in egg reduction rates with albendazole (P=0.024) against *T trichiura* (table E in appendix 2).

An additional limitation of the diagnostic methods (Kato-Katz, McMaster, etc) is their inability to distinguish between *A duodenale* and *N americanus*. Few included studies reported efficacies for specific hookworm species. The overall efficacies might differ according to the species. For example, while both hookworm species are somewhat susceptible to pyrantel pamoate, *N americanus* is reported to be less sensitive.[82] The commonly higher abundance of *N americanus* in Africa than in Asia[83] might have led to the borderline significant difference (P=0.053) in cure rates of pyrantel pamoate in Asia (64%) and Africa (27%; table C in appendix 2).

The network meta-analysis for egg reduction rates was limited by the lack of precision estimates in most of the studies and by the different choices of the measure of central tendency (arithmetic or geometric mean). There is an ongoing debate about advantages and disadvantages of the two systematically different means, while WHO now recommends the arithmetic mean.[18 84] A few, mainly older, studies did not even report which measure of central tendency they used. In the absence of standard errors and confidence intervals, we could not optimise the precision of meta-analytic estimates. Moreover, as arithmetic and geometric means are systematically different, we had to adjust analyses of egg reduction rates for the type of mean.

To deal with potential publication bias, we compared results of smaller and larger studies in an interaction analysis. We might have slightly overestimated the effect of albendazole against hookworm, where the cure rate showed an almost significant negative association with study size (P=0.053). While the cure rates of *A lumbricoides* showed positive or stable associations with study size for all treatments, the rates of *T trichiura* after treatment with albendazole and mebendazole slightly decreased with increasing study size, yet not significantly. Thus, we did not find consistent evidence of publication bias (table J in appendix 2). The small number of available and eligible studies for levamisole is another limitation of our work. Consequently, all estimates relating to levamisole (cure rates, egg reduction rates, and odd ratios) have wide confidence intervals. Nonetheless, we present the first pooled estimates of efficacy for levamisole against hookworm, showing a low average cure rate (10.3%, with an upper 95% confidence limit of 35.2%), which conflicts with the fact that the drug is recommended for the treatment.[3 11]

Clinical implications

Efficacy of anthelmintic drugs is defined by cure rates and egg reduction rates. As both parameters have to be taken into consideration in comparisons of the efficacy of the drugs for each helminth species, the comparison was done qualitatively. Against *A lumbricoides* we found no significant differences, and all drugs had high efficacy. Albendazole had the highest efficacy for treating hookworm infections with significantly higher cure rates, followed by pyrantel pamoate, and lowest efficacy for levamisole and mebendazole when used at single oral doses. With regard to *T trichiura* infections, mebendazole had the highest, yet only moderate, efficacy, with significantly higher egg reduction rates than albendazole. The cure rates of levamisole and pyrantel pamoate did not differ from placebo.

Moreover, after stratification by year, we found a significant decrease in cure rates for albendazole against *T trichiura* (P=0.039) and a remarkable reduction against hookworm (table I in appendix 2). These results were even more pronounced in the interaction analysis. The cure rates for albendazole against *T trichiura* remained significantly lower (P=0.027). Furthermore, egg reduction rates of albendazole (p=0.027) against hookworm and of albendazole (P=0.049) and mebendazole (P=0.014) against *T trichiura* (table K in appendix 2) significantly decreased over time, which might be attributable to drug resistance.[85] Several studies correlated reduced efficacies of benzimidazoles[58 64 70-72 86] with emerging resistance. In 2015, more than a billion people infected with lymphatic filariasis and soil transmitted helminths

RESEARCH

were treated with albendazole,[9] which is causing high drug pressure on parasites and might trigger drug resistance. In veterinary medicine, frequently repeated treatment with benzimidazoles has caused resistance in numerous nematode species.[84 87 88] Resistance to anthelmintic drugs in humans, however, has not yet been shown. While the reduction in efficacy could be explained by emerging resistance, other factors, related to drug regimen, diagnostics, or host and parasite characteristics, might have contributed to the reduction.[18] We evaluated the impact of some potential confounders but did not assess the influence of, for example, drug quality (original versus generic drugs), change in compliance over the years, or the day to day variation in egg excretion.[18 89] Hence, future randomised controlled trials should follow a harmonised design to reduce confounders, as suggested by WHO,[18] which will yield improved summary estimates of efficacy of anthelmintic drugs.

Conclusion

Our data confirm that the most widely used drugs—albendazole and mebendazole—have shortcomings in their efficacy profile, especially against infections with hookworm and *T trichiura*. Alarmingly, the efficacy of albendazole and mebendazole has decreased over time. As the two most widely distributed drugs in preventive chemotherapy—albendazole and mebendazole—have been in use for almost 50 years, the threat of resistance is real and immediate. For careful monitoring of potential resistance, our summary estimates might help to revise current reference figures of efficacy.[18]

There is an imminent need to strengthen efforts to develop new drugs for soil transmitted helminths. Alternatively, old and new drugs—such as tribendimidine, oxantel pamoate, moxidectin, or ivermectin—with different efficacy profiles could be used in combination with the recommended drugs to successfully tackle infections with all three soil transmitted helminths.[12 90] Only with an integrated approach combining improved sanitation, health education,[91-93] and scaling up of research for new anthelmintic drugs and use of drug combinations for preventive chemotherapy will we achieve the ultimate goal to control soil transmitted helminth infections. Furthermore, future randomised controlled trials should follow a harmonised design to reduce confounders and yield improved summary estimates of efficacy of anthelmintic drugs.

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Appendix 1: Study protocol and search terms
Appendix 2: Supplementary tables A-N
Appendix 3: Supplementary figures A-C

Chapter 3

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

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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

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Summary

Background Commonly used drugs for preventive chemotherapy against soil-transmitted helminths (ie, albendazole and mebendazole) show low efficacy against *Trichuris trichiura*. Recent studies with oxantel pamoate revealed good cure rates and high egg-reduction rates against *T trichiura*. We aimed to assess the nature of the dose–response relation to determine the optimum dose.

Methods We did a parallel, randomised, placebo-controlled, single-blind trial with oxantel pamoate in school-aged children (aged 6–14 years) infected with *T trichiura* on Pemba Island, Tanzania. Children were asked to provide two stool samples and children positive for *T trichiura* were eligible to participate in the trial. Children were excluded if they suffered from any systematic illness. Children were randomly assigned to six different oxantel pamoate doses (5–30 mg/kg) or a placebo. Randomisation was stratified by baseline infection intensity using random block sizes of seven and 14. The primary endpoints were cure rates and egg-reduction rates against *T trichiura*, both analysed by available case. Drug safety was assessed 2 h and 24 h after treatment. The trial is registered at www.isrctn.com, number ISRCTN86603231.

Findings Between Oct 14, and Nov 28, 2014, we enrolled 480 participants and randomly assigned 350 children to the different oxantel pamoate doses or the placebo. 5 mg/kg oxantel pamoate was the minimum effective dose (10 of 46 children cured [cure rate 22%, 95% CI 11–36]; egg-reduction rate 85·0%, 64·5–92·9). An increased probability of being cured and reduced egg counts with escalating doses was recorded. At 25 mg/kg oxantel pamoate 27 of 45 children were cured (cure rate 60%, 95% CI 44–65) with an egg-reduction rate of 97·5% (94·4–98·9), and at 30 mg/kg 27 of 46 children were cured (59%, 43–73) with an egg-reduction rate of 98·8% (96·8–99·6). Oxantel pamoate was well tolerated across all treatment groups; only mild adverse events were reported by the participants 2 h (27 [10%]) and 24 h (12 [4%]) after treatment.

Interpretation Our dose-finding study revealed an excellent tolerability profile of oxantel pamoate in children infected with *T trichiura*. An optimum therapeutic dose range of 15–30 mg/kg oxantel pamoate was defined. With a weight independent dose of 500 mg oxantel pamoate 95% of children aged 7–14 years in sub-Saharan Africa would receive doses of 11·7–32·0 mg/kg. Future research should include studies with oxantel pamoate in younger children and on different continents with the ultimate goal to be able to add oxantel pamoate to soil-transmitted helminth control programmes.

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See Comment page 5

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Introduction

The global burden of infections with soil-transmitted helminths is substantial. According to figures from 2010,[1] 5·3 billion people are at risk and 1·5 billion people are infected with soil-transmitted helminths, in brief: 820 million people are infected with *Ascaris lumbricoides*, 440 million with hookworm, and 470 million with *Trichuris trichiura*.[2–4] Soil-transmitted helminth infections mostly occur in tropical and subtropical regions with the highest prevalence in Asia and Africa.

WHO announced the goal of reducing the prevalence of moderate and heavy soil-transmitted helminth infections in preschool and school-aged children to a level at which they would not be deemed a public health problem any longer.[5] Preventive chemotherapy with the two benzimidazoles (albendazole and mebendazole) is the most widely used and cost-effective strategy against *A lumbricoides*, hookworm, and *T trichiura* infections.[6] Annually, about 1 billion tablets of albendazole (against soil-transmitted helminth infections and lymphatic filariasis) and 200 million tablets of mebendazole are donated by GlaxoSmithKline and Johnson & Johnson, respectively.[6,7] Albendazole and mebendazole have excellent cure rates against *A lumbricoides*; however, albendazole is more effective against hookworm and both treatments revealed only low-to-moderate efficacy against *T trichiura* when given a single oral dose, in particular in high infection intensity settings.[8–10] Hence, research for the development of new drugs and alternative treatments is urgently needed[8,9,11] to reach the ambitious goal of WHO.[5]

Oxantel pamoate is a pyrimidine derivate developed from pyrantel, with excellent activity against *T trichiura*. The drug causes spastic paralysis of nematodes because of the selective gating of the acetylcholine receptor ion

Research in context

Evidence before this study

We searched PubMed, Embase, and Google Scholar for studies published between Jan 1, 1970, and May 1, 2015. All published articles in any language with a combination of the terms "*Trichuris trichiura*", "drug", "oxantel pamoate", and "dose" were included. Our literature search identified a systematic review and meta-analysis, and several clinical trials, and confirmed the low efficacy of the current standard drugs (ie, albendazole and mebendazole) against *T trichiura*. To overcome this unsatisfactory situation the old anthelminthic oxantel pamoate was reconsidered in two recent clinical trials. At present, oxantel pamoate presents the most efficacious drug against *T trichiura*. Despite promising results, the optimum dose and the dose–response relation has not been assessed.

Added value of this study

This study continued the recent successful work on oxantel pamoate. The results of our parallel, randomised, controlled, dose-ranging study, confirm the good trichuricidal activity of oxantel pamoate. Furthermore, this trial provides the first data on the efficacy of six different oxantel pamoate doses against *T trichiura*. The highest cure rate was recorded with 25 mg/kg oxantel pamoate. We identified an optimum dose range of 15–30 mg/kg, which had excellent egg-reduction rates. Only a low number of mild adverse events were reported among the different treatment groups. Preventive chemotherapy is the method of choice to control infections with soil-transmitted helminths and is implemented in large parts of the world. To facilitate the delivery of oxantel pamoate, we determined its weight-independent dose. Our calculations gave a dose of 500 mg oxantel pamoate for sub-Saharan African children in preventive chemotherapy programmes.

Implications of all the available evidence

By 2020, WHO aims to achieve a 75% treatment coverage in all countries in which soil-transmitted helminths are a public health problem. Albendazole and mebendazole are the predominant drugs used for preventive chemotherapy but they have limitations. The development of an efficacious drug against *T trichiura*, which could be added to the small armamentarium, is therefore of highest priority. Our data suggest that in large-scale preventive chemotherapy programmes a weight-independent dose of 500 mg oxantel pamoate in sub-Saharan African children could be used, but further safety and efficacy trials are necessary.

channels on nerves and muscles.[12] Oxantel pamoate is marketed in combination with pyrantel pamoate (Quantrel) at a recommended dose of 10–20 mg/kg for soil-transmitted helminth infections in Colombia and Peru. In 1976, cure rates of between 93% and 100% were reported,[13,14] treating patients with 20 mg/kg oxantel pamoate. With half of the dose (10 mg/kg), still 56·6% and 77% of the participants were cured.[13,14] In 2014, Speich and colleagues[9] reported a lower cure rate of 26·3% with an administered dose of 20 mg/kg. Oxantel pamoate is poorly absorbed by human beings and hence, all studies described mainly mild adverse events.[9,11,13–17]

However, proper dose-finding studies with oxantel pamoate have not been done. Determining the adequate dose and characterising the drug's dose–response relation are a crucial part of clinical drug development. We did a parallel, randomised, placebo-controlled trial to compare the efficacy of six different oxantel pamoate doses (between 5 mg/kg and 30 mg/kg) to assess the nature of the dose–response relation and determine the optimum dose.

Methods

Study design and participants

This parallel, randomised, placebo-controlled, dose-finding, single-blinded trial was conducted from Oct 14, to Nov 28, 2014, on Pemba Island, Tanzania. Two primary schools were chosen, Matale and Mbuzini.

Children aged 6–14 years were asked to provide two stool samples and children positive for *T trichiura* were eligible to participate in this trial. At the initial clinical assessment, a study physician physically examined all eligible children and obtained an oral medical history by active questioning. Children were excluded if they suffered from any systematic illness (eg, clinical malaria or hepatosplenic schistosomiasis).

Ethical approval was obtained from the Zanzibar Medical Research and Ethical Committee (ZAMREC, reference number 0002/August/2014) and from the Ethics Committee of Northern and Central Switzerland (EKNZ, reference number 2014-315). Before enrolment written informed consent was obtained from parents or legal guardians of the invited children and the children provided verbal assent.

Randomisation and masking

We used a computer-generated stratified (by baseline infection intensities) block randomisation code (varying block size of either seven or 14) provided by an independent statistician. Study participants entitled for treatment were allocated to the placebo or one of six oxantel pamoate treatment groups: 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, and 30 mg/kg. Only the study site investigator was aware of the treatment assignments; children and laboratory technicians doing the diagnostics were masked.

400 mg and 100 mg oxantel pamoate tablets[18] and identical matching 400 mg placebo tablets were manufactured at the University of Basel. Drug quality was assured for all products and approved by the Zanzibar Food and Drug Board (reference number MMBLZ/15/2014) for use in human beings. Oxantel pamoate was given in

50 mg (half of a 100 mg tablet) increments, according to the calculated dose per kg of bodyweight.

Procedures

Before the beginning of the study, permission to conduct the trial at the two schools was obtained from the headmasters. In an information meeting held with parents or legal guardians of the children from class 1 to 5, the purpose and procedures of the study including potential benefits and risks were explained in detail.

For the baseline, two stool samples, labelled with unique identification numbers were collected on two consecutive days and transferred to the Public Health Laboratory-Ivo de Carneri (PHL-IdC), Chake Chake, Tanzania. From each stool sample, duplicated Kato-Katz thick smears were prepared using 41·7 mg templates.[19] The Kato-Katz thick smears were quantitatively examined under a light microscope for *T trichiura*, *A lumbricoides*, and hookworm eggs by six experienced laboratory technicians. To avoid overclearing of hookworm eggs, the Kato-Katz thick smears were read within 1 h after the preparation on the collection day.[20] 10% of all slides were randomly chosen and re-examined for quality control.[21] Deviating results were read a third time and discussed with the laboratory technicians until agreement was reached.

Before treatment, clinical signs and symptoms of all children were investigated with a standardised and previously used questionnaire. Height was measured with a standard meter (to the nearest 1 cm) and weight with an accurate electronic balance (to the nearest 0·1 kg). Adverse events (gastrointestinal and systemic symptoms) were recorded by active questioning and grading symptoms at two points after treatment (2 h and 24 h) and mitigating drugs were provided if necessary. To assess treatment efficacy, another two stool samples were collected between 20 days and 26 days after treatment for the follow-up. At the end of the study, all children from the two schools received 400 mg albendazole according to national guidelines[22,23] and participating children still infected with *T trichiura* obtained oxantel pamoate at a dose of 20 mg/kg.

Outcomes

The primary endpoints were cure rates and egg-reduction rates against *T trichiura* at follow-up. Secondary endpoints were cure rates and egg-reduction rates against concomitant nematode infections (*Ascaris lumbricoides* and hookworm) and drug safety (assessed 2 h and 24 h after treatment).

Statistical analysis

Since the existence of a drug effect is well known for oxantel pamoate, the main aim of this study was to understand the dose–response relation. Based on earlier studies[9,11] we assumed the following cure rates for the six treatment oxantel pamoate groups: 20% (5 mg/kg), 30% (10 mg/kg), 40% (15 mg/kg), 50% (20 mg/kg), 55% (25 mg/kg), and 60% (30 mg/kg). Initial computer simulation models showed that with 40 children enrolled in each of the seven study groups the dose–response prediction model will have a median precision (one half length of the 95% CIs) of 5% points, assuming the cure rates mentioned above.

We assumed a prevalence of 80% *T trichiura* infection based on two recent trials in nearby settings.[9,11] The sample size was increased by 25% to account for

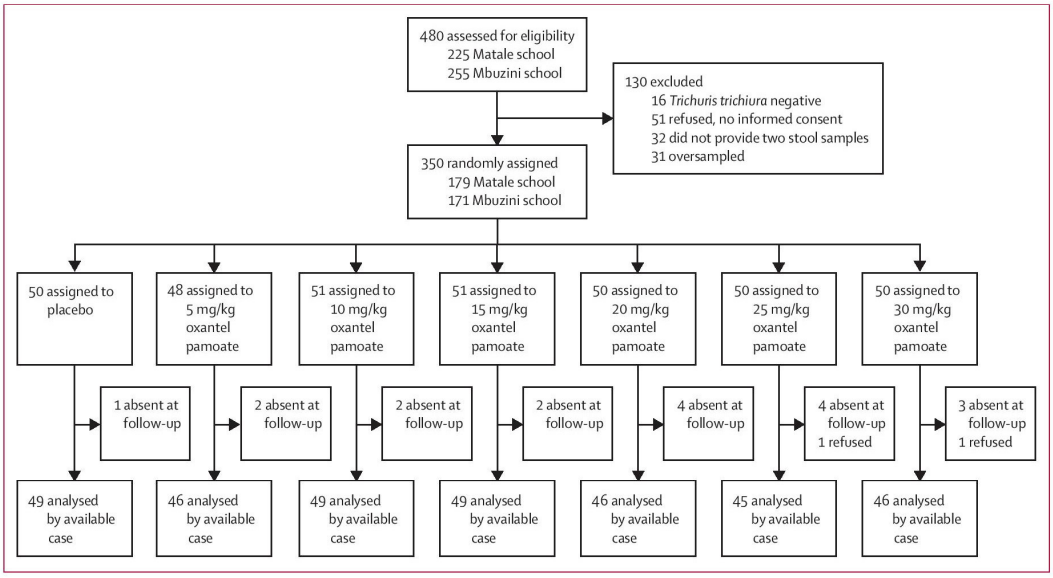

Figure 1: **Trial profile**

	Placebo (n=50)	Oxantel pamoate						Total (n=350)
		5 mg/kg (n=48)	10 mg/kg (n=51)	15 mg/kg (n=51)	20 mg/kg (n=50)	25 mg/kg (n=50)	30 mg/kg (n=50)	
Age (years)	10·6 (1·9)	10·6 (1·7)	10·2 (1·8)	10·4 (1·7)	10·5 (1·6)	10·7 (1·6)	10·7 (1·8)	10·5 (1·7)
Girls	33 (66%)	28 (58%)	26 (51%)	29 (57%)	30 (60%)	28 (56%)	25 (50%)	199 (57%)
Matale school	27 (54%)	24 (50%)	26 (51%)	25 (49%)	24 (48%)	25 (50%)	28 (56%)	179 (51%)
Weight (kg)	28·8 (6·9)	28·6 (5·2)	27·3 (5·0)	28·4 (5·6)	28·7 (6·9)	27·7 (5·5)	28·7 (6·1)	28·3 (5·9)
Height (cm)	133·7 (10·1)	134·0 (8·1)	131·5 (9·1)	133·4 (8·3)	133·8 (9·3)	133·2 (9·9)	132·9 (9·0)	133·2 (9·1)
Trichuris trichiura								
Infected children	50 (100%)	48 (100%)	51 (100%)	51 (100%)	50 (100%)	50 (100%)	50 (100%)	350 (100%)
EPG geometric mean	618 (3·9)	537 (3·4)	595 (4·9)	539 (4·2)	538 (4·3)	435 (4·2)	563 (5·3)	544 (4·3)
Infection intensity								
Light (1–999 EPG)	30 (60%)	31 (65%)	31 (61%)	33 (65%)	34 (68%)	33 (66%)	30 (60%)	222 (63%)
Moderate (1000–9999 EPG)	19 (38%)	17 (35%)	19 (37%)	18 (35%)	15 (30%)	17 (34%)	19 (38%)	124 (35%)
Heavy (≥10 000 EPG)	1 (2%)	0	1 (2%)	0	1 (2%)	0	1 (2%)	4 (1%)
Ascaris lumbricoides								
Infected children	21 (42%)	20 (42%)	25 (49%)	23 (45%)	19 (38%)	26 (52%)	22 (44%)	156 (45%)
EPG geometric mean	3139 (10·2)	1265 (10·6)	1826 (8·2)	2657 (9·1)	2083 (7·8)	1899 (12·6)	1906 (10·3)	2038 (9·5)
Infection intensity								
Light (1–4999 EPG)	10 (48%)	14 (70%)	18 (72%)	12 (52%)	15 (79%)	16 (62%)	14 (64%)	99 (63%)
Moderate (5000–49 999 EPG)	11 (52%)	6 (30%)	6 (24%)	10 (43%)	4 (21%)	10 (38%)	7 (32%)	54 (35%)
Heavy (≥50 000 EPG)	0	0	1 (4%)	1 (4%)	0	0	1 (5%)	3 (2%)
Hookworm								
Infected children	15 (30%)	19 (40%)	14 (27%)	18 (35%)	20 (40%)	19 (38%)	20 (40%)	125 (36%)
EPG geometric mean	77 (3·8)	66 (4·5)	52 (3·1)	55 (2·5)	99 (4·3)	88 (3·9)	81 (3·8)	73 (3·7)
Infection intensity								
Light (1–1999 EPG)	15 (100%)	19 (100%)	14 (100%)	18 (100%)	19 (95%)	19 (100%)	20 (100%)	124 (99%)
Moderate (2000–3999 EPG)	0	0	0	0	1 (5%)	0	0	1 (1%)

Data are mean (SD) or n (%) unless otherwise indicated. EPG=eggs per g of stool.

Table 1: **Baseline characteristics of children infected with *T trichiura* stratified by oxantel pamoate treatment group**

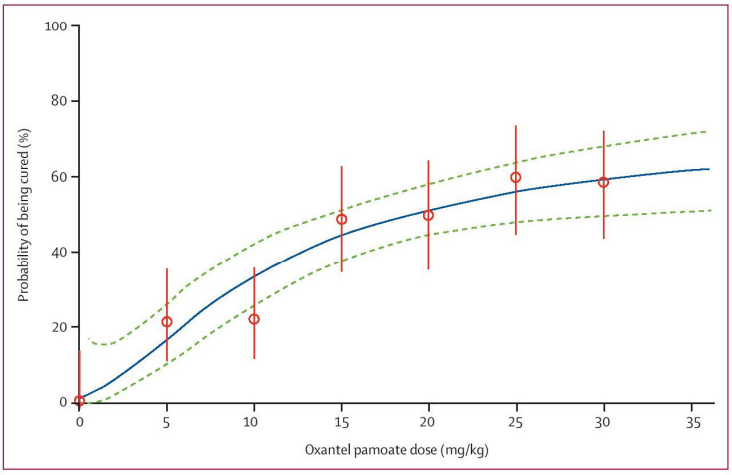

Figure 2: **E_{max} model for the cure rates of the placebo and six different oxantel pamoate doses against *Trichuris trichiura* in school-aged children**

See Online for appendix

potential loss to follow-up and hence 480 children were enrolled for detecting at least 350 children infected with *T trichiura*.

Data were double-entered into a database (Access 2003, Microsoft), compared with EpiInfo version 3.3.2 (Centre for Disease Control and Prevention; Atlanta, GA, USA), and analysed with Stata version 12.1 (Stata Corporation; College Station, TX, USA) and R version 3.0.2 (www.r-project.org).

An available case analysis was done including all children who did not miss treatment and provided two stool samples (one case with only one stool sample was included) at the follow-up. For the primary objective the nature of the dose–response relation was determined assuming E_{max} models using the DoseFinding package (version 0.9-12) of the statistical software environment R. The dose–response curve was estimated for cure rate and egg-reduction rate. A sensitivity analysis was done to assess the potential effect of children lost to follow-up. We reassessed the dose–response curve treating once all missing data as treatment failure and once as treatment success (appendix). The minimum effective dose was assessed using the bias reduced logistic regression to account for quasi separation. Since E_{max} models are monotonic, the maximum effective dose was approximated as the dose that elicits the lower confidence limit of the predicted maximum.

For the primary endpoint and secondary endpoints the cure rates were calculated as the percentage of egg-

positive children at baseline, who become egg-negative after treatment at follow-up. The number of eggs per g of stool was assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears (from baseline and follow-up separately) and multiplying this number by a factor of six. The classification of the infection intensities followed WHO cutoffs.[24]

Geometric mean egg counts were calculated according to earlier guidelines of WHO[24] for the seven different treatment groups before and after treatment to determine the corresponding egg-reduction rate (ERR=(1−[mean at follow-up / mean at baseline])×100). Ordinary non-parametric bootstrap resampling within each trial group (2000 replicates) was used to calculate 95% CIs for the egg-reduction rates.

The mean weight distributions of sub-Saharan African children (boys and girls) aged 7–14 years were studied[25,26] to calculate a weight-independent dose of oxantel pamoate. The trial is registered at www.isrctn.com, number ISRCTN86603231.

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

	Placebo	Oxantel pamoate					
		5 mg/kg	10 mg/kg	15 mg/kg	20 mg/kg	25 mg/kg	30 mg/kg
Trichuris trichiura							
Children positive before treatment	49	46	49	49	46	45	46
Children cured after treatment	0	10 (22%, 11 to 36; p=0·02)*	11 (22%, 12 to 37; p=0·02)*	24 (49%, 34 to 64; p=0·002)*	23 (50%, 35 to 65; p=0·002)*	27 (60%, 44 to 74; p=0·0007)*	27 (59%, 43 to 73; p=0·0008)*
Children cured/total with infection							
From light infection	0/30	8/30 (27%)	9/29 (31%)	19/33 (58%)	18/32 (56%)	21/31 (68%)	18/27 (67%)
From moderate infection	0/18	2/16 (13%)	2/19 (11%)	5/16 (31%)	5/13 (38%)	6/14 (43%)	9/18 (50%)
From heavy infection	0/1	0/0	0/1	0/0	0/1	0/0	0/1
EPG geometric mean							
Before treatment	611 (3·9)	547 (3·3)	627 (4·9)	515 (4·2)	519 (4·3)	399 (4·2)	608 (5·4)
After treatment	400 (4·0)	82 (14·9)	85 (15·9)	15 (18·6)	12 (16·0)	10 (22·2)	7 (16·8)
Egg-reduction rate (95% CI)	34·5% (6·4 to 56·8)	85·0% (64·5 to 92·9)	86·4% (75·0 to 92·7)	97·1% (92·2 to 98·7)	97·7% (95·3 to 98·8)	97·5% (94·4 to 98·9)	98·8% (96·8 to 99·6)
Ascaris lumbricoides							
Children positive before treatment	20	20	25	22	18	21	21
Children cured after treatment	3 (15%, 3 to 38)	9 (45%, 23 to 69)	7 (28%, 12 to 49)	8 (36%, 17 to 59)	4 (22%, 6 to 48)	5 (24%, 8 to 47)	7 (33%, 15 to 57)
EPG geometric mean							
Before treatment	2781 (10·1)	1281 (10·6)	1842 (8·2)	3006 (8·9)	2172 (8·2)	2590 (9·1)	1857 (10·9)
After treatment	1368 (32·3)	51 (77·3)	457 (51·6)	240 (82·2)	331 (44·2)	721 (57·5)	268 (67·2)
Egg-reduction rate (95% CI)	50·8% (−18·9 to 86·5)	96·0% (81·7 to 99·2)	75·2% (36·0 to 89·8)	92·0% (75·2 to 98·2)	84·8% (40·3 to 96·4)	72·2% (7·6 to 94·5)	85·6% (42·5 to 97·3)
Hookworm							
Children positive before treatment	14	17	13	17	18	18	19
Children cured after treatment	3 (21%, 5 to 51)	8 (47%, 23 to 72)	3 (23%, 5 to 54)	6 (35%, 14 to 62)	6 (33%, 13 to 59)	5 (28%, 10 to 53)	3 (16%, 3 to 40)
EPG geometric mean							
Before treatment	89 (3·6)	48 (3·2)	57 (3·1)	52 (2·6)	75 (3·3)	91 (4·1)	94 (3·4)
After treatment	51 (11·6)	9 (16·2)	41 (11·2)	16 (10·4)	17 (9·9)	32 (11·4)	59 (9·5)
Egg-reduction rate (95% CI)	42·7% (−50·8 to 77·9)	81·3% (28·8 to 94·5)	28·1% (−142·8 to 82·7)	69·2% (10·6 to 87·8)	77·3% (56·3 to 89·5)	64·8% (4·8 to 90·4)	37·2% (−51·5 to 70·1)

Data are n, n/N (%), n (%, 95% CI), or geometric mean (SD) unless otherwise indicated. EPG=egg per g of stool. *p values derived from logistic regression with placebo as reference to determine the minimum effective dose.

Table 2: **Cure rates and egg-reduction rates of the placebo and the different oxantel pamoate dose groups**

responsibility for the decision to submit for publication.

Results

Of 480 children invited to participate, 397 children gave informed consent and provided two stool samples at baseline (figure 1). Of these, 381 (96%) were infected with *T trichiura*, 173 (44%) were infected with *A lumbricoides*, and 147 (37%) were infected with hookworm. Triple infections were recorded in 88 (22%) children. We randomly assigned 350 children infected with *T trichiura* to the seven treatment groups; 31 children were excluded due to oversampling. 18 children were absent at follow-up and two refused to provide stool samples (figure 1).

Demographic and parasitological baseline data for all treated children are presented in table 1. All treatment groups were well balanced by infection intensities according to WHO cutoffs,[24] with respect to age, sex, weight, and height. 222 (63%) children were classified with a light *T trichiura* infection (<1000 eggs per g of stool), 124 (35%) had a moderate infection (1000–9999 eggs per g of stool), and four children (1%) were heavily infected (≥10 000 eggs per g of stool). Children infected with *A lumbricoides* were classified as light (99 children, 63%), moderate (54 children, 35%), and heavy infections (three children, 2%). Hookworm infections were predominantly diagnosed as light (124 children, 99%).

The estimated dose–response curve of the E_{max} model is shown in figure 2. Consistent with the E_{max} model, the observed cure rates generally increased with an increased dose (table 2). Ten of 46 children were cured with the lowest dose of 5 mg/kg oxantel pamoate (cure rate 22%, 95% CI 11–36) and differed significantly from the placebo group in which none of the children were cured (p=0·02). Therefore, 5 mg/kg represents the minimum effective dose, which is in good agreement with the E_{max} model. The highest cure rates were recorded for 25 mg/kg (27 of 45 cured; cure rate 60%, 95% CI 44–74) and 30 mg/kg oxantel pamoate (27 of 46; 59%, 43–73). Cure rates for all doses are shown in table 2. No noteworthy differences were recorded after adjusting for sex, age, height, weight, and schools.

The lowest two oxantel pamoate doses resulted in egg-reduction rates of 85·0% (95% CI 64·5–92·9) for 5 mg/kg and 86·4% (75·0–92·7) for 10 mg/kg. Doses of 15 mg/kg to 30 mg/kg oxantel pamoate achieved similar high egg-reduction rates (15 mg/kg: 97·1%, 95% CI 92·2–98·7; 30 mg/kg: 98·8%, 96·8–99·6). The E_{max} model predicted a dose of 17 mg/kg for an egg-reduction rate of 95% and 27 mg/kg for an egg-reduction rate of 98% (appendix). The placebo group had an egg-reduction rate of 34·5% (95% CI 6·4–56·8). Arithmetic means of the egg counts are presented in the appendix.

Although the number of children without follow-up data was relatively small, we re-estimated the dose–response curve making different assumptions about the missing data. Evidently the estimated cure rates were lower in scenarios with treatment failure and higher with treatment success. However, the predictions differed only slightly from the original estimate (missing data omitted). The curves are provided in the appendix. We also assessed different dose–response models in addition to the E_{max} model (linear, log-linear, sigmoid-E_{max}, and logistic). Visual inspection revealed that the prespecified E_{max} model fits best to the recorded data.

We calculated a weight-independent dose of 500 mg oxantel pamoate for sub-Saharan African children (figure 3). With this dose, 95% of sub-Saharan African school-aged children will receive a minimum of 11·7 mg/kg and a maximum of 32·0 mg/kg oxantel pamoate.

We recorded no substantial effect of oxantel pamoate dose against *A lumbricoides* and hookworm. Against *A lumbricoides* cure rates of 22–45% and egg-reduction rates of 72–96% (table 2) were recorded. However, the CIs were broad and not different from the placebo group (cure rate 15% and egg-reduction rate 51%). Additionally, there was no indication of a dose–response relation. A similar picture was recorded for hookworm (cure rates 16–47% and egg-reduction rates 28–81%).

Before treatment, overall 15 children (5%, ranging from one to five children among the different treatment groups) suffered from mild clinical symptoms, with three children reporting more than one mild symptom. 2 h after treatment, overall 27 children (10%) reported mild adverse events (seven children had more than one mild adverse event; table 3). 24 h after treatment the adverse event rate decreased to 4% (12 children). For comparison, two (4%) placebo-treated children reported a symptom before treatment (one child had three symptoms), whereas four children (8%) reported one or more adverse events 2 h and 24 h after treatment. Any moderate or

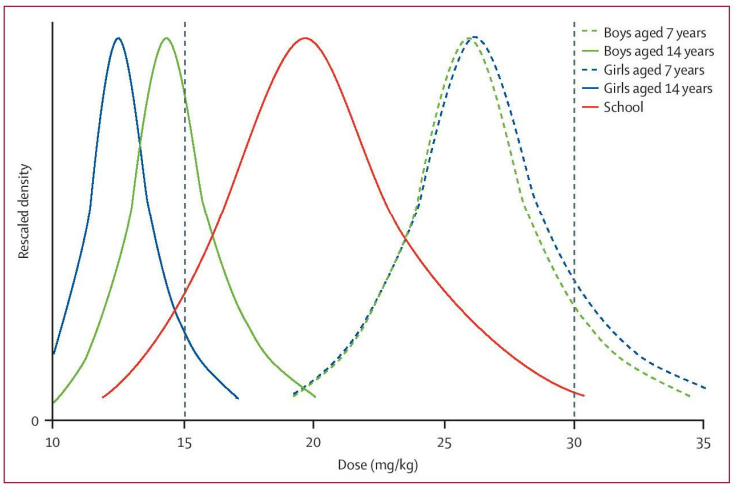

Figure 3: **Distribution of the estimated dose of oxantel pamoate in sub-Saharan African school-aged children (7–14 years) after a 500 mg oxantel pamoate dose**
The densities from the interpolated data[26] have been rescaled to have maxima at the same height. Dashed grey lines show the optimum dose range of 15–30 mg/kg oxantel pamoate.

	Placebo (n=49)	Oxantel pamoate						
		5 mg/kg (n=46)	10 mg/kg (n=49)	15 mg/kg (n=49)	20 mg/kg (n=46)	25 mg/kg (n=45)	30 mg/kg (n=46)	Total (n=281)
Symptoms present before treatment								
Mild	2 (4%)	2 (4%)	5 (10%)	3 (6%)	1 (2%)	2 (4%)	2 (4%)	15 (5%)
Abdominal cramps	1 (2%)	1 (2%)	1 (2%)	0	0	0	1 (2%)	3 (1%)
Headache	1 (2%)	0	2 (4%)	1 (2%)	1 (2%)	0	1 (2%)	5 (2%)
Symptoms present 2 h after treatment								
Mild	4 (8%)	6 (13%)	4 (8%)	2 (4%)	5 (11%)	6 (13%)	4 (9%)	27 (10%)
Abdominal cramps	2 (4%)	3 (7%)	1 (2%)	1 (2%)	2 (4%)	3 (6%)	4 (9%)	14 (5%)
Headache	1 (2%)	1 (2%)	2 (4%)	1 (2%)	2 (4%)	2 (4%)	0	8 (3%)
Symptoms present 24 h after treatment								
Mild	4 (8%)	1 (2%)	2 (4%)	1 (2%)	3 (7%)	2 (4%)	3 (7%)	12 (4%)
Abdominal cramps	2 (4%)	0	2 (4%)	0	1 (2%)	1 (2%)	2 (4%)	6 (2%)
Headache	0	1 (2%)	0	0	2 (4%)	1 (2%)	1 (2%)	5 (2%)

Data are n (%). All adverse events were mild, no moderate or serious adverse events were reported by any child.

Table 3: **Adverse events assessed at two timepoints in children treated with different doses of oxantel pamoate**

serious adverse events were reported by all children at both timepoints. Only slight differences were recorded for the six treatment groups.

Before treatment among all mentioned clinical symptoms, headache was the most frequent symptom reported by six (2%) of 330 children. Of all adverse events for the different oxantel pamoate treatment groups, abdominal cramps were most commonly reported 2 h (n=14, 5%) and 24 h (n=6, 2%) after treatment.

Discussion

WHO aims to achieve a 75% treatment coverage of school-aged children at risk of soil-transmitted helminths by 2020 to eliminate morbidity.[5,6] However, both albendazole and mebendazole have only a poor-to-moderate effect on *T trichiura*, particularly in high endemic areas.[8,10,11] Oxantel pamoate presents the most promising alternative drug against *T trichiura* infections and has shown good results in recent studies.[9,11] To our knowledge, this is the first dose-finding study on the safety and efficacy of oxantel pamoate against infections with *T trichiura*.

Our results confirm the good trichuricidal activity of oxantel pamoate. Oxantel pamoate showed a good tolerability profile with only a few mild adverse events reported at all doses. Our study identified 5 mg/kg as the minimum effective dose. 22 mg/kg was modelled in this trial as the maximum effective dose, which is in good agreement with the recorded maximum cure rate of 25 mg/kg, at which the dose–response relation plateaus. In comparison, Quantrel is recommended at a dose of 10–20 mg/kg. By contrast with Speich and colleagues,[9] our result for a single dose of 20 mg/kg had a higher cure rate and egg-reduction rate (cure rate 26·3% *vs* 50%; egg-reduction rate 93·2% *vs* 97·7%); however, our baseline infection intensity was lower. A difference in baseline infection intensities affects the proportion of cured children and complicates the comparison of cure rates from different studies.[10]

Our study identified 15–30 mg/kg oxantel pamoate as the optimum dose range. At these doses, high egg-reduction rates of 97·1–98·8% were recorded with only few, mild adverse events. Egg-reduction rate is obviously a key outcome parameter as it shows morbidity reduction. Given this broad efficacious range we aimed to determine an optimum weight-independent dose to facilitate preventive chemotherapy programmes. Based on our calculations we suggest a weight-independent dose of 500 mg oxantel pamoate for sub-Saharan African children. To be able to include younger school-aged children (aged 5 years and 6 years) further trials are necessary to investigate the safety of doses as high as 40 mg/kg oxantel pamoate (10% of girls in sub-Saharan Africa aged 5 years would receive at least 39·7 mg/kg). Of note, a proper dose-finding study was never carried out for albendazole. Albendazole's most commonly used 400 mg dose was based on a pragmatic approach—namely, a simple extrapolation from the most effective dose in animals.[27]

In the placebo group, none of the children were cured. There was a 34·5% reduction in geometric mean and 49·5% in arithmetic mean eggs counts. After stratification according to baseline infection intensities, children with a light infection intensity (n=30) had a geometric mean egg-reduction rate of 0%, whereas children characterised by a moderate (n=18) and heavy (n=1) infection intensity had a combined egg-reduction rate of 64·8% (appendix). These high egg-reduction rates might be explained by the diagnostic limitations of the Kato-Katz method (ie, low accuracy in correctly reading slides with high egg counts)[28] and a high variability of egg shedding at different timepoints.[29] Moreover, two extreme outliers at baseline (egg count 26 220 and 9204) with a low egg count at follow-up were found. Removing these two participants from the analysis an egg-reduction rate of 25·6% (95% CI −2·3 to 47·0) was obtained, which is not significantly different to zero.

In agreement with our two recent trials with oxantel pamoate[9,11] the drug showed only low efficacy against *A lumbricoides* and hookworm. However, as shown by the wide CIs the number of infected children at baseline was too small and therefore statistical power was low.

Few adverse events were reported for all six treatment groups regardless of the received dose. Clinical symptoms before treatment were similar in numbers to the adverse events recorded 1 day after treatment (15 *vs* 12 children) for children of the six oxantel pamoate treatment groups. 2 h after treatment the adverse event rate slightly increased and 27 (10%) of 281 children complained of symptoms. Compared with a recent study,[9] children in our treatment group (20 mg/kg oxantel pamoate) reported less adverse events 2 h (11% *vs* 13%) and 24 h (7% *vs* 17%) after treatment.

Further trials with oxantel pamoate in younger children and on different continents are necessary for developing a globally applicable oxantel pamoate weight-independent dose, which ultimately could be used in preventive chemotherapy programmes in combination with albendazole against the three major soil-transmitted helminths.

Contributors

WM, BS, MA, JHa, and JK designed the study. MP and JHu supervised the formulation and manufacturing of the oxantel pamoate tablets. WM, SMAl, SMAm, MA, and JK implemented the study. WM, JHa, and JK analysed and interpreted the data. WM and JK wrote the first draft of the manuscript. BS, MA, and JHa revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

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Chapter 4a

Efficacy and safety of tribendimidine, tribendimidine plus ivermectin, tribendimidine plus oxantel pamoate, and albendazole plus oxantel pamoate against hookworm and concomitant soil-transmitted helminth infections in Tanzania and Côte d'Ivoire: a randomised, controlled, single-blinded, non-inferiority trial

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Summary

Background Preventive chemotherapy is the current strategy to control soil-transmitted helminth infections (caused by *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*). But, to improve efficacy and avoid emerging resistance, new drugs are warranted. Tribendimidine has shown good anthelmintic efficacy and is therefore a frontrunner for monotherapy and combination chemotherapy.

Methods We did a randomised, controlled, single-blinded, non-inferiority trial on Pemba Island, Tanzania, and in Côte d'Ivoire. We recruited adolescents aged 15–18 years from four primary schools on Pemba, and school attendees and non-schoolers from two districts in Côte d'Ivoire. Only hookworm-positive participants were randomly assigned (1:1:1:1) to single, oral doses of tribendimidine 400 mg plus placebo (tribendimidine monotherapy), tribendimidine 400 mg plus ivermectin 200 µg/kg, tribendimidine 400 mg plus oxantel pamoate 25 mg/kg, or albendazole 400 mg plus oxantel pamoate 25 mg/kg. Randomisation was done via a computer-generated list in block sizes of four or eight. Participants were asked to provide two stool samples on 2 consecutive days at baseline and again 14–21 days at follow-up. The primary outcome was the difference in egg-reduction rates (ERRs; ie, the geometric mean reduction) in hookworm egg counts between treatment groups, measured by the Kato-Katz technique. Differences in coadministrated treatment groups were assessed for non-inferiority with a margin of –3% to albendazole plus oxantel pamoate based on the available-case population, analysed by intention to treat. Safety was assessed 3 h and 24 h after treatment. This study is registered with ISRCTN (number 14373201).

Findings Between July 26, and Dec 23, 2016, we treated 636 hookworm-positive participants, and outcome data were available for 601 participants (151 assigned to tribendimidine monotherapy, 154 to tribendimidine plus ivermectin, 148 to tribendimidine plus oxantel pamoate, and 148 to albendazole plus oxantel pamoate). Tribendimidine plus ivermectin was non-inferior to albendazole plus oxantel pamoate (ERRs 99·5% [95% CI 99·2–99·7] *vs* 96·0% [93·9–97·4]; difference 3·52 percentage points [2·05–5·65]). Likewise, tribendimidine plus oxantel pamoate was non-inferior to albendazole plus oxantel pamoate (ERRs 96·5% [95% CI 94·9 to 97·6] *vs* 96·0% [93·9 to 97·4]; difference 0·48 percentage points [–1·61 to 2·88]). 3 h after treatment, headache (n=50 [8%]) and vertigo (n=37 [6%]) were the most widely reported symptoms; 24 h after treatment, 50 (8%) patients reported vertigo and 41 (7%) reported headache. Mainly mild adverse events were reported with peak numbers (n=111 [18%]) at 24 h after treatment. Three participants had moderate adverse events 3 h after treatment: two (<1%) had vertigo and one (<1%) had headache, and two had moderate adverse events 24 h after treatment: one (<1%) had vomiting and one (<1%) had vomiting plus diarrhoea.

Interpretation Tribendimidine in combination with either ivermectin or oxantel pamoate had a similar, non-inferior efficacy profile as albendazole plus oxantel pamoate, hence tribendimidine will be a useful addition to the depleted anthelmintic drug armamentarium.

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Introduction

Soil-transmitted helminth infections are among the most common intestinal infections around the world and are caused by the nematodes *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura*. An estimated 1·5 billion people are infected with at least one soil-transmitted helminth species.[1] The estimated

Research in context

Evidence before this study

We searched PubMed for all articles published before June 1, 2017, with a combination of the search terms "hookworm", "tribendimidine", and "efficacy", without language restrictions. Clinical data about the efficacy of tribendimidine against soil-transmitted helminth infections exist only from China, and no published clinical data from other countries and continents exist.

Added value of this study

This study provides the first clinical data outside China for the efficacy of tribendimidine alone and in coadministration with ivermectin and oxantel pamoate against soil-transmitted helminth infections in two African countries (Tanzania and Côte d'Ivoire). The results from this randomised, controlled, single blinded, non-inferiority trial confirmed the good safety profile of tribendimidine, with only a few and mainly mild adverse events reported. The efficacy profile of tribendimidine resembles albendazole, the current standard drug against hookworm infections. Tribendimidine coadministered with ivermectin provided high efficacy against hookworm infection, indicating synergism. To tackle infections from all three soil-transmitted helminths, coadministration of tribendimidine plus oxantel pamoate showed promising results.

Implications of all the available evidence

The current strategy to control soil-transmitted helminth infections is preventive chemotherapy—ie, annual or biannual treatment of at-risk populations. For more than three decades, the two benzimidazoles (albendazole and mebendazole) have been the only drugs used in this area, leading to a high drug pressure and the risk of emerging benzimidazole resistance. Tribendimidine, which is currently under assessment towards approval by the US Food and Drug Administration, is the most advanced drug in the anthelmintic pipeline, and our data suggest that it will be a useful addition in preventive chemotherapy programmes.

global burden of such infections was 3·4 million disability-adjusted life-years in 2015.[2]

To reduce the morbidity caused by soil-transmitted helminths, the current control strategy is preventive chemotherapy—ie, regular (annual or biannual) administration of drugs to at-risk populations. One of WHO's goals for this disease area is to provide effective drugs to 75% of all at-risk populations by 2020.[3] In 2015, an estimated 59·5% of people in need of preventive chemotherapy were treated.[4] For the past three decades, mainly two benzimidazoles—albendazole and mebendazole—have been used. However, these drugs have shortcomings in their efficacy profiles of single-dose regimens against hookworm (mebendazole) and *T trichiura* (both albendazole and mebendazole).[5] And because of their long-standing use, the threat of benzimidazole resistance increases, as shown in veterinary medicine.[6] Alarmingly, there are only a few drugs that could serve as backups.

The Chinese drug tribendimidine is the most advanced therapy in the anthelmintic drug pipeline and is currently under assessment towards approval by the US Food and Drug Administration (FDA).[7] Tribendimidine, a B-subtype selective nicotinic acetylcholine receptor agonist, has a different mode of action compared with commonly used anthelmintic drugs.[8] Up to now, published data for the clinical use of tribendimidine have been restricted to Asia. The drug was discovered in the early 1980s, and approved for human use by the Chinese FDA in 2004,[9] hence its safety and efficacy is supported by several decades of clinical research and approved use. The efficacy profile of tribendimidine against soil-transmitted helminths is similar to albendazole—it has high efficacy against *A lumbricoides* and hookworm and low efficacy against *T trichiura*.[9] Additionally, tribendimidine has shown activity against several other parasitic diseases, such as those caused by *Enterobius vermicularis*, *Taenia* spp, *Opistorchis viverrini*, and *Clonorchis sinensis*.[9]

Tribendimidine could complement albendazole in the parasitic drug armamentarium to reduce drug pressure or serve as a backup in case of benzimidazole resistance. Neither tribendimidine nor current standard drugs are capable of successfully curing all three soil-transmitted helminths at a single dose. Hence, to broaden the range of efficacies and successfully treat hookworm and *T trichiura* infections, drug coadministrations will have an important role. The aim of our study was to investigate tribendimidine as an alternative to albendazole, and to assess tribendimidine coadministration with ivermectin or oxantel pamoate as therapy against all three soil-transmitted helminth infections.

Methods

Study design and participants

We did a randomised, controlled, single blinded, non-inferiority trial in Pemba Island in Tanzania and Agboville district in Côte d'Ivoire. Ethical approval was granted by the Zanzibar Medical Research and Ethical Committee in Tanzania (ZAMREC/0001/APRIL/016), the Comité National d'Ethique et de la Recherche in Côte d'Ivoire (083/MSHP/CNER-kp) and the Ethics Committee of Northwestern and Central Switzerland (EKNZ UBE-15/35).

We invited adolescents aged 15–18 years to participate in the study. In Tanzania, adolescents were recruited via secondary schools (Wingwi, Mizingani, Wesha, and Tumbe); in Côte d'Ivoire, they were recruited from

Articles

schools and non-schoolers from the districts Azaguié and Rubino. Participants were asked to provide two stool samples and only participants who tested positive for hookworm were eligible. Participants with any systematic illness (eg, clinical malaria, cancer, diabetes, or asthma) were excluded. Detailed inclusion and exclusion criteria are in the appendix (p 3). All parents or legal guardians, signed a written informed consent form and participants provided verbal (Tanzania) or written (Côte d'Ivoire) assent. Before the baseline screening, permission to do the trial was obtained from the school headmasters (Tanzania) or village chiefs (Côte d'Ivoire).

See Online for appendix

Randomisation and masking

Participants were randomly assigned (1:1:1:1) to tribendimidine 400 mg plus placebo (tribendimidine monotherapy), tribendimidine 400 mg plus ivermectin 200 µg/kg, tribendimidine 400 mg plus oxantel pamoate 25 mg/kg, or albendazole 400 mg plus oxantel pamoate 25 mg/kg. An independent statistician did the randomisation via a computer-generated list, block-randomised in sizes of four or eight and stratified according to baseline-infection intensity (light or moderate plus heavy infections). Participants, laboratory technicians, and field technicians were masked to treatment. The different

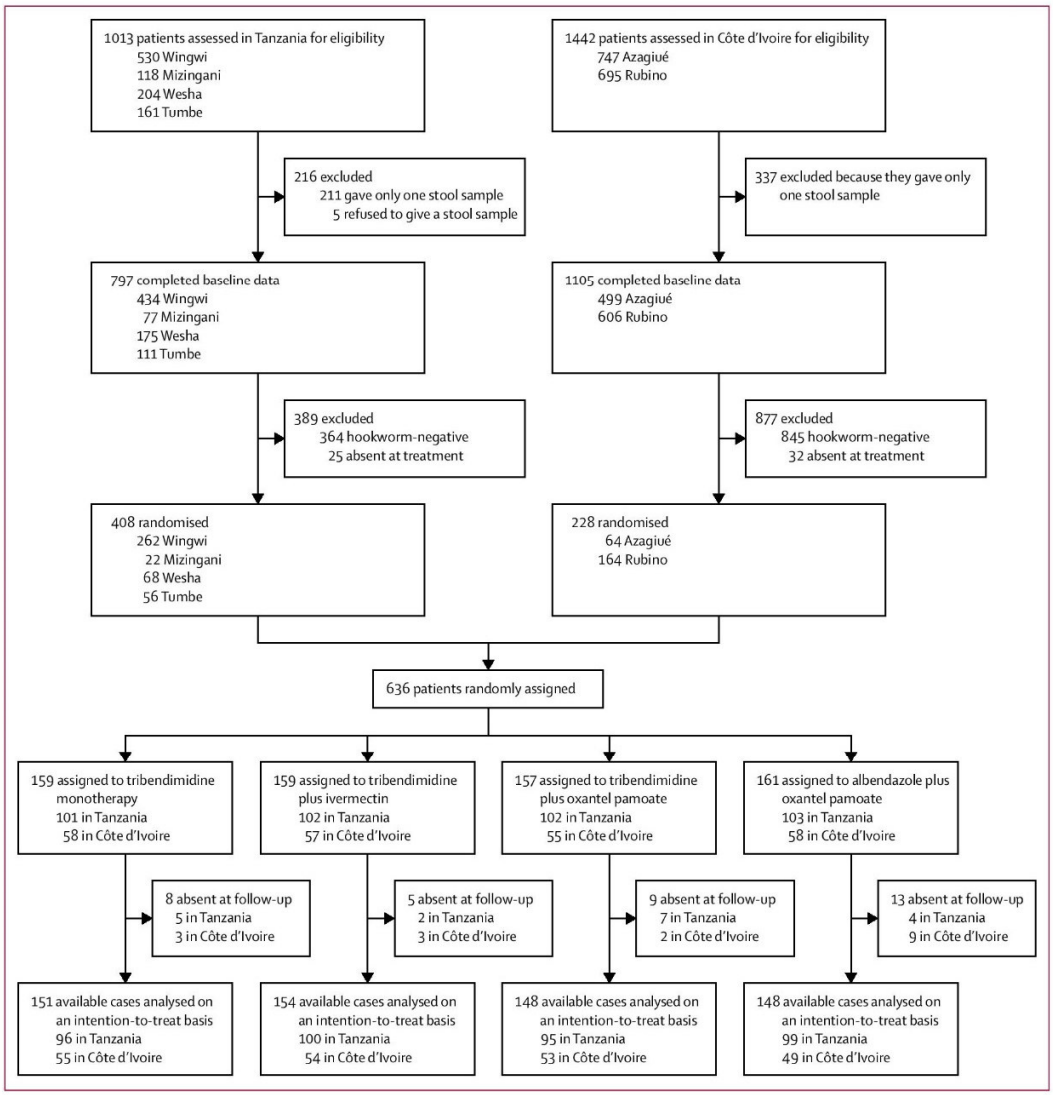

Figure 1: **Trial profile**

treatments were prepacked by two independent pharmacists in plastic bags and labelled with a unique identification code according to the randomisation list. Each treatment consisted of at least two tablets. However, participants and the nurses administering the treatment could have recognised the different treatments because of the different shape and size of the tablets.

Procedures

All drugs and placebo were given as oral tablets at one timepoint. Oxantel pamoate 400 mg tablets and matching placebo tablets were manufactured at the University of Basel.[10] Tribendimidine 400 mg was donated by Shandong Xinhua (Zibo, Shandong, China). Albendazole (Zentel 400 mg) was purchased from GlaxoSmithKline and ivermectin (Mectizan 3 mg) from Merck.

Participants were asked to provide two stool samples on 2 consecutive days for baseline data. All stool samples were analysed in the Public Health Laboratory-Ivo de Carneri (Tanzania) or in the hospitals of Azaguié and Rubino (Côte d'Ivoire) by experienced laboratory technicians. Stool samples were prepared with a duplicate Kato-Katz thick smear using a 41·7 mg template, and quantitatively examined under a light microscope for helminth eggs.[11] The reading was done within 1 h after preparing the slides to avoid overclearing of hookworm eggs.[12] To maintain a high diagnostic quality, 10% of all slides were randomly selected and re-read for *A lumbricoides and T trichiura*, and if discrepancies occurred with the previous reading the results were discussed until a consensus was reached.[13] Stool samples were additionally analysed with FECPAK^{G2}, a new diagnostic tool (Techion Group Limited, Dunedin, New Zealand), and compared with the readings obtained with the Kato-Katz technique (data will be presented elsewhere).

Before enrolment, participants were physically examined and actively questioned about their medical history by the study physician. Participants were followed up 14–21 days after the treatment and asked to provide another two stool samples. Participants remaining positive for any soil-transmitted helminths were treated with a standard dose of albendazole 400 mg according to national guidelines. In Côte d'Ivoire, blood was collected for pharmacokinetic studies (these data will be presented elsewhere).

Outcomes

The primary outcome was the differences in egg-reduction rates (ERRs; ie, reduction of the geometric mean) in hookworm egg counts between treatment groups, measured by the Kato-Katz technique. Secondary outcomes were safety, cure rates, and ERRs against *T trichiura* and *A lumbricoides*. Data for faecal egg counts determined by FECPAK^{G2} and pharmacokinetic variables were also obtained and will be presented elsewhere. Adverse events were assessed by active questioning and grading of the severity at 3 h and 24 h after treatment.

	Tribendimidine monotherapy (n=159)	Tribendimidine plus ivermectin (n=159)	Tribendimidine plus oxantel pamoate (n=157)	Albendazole plus oxantel pamoate (n=161)
Age (years)	15·8 (0·9)	15·9 (1·0)	15·9 (1·0)	15·8 (1·0)
Sex				
Boys (%)	74 (47%)	65 (41%)	84 (54%)	75 (47%)
Girls (%)	85 (53%)	94 (59%)	73 (46%)	86 (53%)
Tanzanian schools or health districts				
Wingwi	64 (40%)	67 (42%)	66 (42%)	65 (40%)
Mizingani	7 (4%)	4 (2%)	5 (3%)	6 (4%)
Wesha	16 (10%)	17 (11%)	18 (12%)	17 (11%)
Tumbe	14 (9%)	14 (9%)	13 (8%)	15 (9%)
Côte d'Ivoire schools or health districts				
Azaguié	19 (12%)	18 (11%)	17 (11%)	10 (6%)
Rubino	39 (25%)	39 (25%)	38 (24%)	48 (30%)
Weight (kg)	47·9 (7·8)	48·9 (8·7)	48·2 (8·3)	48·5 (8·4)
Height (cm)	157·7 (8·7)	157·6 (9·8)	158·3 (13·6)	158·5 (9·8)
Hookworm infections				
Number of participants infected	159 (100%)	159 (100%)	157 (100%)	161 (100%)
Geometric mean number of eggs per g of stool	179·6	163·4	189·5	190·9
Light-intensity infection*	153 (96%)	153 (96%)	153 (97%)	154 (96%)
Moderate-intensity infection†	6 (4%)	4 (3%)	4 (3%)	3 (2%)
Heavy-intensity infection‡	0	2 (1%)	0	4 (2%)
Trichuris trichiura infections				
Number of participants infected	102 (64%)	106 (67%)	101 (64%)	103 (64%)
Geometric mean number of eggs per g of stool	735·6	646·1	693·9	751·0
Light-intensity infection§	56 (55%)	64 (60%)	61 (60%)	53 (51%)
Moderate-intensity infection¶	44 (43%)	41 (39%)	38 (38%)	50 (49%)
Heavy-intensity infection‖	2 (2%)	1 (<1%)	2 (2%)	0
Ascaris lumbricoides infections				
Number of participants infected	74 (47%)	77 (48%)	75 (48%)	79 (49%)
Geometric mean number of eggs per g of stool	2829·8	4255·8	2456·8	494·0
Light-intensity infection**	37 (50%)	32 (41%)	36 (48%)	35 (44%)
Moderate-intensity infection††	34 (46%)	39 (51%)	37 (49%)	40 (51%)
Heavy-intensity infection‡‡	3 (4%)	6 (8%)	2 (3%)	4 (5%)

Data are mean (SD) and n (%), unless otherwise stated. *1–1999 eggs per g of stool. †2000–3999 eggs per g of stool. ‡≥4000 eggs per g of stool. §1–999 eggs per g of stool. ¶1000–9999 eggs per g of stool. ‖≥10 000 eggs per g of stool. **1–4999 eggs per g of stool. ††5000–49 999 eggs per g of stool. ‡‡≥50 000 eggs per g of stool.

Table 1: **Baseline characteristics of the intention-to-treat population**

Statistical analysis

We ran a series of computer simulations with artificial data and data from previous trials[14–17] to determine the required sample size. We calculated that with a sample size of 140 participants per treatment arm, the study would have 80% power to test the primary non-inferiority hypothesis that the ERRs for the comparator combination treatments (tribendimidine with ivermectin or oxantel pamoate) were non-inferior to the currently most

Articles

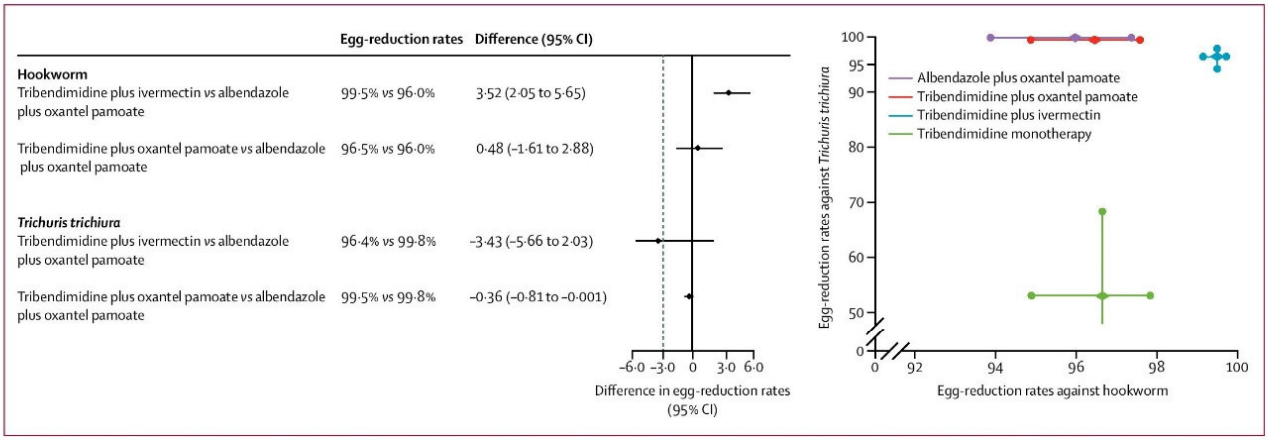

Figure 2: **Egg-reduction rates**
Data shown are differences in egg-reduction rates (95% CIs; left panel) and direct comparison of ERRs (95% CIs; right panels) against hookworm and *Trichuris trichiura* with tribendimidine plus ivermectin and tribendimidine plus oxantel pamoate versus albendazole plus oxantel pamoate.

efficacious treatment (albendazole plus oxantel pamoate)[15,17] and close to 97%. On the basis of expert opinion, we postulated that a difference in ERRs of 3 percentage points could be assumed as clinically equivalent and set the non-inferior margin at this level. To account for loss to follow-up, we increased the sample size by 15% to 160 participants per treatment and enrolled them (2:1) in Tanzania (n=400) versus Côte d'Ivoire (n=240). Considering previous studies, a prevalence of 40% was estimated for both settings.[15–18]

For baseline characteristics, the average egg count of the four Kato-Katz slides was multiplied by 24 to determine the egg count per g (EPG) of stool. WHO guidelines[19] were used for infection-intensity cutoffs and the calculations of ERR—the percentage reduction of geometric mean EPG at follow-up compared with baseline:

$$ERR = \left(1 - \frac{e^{\frac{1}{n}\sum \log\left(EPG_{follow\text{-}up}+1\right)} - 1}{e^{\frac{1}{n}\sum \log\left(EPG_{baseline}+1\right)} - 1}\right) * 100$$

Following intention-to-treat principles, the primary analysis tested non-inferiority on the available case population including participants with primary outcome data as randomised. The 95% CIs of the ERRs and of the differences between ERRs were constructed using a bootstrap-resampling approach with 5000 replications.[20] Non-inferiority between the comparator and the standard treatment was established if the lower bound of the CI of the difference in ERRs rates was above −3 percentage points. Post-hoc analyses not specified in the protocol included superiority of tribendimidine monotherapy versus coadministration, whereby p values were obtained by permutation tests. Cure rates were analysed using unadjusted and adjusted logistic regression models. A database (Access 2003, Microsoft) was created and all data were entered twice, compared with EpiInfo version 3.3.2, and analysed with Stata version 14.0. This trial is registered at ISRCTN (number 14373201).

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

Between July 26, and Dec 23, 2016, we assessed 2455 adolescents for eligibility (figure 1). Of 693 hookworm-positive participants, 57 did not present for treatment. 636 hookworm-positive participants received treatment (408 in Tanzania and 228 in Côte d'Ivoire). Most of the 412 participants co-infected with *T trichiura* and 305 co-infected with *A lumbricoides* were from Tanzania. Since all patients completed the study according to the protocol the available case population was identical to the per-protocol population. Baseline characteristics and ERRs based on arithmetic means are in the appendix (pp 4–9). Additionally, efficacy is presented, using all missing data once as treatment failure and once as treatment successes in the appendix (p 10).

Treatment arms were well balanced in terms of age, sex, weight, height, and baseline infection intensity for any helminths and no noteworthy between-group differences occurred (table 1). Demographic and baseline laboratory characteristics for the 636 treated participants are stratified by country in the appendix (pp 4–6). The number of days until the first follow-up sample was taken was balanced among the treatment arms.

Outcome data were available for 601 participants (151 assigned to tribendimidine monotherapy, 154 to

tribendimidine plus ivermectin, 148 to tribendimidine plus oxantel pamoate, and 148 to albendazole plus oxantel pamoate). Tribendimidine plus ivermectin was non-inferior to albendazole plus oxantel pamoate for ERRs (99·5% [95% CI 99·2–99·7] *vs* 96·0% [93·9–97·4]; difference 3·52 percentage points [2·05–5·65]; figure 2, table 2), while cure rates were higher with tribendimidine plus ivermectin (figure 3, table 2). Non-inferiority was reached for tribendimidine plus oxantel pamoate and albendazole plus oxantel pamoate comparing ERRs (96·5% [95% CI 94·9 to 97·6] *vs* 96·0% [93·9 to 97·4]; difference 0·48 percentage points [–1·61 to 2·88] for tribendimidine plus oxantel pamoate), and cure rates did not differ. For *T trichiuria*, tribendimidine plus ivermectin did not reach non-inferiority compared with albendazole plus oxantel pamoate and cure rates were lower (figures 2, 3). Tribendimidine plus oxantel pamoate achieved non-inferiority compared with albendazole plus oxantel pamoate for T trichuria (table 2, figure 2), but cure rates were lower. Overall, 291 participants infected with *A lumbricoides*, mainly from Tanzania (n=282), with primary outcome data were analysed. ERRs reached almost 100% in all treatment arms and cure rates ranged from 93·6% to 98·7% with no differences among the treatment arms (table 2).

All participants were questioned for adverse events 3 h after treatment, while 31 were missing for the 24 h post-treatment interview (figure 4). Adverse events were mainly mild (table 3) and no serious adverse events were reported. Fewer participants reported adverse events 3 h (16%) and 24 h (18%) after treatment compared with symptoms before treatment (21%). Slightly more participants treated with one of the tribendimidine coadministrations reported adverse events—ie, 20% treated with tribendimidine plus oxantel pamoate after 3 h and 22% with tribendimidine plus ivermectin 24 h after treatment. The lowest numbers of adverse events at 3 h (12%) and 24 h (9%) after treatment were documented for albendazole plus oxantel pamoate.

Before treatment, the most common symptoms were headache (n=60) and abdominal cramps (n=55). 3 h after treatment, headache was still the most widely reported symptom (n=50), followed by vertigo (n=37). 1 day after treatment, 50 participants indicated vertigo and 41 headache. Results varied only slightly between both countries (appendix pp 11–13). Mainly mild adverse events were reported with peak numbers (n=111 [18%]) at 24 h after treatment. Three participants had moderate adverse events 3 h after treatment: two (<1%) had vertigo and one (<1%) had headache, and two had moderate adverse events 24 h after treatment: one (<1%) had vomiting and one (<1%) had vomiting plus diarrhoea. 2 days after treatment, none of the participants had any adverse events.

In a post-hoc analysis superiority of the two coadministrations over tribendimidine monotherapy was assessed. Against hookworm, the coadministration of tribendimidine plus ivermectin was superior over tribendimidine monotherapy in terms of ERR (difference 2·84 percentage points, 95% CI 1·63–4·50, p<0·0001) and cure rates (odds ratio [OR] 0·22, 95% CI 0·12–0·38, p<0·0001). Tribendimidine plus oxantel pamoate was not superior to tribendimidine for ERRs (difference –0·19 percentage points, 95% CI –2·22 to –1·83, p=0·85) and cure rates (OR 1·04, 95% CI 0·82 to 1·32, p=0·99).

The efficacy of tribendimidine plus ivermectin against *T trichiura* was superior to tribendimidine monotherapy in terms of ERRs (difference 43·30 percentage points, 95% CI 28·70–63·70, p<0·0001) and cure rates (OR 0·14, 95% CI 0·05–0·32, p<0·0001). Tribendimidine plus oxantel pamoate was superior compared with tribendimidine in terms of ERRs (difference 46·40 percentage points, 95% CI 31·56–66·76, p<0·001) and cure rates (OR 0·18, 95% CI 0·10–0·28, p<0·0001).

	Tribendimidine monotherapy (n=151)	Tribendimidine plus ivermectin (n=154)	Tribendimidine plus oxantel pamoate (n=148)	Albendazole plus oxantel pamoate (n=148)
Hookworm				
Number of participants positive for infection				
Before treatment	151 (100%)	154 (100%)	148 (100%)	148 (100%)
After treatment	70 (46%)	24 (16%)	71 (48%)	77 (52%)
Cure rate	53·6% (45·4–61·8)	84·4% (77·7–89·8)	52·0% (43·7–60·3)	48·0% (39·7–56·3)
Geometric mean number of eggs per g of stool				
Before treatment	183·1	165·7	192·1	194·6
After treatment	6·1	0·8	6·8	7·8
Egg-reduction rate	96·7% (94·9–97·8)	99·5% (99·2–99·7)	96·5% (94·9–97·6)	96·0% (93·9–97·4)
Trichuris trichiura				
Number of participants positive for infection				
Before treatment	97 (64%)	104 (68%)	95 (64%)	99 (67%)
After treatment	89 (59%)	69 (45%)	32 (22%)	17 (11%)
Cure rate	8·2% (3·6–15·6)	33·7% (24·7–43·6)	66·3% (55·9–75·7)	82·8% (73·9–89·7)
Geometric mean number of eggs per g of stool				
Before treatment	771·3	642·8	715·0	753·9
After treatment	362·0	23·1	3·8	1·3
Egg-reduction rate	53·1% (32·0–68·3)	96·4% (94·3–97·8)	99·5% (99·1–99·7)	99·8% (99·7–99·9)
Ascaris lumbricoides				
Number of participants positive for infection				
Before treatment	72 (48%)	76 (49%)	70 (47%)	78 (53%)
After treatment	1 (1%)	1 (1%)	4 (3%)	5 (3%)
Cure rate	98·6% (92·5–100·0)	98·7% (92·9–100·0)	94·3% (86·0–98·4)	93·6% (85·7–97·9)
Geometric mean number of eggs per g of stool				
Before treatment	2817·2	4153·7	2663·4	4826·2
After treatment	0·03	0·03	0·5	0·6
Egg-reduction rate	>99·99% (>99·99–100·0)	>99·99% (>99·99–100·0)	99·98% (99·93–100·0)	99·99% (99·96–100·0)

Data are n (%) or % (95% CI), unless otherwise stated.

***Table 2*: Efficacy outcomes in available cases**

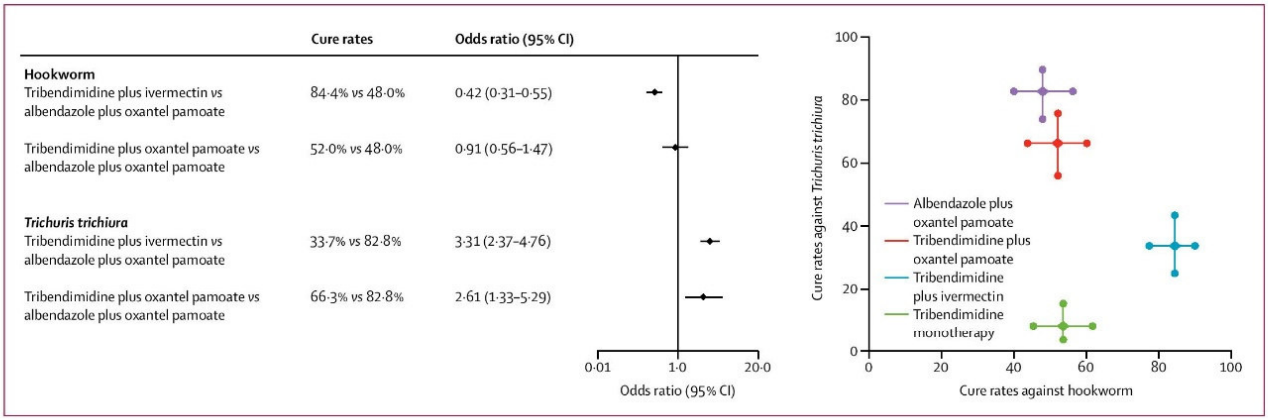

Figure 3: **Cure rates**
Data shown are odds ratios of cure rates (95% CIs; left panel) and direct comparison of cure rates (right panel) against hookworm and *Trichuris trichiura* of tribendimidine plus ivermectin and tribendimidine plus oxantel pamoate versus albendazole plus oxantel pamoate.

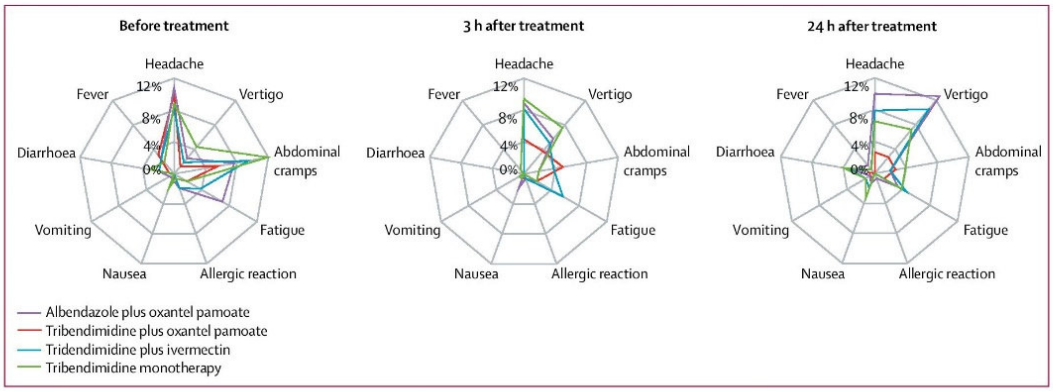

Figure 4: **Symptoms before treatment and adverse events 3 h and 24 h after treatment**
Spider plots showing percentage of reported mild clinical symptoms (before treatment) and adverse events 3 h and 24 h after treatment for the four treatment groups.

	Pre-treatment			3 h after treatment			24 h after treatment		
	Tanzania	Côte d'Ivoire	Total	Tanzania	Côte d'Ivoire	Total	Tanzania	Côte d'Ivoire	Total
Tribendimidine monotherapy	8/101 (8%)	29/58 (50%)	37/159 (23%)	8/101 (8%)	15/58 (26%)	23/159 (15%)	23/96 (24%)*	7/57 (12%)	30/153 (20%)
Tribendimidine plus ivermectin	10/102 (10%)	21/57 (37%)	31/159 (20%)	13/102 (13%)	14/57 (25%)	27/159 (17%)	26/100 (26%)	8/52 (15%)	34/152 (22%)
Tribendimidine plus oxantel pamoate	7/102 (7%)	30/55 (55%)	37/157 (24%)	14/102 (14%)	17/55 (31%)†	31/157 (20%)	27/99 (27%)	6/51 (12%)	33/150 (22%)
Albendazole plus oxantel pamoate	7/103 (7%)	19/58 (33%)	26/161 (16%)	12/103 (12%)	8/58 (14%)	20/161 (12%)	14/97 (14%)‡	0/53	14/150 (9%)
Total	32/408 (8%)	99/228 (43%)	131/636 (21%)	47/408 (12%)	54/228 (24%)	101/636 (16%)	90/392 (23%)	21/213 (10%)	111/605 (18%)

Data are n/N (%). Participants were treated with tribendimidine, tribendimidine plus ivermectin, tribendimidine plus oxantel pamoate, and albendazole plus oxantel pamoate and assessed at three timepoints (pre-treatment, and 3h and 24 h after treatment) in Tanzania and Côte d'Ivoire. *One participant with a moderate adverse event (vomiting). †Three participants with moderate adverse events (one headache and two vertigo). ‡One participant with moderate adverse events (diarrhoea and vomiting).

Table 3: **Number of participants with mild clinical symptoms or adverse events**

Discussion

Coadministration of tribendimidine plus ivermectin had the highest efficacy against hookworm. Tribendimidine in combination with other drugs had a similar, non-inferior efficacy profile as albendazole plus oxantel pamoate and would therefore be a useful addition to the anthelmintic drug armamentarium. Nearly a billion albendazole tablets are distributed every year in the framework of preventive chemotherapy against soil-transmitted helminth infections and lymphatic filariasis.[4] Albendazole has been in clinical use for almost half a century and as coverage of preventive chemotherapy further expands, emergence of anthelmintic resistance is likely to occur as evidenced in veterinary medicine.[6] To avoid or delay the emergence of drug resistance, new drugs should be discovered and developed to increase the armamentarium of drugs. In the absence of alternative treatments the use of drug combinations or coadministrations is a possible strategy to delay the occurrence of drug resistance. Moreover, drug combinations offer the benefit of an increased and broader spectrum of efficacy.

The Chinese drug tribendimidine is the frontrunner to complement or replace albendazole. Since a wealth of clinical data are already available,[9] tribendimidine is being assessed towards approval by the US FDA.[7] We present the first published data on the efficacy of tribendimidine against soil-transmitted helminth infections outside Asia, acquired in the framework of a randomised trial in two countries—Tanzania and Côte d'Ivoire. Against hookworm, our results confirmed the high ERRs of tribendimidine monotherapy reported by studies from China, while cure rates were lower as in previous studies,[9,21] but in line with findings from Steinmann and colleagues[22] in the Yunnan Province, China. No difference in ERRs between tribendimidine plus oxantel pamoate and albendazole plus oxantel pamoate against hookworm and *A lumbricoides* was observed. Hence, our data confirmed that tribendimidine had a similar efficacy profile as albendazole.

Tribendimidine plus ivermectin reached the highest ERRs against hookworm among all treatment arms and was non-inferior to the currently most efficacious treatment albendazole plus oxantel pamoate. ERRs based on arithmetic mean were generally lower in comparison with the geometric mean; however, tribendimidine plus ivermectin still had the highest ERRs among the four treatment arms (appendix pp 7–9). Tribendimidine plus ivermectin resulted in a several-fold higher cure rate in comparison with only low to moderate cure rates with tribendimidine (53·6%) and ivermectin (33·3%)[23] when given as monotherapy. Therewith, our data confirm the synergism of the co-administration of tribendimidine and ivermectin against hookworm reported by Wu and Qian.[24]

The treatment arms of tribendimidine plus ivermectin or oxantel pamoate were assessed for superiority of ERR and cure rates compared with tribendimidine in a post-hoc analysis. This analysis showed the superiority of tribendimidine plus ivermectin versus tribendimidine monotherapy. However, coadministration of tribendimidine plus oxantel pamoate was not superior for ERR and cure rates compared with tribendimidine monotherapy. This result confirms that oxantel pamoate has little effect on hookworm, which is in line with the efficacy findings of two previous studies (cure rates 11% and 33%, ERRs 37% and 77%).[15,16]

Preventive chemotherapy is limited by the absence of drugs with high efficacy against all three soil-transmitted helminths.[5] As mentioned, the drugs used exclusively in these programmes (albendazole and mebendazole) and the potential alternative tribendimidine have poor efficacy against *T trichiura*. Oxantel pamoate, a drug licensed for the treatment of soil-transmitted helminth infections several decades ago has proven high trichuricidal activity in previous trials.[15–17] Here we have shown that oxantel pamoate is not only an excellent partner drug for albendazole, in line with earlier findings,[15,17] but also in coadministration with tribendimidine high efficacy can be reached against any soil-transmitted helminth species.

Against *T trichiura*, there was no difference in ERRs between both coadministrated treatments, while albendazole plus oxantel pamoate had a higher cure rate than tribendimidine plus oxantel pamoate. On the basis of results from a dose-finding study,[16] we increased the oxantel pamoate dose from 20 mg/kg to 25 mg/kg. The slight dose increase might explain the higher ERRs and cure rates for albendazole plus oxantel pamoate in this study compared with two studies[15,17] using a lower dose (ERRs 96·0% and 99·2%, cure rates 31·2% and 68·5%).

Our study had several limitations. Efficacy results against *T trichiura* and *A lumbricoides* were mainly based on data from Tanzania, because only a few participants from Côte d'Ivoire had a co-infection. To confirm the results, further studies should focus on co-endemic settings. Furthermore, in this study most hookworm baseline infections were light. Considering that low infection intensities affect the efficacy,[25] our results might not be generalisable to high-transmission settings. Moreover, the sensitivity of Kato-Katz is reduced in low infection intensity settings.[26] To partly account for the lowered sensitivity, we collected additional stool samples compared with current WHO recommendations.[19]

Clearly a double-blind design—which is commonly used in clinical trials—would have improved the study. However, this trial included four different drugs, which were administered weight dependently (ivermectin and oxantel pamoate) and independently (tribendimidine and albendazole). By including matching placebos in each treatment arm, participants would have to swallow multiple tablets for each treatment and placebo. Apart from ethical considerations, this would have led to unnecessary complications and high dropouts.

Before this study, in-vitro studies with the human recombinant cytochromes P450 and in-vivo studies

were done for the tribendimidine coadministrations, as described for albendazole plus oxantel pamoate.[27] No interactions were observed, indicating the safe use of these coadministrations (results will be published elsewhere). Indeed, adverse events were predominantly mild, with only three moderate adverse events observed at each time-point after treatment and no serious adverse events. All adverse events were resolved within 48 h after treatment. Adverse events 3 h and 24 h after treatment were similar to studies with tribendimidine from China,[9] Laos,[28] and recent studies with oxantel pamoate from Tanzania.[15–17] No difference in adverse events occurred between tribendimidine monotherapy and in coadministration with ivermectin, as shown in a previous study.[21] In Côte d'Ivoire, more participants reported symptoms before treatment than 24 h after treatment, in line with an earlier study in this country.[29] The large decrease in numbers of symptoms before versus after treatment might be explained by a perception bias; the participant's perceived improvement was driven by the expectations after treatment.

In conclusion, we showed that tribendimidine had a good safety profile and similar efficacy against hookworm—as shown in China[9]—in participants from two African countries. Tribendimidine showed a similar efficacy profile as albendazole. The co-administration of tribendimidine with ivermectin had high efficacy against hookworm, hinting at synergism. To tackle all three soil-transmitted helminths at once, tribendimidine could be combined with oxantel pamoate. Hence, we recommend tribendimidine to complement albendazole in preventive chemotherapy interventions, to decrease drug pressure on soil-transmitted helminths and avoid the emergence of drug resistance against benzimidazoles. Moreover, the coadministration of tribendimidine with ivermectin or oxantel pamoate could broaden the spectrum of activity.

Contributors

WM, JHa, and JK designed the study. MP and JHu formulated and manufactured the oxantel pamoate tablets. WM, JC, RBY, SMAl, SMAm, AKA, MA and JK did the study. WM, JHa, and JK analysed and interpreted the clinical data. WM and JK wrote the first draft and JHa revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

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Chapter 4b

Efficacy and tolerability of triple drug therapy with albendazole, pyrantel pamoate, and oxantel pamoate compared with albendazole plus oxantel pamoate, pyrantel pamoate plus oxantel pamoate, and mebendazole plus pyrantel pamoate and oxantel pamoate against hookworm infections in school-aged children in Laos: a randomised, single-blind trial

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Efficacy and tolerability of triple drug therapy with albendazole, pyrantel pamoate, and oxantel pamoate compared with albendazole plus oxantel pamoate, pyrantel pamoate plus oxantel pamoate, and mebendazole plus pyrantel pamoate and oxantel pamoate against hookworm infections in school-aged children in Laos: a randomised, single-blind trial

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Summary

Background Albendazole and mebendazole are commonly used to control hookworm, but have shortcomings in their efficacy profiles. We assessed whether triple drug therapy (TDT) with albendazole, pyrantel pamoate, and oxantel pamoate was more effective than the co-administration of two drugs for the treatment of hookworm infections.

Methods A randomised, single-blind trial was done from Sept 27 until Nov 17, 2017, in Laos. Children (6–15 years) from six schools were invited to participate. Hookworm-positive children were randomly assigned (2:2:1:1) by a computer stratified list (block sizes of six and 12) to TDT with albendazole (400 mg), pyrantel pamoate (20 mg/kg), and oxantel pamoate (20 mg/kg); albendazole plus oxantel pamoate; pyrantel pamoate plus oxantel pamoate; or mebendazole (500 mg) combined with both pyrantel pamoate and oxantel pamoate (used as proof of concept to compare the two TDTs). Two stool samples were collected at baseline and follow-up (17–30 days after treatment) and analysed with the Kato-Katz method. The primary outcome was the proportion of hookworm egg-negative children at follow-up in all Kato-Katz slides (cure rate [CR]) in the TDT with albendazole, pyrantel pamoate, and oxantel pamoate group compared with the albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate groups. Secondary outcomes were tolerability 3 h and 24 h after treatment, egg reduction rates (ERRs) against hookworm, and efficacy against concomitant soil-transmitted helminth infections. Participating children and field and laboratory technicians were masked to treatment allocation. All children with follow-up data were included in the primary analysis. This trial is registered with ClinicalTrials.gov, number NCT03278431.

Findings 1529 children were assessed for eligibility, of whom 533 provided complete baseline data and 414 provided complete outcome data. The CR was higher for the TDT albendazole, pyrantel pamoate, and oxantel pamoate (116 [84%] of 138) than with albendazole plus oxantel pamoate (73 [53%] of 138; odds ratio 4·7, 95% CI 2·7–8·3; p<0·0001) and pyrantel pamoate plus oxantel pamoate (36 [52%] of 69; 4·8, 2·5–9·3; p<0·0001). The geometric ERR of the TDT albendazole, pyrantel pamoate, and oxantel pamoate (99·9%) was higher than that for albendazole plus oxantel pamoate (99·0%; difference in ERR 0·9 percentage points, 95% CI 0·5–1·4), and pyrantel pamoate plus oxantel pamoate (99·2%; 0·7 percentage points, 0·3–1·3). Adverse events were reported by six (1%) children 3 h and none 24 h after treatment, without any difference across treatment groups.

Interpretation TDT with albendazole, pyrantel pamoate, and oxantel pamoate could make a difference, in particular in the context of soil-transmitted helminth elimination. Pyrantel pamoate might be a useful alternative to prevent benzimidazole resistance; however, larger trials are needed to confirm this finding.

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Introduction

About 1·5 billion people are infected with one of the three soil-transmitted helminths (STHs): *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura*.[1] STH infections are a major public health problem in poor and vulnerable populations, with the highest prevalence in Asia, followed by sub-Saharan Africa and the Americas.[1] The global burden

Articles

Research in context

Evidence before this study

Of the most widely used anthelmintic drugs albendazole and mebendazole, only albendazole has a moderate efficacy against hookworm and both show low performance against *Trichuris trichiura* when used as a single dose. Several co-administrations have been assessed, which revealed a broad spectrum of efficacy particularly against *T trichiura* infections. We searched PubMed for clinical trials on triple drug therapy for soil-transmitted helminthiasis before Nov 1, 2017, using different combinations of the following search terms: "hookworm", "albendazole", "pyrantel pamoate", "oxantel pamoate" , "mebendazole", "triple drug therapy", and "efficacy". Two preliminary studies using triple drug therapies were identified: one with albendazole, pyrantel pamoate, and oxantel pamoate (1989) and one with mebendazole, pyrantel pamoate, and oxantel pamoate (1990). However, neither study used the doses recommended by WHO.

Added value of this study

In this study, to our knowledge for the first time, the triple drug therapies albendazole, pyrantel pamoate, and oxantel pamoate, and mebendazole, pyrantel pamoate, and oxantel pamoate were thoroughly assessed. Triple drug therapy with albendazole, pyrantel pamoate, and oxantel pamoate had greater efficacy against hookworm than the co-administrations albendazole with oxantel pamoate and pyrantel pamoate with oxantel pamoate, with no differences among the co-administrations.

Implication of all available evidence

Almost 1 billion albendazole or mebendazole treatments are administered annually to at-risk populations in preventive chemotherapy programmes against soil-transmitted helminthiasis and lymphatic filariasis. The success of preventive chemotherapy is threatened by the development of drug resistance and by the moderate to low efficacy of the current drugs against hookworm and *T trichiura*. Apart from the discovery of new drugs, the combination of two or more drugs could improve efficacy and prevent resistance. The triple drug therapy albendazole, pyrantel pamoate, and oxantel pamoate might have a pivotal role in soil-transmitted helminth elimination. Pyrantel pamoate might be a useful treatment alternative against hookworm and in co-administration with oxantel pamoate against any soil-transmitted helminth in case of benzimidazole resistance.

of STH infections reached 3·3 million disability-adjusted life-years in 2016.[2]

To control the burden caused by moderate and heavy STH infections, preventive chemotherapy—that is, the administration of anthelmintic drugs in a single dose regimen, to at-risk populations—is the current strategy.[3] Although pyrantel pamoate and levamisole are on the WHO's Model Lists of Essential Medicines against intestinal helminth infections,[4] primarily albendazole and mebendazole are used for preventive chemotherapy programmes. Before and after pyrantel pamoate was added to WHO's Model Lists of Essential Medicines in 1983, its efficacy, as monotherapy and in combination with oxantel pamoate, was investigated in several clinical trials in human beings, including at different dose regimens.[5,6] Nonetheless, pyrantel pamoate was gradually replaced by albendazole and mebendazole, primarily because they are available as a weight-independent dose,[7] which is a major advantage for large-scale preventive chemotherapy programmes.[8]

Each year, almost 1 billion doses of albendazole or mebendazole are distributed in preventive chemotherapy programmes against soil-transmitted helminthiasis and lymphatic filariasis.[9] In 2016, about 166 million preschool-aged children and 467 million school-aged children were treated in STH preventive chemotherapy programmes, resulting in global coverage of 51% and 69%, respectively.[9] The goal of WHO is to expand the coverage of preventive chemotherapy to reach 75% of preschool and school-aged children in need of treatment, to reduce the burden caused by moderate and heavy infections by 2020.[3] Recent findings from a systematic literature review and network meta-analysis[5] showed that the efficacy of albendazole and mebendazole against hookworm and *T trichiura* has decreased over time, which might hint at anthelmintic drug resistance, although study confounders might have affected this result. Hence, novel therapeutic options should be assessed to fill the gap in the depleted anthelmintic drug pipeline.[10,11]

At present, no drug has high efficacy against all three STH species.[5] Hence, co-administration of drugs with different efficacy profiles is recommended to increase and broaden the spectrum of anthelmintic efficacy. Moreover, combining drugs with different modes of action could protect from the selection of drug resistance and, thus, extend the lifespan of effective and available anthelmintic drugs. Several studies in the past 5 years have assessed different co-administrations and found that albendazole plus oxantel pamoate is the treatment with highest and broadest efficacy against any STH.[12–14] After treatment with albendazole and oxantel pamoate, about half of hookworm-positive participants were cured (cure rate [CR] 45·5–51·4, egg reduction rate [ERR] 90·9–96·0), whereas the efficacy against *T trichiura* varied among the three clinical trials and was affected by baseline infection intensity (CR 31–83%, ERR 96·0–99·8).[12–14]

The combination of pyrantel pamoate and oxantel pamoate was approved for human use in Colombia, Peru,

and the Philippines; however, it is no longer produced.[15] Although both drugs are selective nicotinic acetylcholine receptor agonists, pyrantel pamoate acts on the L-subtype, whereas oxantel pamoate targets the N-subtype.[16] This combination has been widely tested, primarily using 10 mg/kg doses. In an analysis of 20–23 trials, the CR was high against *A lumbricoides* (96%) and moderate against hookworm (73%) and *T trichiura* (61%).[6]

In two preliminary studies,[17,18] efficacy against hookworm and *T trichiura* was improved by triple drug therapies (TDTs) with albendazole or mebendazole in combination with pyrantel pamoate and oxantel pamoate. TDTs are widely used in many therapeutic areas, including lymphatic filariasis,[19] but have not been explored systematically in randomised controlled trials for the treatment of STH infection.

The aim of this study was to comparatively assess the efficacy of TDT with albendazole, pyrantel pamoate, and oxantel pamoate, and the two co-administrations albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate against hookworm infections. Moreover, we included a treatment arm of TDT with mebendazole, pyrantel pamoate, and oxantel pamoate as proof of concept to compare the activity of the two TDTs.

Methods

Study design and participants

We did this randomised, controlled, single-blind trial from Sept 27 until Nov 17, 2017, in Luang Prabang, Laos. Children (aged 6–15 years) from three primary (Nayang, Nakhone, and Phonmany) and three secondary schools (Xonphuao, Nayang, and Namthouama) were invited to participate. Children were deemed eligible if they provided two stool samples and were hookworm positive (eggs per gram of stool [EPG] >100), passed a physical and clinical examination, had no chronic illness, had no anthelmintic treatment in the past 4 weeks, and, if female, were not pregnant (≥12 years).

Before study start, the purpose, procedure, and potential risks and benefits of the study were explained to all eligible children and their parents or legal guardians. Parents or legal guardians were asked to sign a written consent and children an assent (≥12 years) according to Lao law. Before study start, permission for fieldwork was obtained from the Lao Ministry of Health, the Provincial Health Office, the District Health Office, and the District Office for Education and Sport. Ethical approval for the trial was granted by the National Ethics Committee for Health Research, Lao Ministry of Health (reference number 083/NECHR), and by the Ethics Committee of Northwestern and Central Switzerland (reference EKNZ ID:2017-00375).

Randomisation and masking

A computer-generated randomisation list with randomly selected block of sizes of six and 12 was provided by an independent statistician. The randomisation list was stratified according to the baseline infection intensity into light and moderate or heavy infections based on official WHO cutoffs.[20] Children were randomly assigned (2:2:1:1) to one of the four treatment arms: albendazole, pyrantel pamoate, and oxantel pamoate; albendazole plus oxantel pamoate; pyrantel pamoate plus oxantel pamoate; and mebendazole, pyrantel pamoate, and oxantel pamoate. Albendazole (Zentel, GlaxoSmithKline, London, UK) was used at a dose of 400 mg, mebendazole (Vermox, Janssen, Beerse, Belgium) at 500 mg, and pyrantel pamoate (125 mg tablets; Combantrin, Teofarma, Pavia, Italy) and oxantel pamoate (400 mg tablets manufactured at the University of Basel, Basel, Switzerland[21]) at 20 mg/kg bodyweight.

Participating children and field and laboratory technicians were masked to treatment allocation; only the investigator administering the drugs was aware of treatment allocation. Before study start, the drugs were prepared in plastic bags and labelled with a unique identification number by two independent pharmacists. Because of the large number of tablets administered, we did not include matching placebos. Hence, in theory children might have recognised the treatment because of the differing shape, colour, and number of tablets. However, the number of oxantel pamoate and pyrantel pamoate tablets varied depending on the weight of the children and they were probably unaware about the appearance of the specific drugs.

Procedures

All eligible children were asked to provide a stool sample and from all hookworm-positive children an additional sample was collected within 5 days. The stool samples were transported to the Nambak District Hospital (Nambak, Laos) for examination by the Kato-Katz method by experienced laboratory technicians. A 41·7 mg template was used for preparation of the Kato-Katz thick smear. Within 1 h after preparation, to avoid over-clearing of hookworm eggs, the slides were analysed quantitatively for helminth eggs under a light microscope.[22] To maintain high diagnostic standards, 10% of the slides were randomly re-read by the co-investigator (WM or SS) for *A lumbricoides* and *T trichiura* and discussed in case of discordance.[23] Because of the restricted time for analysing hookworm, one of the two slides was read by the co-investigator (WM or SS) and cross-checked with the second slide result to maintain high quality.

Before treatment, experienced physicians clinically and physically examined each eligible child for any acute or chronic illness. Medical history was assessed by active questioning using a standard questionnaire. Height was measured with a stadiometer (to the nearest 0·1 cm), weight with an electronic balance (to the nearest 0·1 kg), axillary temperature with an electric thermometer (to the nearest 0·1°C), and girls (age ≥12 years) were examined for pregnancy by taking a urine sample (ORANGE TEST, Artron Laboratories, Burnaby, BC, Canada). After

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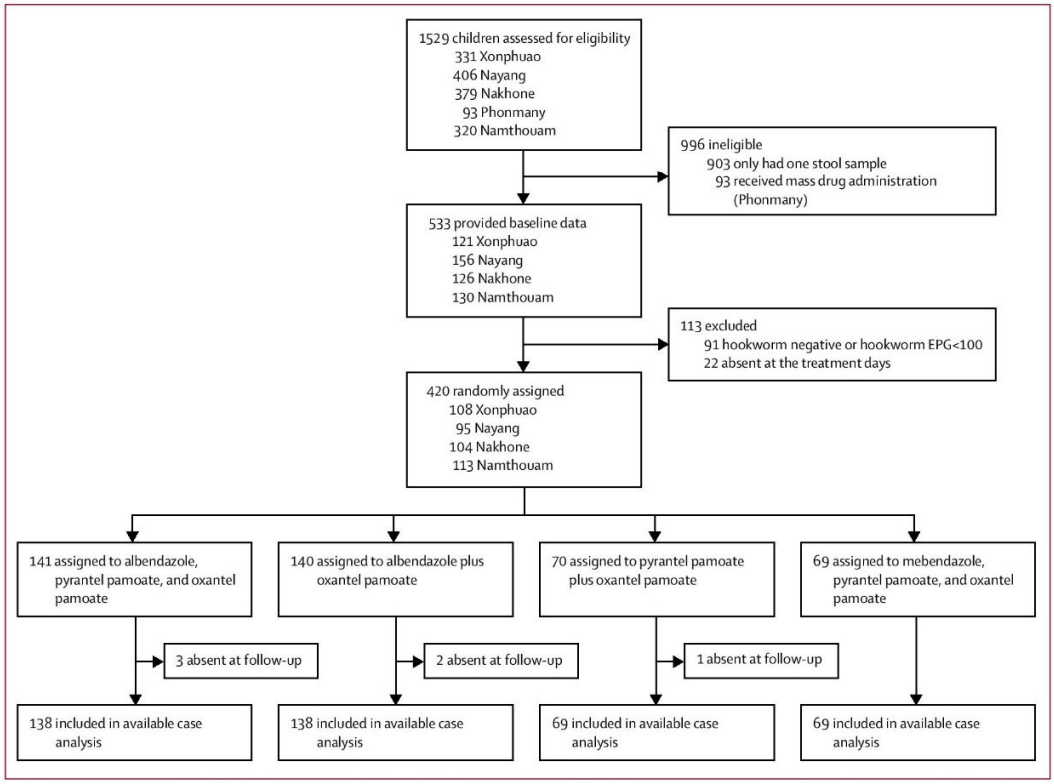

Figure 1: **Trial profile**
EPG=eggs per gram of stool.

treatment, children were monitored for 3 h. At 3 h and 24 h after treatment, children were actively questioned for adverse events by the study physicians. To assess treatment efficacy, another two stool samples were collected at follow-up, 17–30 days after treatment. Children remaining positive for any STH species were treated according to Laos national guidelines.[24]

Outcomes

The primary outcome was CR against hookworm after treatment measured by the Kato-Katz method. Secondary outcomes were tolerability at 3 h and 24 h after treatment, and efficacy against hookworm in terms of ERRs, and CRs and ERRs against *T trichiura* and *A lumbricoides*.

Statistical analysis

The primary hypothesis was that TDT with albendazole, pyrantel pamoate, and oxantel pamoate would have a higher efficacy than the co-administrations albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate. We assumed CRs of 70% for TDT with albendazole, pyrantel pamoate, and oxantel pamoate, 50% for albendazole plus oxantel pamoate, and 35% for pyrantel pamoate plus oxantel pamoate against hookworm, on the basis of previous clinical trial data and expert opinions. To detect a difference with 90% power at a 5% two-sided significance, 121 children were required for the TDT with albendazole, pyrantel pamoate, and oxantel pamoate group and the albendazole plus oxantel pamoate group, and 38 children for the pyrantel pamoate plus oxantel pamoate group. However, we included 60 children in the pyrantel pamoate plus oxantel pamoate group to ensure balanced baseline characteristics after randomisation. As proof of concept, 60 children were allocated to TDT with mebendazole, pyrantel pamoate, and oxantel pamoate to assess efficacy. To account for potential loss to follow-up, the sample size was increased by 15% and resulted in a total of 420 children.

All children with follow-up data were included in the primary available case analysis. A sensitivity analysis was done to assess the potential effect of children lost to follow-up, with all missing data interpreted both as treatment failures and as treatment successes.

The mean egg count of the four Kato-Katz thick smears was multiplied by 24 to calculate the EPG. For the primary analysis, the CR was defined as the percentage of hookworm-positive children who were negative at follow-up (ie, no eggs in all Kato-Katz slides) in the

available case population, and odds ratios (ORs) were calculated using logistic regression unadjusted (primary analysis) and adjusted for age and sex. The ERR (ie, the percentage of mean reduction in egg count at follow-up compared with baseline) was calculated using the geometric mean and arithmetic mean. The geometric mean was calculated as follows:

$$ERR = \left(1 - \frac{e^{\frac{1}{n}\sum \log(EPG_{follow\text{-}up}+1)} - 1}{e^{\frac{1}{n}\sum \log(EPG_{baseline}+1)} - 1}\right) \times 100$$

To calculate the CIs for ERRs, a bootstrap resampling approach with 2000 replications was applied.[25] p values were calculated using a permutation test. No adjustment was made for multiple testing. All data were entered twice into a database (Microsoft Access 2003) and compared with EpiInfo version 3.3.2. Statistical analysis was done using Stata version 14.0 and R version 3.0.2.

This trial is registered with ClinicalTrials.gov, number NCT03278431.

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

1529 children were assessed for eligibility (figure 1), 533 of whom had complete baseline data (two stool samples). 113 children were excluded: 91 were hookworm negative or had a hookworm EPG less than 100 and 22 were absent at the treatment days. Thus, 420 hookworm-positive children were randomly assigned to one of the four treatment groups. Six children were lost to follow-up and 414 had complete outcome data. The first follow-up sample was taken between 17 days and 28 days after treatment and the second between 18 days and 30 days. All children completed the trial according to the protocol and therefore the available case population was identical to the per-protocol population (appendix). Treatment arms were well balanced according to age, sex, weight, height, and hookworm baseline infection intensity (table 1).

	Albendazole, pyrantel pamoate, and oxantel pamoate (n=141)	Albendazole plus oxantel pamoate (n=140)	Pyrantel pamoate plus oxantel pamoate (n=70)	Mebendazole, pyrantel pamoate, and oxantel pamoate (n=69)
Age (years)	12·3 (1·8)	12·4 (1·6)	12·4 (1·8)	12·2 (1·6)
Sex				
Boys	71 (50%)	74 (53%)	40 (57%)	43 (62%)
Girls	70 (50%)	66 (47%)	30 (43%)	26 (38%)
School				
Xonphuao	36 (26%)	36 (26%)	18 (26%)	18 (26%)
Nayang	32 (23%)	32 (23%)	17 (24%)	14 (20%)
Nakhone	36 (26%)	35 (25%)	16 (23%)	17 (25%)
Namthouam	37 (26%)	37 (26%)	19 (27%)	20 (29%)
Weight (kg)	36·4 (9·1)	36·5 (8·9)	34·8 (7·6)	35·2 (7·2)
Height (cm)	143·2 (10·1)	142·3 (14·1)	140·5 (14·7)	144·2 (9·8)
Hookworm				
Infected children	141 (100%)	140 (100%)	70 (100%)	69 (100%)
EPG geometric mean	690·8	696·9	656·5	703·1
Infection intensity				
Light (1–1999 EPG)	112 (79%)	112 (80%)	56 (80%)	56 (81%)
Moderate 2000–3999 EPG)	15 (11%)	20 (14%)	10 (14%)	9 (13%)
Heavy (≥4000 EPG)	14 (10%)	8 (6%)	4 (6%)	4 (6%)
Trichuris trichiura				
Infected children	43 (30%)	52 (37%)	31 (44%)	26 (38%)
EPG geometric mean	66·9	83·4	76·2	47·9
Infection intensity				
Light (1–999 EPG)	42/43 (98%)	48/52 (92%)	30/31 (97%)	25/26 (96%)
Moderate (1000–9999 EPG)	1/43 (2%)	4/52 (8%)	1/31 (3%)	1/26 (3%)
Heavy (≥10 000 EPG)	0	0	0	0
Ascaris lumbricoides				
Infected children	22 (16%)	24 (17%)	13 (19%)	15 (22%)
EPG geometric mean	3361·5	2708·3	3665·5	2718·8
Infection intensity				
Light (1–4999 EPG)	15/22 (68%)	16/24 (67%)	6/13 (46%)	11/15 (73%)
Moderate (5000–49 999 EPG)	5/22 (23%)	6/24 (25%)	7/13 (54%)	3/15 (20%)
Heavy (≥50 000 EPG)	2/22 (9%)	2/24 (8%)	0	1/15 (7%)

Data are mean (SD), number (%), or n/N (%), unless otherwise stated. Some percentages do not add up to 100 because of rounding. EPG=eggs per gram of stool.

Table 1: **Demographics and baseline characteristics**

Against hookworm, the CR of TDT with albendazole, pyrantel pamoate, and oxantel pamoate (84·1%) was significantly higher than that of albendazole plus oxantel pamoate (52·9%; OR 4·7, 95% CI 2·7–8·3; p<0·0001) and pyrantel pamoate plus oxantel pamoate (52·2%; 4·8, 2·5–9·3; p<0·0001; table 2). No significant differences were found between the OR of the unadjusted logistic regression and after adjusting for age and sex (data not shown). The highest geometric ERR against hookworm occurred with TDT with albendazole, pyrantel pamoate, and oxantel pamoate (99·9%), compared with albendazole plus oxantel pamoate (99·0%; difference 0·9 percentage points, 95% CI 0·5–1·4) and pyrantel pamoate plus oxantel pamoate (99·2%; 0·7 percentage points, 0·3–1·3). The arithmetic ERR was higher for TDT with albendazole, oxantel pamoate, and pyrantel pamoate (98·4%) than for albendazole plus oxantel pamoate (91·0%; difference 7·4 percentage points, 95% CI 3·4 to 12·0), but was not significantly different from pyrantel pamoate plus oxantel pamoate (96·3%; 2·1 percentage points, –0·4 to 5·1).

All children with a *T trichiura* infection were cured after treatment with albendazole plus oxantel pamoate

See Online for appendix

	Albendazole, pyrantel pamoate, and oxantel pamoate (n=138)	Albendazole plus oxantel pamoate (n=138)	Pyrantel pamoate plus oxantel pamoate (n=69)	Mebendazole, pyrantel pamoate, and oxantel pamoate (n=69)
Hookworm				
Children positive for infection				
Before treatment	138	138	69	69
After treatment	22	65	33	21
Cure rate (95% CI)	84·1% (76·9–89·7)	52·9% (44·2–61·4)	52·2% (39·8–64·4)	69·6% (57·3–80·1)
Children cured/total number with infection (%)				
From light infection	98/111 (88%)	59/110 (54%)	30/55 (55%)	41/56 (73%)
From moderate infection	8/15 (53%)	11/20 (55%)	4/10 (40%)	6/9 (67%)
From heavy infection	10/12 (83%)	3/8 (38%)	2/4 (50%)	6/9 (67%)
EPG geometric mean				
Before treatment	671·4	706·9	671·4	703·8
After treatment	0·9	7·2	5·6	2·5
Egg reduction rate (95% CI)	99·9% (99·8–99·9)	99·0% (98·5–99·4)	99·2% (98·5–99·6)	99·6% (99·3–99·8)
EPG arithmetic mean				
Before treatment	1373·7	1269·2	1301·0	1456·7
After treatment	22·0	114·1	48·1	99·4
Egg reduction rate (95% CI)	98·4% (96·7–99·4)	91·0% (85·9–94·5)	96·3% (93·4–98·0)	93·2% (86·3–98·7)
Trichuris trichiura				
Children positive for infection				
Before treatment	43	51	31	26
After treatment	3	0	8	3
Cure rate (95% CI)	93·0% (80·9–98·5)	100·0% (93·0–100·0)	74·2% (55·4–88·1)	88·5% (69·8–97·6)
EPG geometric mean				
Before treatment	68·1	88·8	77·4	49·1
After treatment	0·3	0	1·6	0·6
Egg reduction rate (95% CI)	99·6% (98·4–100·0)	100·0%	97·9% (95·1–99·2)	98·8% (96·8–100·0)
EPG arithmetic mean				
Before treatment	181·1	376·4	209·8	159·9
After treatment	9·9	0	51·7	11·3
Egg reduction rate (95% CI)	94·5% (87·5–100·0)	100·0%	75·4% (58·1–98·1)	92·9% (86·0–100·0)
Ascaris lumbricoides				
Children positive for infection				
Before treatment	22	24	13	15
After treatment	2	1	0	0
Cure rate (95% CI)	90·9% (70·8–98·9)	95·8% (78·9–99·9)	100·0% (75·3–100·0)	100·0% (78·2–100·0)
EPG geometric mean				
Before treatment	3363·8	2710·3	3670·3	2720·3
After treatment	0·5	0·2	0	0
Egg reduction rate (95% CI)	>99·9% (99·9–100·0)	>99·9% (99·9–100·0)	100·0%	100·0%
EPG arithmetic mean				
Before treatment	13531·4	18221·0	13062·0	21041·2
After treatment	8·2	5·0	0	0
Egg reduction rate (95% CI)	>99·9% (>99·9–100·0)	>99·9% (>99·9–100·0)	100·0%	100·0%

Data are number (%), unless otherwise stated. EPG=eggs per gram of stool.

Table 2: **Efficacy outcomes in the available case analysis**

(CR 100·0%; table 2). Three children treated with TDT with albendazole, pyrantel pamoate, and oxantel pamoate remained *T trichiura* positive after treatment, which resulted in a CR of 93·0% (95% CI 80·9–98·5), a geometric ERR of 99·6% (98·4–100·0), and an arithmetic ERR of 94·5% (87·5–100·0). Efficacy was lower for pyrantel pamoate plus oxantel pamoate, with a CR of 74·2% (95% CI 55·4–88·1), geometric ERR of 97·9% (95·1–99·2), and arithmetic ERR of 75·4% (58·1–98·1).

Pyrantel pamoate plus oxantel pamoate cured all children with an *A lumbricoides* infection (CR 100·0%; table 2). One child remained positive for *A lumbricoides* after treatment with albendazole plus oxantel pamoate (CR 95·8%, 95% CI 78·9–99·9) and two children after TDT with albendazole, pyrantel pamoate, and oxantel pamoate (90·9%, 70·8–98·9); for both treatments the geometric and arithmetic ERRs were higher than 99·9%.

In the comparison of the two TDTs, the CR was higher for TDT with albendazole, pyrantel pamoate, and oxantel pamoate against hookworm than for TDT with mebendazole, pyrantel pamoate, and oxantel pamoate (84·1% *vs* 69·6%; OR 2·3, 95% CI 1·2–4·6; p=0·017; table 2). Similarly, the geometric ERR of albendazole, pyrantel pamoate, and oxantel pamoate was higher than that for TDT with mebendazole, pyrantel pamoate, and oxantel pamoate (geometric ERR 99·9% *vs* 99·6%; difference in ERRs 0·2 percentage points, 95% CI <0·1 to 0·5), but the difference between arithmetic ERRs was not significant (98·4% *vs* 93·2%, 5·2 percentage points, –1·1 to 12·2). Against *T trichiura*, similar efficacy was observed for both TDTs, with a CR of 88·5% (95% CI 69·8–97·6), a geometric ERR of 98·8% (96·8–100·0), and an arithmetic ERR of 92·9% (86·0–100·0) for TDT with mebendazole, pyrantel pamoate, and oxantel pamoate. All *A lumbricoides*-infected children were cured after TDT with mebendazole, pyrantel pamoate, and oxantel pamoate.

Before treatment, 44 (10%) children reported symptoms (figure 2; table 3; appendix), with headache (n=28), stomach pain (n=9), and itching (n=6) most often reported. 3 h after treatment, six (1%) children reported adverse events, including mild dizziness (n=3), mild (n=1) and moderate (n=1) stomach pain, and both moderate headache and mild dizziness (n=1). The highest number of adverse events (n=4) were reported by three children after treatment with albendazole plus oxantel pamoate. 24 h after treatment, all adverse events had resolved.

Discussion

In our study, TDT with albendazole, pyrantel pamoate, and oxantel pamoate reached higher efficacy in terms of CR and geometric ERR than the two co-administrations. Although the CR of TDT with albendazole, pyrantel pamoate, and oxantel pamoate was higher by about 30 percentage points, the ERR was only slightly, yet clinically relevantly, increased compared with albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate. Moreover, in children harbouring light infection intensities, a substantially higher CR of 88% was observed with TDT with albendazole, pyrantel pamoate, and oxantel pamoate compared with the other treatments. Once the strategy against STH moves from control towards elimination, TDT with albendazole, pyrantel pamoate, and oxantel pamoate might make a difference, similar to in the elimination of lymphatic filariasis. WHO guidelines for lymphatic filariasis were recently adapted

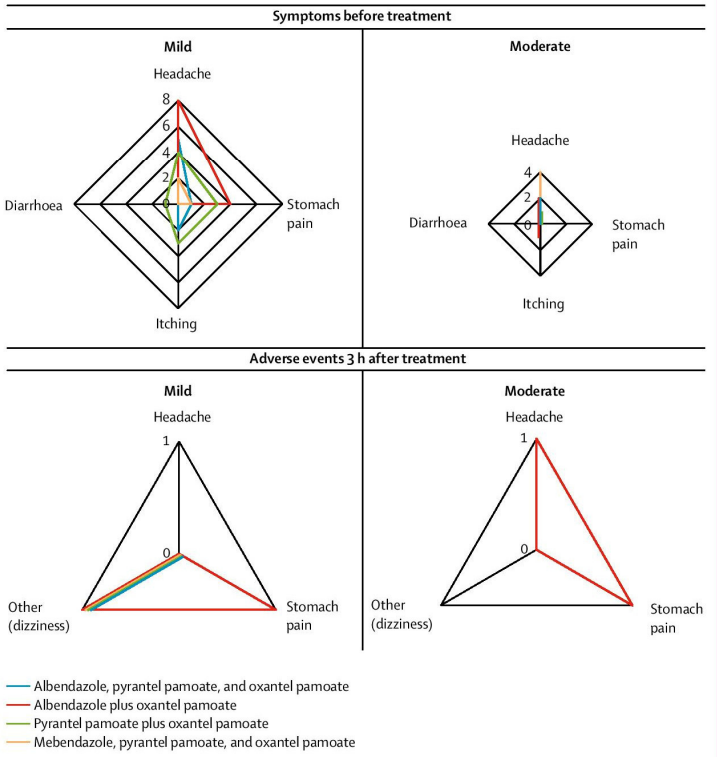

Figure 2: **Symptoms before and adverse events 3 h after treatment**
None of the children had adverse events 24 h after treatment.

	Number of children with mild/moderate symptoms				Number (%) of children with any symptoms
	Albendazole, pyrantel pamoate, and oxantel pamoate	Albendazole plus oxantel pamoate	Pyrantel pamoate plus oxantel pamoate	Mebendazole, pyrantel pamoate, and oxantel pamoate	
Symptoms before treatment					
Number of children	141	140	70	69	420
Headache	5/2	8/2	4/1	2/4	28 (7%)
Stomach pain	1/0	4/0	3/0	1/0	9 (2%)
Itching	2/0	0/1	3/0	0/0	6 (1%)
Diarrhoea	0/0	0/0	1/0	0/0	1 (<1%)
Total	8/2	12/3	11/1	3/4	44 (10%)
Adverse events 3 h after treatment					
Number of children	138	138	69	69	414
Headache	0/0	0/1*	0/0	0/0	1 (<1%)
Stomach pain	0/0	1/1	0/0	0/0	2 (<1%)
Other (dizziness)	1/0	1*/0	1/0	1/0	4 (1%)
Total	1/0	2/2	1/0	1/0	6 (1%)

All adverse events disappeared after 24 h. *One child reported moderate headache and mild dizziness.

Table 3: **Symptoms before treatment and adverse events 3 h after treatment**

and the TDT ivermectin, diethylcarbamazine, and albendazole is now the treatment of choice.[19]

The efficacy of TDT with albendazole, pyrantel pamoate, and oxantel pamoate was assessed in a previous study more than 30 years ago, in which slightly higher CRs (90·3% and 92·6%) and similar ERRs (99·8% and 99·3%) against hookworm were reported, regardless of lower albendazole doses (150 mg and 300 mg) compared with the 400 mg dose in our study.[17]

Powered as a proof-of-concept analysis, albendazole and mebendazole combined with pyrantel pamoate plus oxantel pamoate were compared in the present study. The efficacy of the albendazole TDT was higher against hookworm in terms of CR and geometric ERR. Our results might reflect the higher efficacy of the single-drug albendazole compared with mebendazole[5] and are in line with findings from two studies from the 1980s,[17,18] which suggested a lower efficacy (CR 67% and ERR 86%) for mebendazole, pyrantel pamoate, and oxantel pamoate compared with the albendazole TDT.

Albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate had comparable efficacy against hookworm; however, our study was not designed to show equivalence between the two co-administrations. Nonetheless, this finding highlights the potential of pyrantel pamoate since it is a safe drug,[6] is produced by several pharmaceutical companies, and provides an alternative treatment option in case of benzimidazole-resistant hookworm infections. Currently, WHO recommends a single dose of 10 mg/kg pyrantel pamoate for light hookworm infections.[26] Pyrantel pamoate alone or in co-administration with oxantel pamoate was investigated in the 1970–80s with different dose regimens against infections with hookworm (pyrantel pamoate alone) and in co-administration with oxantel pamoate against infections with hookworm and *T trichiura*. For pyrantel pamoate monotherapy, inconclusive results were obtained using different doses.[6] However, findings from an almost half a decade old dose-finding study for pyrantel pamoate against hookworm infections suggested an increased ERR for higher doses,[27] and based on a more recent dose-finding study for oxantel pamoate against *T trichiura* infections,[28] a dose regimen of 20 mg/kg for both drugs was chosen for this study. Yet, the inconsistent results for pyrantel pamoate alone and in co-administration with oxantel pamoate in previous studies call for a thorough dose-finding study for pyrantel pamoate, as was done for oxantel pamoate,[28] which furthermore allow the development of a weight-independent dose of pyrantel pamoate for preventive chemotherapy.

The results against hookworm were limited by the inability of the Kato-Katz method to distinguish between the two hookworm species *N americanus* and *A duodenale*. However, ongoing work from PCR analysis suggests that *N americanus* was the predominant species in our study (data not shown). Both species are susceptible for pyrantel pamoate; however, *A duodenale* is more sensitive according to a study by Kale and colleagues from 1982.[29] Another limitation of our trial was that only a few *T trichiura* co-infections were observed. We confirmed the high trichuricidal activity of albendazole plus oxantel pamoate, to our knowledge for the first time, in an Asian setting, compared with three recent trials in Africa.[12–14] All children positive for *T trichiura* were cured with albendazole plus oxantel pamoate, which is probably associated with the low infection intensity of the children. Similarly, among the trials in the African settings, efficacy was greater for lower[13] compared with higher baseline infection intensity.[12] A lower efficacy was shown for pyrantel pamoate plus oxantel pamoate, which is not unexpected, since single-dose pyrantel pamoate has a lower efficacy against *T trichiura* than albendazole.[5]

Overall, in this study only six children had mild or moderate (headache and stomach pain) adverse events and all adverse events disappeared 1 day after treatment. Three (2%) children reported adverse events 3 h after treatment with albendazole plus oxantel pamoate, compared with 8–12% in the African trials.[12–14] Our findings are in line with low or absence of adverse events reported in clinical trials including pyrantel pamoate plus oxantel pamoate[30] and the two TDTs.[17,18]

In conclusion, TDT with albendazole, pyrantel pamoate, and oxantel pamoate showed higher efficacy than the co-administrations albendazole plus oxantel pamoate and pyrantel pamoate plus oxantel pamoate and might become a key treatment for STH control and elimination. Although pyrantel pamoate is readily available on the market, oxantel pamoate needs to be developed and registered by a stringent regulatory authority. Our study confirmed that pyrantel pamoate plus oxantel pamoate is a valuable candidate in preventive chemotherapy programmes to avoid the development of drug resistance.

Contributors

WM, SS, JHa, and JK planned and designed the study. The oxantel pamoate tablets were manufactured and produced by MP and JHu. WM, SS, SX, BB, and JK did the study. WM, JHa, and JK analysed and interpreted the data. WM and JK wrote the first draft and JHa revised the manuscript. All authors read and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

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Chapter 4c

Efficacy and reinfection with soil-transmitted helminths 18-weeks post-treatment with albendazole-ivermectin, albendazole-mebendazole, albendazole-oxantel pamoate and mebendazole

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RESEARCH Open Access

Efficacy and reinfection with soil-transmitted helminths 18-weeks post-treatment with albendazole-ivermectin, albendazole-mebendazole, albendazole-oxantel pamoate and mebendazole

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Abstract

Background: Preventive chemotherapy with albendazole or mebendazole is the current strategy to control soil-transmitted helminth (STH) infections (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*). STH reinfections, in particular *A. lumbricoides* and *T. trichiura* occur rapidly after treatment with the standard drugs. However, their low efficacy against *T. trichiura*, made an accurate assessment of reinfection patterns impossible.

Methods: In 2013 a randomised controlled trial was conducted on Pemba Island, Tanzania. School-aged children diagnosed positive for *T. trichiura*, were randomly allocated to (i) albendazole-ivermectin; (ii) albendazole-mebendazole; (iii) albendazole-oxantel pamoate; or (iv) mebendazole. Here we report the efficacy [cure rates (CR) and egg-reduction rates (ERR)], reinfection rates and new infections determined 18 weeks post-treatment.

Results: For a total of 405 children complete baseline and follow-up data were available. Similar to the efficacy determined after 3 weeks, 18 weeks after treatment albendazole-oxantel pamoate showed a significantly higher efficacy against *T. trichiura* (CR: 54.0 %, 95 % CI: 43.7–64.0; ERR: 98.6 %, 95 % CI: 97.8–99.2) compared to the other treatment arms. Children treated with albendazole-oxantel pamoate or albendazole-ivermectin had fewer moderate infections compared to children treated with albendazole. The reinfection rates 18 weeks post-treatment among all treatment arms were 37.2 % for *T. trichiura* (95 % CI: 28.3–46.8), 34.6 % for *A. lumbricoides* (95 % CI: 27.3–42.3) and 25.0 % for hookworms (95 % CI: 15.5–36.6).

Conclusion: The moderate reinfection rates with STHs 18 weeks post-treatment support the concept of regular anthelminthic treatment in highly endemic settings. Combination chemotherapy might achieve decreased morbidity in children since in the albendazole plus oxantel pamoate and albendazole plus ivermectin treatment arms only few moderate *T. trichiura* infections remained. Further trials should investigate the long term efficacy of albendazole-oxantel pamoate (i.e. 6 and 12 month post-treatment) and after several rounds of treatment in order to develop recommendations for appropriate control approaches for STH infections.

Trial registration: Current Controlled Trials ISRCTN80245406

Keywords: *Trichuris trichiura*, *Ascaris lumbricoides*, Hookworm, Randomised controlled trial, Reinfection, Oxantel pamoate

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Background

The most common soil-transmitted helminths (STH; *Ascaris lumbricoides*, hookworms and *Trichuris trichiura*) infect approximately 1.5 billion people [1] worldwide with the highest prevalences in Asia and Africa. School-aged children living in the least developed settings, lacking clean water and sanitation facilities are primarily affected by *A. lumbricoides* and *T. trichiura*, while hookworm infections mostly occur in adults [2, 3]. Untreated, chronically infected children might suffer from anemia, malnutrition and impairments in cognitive and physical development [2]. The burden of soil-transmitted helminthiasis has been estimated as 5.3 million disability-adjusted life years [1]. Large scale distribution of anthelminthic drugs without prior diagnosis ("preventive chemotherapy") mainly given to school-aged children, is the current strategy to control morbidity [4]. The most common anthelminthic drugs are albendazole and mebendazole [5]. Both drugs have high efficacy against *A. lumbricoides*, while only albendazole reveals a good performance in the treatment of hookworm infections. For the treatment of *T. trichiura* both drugs show poor cure rates in single-dose regimen [6].

Preventive chemotherapy does not avert reinfections as demonstrated in earlier studies [7, 8]. Six to 12 months after treatment with albendazole or mebendazole, the prevalence of *A. lumbricoides* reached the pretreatment level [9–11], while hookworm reinfection is slow [9]. However, it is difficult to accurately estimate the reinfection rate of *T. trichiura* since, as mentioned above, the efficacy of the benzimidazoles against *T. trichiura* is low [6], in particular when children suffer from high infection intensity [12].

In a clinical trial conducted in 2013 on Pemba Island, Tanzania, we examined the efficacy of three drug combinations (i.e. albendazole-ivermectin, albendazole-mebendazole, and albendazole-oxantel pamoate) versus mebendazole against *T. trichiura* and concomitant STH infections [13]. In brief, the combination albendazole-oxantel pamoate revealed a significantly higher cure rate (CR) (68.0 %) and egg-reduction rate (ERR 99.2 %) against *T. trichiura* than the other treatment regimens. This high efficacy might allow drawing better conclusions on reinfection with *T. trichiura* and ultimately to develop recommendations for control efforts.

The aim of the present study was to investigate whether the efficacy of albendazole-oxantel pamoate remains superior to the other combinations 18 weeks post-treatment and to monitor reinfection patterns of *T. trichuris*, *A. lumbricoides* and hookworms.

Methods

Study oversight

The presented data derive from a randomised controlled trial conducted among school-aged children on Pemba Island, Tanzania [13]. Ethical clearance was obtained from the Ministry of Health and Social Welfare in Zanzibar, Tanzania (ZAMREC, reference no. 0001/June/13) and from the ethics committee of Basel, Switzerland (EKBB reference no. 123/13). The trial was registered with www.isrctn.com (identifier: ISRCTN80245406). Prior to the study start, written informed consent was obtained from the parents or legal guardians and verbal assent from the participating children.

Study procedures and patients

The clinical trial was conducted from September 2013 to January 2014. Study setting and trial procedures are presented elsewhere [13]. In brief, school-aged children diagnosed positive for *T. trichiura* were randomly assigned to one of the following treatment arms: (i) albendazole (400 mg) plus ivermectin (200 μg/kg); (ii) albendazole (400 mg) plus mebendazole (500 mg); (iii) albendazole (400 mg) plus oxantel pamoate (20 mg/kg); and (iv) mebendazole (500 mg). All children were invited 3 and 18 weeks after treatment to provide stool samples on two consecutive days for the diagnosis of STH infections. Duplicate Kato-Katz thick smears were prepared from each stool sample using 41.7 mg templates [14] and quantitatively examined by experienced laboratory technicians for eggs of *T. trichiura*, hookworms and *A. lumbricoides*. Slides were read under a light microscope within 60 min after preparation to avoid overclearing of hookworm eggs [15]. Ten percent of the Kato-Katz thick smears were randomly chosen and re-examined to assure high quality of the microscopic diagnosis [16]. In case of discrepancies, the slides were read again and discussed until consensus was reached. At the end of the study, all children remaining infected from the two schools (Mchangamdogo and Shungi) received treatment according to national guidelines [17].

Statistical analysis

Prevalence of infection with *T. trichiura*, *A. lumbricoides* and hookworms was calculated for each treatment arm at baseline, 3 and 18 weeks post-treatment for all children with a complete dataset (2 stool samples at each time point). Differences among treatment arms in CRs 3 weeks and extended CRs (extended CR: children positive at baseline and negative at both follow-ups) 18 weeks post-treatment against *T. trichiura*, *A. lumbricoides* and hookworms were assessed using logistic regression.

Geometric means (GM) for eggs per gram (EPG) were calculated by adding 1 to each count (to take the logarithm in case of EPG = 0), taking the GM and subtracting 1 ($GM = \exp((\Sigma \log(EPG + 1))/n) - 1$) [18]. Bootstrap resampling method with 10,000 replicates was used for calculating the 95 % confidence intervals (CI) for the ERRs. We assumed non-overlapping CI as statistical

significant difference in ERRs. All arithmetic means (AM) are presented in the Additional file 1: Table S1.

Reinfection rates were defined as children positive at baseline, negative 3 weeks and positive 18 weeks post-treatment. New infections were defined as children negative at baseline and 3 weeks after treatment and positive 18 weeks post-treatment. As all children were by design positive for *T. trichiura*, new infections according to our definition, were only observed for *A. lumbricoides* and hookworms. Children negative at baseline and positive 3 weeks later were not included in these calculations (excluded for *A. lumbricoides*: 4 out of 405 children; and for hookworm: 27 out of 405 children).

Results

Efficacy of drug combinations against *T. trichiura* 3 and 18 weeks post-treatment

In total 405 children infected with *T. trichiuria* were allocated to four different treatment regimens and provided all six stool samples (i.e. duplicate stool samples at baseline, 3 and 18 weeks follow-up; Fig. 1). The efficacies 3 weeks post-treatment observed with the different treatment regimens are presented elsewhere [13] and summarised in Table 1. The CRs documented 18 weeks post-treatment were 54.0 % (43.7–64.0) for albendazole-oxantel pamoate, 20.0 % (12.7–29.2) for albendazole-ivermectin, 13.9 % (7.8–22.2) for albendazole-mebendazole and 10.6 % for mebendazole (5.4–18.1). At the second follow-up, the efficacy of the other treatment arms remained significantly lower compared to albendazole-oxantel pamoate in terms of CR (*P*-values in Table 1) and ERR (98.6, CI: 97.8–99.2) compared to the other treatments at the second follow up. Considering the arithmetic ERRs, albendazole combined with ivermectin and albendazole-oxantel pamoate were significantly higher compared to the other treatments 18 weeks post-treatment (Additional file 1: Table S1).

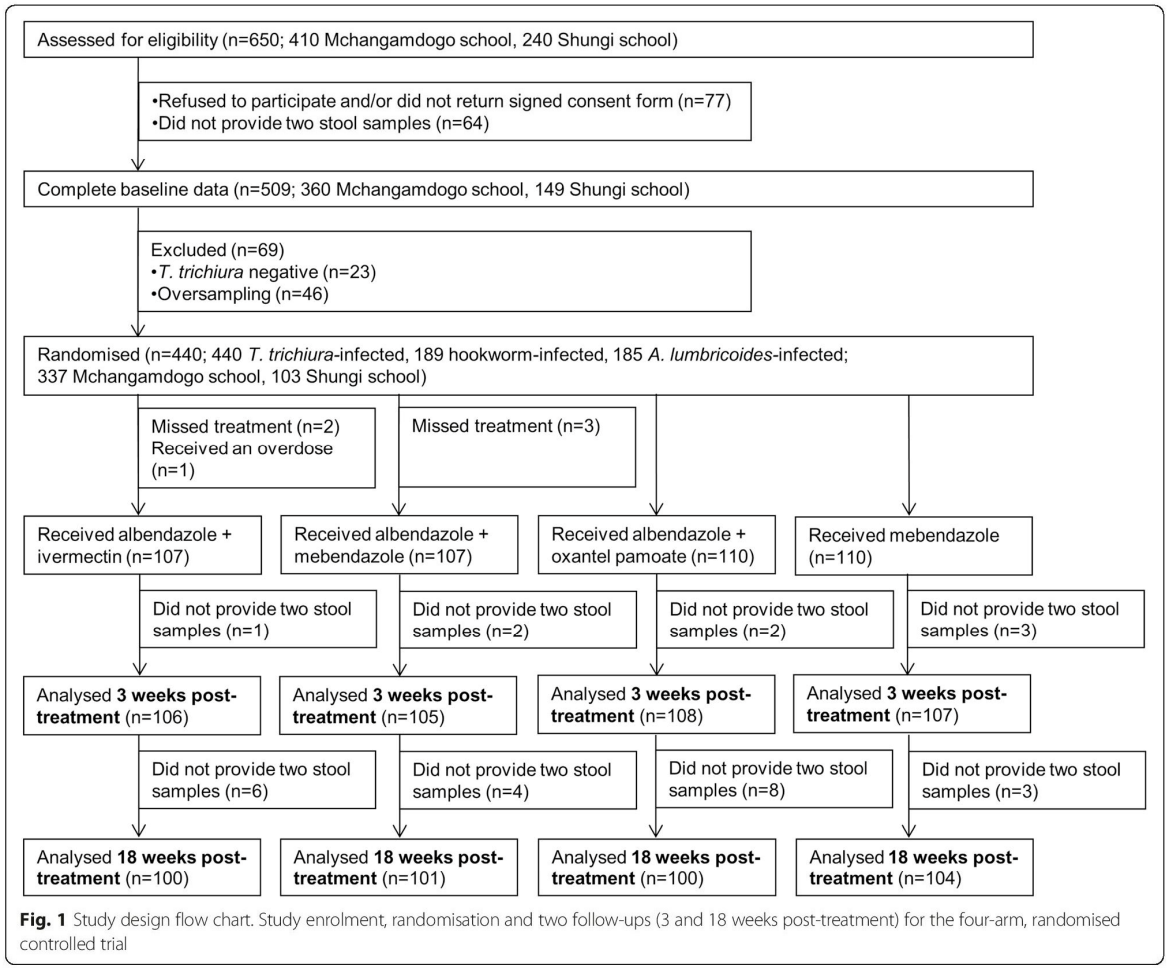

Fig. 1 Study design flow chart. Study enrolment, randomisation and two follow-ups (3 and 18 weeks post-treatment) for the four-arm, randomised controlled trial

Table 1 Cure rates (CR), extended CRs, egg-reduction rates (ERR), extended ERRs and reinfection data for the four different treatments against *T. trichiura* infections. Results of baseline and 3 weeks follow-up have been reported elsewhere [13]

Trichuris trichiura					
	Weeks post-treatment	Albendazole – ivermectin (n = 100)	Albendazole – mebendazole (n = 101)	Albendazole – oxantel pamoate (n = 100)	Mebendazole (n = 104)
Children positive before treatment (%)		100 (100)	101 (100)	100 (100)	104 (100)
No. of children cured (CR, 95 % CI)	3 weeks	28 (28.0, 19.5–37.9, $p < 0.001$)*	9 (8.9, 4.2–16.2, $p < 0.001$)*	68 (68.0, 57.9–77.0)	8 (7.7, 3.4–14.6, $p < 0.001$)*
No. of children negative (extended CR, 95 % CI)	18 weeks	20 (20.0, 12.7–29.2, $p < 0.001$)*	14 (13.9, 7.8–22.2, $p < 0.001$)*	54 (54.0, 43.7–64.0)	11 (10.6, 5.4–18.1, $p < 0.001$)*
Geometric mean: EPG	Baseline	489.9	390.0	471.3	467.8
	3 weeks	25.0	176.0	3.7	207.8
	18 weeks	47.8	128.1	6.6	158.8
ERR (95 % CI)	3 weeks	94.9 (92.3–96.7)	54.9 (38.3–67.7)	99.2 (98.6–99.6)[a]	55.6 (40.0–68.0)
Extended ERR (95 % CI)	18 weeks	90.2 (85.3–93.6)	67.2 (51.9–77.9)	98.6 (97.8–99.2)[a]	66.1 (52.0–76.6)
No. of children positive (prevalence, 95 % CI)	Baseline	100 (100, –)	101 (100, –)	100 (100, –)	104 (100, –)
	3 weeks	72 (72.0, 62.1–80.5)	92 (91.1, 83.8–95.8)	32 (32.0, 23.0–42.1)	96 (92.3, 85.4–96.6)
	18 weeks	80 (80.0, 70.8–87.3)	87 (86.1, 77.8–92.2)	46 (46.0, 36.0–56.3)	93 (89.4, 81.9–94.6)
Reinfections (%, 95 % CI)	18 weeks	15/28 (53.6, 33.9–72.5)	2/9 (22.2, 2.8–60.0)	21/68 (30.9, 20.2–43.6)	4/8 (50.0, 15.7–84.3)

Data are n (%, 95 % CI) unless otherwise indicated. EPG = egg per gram of stool. *Significantly lower CR compared to albendazole-oxantel pamoate (*P*-values derived from logistic regression)
[a]Significantly higher ERR compared to other treatment arms (no overlapping confidence interval assumption)

Baseline infection intensities among treatment arms were equally balanced; 70.9 % of all children harboured light, 28.6 % moderate and 0.5 % heavy infections, stratified according to The World Health Organisation (WHO) cut-offs [18]. The number of children with light, moderate and heavy infection intensities at baseline, 3 and 18 weeks post-treatment for each treatment arm are summarised in Fig. 2. Albendazole plus ivermectin and albendazole plus oxantel pamoate caused a higher reduction of moderate *T. trichiura* infections 3 and 18 weeks post-treatment compared to albendazole-mebendazole and mebendazole. At the 18 weeks follow-up, the number of moderate infections remained higher for albendazole-mebendazole ($n = 16$) and mebendazole ($n = 17$) unlike albendazole-ivermectin ($n = 2$) and albendazole-oxantel pamoate ($n = 3$; see Fig. 2).

T. trichiura reinfection dynamics

For *T. trichiura* the prevalence at baseline was 100 % by design, as only *T. trichiura*-positive children were included ($n = 405$). In total 42 of 113 children (37.2 %, 28.3–46.8), were reinfected with *T. trichiura* 18 weeks after treatment. All reinfections were of mild infection intensity (Table 1, Fig. 2).

Efficacy against *A. lumbricoides* and reinfection dynamics

At baseline, 169 (41.7 %) out of 405 children were infected with *A. lumbricoides*. All treatment arms achieved high CRs at the first follow up (Table 2) [13]. CRs decreased for all treatment arms 18 weeks post-treatment, ranging from 60.5 % for mebendazole up to 69.6 % for albendazole-ivermectin. While at the first follow-up nearly all eggs were cleared (ERR 99.8–100.0 %), 18 weeks after treatment the ERRs remained significantly lower (99.0–99.2 %), except for albendazole-ivermectin (99.7 %).

In total, 57 of the 165 (34.6 %; 27.3–42.3) cured children were found to be reinfected 18 weeks after being treated. Reinfections included light ($n = 41$) and moderate ($n = 16$) infections (Table 2, Fig. 3). In total 58 out of 232 (25.0 %; 19.7–31.1) children had acquired a new light ($n = 50$) or moderate ($n = 8$) *A. lumbricoides* infection.

Efficacy against hookworms and reinfection dynamics

Hookworm prevalence among the children included in the trial was 42.5 % (233 of 405 children). At the second follow-up, slightly higher CRs were observed in all treatment groups compared to 3 weeks post-treatment: 54.0 % for albendazole-oxantel pamoate, 53.5 % for albendazole-mebendazole, 50.0 % for albendazole-ivermectin and 34.5 % for mebendazole (Table 3). At this examination

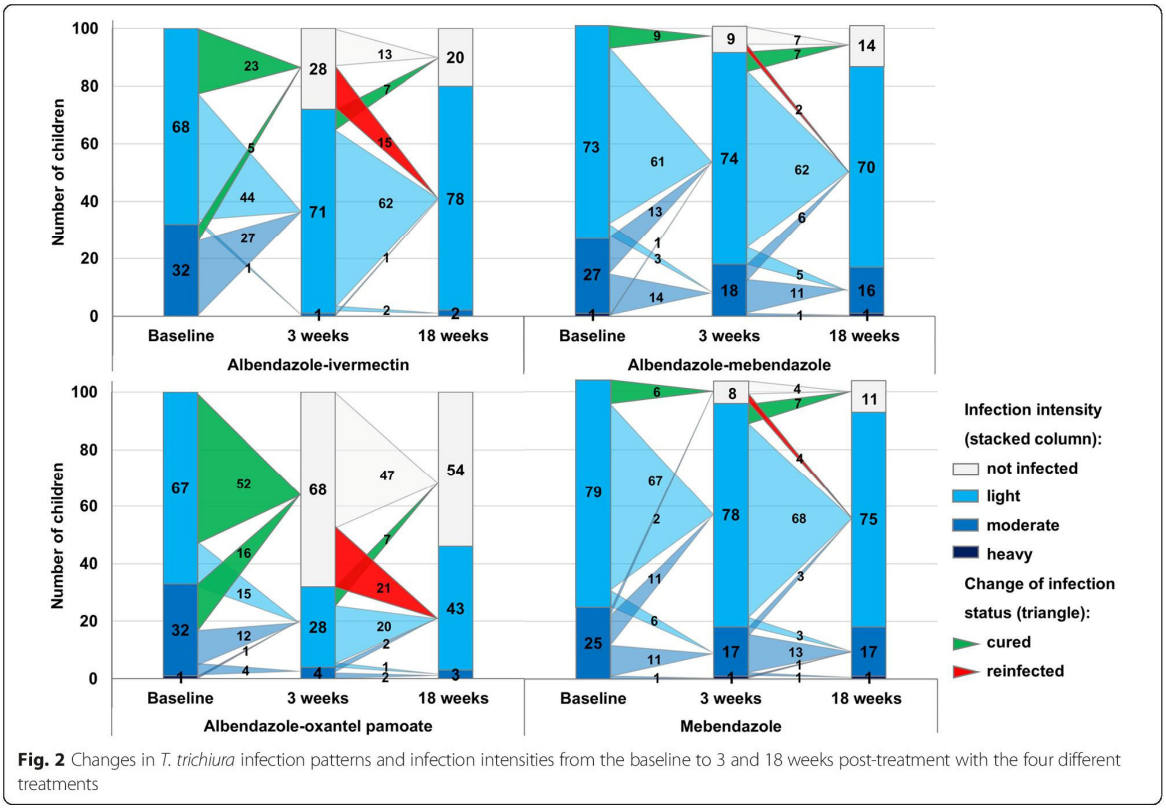

Fig. 2 Changes in *T. trichiura* infection patterns and infection intensities from the baseline to 3 and 18 weeks post-treatment with the four different treatments

Table 2 Cure rates (CR), extended CRs egg-reduction rates (ERR), extended ERRs and reinfection data for the four different treatments against *A. lumbricoides* infections. Results of baseline and 3 weeks follow-up have been reported elsewhere [13]

Ascaris lumbricoides					
	Weeks post-treatment	Albendazole – ivermectin ($n = 100$)	Albendazole – mebendazole ($n = 101$)	Albendazole – oxantel pamoate ($n = 100$)	Mebendazole ($n = 104$)
Children positive before treatment (%)		46 (46.0)	36 (35.6)	44 (44.0)	43 (41.3)
No. of children cured (CR, 95 % CI)	3 weeks	45 (97.8, 88.5–99.9)	36 (100, 90.3–100.0)	43 (97.7, 88.0–99.9)	41 (95.4, 84.2–99.4)
No. of children negative (extended CR, 95 % CI)	18 weeks	32 (69.6, 54.2–82.3)	25 (69.4, 51.9–83.7)	27 (61.4, 45.5–75.6)	26 (60.5, 44.4–75.0)
Geometric mean: EPG	Baseline	2,385.8	1,195.3	1,503.4	1,095.2
	3 weeks	0.1	0.0	0.2	0.4
	18 weeks	6.9	9.1	12.5	10.6
ERR (95 % CI)	3 weeks	99.9 (99.9–100.0)	100 (–)	99.9 (99.9–100)	99.9 (99.8–100)
Extended ERR (95 % CI)	18 weeks	99.7 (99.1–99.9)	99.2 (97.5–99.8)[a]	99.2 (97.1–99.8)[a]	99.0 (97.7–99.6)[a]
No. of children positive (prevalence, 95 % CI)	Baseline	46 (46.0, 36.1–55.9)	36 (35.6, 26.1–45.1)	44 (44.0, 34.1–53.9)	43 (41.3, 31.7–51.0)
	3 weeks	1 (1.0, −1.0–3.0)	2 (2.0, −0.8–4.7)	3 (3.0, −0.4–6.4)	2 (2.0, −0.8–4.6)
	18 weeks	24 (24.0, 15.5–32.5)	28 (27.7, 18.8–36.6)	33 (33.0, 23.6–42.4)	33 (31.7, 22.6–40.8)
Reinfections (%, 95 % CI)	18 weeks	14/45 (31.1, 18.2–46.6)	11/36 (30.6, 16.3–48.1)	16/43 (37.2, 23.0–53.3)	16/41 (39.0, 24.2–55.5)
New infections (%, 95 % CI)	18 weeks	10/54 (18.5, 9.3–31.4)	17/63 (27.0, 16.6–39.7)	15/54 (27.8, 16.5–41.6)	16/61 (26.2, 15.8–39.1)

Data are n (%, 95 % CI) unless otherwise indicated. EPG = egg per gram of stool
[a]Significantly lower ERR compared to the 3 weeks ERR (no overlapping confidence interval assumption)

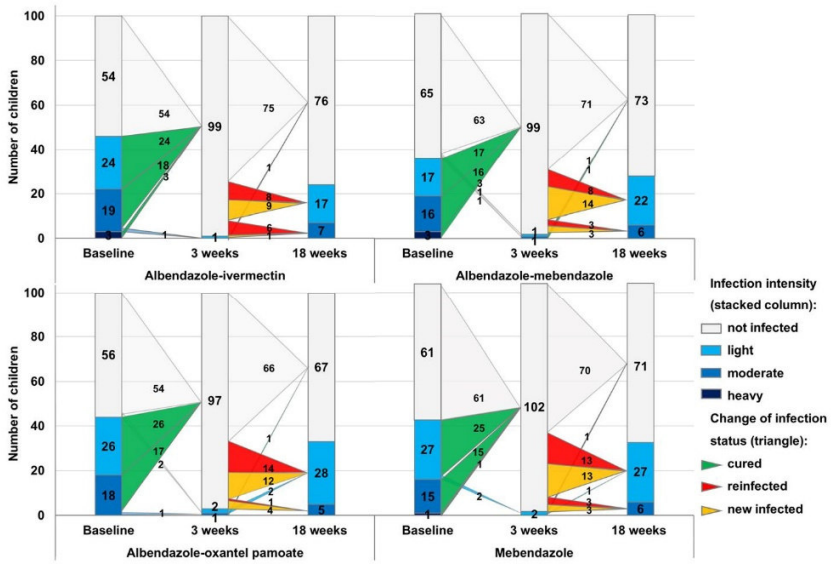

Fig. 3 Changes in *A. lumbricoides* infection patterns and infection intensities from the baseline to 3 and 18 weeks post-treatment with the four different treatments

Table 3 Cure rates (CR), extended CRs, egg-reduction rates (ERR), extended ERRs and reinfection data for the four different treatments against hookworms infections. Results of baseline and 3 weeks follow-up have been reported elsewhere [13]

Hookworms					
	Weeks post-treatment	Albendazole – ivermectin ($n = 100$)	Albendazole – mebendazole ($n = 101$)	Albendazole – oxantel pamoate ($n = 100$)	Mebendazole ($n = 104$)
Children positive before treatment (%)		38 (38.0)	43 (42.6)	50 (50.0)	41 (39.4)
No. of children cured (CR, 95 % CI)	3 weeks	17 (44.7, 28.6–61.7)	21 (48.8, 33.3–64.5, $p = 0.022$)*	24 (48.0, 33.7–62.6, $p = 0.023$)*	10 (24.4, 12.4–40.3)
No. of children negative (extended CR, 95 % CI)	18 weeks	19 (50.0, 33.4–66.6)	23 (53.5, 37.7–68.8)	27 (54.0, 39.3–68.2)	14 (34.2, 20.1–50.6)
Geometric mean: EPG	Baseline	113.1	139.8	87.2	80.0
	3 weeks	6.1	8.3	7.1	31.8
	18 weeks	6.3	6.3	6.6	20.7
ERR (95 % CI)	3 weeks	94.6 (89.2–97.6)[a]	94.1 (88.7–97.0)[a]	91.9 (85.0–95.8)[a]	60.3 (27.8–79.2)
Extended ERR (95 % CI)	18 weeks	94.4 (88.8–97.5)[a]	95.5 (91.5–97.8)[a]	92.4 (85.4–96.3)	74.1 (52.8–86.4)
No. of children positive (prevalence, 95 % CI)	Baseline	38 (38.0, 28.3–47.7)	43 (42.6, 32.8–52.4)	50 (50.0, 40.0–60.0)	41 (39.4, 29.9–49.0)
	3 weeks	26 (26.0, 17.3–34.7)	26 (25.7, 17.1–34.4)	31 (31.0, 21.8–40.2)	44 (42.3, 32.7–52.0)
	18 weeks	27 (27.0, 18.1–35.9)	32 (31.7, 22.5–40.9)	34 (34.0, 24.6–43.4)	39 (37.5, 28.0–47.0)
Reinfections (%, 95 % CI)	18 weeks	5/17 (29.4, 10.3–56.0)	4/21 (19.0, .4–41.9)	6/24 (25.0, 9.8–46.7)	3/10 (30.0, 6.7–65.4)
New infections (%, 95 % CI)	18 weeks	6/57 (10.5, 4.0–21.5)	9/54 (16.7, 7.9–29.3)	8/45 (17.8, 8.0–32.1)	6/50 (12.0, 4.5–24.3)

Data are n (%, 95 % CI) unless otherwise indicated. EPG = egg per gram of stool
*Significantly higher CR compared to mebendazole (*P*-values derived from logistic regression)
[a]Significantly higher ERR compared to mebendazole (no overlapping confidence interval assumption)

time point albendazole-ivermectin (94.4 %; 88.8–97.5) and albendazole-mebendazole (95.5 %; 91.5–97.8), achieved significantly higher ERRs in comparison to mebendazole (74.1 %; 52.8–86.4). The arithmetic ERRs showed comparable results (Additional file 1: Table S1).

Eighteen weeks post-treatment 18 out of 72 (25.0 %; 15.5–36.6) children were reinfected with hookworms. All infections were of mild intensity (Table 3, Fig. 4). In total, 29 out of 206 children (14.1 %, CI 9.6–19.6) had acquired a new infection, ranging from 10.5 % (albendazole-ivermectin) to 17.8 % (albendazole-oxantel pamoate).

Discussion

In preventive chemotherapy control programs albendazole and mebendazole are the treatment of choice against infections with all three STHs, yet both reveal a poor efficacy against *T. trichiura* [6]. In the recent past, oxantel pamoate has emerged as frontrunner for the treatment of infections with *T. trichiura* [13, 19, 20]. In more detail, in our recent studies albendazole-oxantel pamoate revealed good CRs and high ERRs, while mebendazole achieved CRs of 11.8 % [19] and 8.4 % [13] against *T. trichiura* infection. This low efficacy of mebendazole in the Pemba setting might be due to the occurrence of drug resistance, although molecular studies were not carried out to demonstrate it. In this scenario that is common also to other STH endemic areas, the need of new drug combinations in order to expand the armamentarium of treatments available for preventive chemotherapy strategy is of utmost importance [21].

In addition, albendazole-oxantel pamoate achieved a high reduction of moderate *T. trichiura* infections (persisting at 18 weeks post-treatment) in contrast to mebendazole. Note that, the goal of preventive chemotherapy is to reduce the morbidity from STH in pre- and school-aged children, by decreasing moderate and heavy infection intensities to a level below 1 % [22]. The combination albendazole-oxantel pamoate might contribute to achieve this goal.

Infections after treatment re-appear fast, particularly for *A. lumbricoides* and hence have a huge impact on the success of preventive chemotherapy [9]. This study presents detailed insights about the impact of drug combinations including the effective oxantel pamoate combination on the reinfection dynamics of the three STHs in a highly endemic area on Pemba Island, Tanzania. Earlier studies on reinfection struggled with the low CR

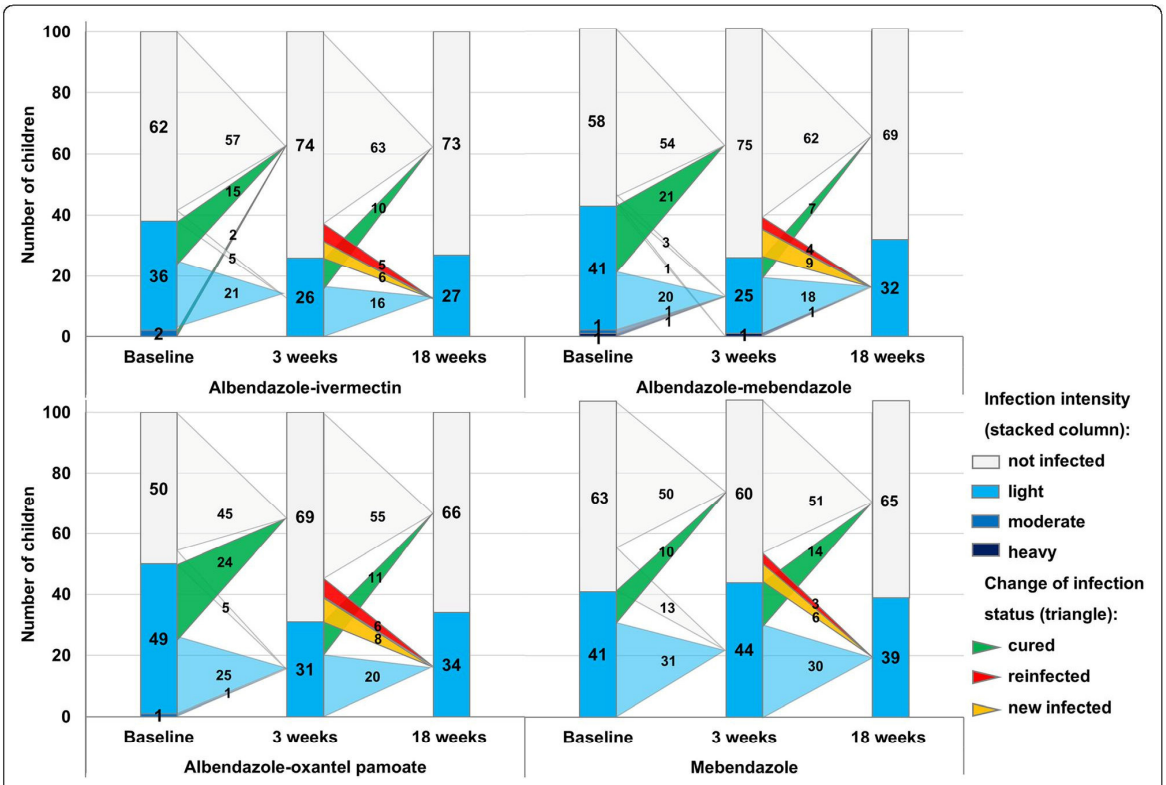

Fig. 4 Changes in hookworms infection patterns and infection intensities from the baseline to 3 and 18 weeks post-treatment with the four different treatments

of the standard drugs against *T. trichiura* which complicated drawing sound conclusions against this helminth species [9, 23].

Previous studies on reinfection mainly described the reacquired level of infection after treatment, in comparison to the pre-treatment level and presented the prevalence risk ratio [9, 24, 25]. With the focus only on prevalence before and after treatment, new infections are falsely considered as reinfection. Furthermore, prevalence risk ratios are strongly influenced by the achieved CRs of the treatments. Hence, in this study we distinguish and present both; reinfections rates (positive children at baseline, negative at the 3 week examination point and positive 18 weeks after treatment) and new infections (children with an infection exclusively 18 weeks post-treatment). Note that, children negative at baseline and positive 3 weeks later, which was observed for *A. lumbricoides* and hookworms, were not considered as new infections. We assume, that these children either harboured a non-patent infection or were wrongly diagnosed as negative at baseline [26]. We are confident that our differentiation between reinfections and new infections holds true given that the majority of *A. lumbricoides* and hookworm infections were of moderate intensities at baseline. However, obviously the low sensitivity of the Kato-Katz technique mainly for light infection intensities [26–28] reflects a general limitation of our study. Please note that in the present study four slides were examined by Kato-Katz. However, even when examining multiple thick smears the Kato-Katz method only reaches moderate sensitivity [29]. Therefore our results (i.e. re-infection, new infection) have to be interpreted with caution.

Earlier studies on reinfection with *T. trichiura* reported prevalence to pre-treatment level between 6 and 12 months [9–11, 24, 30]. In our study, the overall reinfection rate 18 weeks post-treatment for *T. trichiura* was 37.2 %, which is comparable to the 3 month post treatment prevalence risk ratio of 36.0 % reported by Sinniah [31] and 39.7 % by Al-Mekhlafi et al. [24]. A similar reinfection rate was observed for *A. lumbricoides* (34.6 %), which is in agreement with the 3 month post-treatment data of Jia et al. [9]. On the other hand, Yap et al. [30] documented a higher reinfection rate 4 months after treatment. Additionally, 58 (25 %) of the children negative at baseline acquired a new *A. lumbricoides* infection 18 weeks post-treatment. Interestingly, 24 children

(6.0 %) who were negative 3 weeks post-treatment harboured already a moderate infection 18 weeks post-treatment, indicating the fast reinfection potential of *A. lumbricoides* [22].

The three drug combinations cured nearly half of the hookworm-infected children 3 weeks post-treatment, while as expected mebendazole achieved only low CRs [32–35]. Surprisingly, the extended CRs and ERRs among all treatment arms (except the ERR from albendazole-ivermectin) increased at the second compared to the first follow-up. For example, in the mebendazole group, 14 children identified as hookworm-positive 3 weeks post-treatment were negative at the second follow up. This finding is most likely due to a diagnostic issue, i.e. the low sensitivity of Kato-Katz for low egg-counts [36] and a delayed reading of the microscope slides [15, 37], which could have led to fluctuations of Kato-Katz results. Overall, the reinfection rate was slower and less new infections with hookworms were observed compared to *A. lumbricoides* and *T. trichiura*, which is in agreement with other studies [9].

Conclusions

In conclusion, our study has reconfirmed the excellent efficacy of an albendazole-oxantel pamoate combination against *T. trichiura* infections. This combination could become a key element in controlling STH infections, especially in highly endemic settings. Further trials, should evaluate reinfection rates with oxantel pamoate six and 12 month after treatment and ideally after several rounds of treatment.

The moderate reinfection rate observed for *T. trichiura* and *A. lumbricoides* is worrying. This finding supports the necessity of an integrated control approaches including regular treatment, improved sanitation and health education [38–41], in order to reduce the burden of STH infections.

Additional file

Additional file 1: Table S1. Arithmetic mean egg per gram (EPG), egg-reduction rates (ERR) and extended ERRs for the four different treatments against *T. trichiura*, *A. lumbricoides* and hookworms infections. (DOCX 17 kb)

Abbreviations

AM: arithmetic mean; CR: cure rate; DALYs: disability-adjusted life years; ERR: egg-reduction rate; GM: geometric mean; STH: soil-transmitted helminth; WHO: World Health Organisation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BS, MA, JHa and JK designed the study. BS, WM, SMAl, SMAm and JK implemented the study. BS, WM, JHa and JK analysed and interpreted the data. BS, WM and JK wrote the first draft of the report, and MA and JHa reviewed it. All authors read and approved the final manuscript.

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Chapter 5a

Diagnostic comparison between FECPAK^{G2} and the Kato-Katz method for analysing soil-transmitted helminth eggs in stool

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RESEARCH ARTICLE

Diagnostic comparison between FECPAK^{G2} and the Kato-Katz method for analyzing soil-transmitted helminth eggs in stool

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Abstract

Background

Over one billion people are infected with soil-transmitted helminths (STH), i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*. For estimating drug efficacy and monitoring anthelminthic drug resistance, accurate diagnostic methods are critical. FECPAK^{G2} is a new remote-diagnostic tool used in veterinary medicine, which produces an image of the stool sample that can be stored on an internet cloud. We compared for the first time FECPAK^{G2} with the recommended Kato-Katz method.

Methodology/Principal findings

Two stool samples were collected from adolescent participants (age 15–18 years) at baseline and 14 to 21 days after treatment in the framework of a randomized clinical trial on Pemba Island, Tanzania. Stool samples were analyzed with different diagnostic efforts: i) one or ii) two Kato-Katz thick smears from the first sample, iii) two Kato-Katz thick smears from two samples and iv) FECPAK^{G2} from the first sample. Parameters were calculated based on a hierarchical Bayesian egg count model.

Complete data for all diagnostic efforts were available from 615 participants at baseline and 231 hookworm-positive participants at follow-up. At baseline FECPAK^{G2} revealed a sensitivity of 75.6% (72.0–77.7) for detecting *A. lumbricoides*, 71.5% (67.4–95.3) for hookworm and 65.8% (64.9–66.2) for *T. trichiura*, which was significantly lower (all $p<0.05$) than any of the Kato-Katz methods and highly dependent on infection intensity. Despite that the egg counts based on FECPAK^{G2} were relatively lower compared to Kato-Katz by a ratio of 0.38 (0.32–0.43) for *A. lumbricoides*, 0.36 (0.33–0.40) for hookworm and 0.08 (0.07–0.09) for *T. trichiura*, the egg reduction rates (ERR) were correctly estimated with FECPAK^{G2}.

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Competing interests: We have read the journal's policy and the authors of this manuscript have the following competing interests: GJM is managing director of Techion Group Ltd, which developed FECPAKG2. He was not involved in the study design, implementation, analyzing and interpreting of the data, to prevent any conflict of interest.

Conclusions/Significance

The sensitivity to identify any STH infection was considerably lower for FECPAK^{G2} compared to Kato-Katz. Following rigorous development, FECPAK^{G2} might be an interesting tool with unique features for epidemiological and clinical studies.

Author summary

About 1.5 billion people are infected with soil-transmitted helminths (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*). Since morbidity correlates with the number of worms harbored by an infected individual, WHO aims to reduce moderate and heavy infections in pre- and school-aged children by 2020. The cornerstone of estimating the prevalence, assessing drug efficacy and monitoring drug resistance are accurate diagnostic tools. The currently recommended Kato-Katz, has some major disadvantages like a short processing window and low sensitivity and new diagnostic tools are needed. FECPAK^{G2} is an online, remote location tool developed for counting nematode eggs in sheep, cattle, equine and Camelids fecal samples. The output of the system is an image of the sample, which is saved and uploaded onto an internet cloud. This offers new options particularly for low resource settings. We tested FECPAK^{G2} for the first time for analyzing human stool in a randomized controlled trial. We observed a baseline sensitivity of 75.6% for detecting *A. lumbricoides*, 71.5% for hookworm and 65.8% for *T. trichiura* and an increased sensitivity for moderate infection intensities. Despite lower sensitivity and egg counts, FECPAK^{G2} was able to correctly estimate egg reduction rates. Following further development, FECPAK^{G2} might become an important tool for soil-transmitted helminth control programs, epidemiological and clinical studies.

Introduction

Approximately 1.5 billion people are infected with the soil-transmitted helminths (STH) *Ascaris lumbricoides*, hookworm and/or *Trichuris trichiura* [1]. While the majority of light infections remain asymptomatic, moderate and heavy infections are responsible for a considerable health burden, including growth stunting, intellectual retardation, cognitive and educational deficits, malnutrition and iron-deficiency anemia [2,3]. The estimated global STH burden was 3.3 million disability adjusted life-years in 2016 [4]. Large scale distribution of anthelminthic drugs (i.e. albendazole and mebendazole) to at-risk populations in preventive chemotherapy programs is the current strategy against STH infections [5]. The ultimate goal of the World Health Organization (WHO) is to reduce burden caused by moderate and heavy infections [5].

For estimating prevalence of soil-transmitted helminthiasis, assessing infection intensities, evaluating drug efficacy and monitoring drug resistance, accurate diagnostic methods are essential [5–7]. The currently recommended Kato-Katz method has already been in use for decades [8,9]. The advantages of Kato-Katz are its low cost, short sample preparation time, simple handling and the need of only basic equipment [8,10]. However, the method has a low sensitivity for low STH infection intensities, hookworm eggs disappear after one hour and samples and slides for hookworm cannot be stored [11–13]. The sensitivity can be improved by analyzing multiple Kato-Katz thick smears from several samples [12,14] or by analyzing an increased amount of stool as it is done by the FLOTAC (1 gram) or Mini-FLOTAC (2/10 gram) system [15,16].

Once the strategy is moving towards transmission control and STH elimination, an increased sensitivity of the diagnostic method of choice is crucial [6]. Nowadays, several molecular tools are available to diagnose STH infections. Although these tools show increased sensitivity, they are time consuming, require costly laboratory equipment and highly skilled laboratory technicians [17,18]. Therefore, the research on new diagnostic tools is necessary, with the aim of developing a fast, simple and cost-effective method for the diagnosis of STH infections. FECPAK^{G2} is an online, remote location, parasite diagnostic system used in veterinary medicine [19]. The first FECPAK system was originally established for counting nematode eggs in sheep fecal samples [20–22]. FECPAK^{G2} is based on the flotation-dilution principle, similar to the McMaster method [23]. The novelty of FECPAK^{G2} is the accumulation of parasite eggs into one viewing area within a fluid meniscus [24,25]. An image of the fecal sample is then captured, is stored offline on a computer and can be uploaded onto a cloud once connected to the internet. Subsequently, the image can be analyzed at any time by specialists around the world.

The aim of the study was to comparatively assess the sensitivity, the associated cure rates (CRs), the egg counts and their related egg reduction rates (ERR) based on FECPAK^{G2} and the Kato-Katz method (i.e. single, double and quadruplicate Kato-Katz). The diagnostic comparison was conducted in the framework of a clinical trial including different tribendimidine co-administrations against hookworm infections on Pemba Island, Tanzania [26].

Methods

Ethics statement

In 2016, a randomized controlled, single-blind, non-inferiority trial evaluating the efficacy of tribendimidine co-administrations, was conducted in Tanzania and Côte d'Ivoire. The presented data on the diagnostic comparison is based exclusively on samples collected in Tanzania [26]. Ethical clearance was obtained from the Zanzibar Medical Research and Ethical Committee in Tanzania (reference ZAMREC/0001/APRIL/016) and the Ethics Committee of Northwestern and Central Switzerland (reference EKNZ UBE-15/35). This trial is registered with ISRCTN registry (number ISRCTN14373201). Written informed consent from parents or legal guardians and verbal assent from participants were obtained prior to the sample collection. At the end of the study, participants remaining positive for any STH were treated with a standard dose albendazole (400 mg) according to national guidelines [27].

Study population

The study was carried out during August and September 2016 on Pemba Island, Tanzania. Details of the clinical trial procedure are described elsewhere [26]. Briefly, adolescents (age 15 to 18) from four different secondary schools (Wingwi, Mizingani, Wesha and Tumbe) were asked to provide two stool samples at baseline. Hookworm positive participants were randomly allocated to the treatment arms: i) tribendimidine (400 mg), ii) tribendimidine (400 mg) plus ivermectin (200 μg/kg), iii) tribendimidine (400 mg) plus oxantel pamoate (25 mg/kg) and iv) albendazole (400 mg) plus oxantel pamoate (25 mg/kg). Another two stool samples were collected 14 to 21 days after treatment at the follow-up visit. Participants, laboratory and field technicians were blinded.

Parasitological methods

Kato-Katz. Fresh stool samples were labelled with a unique identification number and transferred to the Public Health Laboratory-Ivo de Carneri. Of each stool sample, a duplicate

Kato-Katz thick smear using a 41.7 mg template [9], was prepared by experienced laboratory technicians. Between a half and one hour after preparation–to avoid over-clearing of hookworm eggs [13]–the STH eggs were counted using a light microscope. For assuring diagnostic quality, 10% of all Kato-Katz slides were randomly selected, re-examined by the study investigator for *A. lumbricoides* and *T. trichiura* eggs. In case of discordant results the slides were read a third time and discussed until consensus was reached [28].

FECPAK^{G2}. The first stool sample collected at baseline and follow-up was analyzed with FECPAK^{G2}. The standard operational procedure (SOP) manual was adopted for human stool samples by Ayana and colleagues and is made available online [25]. Briefly, three grams from each stool sample were mixed thoroughly with 38 ml tap water using a Fill-FLOTAC [16]. The suspension was transferred to the FECPAK^{G2} sedimenter and tap water was added. After one hour the supernatant was flushed away and 80 ml saturated NaCl flotation solution (density = 1.2) was added to the sediment, giving a total volume of 95 ml, which equates to 0.032 g stool per ml saline. The solution was transferred to the FECPAK^{G2} cylinder, which includes two wire mesh sieves (apertures: outer 425 microns, inner 250 microns) to remove large debris. The two wells of the FECPAK^{G2} cassette were each filled with each 455 μl of the solution which combined contained 0.029 g stool. After 20 minutes, the cassette was placed into the MICRO-I (FECPAK^{G2} imaging unit) and a single image frame of the axisymmetric meniscus of each well was captured [29]. The images were uploaded onto the Microsoft Azure Cloud system (Microsoft Corp., Redmond, WA) via the FECPAK^{G2} software. The mark-up of the images was done by two laboratory technicians on Pemba, using the FECPAK^{G2} software. STH eggs were identified on both images, marked according to the species and the combined total egg count was automatically determined by the FECPAK^{G2} software. Quality control was performed on half of the images in Switzerland using a computer-generated list. An image was classified as insufficient quality and excluded in case of: blurriness, stacking bands, cracked rods, debris, air bubbles, over and under filling of the cassette wells.

Statistical analysis

For each of the following diagnostic method i) one Kato-Katz thick smear of the first sample, ii) two Kato-Katz thick smears of the first sample, iii) quadruplicate Kato-Katz thick smears (two Kato-Katz thick smears of each sample) and iv) FECPAK^{G2} from the first sample, the sensitivity was determined for *A. lumbricoides*, hookworm and *T. trichiura* at baseline and follow-up. The sample size calculated for the clinical trial [26] was deemed sufficient for this diagnostic comparison.

A hierarchical Bayesian egg-count model as described by Bärenbold et al. [30] was applied to individual level data. The Kato-Katz counts were modelled with a negative binomial distribution depending on the daily egg density. The log of the mean egg density at the individual level was assumed to vary normally between days and the mean infection intensities to be gamma distributed in the population with a mean that reflects the mean infection intensity of an infected individual. The model was extended with a negative binomial process, to simulate the data obtained by FECPAK^{G2}, with a linearly reduced daily egg density for the same individual compared to Kato-Katz and an independent over-dispersion parameter of the negative binomial distribution. Sample sensitivity of each test was calculated as the ratio between observed prevalence and estimated true prevalence. We assumed a specificity of more than 98% for Kato-Katz and set an uniform prior for the specificity of FECPAK^{G2}.

The efficacy for each treatment arm in terms of CRs (percentage of egg-negative participants with a previous infection) and ERRs (percentage of arithmetic mean egg count reduction from baseline to follow-up) was calculated according to the four different diagnostic methods

for all baseline positive children. CRs were calculated with imperfect diagnostic methods and an estimate for the true value based on the egg count model was given. Varying sensitivity between baseline and follow-up because of reduced infection intensity, show the following relation to the "true" CRs: $(1 - CR_{True}) = (1 - CR_{observed}) \times \frac{s_{bl}}{s_{fu}}$ which follows from the definition of the cure rate under the assumption of no reinfections happening between baseline and follow-up (S1 Text). For the different diagnostic methods, the sensitivity-ratio between baseline and follow-up was calculated. In case the 95% confidence interval (CI) of the sensitivity-ratio included 1, the apparent CRs were not significantly different from the true CR.

Eggs per gram of stool (EPG) were calculated by multiplying the single and the average of two (duplicate) or four (quadruplicate) Kato-Katz thick smears with a factor of 24. For FECPAK^{G2} the egg counts were multiplied by a factor of 34. The true ERR was based on the reduction from baseline to follow-up of the mean infection intensity estimates from the model. The 95%-confidence intervals (CI) for the apparent ERRs of the treatments for each diagnostic method were obtained using a bootstrap resampling approach with 5000 replications [31].

For the statistical analysis, Stata version 14.0 (Stata Corporation; College Station; Texas, United States of America), OpenBugs version 3.2.3, Stan version 2.16.2, and R version 3.4.1 were used.

Results

Study flow

Stool samples from 1,005 participants were collected (Fig 1). Data of 391 participants were excluded: 142 provided only one stool sample, the sample of 105 participants were not analyzed with FECPAK^{G2} because of technical issues (ID mismatch or not sufficient stool) and FECPAK^{G2} images from 144 from participants were classified as insufficient quality. A total of 615 participants had complete baseline data and 384, 330 and 579 were infected with *A. lumbricoides*, hookworm and *T. trichiura*, respectively (Table 1). Only 25 participants were negative for any STH. From the participants with baseline data, 308 were treated, whereas 285 were hookworm negative and 22 were absent at treatment day. Of 308 participants randomized to treatment 13 participants were lost to follow-up,. from 21 participants the samples were not analyzed with FECPAK^{G2} because of technical issues and the data of 43 participants were excluded because of insufficient quality of the images. Complete follow-up data were available from 231 participants.

True prevalence, sensitivity and specificity

The estimated true baseline prevalence was 64.0% (95% confidence interval [CI] 62.2–67.1) for *A. lumbricoides*, 54.8% (53.1–57.9) for hookworm and 94.7% (94.0–96.0) for *T. trichiura*. At follow-up, prevalence values of 5.5% (4.0–8.5), 44.3% (39.4–50.5) and 52.0% (49.8–54.7) were estimated for *A. lumbricoides*, hookworm and *T. trichiura* respectively (S1 Table).

At baseline, the sensitivity of the quadruplicate Kato-Katz was significantly higher compared to any other method with 97.7% (93.1–99.9) for *A. lumbricoides*, 98.3% (92.7–99.9) for hookworm and 99.5% (98.1–99.9) for *T. trichiura*. In contrast, the sensitivity of FECPAK^{G2} was significantly lower than the single and duplicate Kato-Katz method (all $p<0.05$) with 75.6% (72.0–77.7) for detecting *A. lumbricoides*, 71.5% (67.4–95.3) for hookworm and 65.8% (64.9–66.2) for *T. trichiura*. The specificity estimated for FECPAK^{G2} was 96.9% (94.8–98.9) for *A. lumbricoides*, 91.3% (89.3–93.1) for hookworm and 95.3% (91.8–97.6) for *T. trichiura*. Estimated true prevalence, sensitivities, sensitivity-ratio and egg counts from the 231 participants with complete follow-up data is presented in S1 Table.

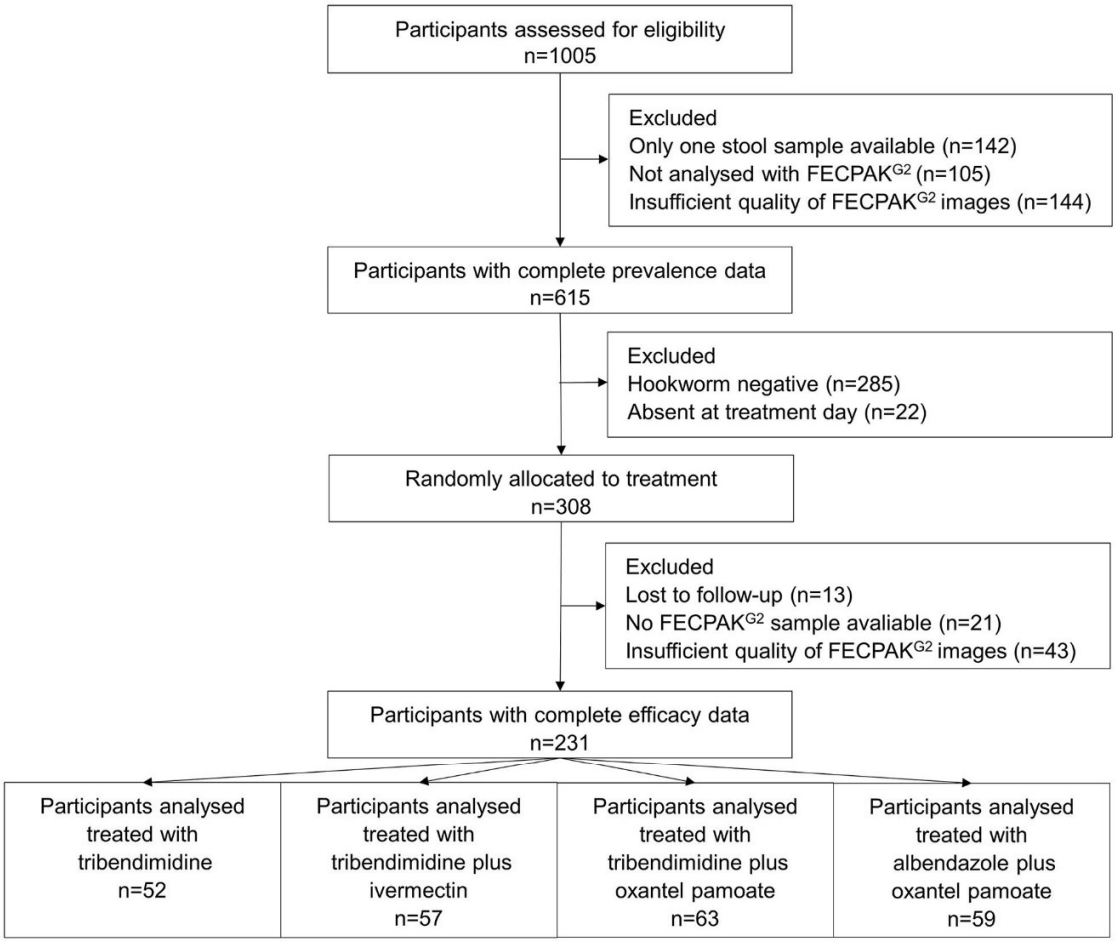

Fig 1. Study flow of stool samples collection and analysis using the single, duplicate, quadruplicate Kato-Katz and FECPAK^{G2}.

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The sensitivity of FECPAK^{G2} was highly dependent on the infection intensity (Fig 2, S2 Table S). For an infection intensity of 100 EPG, the sensitivity of FECPAK^{G2} was as low as 42.9% (37.3–46.9) for *A. lumbricoides*, 56.3% (51.0–61.3) for hookworm and 22.2% (19.9–23.5) for *T. trichiura*. The estimated sensitivity increased for moderate infection intensity according to WHO cut-offs [8] and resulted in 82.0% (78.8–84.5) for *A. lumbricoides* (EPG 5000), 95.6% (94.1–97.3) for hookworm (EPG 2000) and 70.3% (67.6–73.9) for *T. trichiura* (EPG 1000).

Estimation of egg counts

The estimated true mean egg counts according to the model were 18125 EPG (15024–21724) for *A. lumbricoides*, 474 EPG (402–558) for hookworm and 1999 EPG (1762–2252) for *T. trichiura* at baseline (Table 1). Data from the follow up is presented in S1 Table. The EPGs based on FECPAK^{G2} were several times lower at baseline and follow-up compared to the different Kato-Katz sampling efforts. Relative to the Kato-Katz, the egg counts of FECPAK^{G2} were lower by an egg density-ratio (Fig 3, red line) of 0.38 (0.32–0.43) for *A. lumbricoides*, 0.36 (0.33–0.40) for hookworm and 0.08 (0.07–0.09) for *T. trichiura*.

Table 1. Estimated true prevalence, sensitivity and arithmetic mean egg counts from the 615 participants with complete baseline data according to the four different diagnostic methods.

		A. lumbricoides	Hookworm	*T. trichiura*
Estimated true	Prevalence	64.0 (62.2–67.1)	54.8 (53.1–57.9)	94.7 (94.0–96.0)
	Eggs per gram of stool	18125 (15024–21724)	474 (402–558)	1999 (1762–2252)
Single Kato-Katz	No. of positive participants (%)	347 (56.4)	288 (46.8)	553 (89.9)
	Eggs per gram of stool	14361 (12099–16622)	509 (415–603)	1760 (1517–2003)
	Sensitivity	87.8 (83.6–90.7)	85.5 (80.4–88.1)	94.8 (93.3–95.6)
Duplicate Kato-Katz	No. of positive participants %	353 (57.4)	299 (48.6)	559 (90.9)
	Eggs per gram of stool	14175 (11866–16485)	474 (391–556)	1725 (1489–1961)
	Sensitivity	89.8 (85.6–92.3)	89.1 (84.0–91.6)	96.1 (94.7–96.7)
Quadruplicate Kato-Katz	No. of positive participants (%)	384 (62.4)	330 (53.7)	579 (94.2)
	Eggs per gram of stool	13478 (11435–15521)	434 (359–508)	1796 (1544–2048)
	Sensitivity	97.7 (93.1–99.9)	98.3 (92.7–99.9)	99.5 (98.1–99.9)
FECPACK^{G2}	No. of positive participants (%)	297 (48.3)	240 (39.0)	383 (62.3)
	Eggs per gram of stool	3048 (2501–3595)	245 (197–293)	171 (148–194)
	Sensitivity	75.6 (72.0–77.7)	71.5 (67.4–95.3)	65.8 (64.9–66.2)
	Specificity	96.9 (94.8–98.9)	91.3 (89.3–93.1)	95.3 (91.8–97.6)

Numbers in brackets show 95% confidence interval, unless otherwise indicated

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Cure rates

The true CRs estimated by the model and the apparent CRs according to the different diagnostic methods are presented in Fig 4 and S3 Table. According to the sensitivity-ratio (SR), there was no noteworthy difference between the true estimated and the apparent CRs for the quadruplicate Kato-Katz (S3 Table). For FECPAK^{G2} the true estimated CRs for hookworm (SR

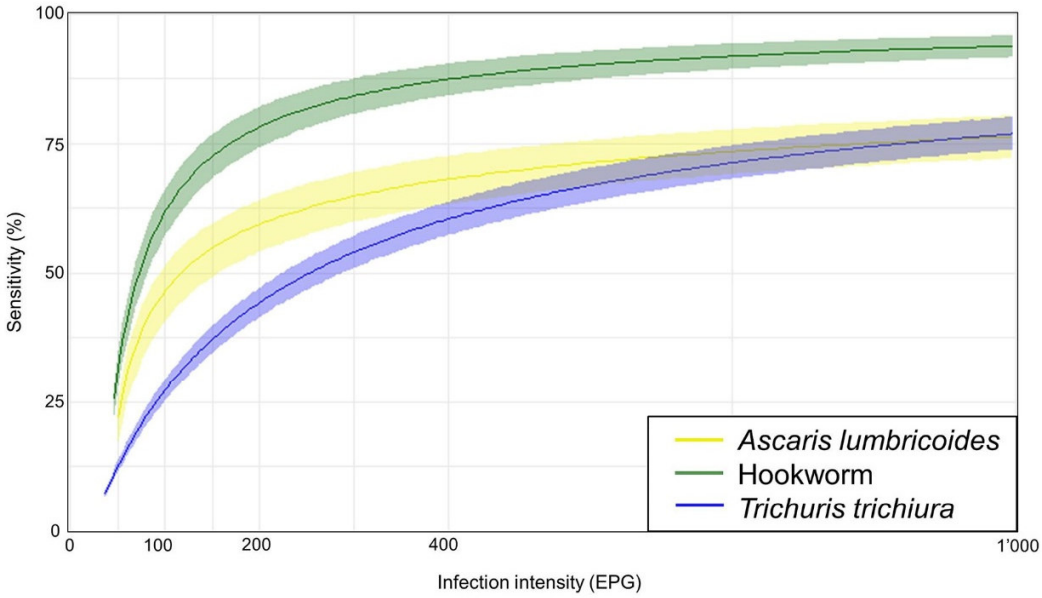

Fig 2. The estimated sensitivity of FECPAK^{G2} based on the infection intensity.

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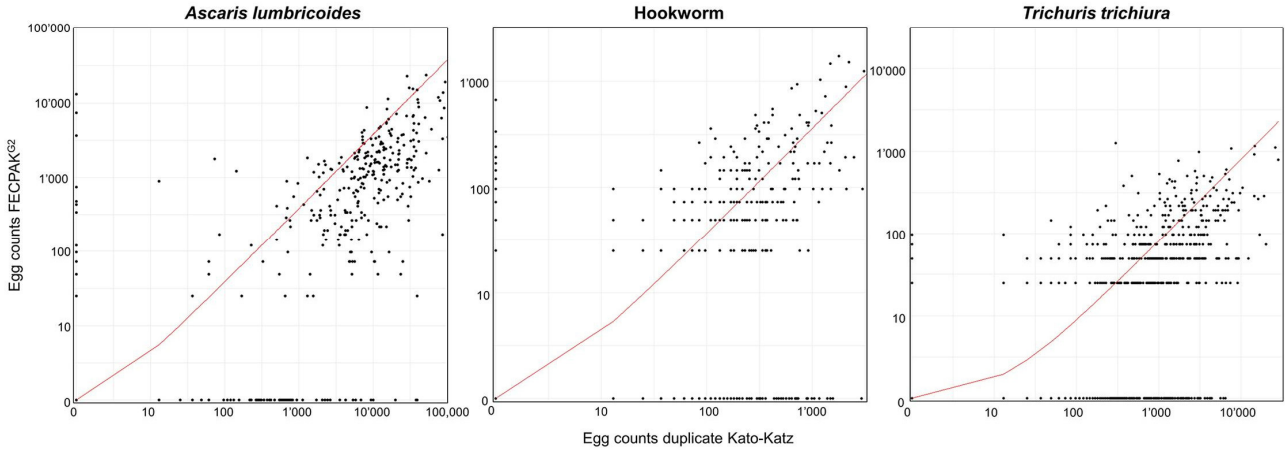

Fig 3. Scatter plot of the egg counts based on FECPAK^{G2} and duplicate Kato-Katz egg counts. Red line indicates egg density-ratio between Kato-Katz and FECPAK^{G2}.

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2.21, 1.88–2.63) and *T. trichiura* (SR 2.06, 1.83–2.36) differed significantly compared to the true estimated CRs. Since the CRs were generally high for *A. lumbricoides* (CR>93%) and most participants were cured, the sensitivity-ratio estimates had a higher uncertainty, included one and no differences among the diagnostic method were observed (SR 1.38, 0.98–2.28, S3 Table).

For tribendimidine or albendazole in combination with oxantel pamoate against hookworm, low true CRs were observed and the apparent CRs decreased with higher Kato-Katz sampling effort. The CRs according to FECPAK^{G2} compared to the true CRs were significantly higher for tribendimidine-oxantel pamoate (82.6%, 68.6–92.2 *versus* 46.3%, 35.2–52.6) and albendazole-oxantel pamoate (82.5%, 67.2–92.7 *versus* 49.2%, 36.7–56.2). Against *T. trichiura*, the difference was particularly pronounced for the treatment arm tribendimidine-ivermectin

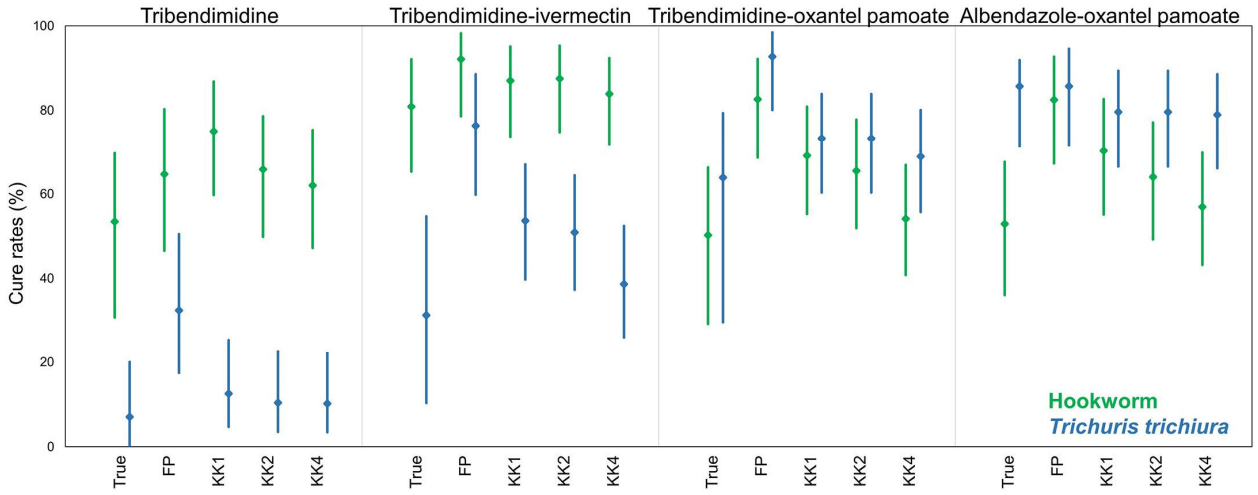

Fig 4. True cure rates (True) and cure rates based on a single (KK1) duplicate (KK2), quadruplicate Kato-Katz (KK4) and FECPAK^{G2} (FP) against hookworm and *T. trichiura* for the four different treatment arms. Cure rates against *A. lumbricoides* are not presented.

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with a true CRs of 34.1% (25.7–37.7), followed by the quadruplicate (38.6%, 26.0–52.4) and duplicate Kato-Katz (50.9%, 37.3–64.4) and a significantly higher CR for FECPAK^{G2} (76.3%, 59.8–88.6). Similar, slightly less pronounced differences were found between the true and the FECPAK^{G2} CRs for tribendimidine monotherapy (5.5%, 1.6–8.5 *versus* 32.4%, 17.4–50.5) and tribendimidine-oxantel pamoate (66.8%, 58.1–71.1 *versus* 92.7%, 80.1–98.5).

Egg reduction rate according to diagnostic methods

No noteworthy difference was observed between the true ERRs and the arithmetic ERRs according to the four diagnostic methods (S4 Table, Fig 5). Despite lower EPGs for FECPAK^{G2} compared to any of the Kato-Katz methods, the ERRs and interval estimates remained similar with one exception. For tribendimidine monotherapy against *T. trichiura*, the true ERR (22.9%, 5.3–50.3) and the ERR determined by FECPAK^{G2} (29.4%, -38.3–66.7), were non-significantly higher compared to the ERRs based on the quadruplicate Kato-Katz (17.6%, -17.1–38.8).

Discussion

New diagnostic tools are required to complement or replace the currently recommended Kato-Katz method [8]. FECPAK^{G2} is a remote-location, online parasite diagnostic system, which is used in veterinary medicine. This is the first study, which compared the FECPAK^{G2} method in human parasitology in the framework of a randomized, clinical trial on Pemba island, Tanzania [26]. We assessed for FECPAK^{G2} several different diagnostic parameters including prevalence, sensitivity and the associated CRs, egg counts, infection intensity and the related reduction in intensity after treatment.

For FECPAK^{G2}, sensitivity was significantly lower compared to single, duplicate and quadruplicate Kato-Katz for identifying any of the STH at baseline and follow-up. However, a lower sensitivity was expected, since FECPAK^{G2} examines only 1/34 of gram of stool compared to 1/24 gram for the single, 1/12 gram for duplicate and 1/12 (day 1) plus 1/12 gram (day 2) for the quadruplicate Kato-Katz. For detecting moderate infection intensities, the

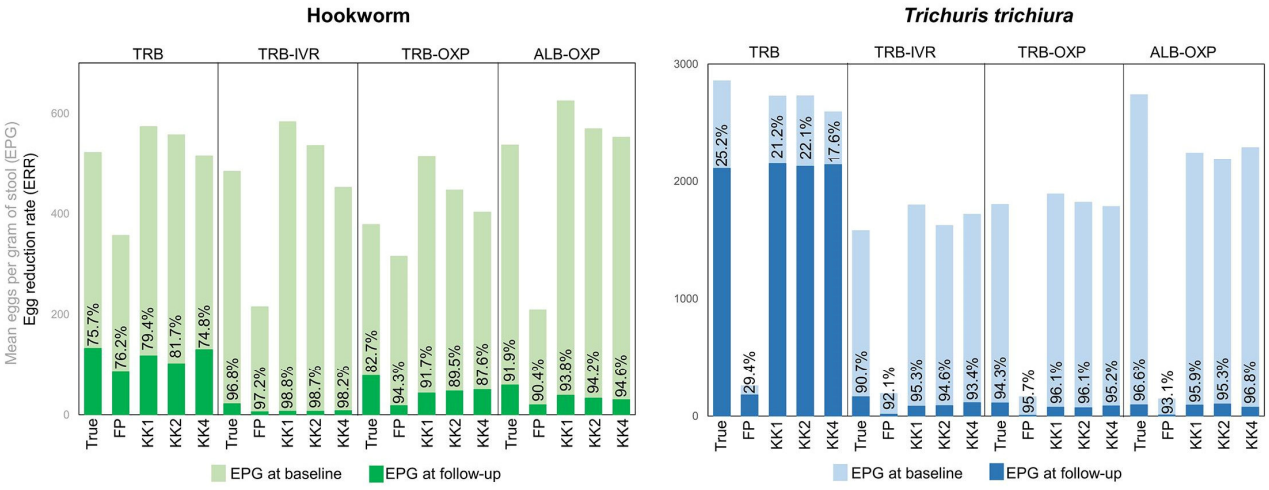

Fig 5. True egg reduction rates (ERR) and ERRs based on single (KK1), duplicate (KK2), quadruplicate Kato-Katz (KK4) and FECPAK^{G2} after treatment with tribendimidine (TRB), tribendimidine-ivermectin (TRB-IVR), tribendimidine-oxantel pamoate (TRB-OXP) and albendazole-oxantel pamoate (ALB-OXP). Egg reduction rates against *A. lumbricoides* are not presented.

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FECPAK^{G2} sensitivity increased to 82.0% for *A. lumbricoides*, 95.6% for hookworm and 70.3% for *T. trichiura*. Similar characteristics have been shown for the Kato-Katz method, i.e. low sensitivity for low infection intensities and high sensitivity for moderate and heavy infections [12].

Since the CRs are a function of the sensitivity, and the sensitivity of FECPAK^{G2} was highly dependent on the infection intensity, the FECPAK^{G2} CRs and the true CRs were significantly different. For example, for tribendimidine-oxantel pamoate the *T. trichiura* infection intensity changed from baseline (true EPG~2000) to follow-up (true EPG~100), which led to a decreased sensitivity from 80.5% (baseline) to 22.2% (follow-up,). Therefore, the CR for FECPAK^{G2} (92.7%) was significantly overestimated compared to the true CR (66.8%) (S3 Table). These results indicate, that in the present form FECPAK^{G2} does not accurately estimate CRs, which was also true for the single and duplicate Kato-Katz.

While the lower sensitivity negatively influenced the CRs, the ERRs remained unchanged, which was already reported by Levecke and colleagues for different Kato-Katz sampling efforts [32]. Similarly, no differences among the diagnostic methods were shown in our study. For instance, the above-mentioned treatment example resulted in a true ERR of 94.3%, which was not significantly different from an ERR of 95.7% with FECPAK^{G2}(S4 Table). While the egg counts with FECPAK^{G2} were generally lower compared to Kato-Katz, the ERRs remained equal. Thus, FECPAK^{G2} might be an interesting tool for monitoring anthelmintic drug efficacy [5].

A lower egg recovery rate from sheep or cattle fecal samples was already observed for the earlier FECPAK system in comparison with FLOTAC, Mini-FLOTAC and McMaster, however, no data about the performance of the new FECPAK^{G2} was available [20,21]. The lower recovery of eggs by FECPAK^{G2} might be due to the inability of detecting unfertilized *A. lumbricoides* eggs and a high extent of debris covering the eggs. To overcome the problem with high debris, a variety of different sized meshes for the FECPAK^{G2} cylinder are currently being tested. In addition, in the FECPAK^{G2} cassette the capillary rise of the aqueous saline generates an axisymmetric meniscus over the cylindrical rod, which converges the eggs on the top of the meniscus [29]. The accumulated eggs remain in a single microscopic field of view and a staged image of the meniscus is taken with the MICRO-I. For increasing the recovery, a vibration function in the MICRO-I might improve the egg accumulation, as suggested by Sowerby and colleagues [29]. Further optical and image processing improvements for the MICRO-I are under development. These improvements will speed up the processing capability of the device and will generate higher quality images that are expected to improve the egg recovery (sensitivity) and accuracy of the image mark-up.

Obviously, the examination of only one cassette and one stool sample with FECPAK^{G2} was a limitation of our study. The collection of two stool samples would account for the day-to-day variation and would increase sensitivity [30]. For example, in this study the sensitivity increased from one analyzed stool sample (single or duplicate Kato-Katz) to two stool samples (quadruplicate Kato-Katz) about 10%-points for *A. lumbricoides* and hookworm. The sensitivity-ratio indicated a weak dependence of the quadruplicate Kato-Katz on infection intensities, which did not induce a significant bias for this study, since the sample size was rather small and precision estimates wide. Nevertheless, the bias might become important in larger studies with higher accuracy. By collecting samples on several days, the sensitivity of FECPAK^{G2} for low infection intensities might improve, which would limit the bias introduced in CR estimates. Hence, the analysis of two cassettes and two stool samples with FECPAK^{G2}, should be the subject of further studies. Additionally, the time for preparing one sample and the costs of FECPAK^{G2} should be compared against current established diagnostic methods.

Other limitations of this study were the loss of samples due to the mixing up of sample IDs, insufficient amount of stool and insufficient quality of many FECPAK^{G2} images. In more

detail, a total of 144 (19.0%) samples at baseline and 43 (14.0%) samples at follow-up were excluded, because of insufficient filling of the cassette or problems associated with the capturing of the image (i.e. blurriness, stacking bands, cracked rods, debris, air bubbles etc.), which was detected only during the mark-up process of the images when sample analysis could not be repeated. With lower numbers of analyzed samples per day, larger number of laboratory technicians, better experience with handling of the FECPAK^{G2} the number of excluded samples might have been lower and hence these factors should be considered in future studies.

Despite the discussed limitations of FECPAK^{G2} at the current stage of development, several advantages are worth highlighting. The most innovative feature is the captured image, which is saved offline, uploaded online onto an internet cloud and analyzed at any later time point. In contrast, the major limitation of Kato-Katz is the disappearance of hookworm eggs one hour after the preparation [13]. Moreover, stool samples cannot be stored [11], which limits the time to control the diagnostic quality [28]. The storage of the FECPAK^{G2} images offers new options, especially for low resource settings. First, diagnostic results of STH can be stored for the first time, analyzed by trained technicians around the world and quality control is not restricted to time. Second, technicians can focus on processing the samples while analysis is done at a later time point, potentially leading to a faster turnaround in laboratories. Third, in case of identification discrepancies, specialist around the world can be consulted, which improves the diagnostic results. Research is ongoing to develop an image-analysis algorithm, which will automatically count the different helminth eggs in the future.

In conclusion, we have assessed for the first time the performance of FECPAK^{G2} in human parasitology, in the framework of a randomized controlled trial. Compared to different Kato-Katz sampling efforts, FECPAK^{G2} showed lower sensitivities and egg recovery rates. The sensitivity increased with higher infection intensities. Further research is required for increasing sensitivity and egg recovery to develop FECPAK^{G2} as a useful addition in the near future to the depleted diagnostic set of tools for STH infections.

Supporting information

S1 Checklist.
(DOCX)

S1 Text.
(DOCX)

S1 Table.
(DOCX)

S2 Table.
(DOCX)

S3 Table.
(DOCX)

S4 Table.
(DOCX)

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Writing – original draft: Wendelin Moser.

Writing – review & editing: Oliver Bärenbold, Greg J. Mirams, Piet Cools, Johnny Vlaminck, Jan Hattendorf, Bruno Levecke, Jennifer Keiser.

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Chapter 5b

Unexpected low soil-transmitted helminth prevalence in the Butha-Buthe district in Lesotho, results from a cross-sectional survey

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Parasites & Vectors

SHORT REPORT Open Access

Unexpected low soil-transmitted helminth prevalence in the Butha-Buthe district in Lesotho, results from a cross-sectional survey

Wendelin Moser[1,2], Niklaus Daniel Labhardt[2,3], Molisana Cheleboi[4], Josephine Muhairwe[5] and Jennifer Keiser[1,2*]

Abstract

Background: Soil-transmitted helminth (STH) infections with *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* affect large parts of the world's population. For the implementation of national STH control programs, e.g. preventive chemotherapy (treatment with albendazole and mebendazole), the spatial distribution and prevalence of STH infections must be known. However, for Lesotho only little data were available and the STH distribution remains largely unknown.

Methods: In early 2016, a cross-sectional parasitological STH survey was conducted including six different primary schools in the Butha-Buthe district of Lesotho. In each school stool samples were collected from 50 children (age 8–14 years) and analysed with a duplicate Kato-Katz thick smear for the presence of *A. lumbricoides*, hookworm and *T. trichiura*.

Results: A total of 301 children provided a stool sample. All children were negative for *A. lumbricoides* and *T. trichiura*. Only two children from one primary school showed a light hookworm infection.

Conclusion: Our data indicate a low prevalence of STH infections in the Butha-Buthe district of Lesotho. Additional parasitological surveys on the prevalence and the spatial distributions of STH infections across the entire country of Lesotho are needed.

Keywords: Soil-transmitted helminths, Neglected tropical diseases, *Ascaris lumbricoides*, Hookworm, *Trichuris trichiura*, Butha-Buthe, Lesotho

Background

Neglected tropical diseases (NTDs) are a group of communicable diseases, which mainly affect poor, rural communities in tropical and sub-tropical regions around the world. Soil-transmitted helminthiasis is an important NTD caused by infections with the nematodes *Ascaris lumbricoides*, hookworm (*Necator americanus* and *Ancylostoma duodenale*) and *Trichuris trichiura*. Around the globe, about 5.3 billion people are living in an area where soil-transmitted helminth (STH) transmission occurs. Approximately 1.5 billion people are infected with at least one of the nematodes, with highest infection numbers for *A. lumbricoides* (820 million), followed by *T. trichiura* (470 million) and hookworm (420 million) [1, 2].

A chronic STH infection causes numerous health conditions including dietary deficiency, physical delayed cognitive development and iron deficiency [3–6]. STH infections are responsible for an estimated burden of 3.4 million disability-adjusted life years (DALYs) [7]. Preventive chemotherapy with repeated administration of albendazole and mebendazole is the current strategy of choice to reduce moderate and heavy STH infections in pre-and school-aged children [8, 9]. Preventive chemotherapy is promoted by the World Health Organization

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(WHO), due to its fast and strong impact on morbidity and simple implementation [10].

In order to guide STH control programs (e.g. preventive chemotherapy), parasitological and epidemiological data are required. In more detail, data on the district and national prevalence and the intensities of infections [11] are necessary to initiate a locally adapted control program. Given that the main public health problems of Lesotho are HIV/AIDS and tuberculosis [12] data on STHs are very scarce. Neither published prevalence data nor maps of Lesotho presenting the spatial and temporal distribution of helminth infections are available [2, 13, 14]. In the WHO NTD country profile for Lesotho of the year 2010 STHs are listed as endemic and approximately half a million pre-and school-aged children are estimated to be in need of preventive chemotherapy. In 2010, about 150,000 pre-school aged children were treated with albendazole or mebendazole. Since 2010, the estimated number of pre- and school-aged children requiring preventive chemotherapy remained unchanged, however the number of tablets administered were not recorded [15, 16].

In early 2015, the Ministry of Health of Lesotho conducted a country wide survey for mapping NTDs [17]. Five schools per district including 50 children per school were examined for STH infections. STH infections were observed to be a significant public health problem for the Butha-Buthe district with highest prevalence for *T. trichiura* (unpublished observations). The aim of our study was to confirm these results generated by the Ministry of Health of Lesotho in the Butha-Buthe district and to present the first time, openly available knowledge on STH prevalence in Lesotho.

Methods

Study area and procedures

This cross-sectional study was conducted from 25 April until 6 May 2016 in Butha-Buthe, the most northern district in Lesotho (Fig. 1). The population of the district slightly exceeds 100,000 inhabitants (Lesotho Bureau of Statistics), of which about 30,000 live in the district's only city, while the remaining inhabitants live in rural, partially very remote mountainous areas. The study was designed based on WHO guidelines for managing helminth control in school-aged children [11]. We aimed to include 50 children aged 8 to 14 from five randomly chosen primary schools in the Seboche catchment area (Marakabei, St. Charles, Lekopa, Lebesa and Khukhune, Fig. 1) and one primary school from the Linakeng catchment area (Damaseka).

On the collection day stool containers labelled with a unique identification number were distributed randomly to 52 eligible children, who were asked to provide a stool on the same morning. An additional two children were invited to participate in the study (total = 52) to account for children who were not able to provide a stool on the same morning. All containers from one school per day were collected and transferred to the laboratory of the St. Charles Seboche Mission Hospital. From each stool sample duplicate Kato-Katz thick smears (41.7 mg each) were prepared [18] and read within 1 h after preparation

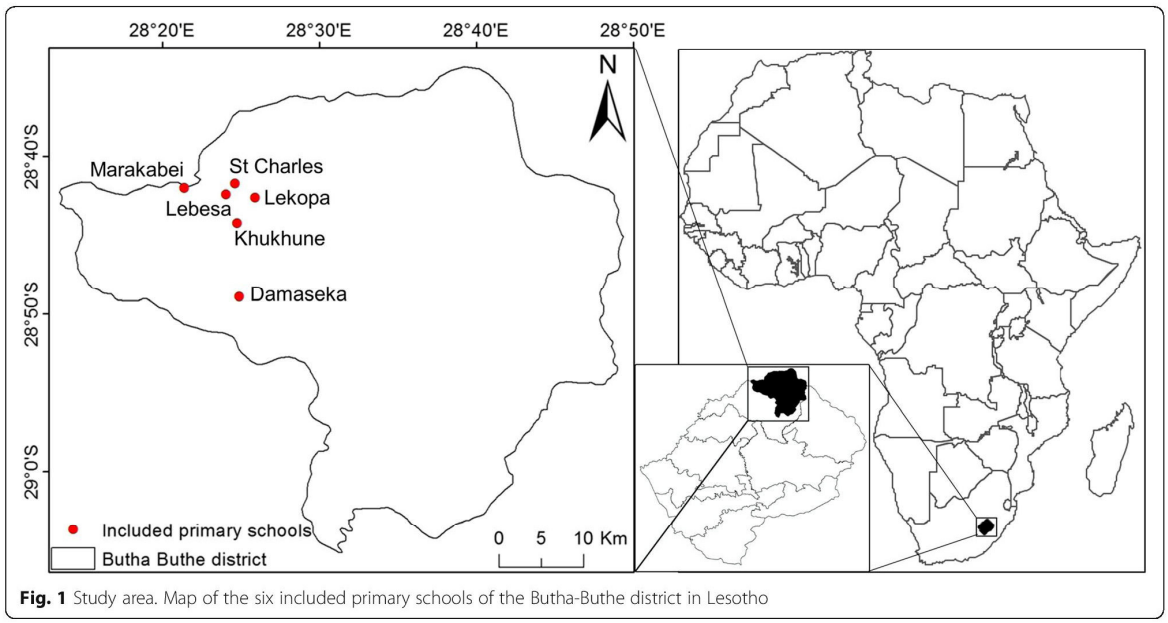

Fig. 1 Study area. Map of the six included primary schools of the Butha-Buthe district in Lesotho

to avoid overclearing of hookworm eggs [19]. The slides were analysed under a light microscope for *A. lumbricoides*, hookworm and *T. trichiura* eggs by three experienced technicians. Subsequently, an independent quality control of sample results (approximately 10%) was conducted. The quality control tolerance margin was chosen according to the suggestion of Speich et al. [20] and in case of discrepancies, the exceeding slides were re-read and discussed until consensus was reached.

An infection was classified as positive if at least one parasite egg in at least one the two Kato-Katz thick smears was present. The eggs counted in both slides were added, divided by two and multiplied by a factor of 24 to obtain egg per gram (epg) of stool values. Infection intensities were stratified into light, moderate and heavy according to WHO cut-offs [21]. The prevalence was calculated as percentage of positive children. All data were analysed using the statistical program STATA Version 14 (Stata Corp. LP; Texas, USA).

Results

A total of 312 children were invited to participate in the study. Of these, 301 children returned a stool sample, while 11 were not able to defecate on the same morning. The aim of collecting 50 samples per school was achieved, except for Khukhune primary school ($n = 49$), where 3 children were not able to provide a stool sample. Fifty samples were collected in the primary schools of St. Charles, Lebesa, Marakabei and Damaseka and 52 in Lekope (Table 1).

The age of included children ranged from 8 to 14 with an average of 10.6 years (Table 1). Slightly more girls (53.8%) were included in the study. The vast majority of participants were visiting grade 4 to 6 (76.1%) with only few children registered in grades 3 and 7 (23.9%).

All studied children from the schools St. Charles, Lebesa, Marakabei, Lekope and Khukhune were negative for *A. lumbricoides*, hookworm and *T. trichiura* (Table 1). From 50 children examined in the Damaseka primary school, two showed a light (epg < 300) hookworm infection (4.0%) and none were infected with *A. lumbricoides* and *T. trichiura*.

Discussion

The most recent estimates from 2010 reported approximately 1.5 billion STH-infected people, with highest infection numbers found in Asia, followed by sub-Saharan Africa [2]. In 2014 the global coverage of preventive chemotherapy with anthelmintic drugs (albendazole and mebendazole) reached 47% [22]. To further increase the treatment coverage and adequately plan and implement STH control programs, knowledge about the spatial distribution and helminth species-specific prevalences are necessary. Despite an ongoing global effort to map the abundance of STH, there are no publicly available data for Lesotho [2, 13, 14]. Official estimates hint that soil-transmitted helminthiasis is a significant public health burden in Lesotho. In more detail, the very first estimate retrieved from the WHO preventive chemotherapy and transmission (PCT) databank, reports a number of 750,000 pre- and school-aged children requiring anthelmintic drugs in Lesotho and dates back to the year 2003 [15]. For the year 2015, the estimates still suggested a high number (520,000) of pre- and school-aged children

Table 1 Characteristics and infections numbers of the included children from the primary schools Marakabei, St. Charles, Lekopa, Lebesa, Khukhune and Damaseka of the Butha-Buthe district in Lesotho

School	St. Charles	Lebesa	Marakabei	Lekopa	Khukhune	Damaseka	Total
Invited children (*n*)	52	52	52	52	52	52	312
Participating children (*n*)	50	50	50	52	49	50	301
Age (yrs) average (± SD)	10.4 ± 1.6	10.5 ± 1.9	11.0 ± 1.7	9.3 ± 1.0	11.0 ± 1.3	11.4 ± 1.6	10.6 (±1.7)
Boys, *n* (%)	28 (56.0)	26 (52.0)	20 (40.0)	25 (48.1)	22 (44.9)	18 (36.0)	139 (46.2)
Grade, *n* (%)							
3	12 (24.0)	11 (22.0)	0 (0)	16 (30.8)	0 (0)	8 (16.0)	47 (15.6)
4	15 (30.0)	11 (22.0)	18 (36.0)	22 (42.3)	10 (20.4)	4 (8.8)	80 (26.6)
5	8 (16.0)	9 (18.0)	11 (22.0)	14 (26.9)	14 (28.6)	13 (26.0)	69 (22.9)
6	15 (30.0)	16 (32.0)	14 (28.0)	0 (0)	16 (32.7)	19 (38.0)	80 (26.6)
7	0 (0)	3 (6.0)	7 (14.0)	0 (0)	9 (18.4)	6 (12.0)	25 (8.3)
Infected children, *n* (%)							
Ascaris lumbricoides	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	0 (0)	0 (0)
Hookworm	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	2 (4.0)	2 (0.7)
Trichuris trichiura	0 (0)	0 (0)	0 (0)	0 (0)	0 (0.0)	0 (0)	0 (0)

in need of preventive chemotherapy for soil-transmitted helminthiasis. In addition, an unpublished survey conducted by the Ministry of health in Lesotho in early 2015 revealed moderate prevalence rates for *A. lumbricoides* and *T. trichiura* infections in the whole of Lesotho. Published data are not available and the exact number of STH-infected children, requiring preventive chemotherapy, remains unknown. We aimed to fill this knowledge gap and present for the first time findings on the prevalence of STHs in Lesotho, specifically in the Butha-Buthe district. According to the above mentioned unpublished survey conducted by the Ministry of Health of Lesotho Butha-Buthe revealed highest prevalence among all districts in Lesotho.

Our study showed no STH infections in school-aged children attending the five primary schools of the Seboche catchment area. Evaluating STH infections in only one catchment area is clearly a limitation of our study. However, in order to verify our negative results, we added one primary school (Damaseka) of another catchment area. The primary school of Damaseka was already involved in the previous, unpublished Ministry of Health survey and revealed high *T. trichiura* prevalence. Despite even the use of a more sensitive diagnostic method compared to the previous NTD survey (duplicated Kato-Katz *vs* direct microscopy) [23, 24] and a quality control, we could not confirm the high *T. trichiura* prevalence, while two children were infected with hookworm. Retrospectively, for settings with such a low prevalence, diagnostic methods with higher sensitivity would have been required, as e.g. a multiple Kato-Katz or polymerase chain reaction (PCR) [24, 25].

Our results are in contrast to the high prevalence reported by the NTD-survey for the Butha-Buthe district conducted in nearby schools and the following points are offered for discussion. First, the prevalence of STHs is highly variable even in small areas and influenced by the difference of locally restricted factors, e.g. geological composition and soil types [26, 27]. Since the Seboche catchment area was not involved in the previous NTD-survey, the difference between the moderate prevalence reported earlier and no infection observed in our study could be attributed to spatial distribution.

Secondly, effective sanitation is in place in the study area. In more detail, during collection of the stool samples in the primary schools information on the availability of sanitation at the school and village level in the Seboche catchment area was obtained. Each of the primary schools of the Seboche catchment area had a clean, well-functioning toilet and all children were encouraged by the teacher to use the toilets, while open defecation is prohibited. Teachers and local collaborators reported that the vast majority of homes around the school are equipped with an adequately working toilet (commonly ventilated pit latrines). Of note, this is not the case in all schools and villages of Lesotho and the Butha-Buthe district. To overcome this problem one large project financed by the Millennium Challenge Cooperation (MCC, USA) currently conducts health trainings, the construction of rural water systems to provide safe drinking water and the construction of ventilated pit latrines in order to improve the health situation in the least developed villages [28, 29], including some adjacent villages of Damaseka (personal observation).

Third, the history of treatment might be responsible for the differing prevalence rates observed by us and the Ministry of Health of Lesotho. However, to our knowledge primarily pre-schoolers are treated in Lesotho. In more detail, to tackle the problem of STH infections in Lesotho, the national guidelines recommend the administration of albendazole or mebendazole twice a year to pre-school aged children (from the age 1 to 5) [30, 31]. Data from the WHO PCT databank report solely the treatment of pre-school aged children in the year 2007 and 2010, while school-aged children were not included in these programs [15]. In subsequent years, albendazole was offered free of charge to pre-school aged children at the health centre level (personal communication). The extend of this deworming program was highly depended on the parents' motivation to get their children treated.

Conclusions

A NTD-survey from 2015 indicated moderate STH prevalence for the Butha-Buthe district, however we could not confirm these findings. Based on our data, STH infections are not a public health problem for the area of Seboche and only marginally for the Linakeng catchment area. It is worth highlighting that we only investigated a handful of schools, which does not allow drawing a general conclusion for the situation for the entire district. To generate further data on the spatial distribution of STH in Butha-Buthe, a random sampling of the entire district is necessary. Subsequently, a detailed national survey should be conducted, which allows revising the national deworming policy.

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Availability of data and materials

All data generated or analysed during this study are included in this article.

Authors' contribution
WM, NDL and JK conceptualized and designed the study. WM, MC and JM conducted the study. WM analysed and interpreted the data. WM wrote the first draft of the paper and JK revised the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Prior to the study start, ethical approval of the Ministry of Health and Ethics Committee of Lesotho (reference: ID62–2016) was obtained. The District Education Officer of Butha-Buthe and the school directors were informed and consented to the study. Information meetings for parents or legal guardians were held and only children with a written consent signed by the parents or legal guardians were included. The study was conducted in collaboration with the Primary Health Care (PHC) team of St. Charles Seboche Mission Hospital and SolidarMed and integrated into routine PHC-activities.

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Chapter 6 - General Discussion

6.1. Rationale and objectives

About 5.3 billion people are living at risk and 1.24 people are infected with at least one of the soil-transmitted helminths (STH), i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* [1]. To control STH infection, the current strategy by the World Health Organization (WHO) is preventive chemotherapy (PC), the administration of anthelminthic drugs to at-risk populations, to reduce the burden caused by moderate and heavy infections [2]. In 2014, more than one billion doses of albendazole or mebendazole were distributed in the framework of STH and lymphatic filariasis control programs [3]. The success of PC is threatened by the low efficacy of the anthelminthic drugs, particularly against *T. trichiura* [4] and the potential development of drug resistance [5]. The main focus of this PhD thesis was to review the performance of the currently available anthelminthic drugs and to further assess the two new drugs, oxantel pamoate and tribendimidine, which are potential candidates for PC.

Almost a decade has passed since the efficacy of anthelminthic drugs have last been reviewed and meta-analysed [6] and new evidence from clinical trials has accumulated in the meantime. Hence, the **first objective** of this PhD thesis was to update the review with a 'network meta-analysis' approach (chapter 2). For estimating anthelminthic drug efficacy, there is an on-going debate about the preferred measurement of central tendency – i.e. arithmetic or geometric mean - for calculating the egg reduction rates (ERR). Among experts, there is no consensus and only vague arguments led to the recommendations of the arithmetic mean by WHO [5]. With a questionnaire including various scenarios of egg burdens and drug efficacies, a panel of helminth experts were asked for their interpretations, however, the work is still on-going and some results are presented within this discussion.

Oxantel pamoate has proven its trichuricidal activity in two recent trials [7,8]. For accelerating the delivery process in PC, a fixed dose of oxantel pamoate would be required. For the **second objective**, we evaluated the optimal dose and assessed a weight-independent dose of oxantel pamoate against *T. trichiura* in the first clinical trial of this PhD thesis (chapter 3).

Combination chemotherapy has two major advantages; i) improved efficacy over single drugs regimens and ii) the delay of drug resistance, since the drugs act on different targets. The **third objective** included two clinical trials with different combinations and a study to assess reinfection after treatment with drug combinations. A new Chinese drug, tribendimidine, has excellent anthelminthic efficacy supported by over a decade of approved use in China [9]. Tribendimidine has a similar efficacy profile compared to albendazole and could complement albendazole in PC to prevent drug resistance or replace albendazole in case of confirmed resistance. In the second clinical trial (chapter 4a), tribendimidine was evaluated in two African settings, as mono- and co-administration therapy (with ivermectin or oxantel pamoate) against hookworm and concomitant helminth infections. In a third clinical trial, the improved efficacy against hookworm of the triple drug therapies (TDT) albendazole-pyrantel-oxantel pamoate compared to the co-administration of albendazole-oxantel pamoate was assessed (chapter 4b). After successful treatment with anthelminthic drugs, reinfection occurs particularly fast for *A. lumbricoides* and *T. trichiura* and somehow slower for hookworm [10]. The low efficacy of the current drugs cumbered the exact *T. trichiura* reinfection measurement among existing studies. Hence, after treatment with different combinations we investigated the reinfection 18 weeks post treatment (chapter 4c).

An accurate diagnostic technique is the base of all research on STH. Since Kato-Katz, the currently recommended method, [11] is used for almost half a decade [5] and has several limitations, new diagnostic tools are warranted. The **fourth objective**, included the diagnostic comparison of Kato-Katz with FECPAK^{G2}, a novel diagnostic tool, developed for veterinary medicine (Chapter 5a). Since past four clinical trials including oxantel pamoate and different combinations [7,8,12,13] were mainly conducted on the same Island (Pemba, Tanzania), a

small epidemiological study was carried out in Lesotho, for estimating the prevalence and evaluating Lesotho as a potential new study site (chapter 5b).

Since the specific topics are already discussed within the publications, I would like to discuss the obtained results in a broader context. Based on the expertise I have gained in the past three and a half years, I would like to offer following points for an in-depth discussion:

1. The advantage of the review and network meta-analysis
2. Application of the review and drug efficacy measurements in the light of current WHO recommendations
3. Review of old and new drugs and combinations
4. Efficacy of the tested drugs
5. Preventive chemotherapy – a debate and outlook
6. Challenges of STH diagnostics

Chapter	Title	Innovation	Validation	Application
2	*Efficacy of the current drugs against soil-transmitted helminths: systematic review and network meta-analysis*	Providing summary estimates of egg reduction rates Using a network meta-analysis approach		The estimates could be used by doctors and global health authorities Adaption of WHO guidelines for monitoring anthelminthic resistance
3	*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*	Different doses of drug oxantel pamoate were assessed in school-aged children		A weight independent dose of 500mg oxantel pamoate should be used for the treatment of children (7-14 years)
4a	*Efficacy and safety of tribendimidine, tribendimidine plus ivermectin, tribendimidine plus oxantel pamoate, and albendazole plus oxantel pamoate against hookworm and concomitant soil-transmitted helminth infections in Tanzania and Côte d'Ivoire: a randomised, controlled, single-blinded, non-inferiority trial*	First published results of tribendimidine against soil-transmitted helminths outside of China Tribendimidine was combined for the first time with other drugs (ivermectin and oxantel pamoate)		Tribendimidine could complement or replace albendazole in preventive chemotherapy In hookworm and *T. trichiura* co-endemic areas, tribendimidine could be combined with oxantel pamoate
4b	*Efficacy and safety of albendazole-pyrantel-oxantel, albendazole-oxantel, pyrantel-oxantel and mebendazole-pyrantel-oxantel against hookworm infections in school-aged children in the Lao People's Democratic Republic: a randomised, single-blinded trial*	To assess the improved efficacy of the triple drug administration albendazole-pyrantel-oxantel pamoate compared to albendazole-oxantel pamoate and pyrantel-oxantel pamoate The first-time evaluation of the currently most efficient co-administration albendazole-oxantel pamoate in an Asian setting.		The improved efficacy of the triple drug administration is clinically irrelevant for preventive chemotherapy Pyrantel pamoate could replace albendazole or mebendazole in case of drug resistance in preventive chemotherapy
4c	*Efficacy and reinfection with soil-transmitted helminths 18-weeks post-treatment with albendazole-ivermectin, albendazole-mebendazole, albendazole-oxantel pamoate and mebendazole*	Evaluation of reinfection after combination treatment with high efficacy against *T. trichiura* Difference between new infection and reinfection after treatment was assessed		Reinfection estimates could help for planning the frequency of preventive chemotherapy
5a	*Diagnostic comparison between FECPAK^{G2} and the Kato-Katz method for quantifying soil-transmitted helminth infections in the framework of a randomized controlled trial*		The first validation of the new diagnostic tool FECPAK^{G2} for analysing soil-transmitted helminth eggs in human stool	After further improvement, FECPAK^{G2} could be used for identifying moderate and heavy infections and assessing egg reduction rates
5b	*Unexpected low soil-transmitted helminth prevalence in the Butha-Buthe district in Lesotho, results from a cross-sectional survey*	Evaluation and publication of STH prevalence data in Lesotho		No preventive chemotherapy is necessary in the Butha-Buthe district in Lesotho.

Table 4. The contribution to the Swiss TPH nexus of innovation, validation and application for each of the chapter.

6.2 Review of the efficacy of current anthelminthic drugs

Almost a decade has passed since the only review and meta-analysis of anthelminthic drug efficacy was published [6]. In the meantime, new evidence from clinical trials has accumulated and new methods for meta-analysing drug efficacy have been developed. Hence, the first objective of this PhD thesis was to update the review about anthelminthic drug efficacy by applying a network meta-analysis and to provide summary estimates of the ERRs, the key summary estimate for anthelminthic drug efficacy. In these chapters, the advantages of the 'network' approach are discussed and our review results are compared to two other reviews.

6.2.1 Advantages of the new review

Anthelminthic drug efficacy is measured qualitatively by cure rates (CR) or quantitatively by egg reduction rates (reduction of egg counts; ERR). Once a country is moving towards elimination, the cure of individuals gains on importance and therewith the CRs are used as an outcome measure. Since there is a long way to go to reach elimination, the current aim of WHO is to reduce the burden of moderate and heavy infections; therefore, the quantitative ERRs are the key measurement of anthelminthic drug efficacy [5]. In the review published during this PhD (chapter 2), ERRs were meta-analysed for the first-time and by applying a network meta-analysis, a larger number of studies could be included. Traditionally, meta-analyses include all clinical trials with direct estimates of an active drug compared to placebo. For example, the review of Keiser and Utzinger [6] meta-analysed all clinical trials with albendazole and placebo or mebendazole and placebo. In contrast, a network meta-analysis is able to provide indirect estimates via the direct estimates of the included studies. In more details, from clinical trials including a direct comparison of drug A *versus* B and other trials with drug A *versus* C, the indirect treatment effect of drug B *versus* C is estimated by the network meta-analysis [14]. With this approach, studies with two or more active drugs can be used, which increased the number of included studies for our review by 11 for CRs and by 13 studies for ERRs. Different models exist among network meta-analysis, summarizing binary variables from studies with two or more

drugs. We have chosen the model from Kessels and colleagues [15] for our review because of a special advantage: the possibility of including clinical trials with only one eligible treatment arm. For each study the model is reconstructing the original dataset based on sample size and case numbers, before pooling the results of all studies with one, two or more treatment arms. This resulted in additional 23 and 16 studies for the meta-analysis of CRs and ERRs, respectively.

Until recently only the review from Keiser and Utzinger [6] existed, whereas in 2017, three different reviews, including ours, were published. A review from Janssen Research & Development [16] about the efficacy of a single-dose mebendazole was published just before our review [17]. Shortly after, WHO released new guidelines for PC, including data from an unpublished, internally conducted, systematic review and meta-analysis [18]. The results of the three reviews are presented in Table 5 and discussed below.

	Mrus et al. 2017, Janssen review [16]		Moser at al. 2017, Swiss TPH [17]	Joseph et al. 2017, unpublished, WHO guidelines [18]	
	All studies	Placebo controlled		All studies	WHO methodology
Ascaris lumbricoides					
Albendazole			98.5	98.7	99.9
Mebendazole	97.9	98.1	98.0	98.3	97.6
Hookworm					
Albendazole			89.6	89.8	92.4
Mebendazole	72.0	61.2	61.0	68.2	76.5
Trichuris trichiura					
Albendazole			49.9	69.7	64.4
Mebendazole	72.5	86.8	66.0	69.0	69.3

Table 5. A comparison of albendazole and mebendazole egg reduction rates from three reviews published in 2017.

The review of Janssen (Mrus et al. [16]) estimated the average ERRs from all identified and only placebo-controlled studies, while WHO (Joseph et al., unpublished [18]) included the average ERRs of epidemiological and clinical studies and in a subgroup, ERRs from studies following the recommended WHO methodology.

For *A. lumbricodies*, similar ERRs were estimated for mebendazole by the three (Janssen, Swiss TPH and WHO) and albendazole by the two reviews (Swiss TPH and WHO). The ERR of mebendazole against hookworm were lowest in the review from Swiss TPH (61.0%) and for the placebo-controlled studies from Janssen (61.2%). Contradictory, 7.2 to 15.5 percentage-points higher ERRs were reported by WHO depending on the included studies. Against *T. trichiura*, highest mebendazole ERRs were summarized by Janssen, while WHO and Swiss TPH

presented similar results. Only Swiss TPH and Janssen presented summary estimates for CRs, whereof the latter review presented lower CRs from all studies against *A. lumbricoides* (96.2% *versus* 92.6%), hookworm (32.5% *versus* 25.5%) and *T. trichiura* (42.1% *versus* 27.6%) (data not presented).

The reviews from WHO and Swiss TPH estimated the efficacy of albendazole and ERRs were found comparable against hookworms. For *T. trichiura*, WHO reported almost 20%-points higher ERR for the main and 15%-points higher ERR for the subgroup analysis, compared to the low ERR of 49.9% estimated by the Swiss TPH.

The review from Janssen and WHO applied a simple pooling approach to summarize the ERRs without any precision estimates. In the main analysis, both included epidemiological and clinical studies with either albendazole or mebendazole, while the subgroup analysis done by WHO included only placebo controlled trials or trials following the WHO recommendations. On the contrary, the review of Swiss TPH included exclusively randomised controlled trials, applied a network meta-analysis, considered the measure of central tendency for estimating the ERRs (i.e. arithmetic, geometric or unknown) and presented precision estimates for the ERRs. To sum up, the advanced statistical methods applied by the review from Swiss TPH led to more credible estimates, compared to the weaker method used for the review from Janssen and WHO. That being said, the leap to the conclusion by the WHO, that the ERRs of albendazole and mebendazole are well above the reference threshold [5] and sufficient to control the morbidity caused by STH infections [18], should be taken with precautions - the ERRs of mebendazole against hookworm and albendazole against *T. trichiura* of the more comprehensive review from the Swiss TPH are considerably lower.

6.3. Application of the review results and new efficacy measures

The two publications, namely the review of the efficacy of current anthelminthic drugs (Chapter 2) and the progressing work about the recommendations of a measure of central tendency for ERRs might have a considerable impact on the current WHO guidelines. Firstly, the estimated ERRs by the review should be considered as reference values for monitoring anthelminthic drug resistance and secondly, the recommended measure of central tendency (i.e. the geometric mean or Hölder mean) should be considered for calculating anthelminthic drug efficacy. The justification will be given in this chapter together with further background on anthelminthic drug resistance.

6.3.1. Anthelminthic drug resistance and application of the review results

With the up-scaling of PC the selective drug pressure on STH populations increases, potentially leading to anthelminthic drug resistance [19]. In veterinary medicine, livestock holders have faced frequent and rapid resistance selection against the major anthelminthic drugs [20,21], including multi-drug resistance in strongyle parasites [22]. For helminths with veterinary importance, resistance against a new drug is already present in a population prior to the first treatment [23]. After treatment, assuming a drug is optimal, all susceptible worms are killed and resistant worms start to produce eggs until re-infection. How fast resistance spreads depends on multiple factors, i.e. treatment frequency, the use of only single drug-regimens, under-dosing, mass treatment, etc. In more detail, high annual deworming frequency or lower frequency over a longer time period is associated with resistance development in animals [24]. Furthermore, farmers used single-drugs in high frequency until the drugs failed after a few years [24]. Under-dosing could lead to resistance, e.g. if animals are treated with same dose independent of their weight or sub-curative doses [25]. The disadvantage of mass treatment is the survival of only resistant worms. By moving the livestock to a new field, which is often done by farmers to

reduce reinfection, resistant worms lack the competition with wild types and resistance spreads faster [26].

While practices described above have led to resistance in veterinary medicine, different treatment approaches are used against human helminthiasis, which might have unintentionally prevented resistance in human medicine until now. In contrast to veterinary medicine where 100% animals are dewormed, mass drug administration for humans only reaches less than 80% of population in need. The remaining people are absent during treatment due to various reasons, such as work, sickness, disregard, pregnancy etc. After the first treatment round the motivation to participate declines in both uninfected and infected people, since infected people likely experience less symptoms. Hereby, the pool of wild type worms remains larger and outcompetes resistant types, and therefore prevents drug resistance in humans [24].

The potential development of resistance is threatening the few available drugs for PC and represents the trade-off between the necessity of up-scaling PC and the increased drug pressure. Current PC programs are reaching mainly school-aged children and to a reduced extend preschool-aged children [27]. However, previous and most recent WHO guidelines include the treatment of young children (24-59 months), non-pregnant adolescent women, women in reproductive age (not pregnant), pregnant women (after first trimester) [18] and adults with an occupational risk [2]. Considering the previous and more recent WHO recommendations, PC should be applied annually (prevalence ≥20%) or biannually (prevalence ≥50%) to almost the entire population in endemic settings. Keeping in mind the fact that the treatment of all animals with anthelminthic drugs led to resistance, the PC recommendations should be applied with precaution.

Obviously, this increases the importance of monitoring anthelminthic drug resistance. Since well-established resistance markers for human STHs do not exist, the change in efficacy (ERRs) of albendazole and mebendazole compared to a reference ERR (for albendazole or mebendazole) could be used to monitor drug resistance according to WHO guidelines [5]. For example, after assessing the ERRs in a given setting they are classified compared to a reference, as satisfactory (equal or higher ERR), doubtful (<10 percentage points below

reference) or reduced (>10%-points below reference, table 6) [5]. In the absence of a systematic review and meta-analysis of ERRs at the time the guidelines were written, the reference ERRs were based on two multi-country studies [5,28,29]. In the light of the WHO recommendations, the ERRs and estimates for the year 1995 and 2015 deriving from our review [17] were interpreted accordingly (Table 6).

		Albendazole		Mebendazole	
		ERR	95%-CI	ERR	95%-CI
A. lumbricoides					
WHO Reference efficacy[1]			**≥95**		**≥95**
Review main estimates[2]		98.5	94.9-100.0	98.0	94.0-100.0
Review estimates for 1995[2]		99.5	94.7-100.0	99.7	93.3-100.0
Review estimates for 2015[2]		95.4	88.2-100.0	94.8*	87.3-100.0
Hookworm					
WHO Reference efficacy[1]			**≥90**		**≥70**
Review main estimates[2]		89.6*	81.9-97.3	61.0*	52.0-69.9
Review estimates for 1995[2]		95.6	85.3-100.0	69.4*	54.3-84.6
Review estimates for 2015[2]		77.1**	62.5-91.7	51.8**	35.3-68.2
T. trichiura					
WHO Reference efficacy[1]	<275 EPG[3]		**≥90**[3]		**≥50**
	<550 EPG[3]		**≥70**[3]		
	<800 EPG[3]		**≥50**[1,3]		
Review main estimates[2]		49.9*	39.0-60.6	66.0	54.6-77.3
Review estimates for 1995[2]		72.6	53.7-91.5	91.4	72.9-100.0
Review estimates for 2015[2]		43.4*	23.5-63.3	54.7	34.6-74.8

Table 6. World Health Organization reference efficacy (bold) for albendazole and mebendazole and efficacy based our systematic review . [1]WHO 2013 [5], [2]Moser et al. 2017 [17], [3]Vercruysse et al. 2011 [19] and Levecke et al. 2012 [30], *doubtful, **reduced

Against *A. lumbricoides*, the ERRs and the estimates for 1995 were well above the reference efficacy for albendazole and mebendazole. Strictly interpreted, the mebendazole estimates for 2015 would be classified as 'doubtful'. For the treatment of hookworm, the efficacy of albendazole (89.6%) and mebendazole (61.0%) are classified as 'doubtful'. Moreover, the estimated albendazole ERR for 2015 is more than 10%-points below the recommendations, classified as 'reduced' efficacy and potentially indicating drug resistance. Since the average *T. trichiura* infection intensity of the included studies was above 800, the reference ≥50 was taken as suggested by two publications [28,30] for albendazole and the ERR and estimates for 2015 were classified as 'doubtful', whereas the ERRs of mebendazole were satisfactory.

One question arises in the light of the reference and estimated ERR from our review [17]; are the doubtful or reduced ERRs of albendazole and mebendazole attributed to anthelminthic drug

resistance or incorrect definition of the reference efficacies? A number of clinical trials reported reduced albendazole and mebendazole efficacy against hookworm and *T. trichiura* [19] and more recently of albendazole against *A. lumbricoides* [31]. However, these studies were either biased, based on wrong assumptions (e.g. for the assumed *A. lumbricoides* treatment failures a wrong follow up period was used) or failed to associate reduced efficacy with the presence of a resistance marker [28,31]. For *Haemonchus contortus*, a nematode with veterinary importance, benzimidazole resistance was associated with a single nucleotide polymorphism (SNPs) in the β-tubulin gene at three positions (codon 200, 167 and 198) [32]. Studies investigating the same SNPs in humans reported the finding of a single SNP in each STH species, e.g. at codon 167 for *A. lumbricoides* and 200 for hookworm and *T. trichiura* [33,34]. After treatment with albendazole an increased frequency of SNPs in codon 200 of *T. trichiura* was observed, however, an association with reduced efficacy could not be confirmed [35]. For human STH, the benzimidazole mode of action and the number and place of SNPs responsible for resistance remain unknown. Hence, in a next step, the particular mode of action in human STH have to be studied to identify the SNPs associated with drugs resistance and with a potential to be used as resistance markers. For the final answer to the above stated question, a study showing reduced drug efficacy directly related to well-established resistance markers are needed, currently being undertaken in a Bill and Melinda Gates funded (Starworms) project. Until the association is proven, the current WHO reference efficacies should be revised based on the review conducted during this PhD-thesis.

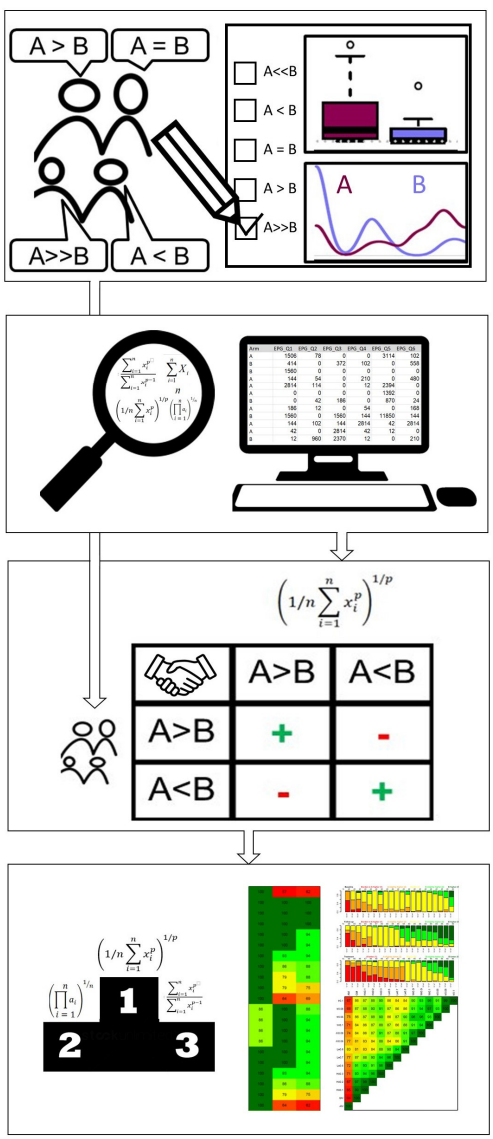
6.3.2. Application of drug efficacy measurement

For calculating the ERRs, the key measurement in anthelminthic drug efficacy, two measurements of central tendency (MCT) are exclusively used; the arithmetic and geometric mean. Both have their limitation, for example, the arithmetic mean is heavily influenced by outliers, which led to decreased ERRs in the study of Speich et al. [8] from 73% compared to 93% without the outlier at follow-up. The disadvantage of the geometric mean is the assumption of homogeneity of the variance between the compared group and the choice of the constant for

taking the logarithm of the zero egg counts at follow-up [36–38], which led to the recommendation of the arithmetic mean by the WHO [5]. Within this PhD thesis, we wanted to address the question about which MCT is preferable by using a completely new approach, however, the work is still in progress and not presented fully in this thesis.

Using a questionnaire including different visualised distributions of helminth egg counts from 7 randomised controlled trials [7,8,12,39–41], we asked a panel of experts (human and veterinary parasitologists/epidemiologists, and biostatisticians) to judge which of the two presented treatment arms is associated with a higher burden and efficacy (Figure 11). Two different definitions were used if the experts judged the worm burden or treatment efficiency higher in a certain trial arm: i) simple majority - more raters considered the burden/treatment efficiency higher compared to the other arm and ii) consent – 90% of the experts rated the burden/efficiency in a certain arm as better or similar, trial arm comparisons with an experts' agreement below 90% were discarded. The response rate was 64% (49 from 76 experts returned a filled-in questionnaire). In addition, we calculated for all trial arms different MCTs - arithmetic, geometric, Hölder (with parameter 0.1-0.9), Lehmer (with parameter 0.1-0.9), truncated (0.02-0.1%) and Winsorized mean (0.02-0.1%). Agreement between raters and MCT was calculated as the proportion of comparisons favouring the same trial arm. The results of both definitions are presented in Figure 11.

Against our expectations, the arithmetic mean showed lowest agreement and ranked last. The different truncations and Winsorizations indicated an improved agreement but were outperformed by the Lehmer mean, while the geometric and the Hölder mean (with parameter 0.2) ranked at the top. Since the Hölder mean only slightly outperformed the geometric mean and since the work is still in progress it remains unclear, whether the slightly better performance of the Hölder mean justifies the increased complexity associated with calculation compared to the geometric mean.



	Raw agreement (all)				Raw agreement (consens)				
	baseline	follow−up	efficacy	mean[95%CI]	baseline	follow−up	efficacy	mean[95%CI]	rank
AM	77	57	61	65 [60−68]	100	57	62	73 [68−75]	25
GM	86	96	91	91 [85−94]	100	100	100	100 [100−100]	5
Hö0.1	91	96	91	93 [85−94]	100	100	100	100 [100−100]	4
Hö0.2	95	96	91	94 [86−96]	100	100	100	100 [100−100]	4
Hö0.3	91	96	87	91 [84−93]	100	100	94	98 [98−98]	6
Hö0.4	91	96	87	91 [84−93]	100	100	94	98 [98−98]	6
Hö0.5	86	91	91	90 [82−91]	100	93	94	96 [95−98]	8
Hö0.6	86	83	83	84 [78−87]	100	86	88	91 [90−93]	15
Hö0.7	82	78	78	79 [75−83]	100	79	88	89 [88−91]	19
Hö0.8	82	74	70	75 [70−78]	100	79	75	85 [82−87]	21
Hö0.9	82	65	65	71 [66−74]	100	64	69	78 [75−79]	23
Le0.1	73	96	83	84 [78−86]	86	100	88	91 [89−96]	16
Le0.2	73	96	87	85 [79−88]	86	100	94	93 [91−98]	13
Le0.3	73	96	87	85 [79−88]	86	100	94	93 [91−98]	13
Le0.4	82	96	87	88 [81−90]	86	100	94	93 [91−98]	12
Le0.5	86	96	91	91 [84−93]	100	100	94	98 [98−98]	6
Le0.6	86	91	91	90 [84−92]	100	100	94	98 [98−98]	7
Le0.7	91	87	87	88 [83−91]	100	93	94	96 [95−96]	9
Le0.8	86	83	78	82 [78−86]	100	86	88	91 [90−93]	15
Le0.9	82	74	70	75 [70−78]	100	79	75	85 [82−87]	21
Wi0.02	82	61	57	66 [61−69]	100	64	62	76 [70−76]	24
Wi0.04	82	78	83	81 [75−84]	86	86	88	86 [83−92]	20
Wi0.06	82	78	78	79 [72−82]	86	86	88	86 [83−92]	21
Wi0.08	82	83	83	82 [75−85]	86	86	88	86 [84−93]	19
Wi0.1	82	83	87	84 [78−87]	86	93	88	89 [87−94]	17
tr0.02	86	70	61	72 [67−75]	100	71	69	80 [76−81]	21
tr0.04	82	83	87	84 [76−86]	86	86	88	86 [83−92]	18
tr0.06	86	78	83	82 [75−85]	100	86	88	91 [89−93]	16
tr0.08	86	83	83	84 [77−87]	86	93	88	89 [87−94]	16
tr0.1	82	83	83	82 [76−85]	86	93	88	89 [87−94]	18

Figure 11. Pictogram of work-flow (left figure) and performance (right figure) of different measures of central tendency for all answers and (first) and answers with high consent, i.e. 90% of the answers with similar of higher burden for the same treatment arm (right).

6.4. Review of old and new drugs

Poor efficacy of the current drugs against *T. trichiura* and additionally, the lack of new drugs in case of benzimidazole resistance, are the major threats to the success of PC. Despite the depleted anthelminthic drug pipeline, a few new drugs are either evaluated by the US Food and Drug Administration (tribendimidine), pushed forward onto the WHO List of Essential Medicine for STH (ivermectin) or under research for their potential in PC programs (albendazole-ivermectin). Since there is already quite some published evidence about the efficacy of tribendimidine, albendazole-oxantel pamoate and albendazole-ivermectin, the systematic review and network meta-analysis (chapter 3) was extended by including studies with the afore mentioned drugs.

6.4.1. Extending the review with new anthelminthic drugs or combinations

The literature search was extended up to 1 September 2017 and the terms “tribendimidine”, “oxantel pamoate”, “ivermectin”, “co-administration” and “combination” were added to the triple MeSH terms of the already published review and network meta-analysis [17]. The search resulted in the inclusion of five published [12,46–49] and one unpublished study including albendazole-oxantel pamoate [46] and several treatment arms of included studies became eligible, i.e. one with tribendimidine [47], two with albendazole-oxantel pamoate [7,8] and 5 with albendazole-ivermectin [8,41,48–50]. For increasing the number of studies, two different oxantel pamoate (20 and 25 mg/kg) and ivermectin (according to height and 200–400 µg) dosages were included and combined. The new network of the extended review is presented in Figure 12.

Against *A. lumbricoides,* the CRs of all newly included treatments were similarly high (>95%) and, the ERRs of each new treatment arm were above 98% (Table 9). For treating hookworm infections, the CRs of albendazole-ivermectin (88.5%, 95% confidence interval, 70.0-96.2) were non-significantly higher compared to tribendimidine (80.8%, 63.3-91.1) and albendazole (77.7%, 70.5-83.6). However, tribendimidine (92.4%, 75.2-100.0) showed marginally increased ERRs in contrast to albendazole monotherapy (89.9%, 81.7-98.1), while the ERRs of the combination

albendazole with oxantel (85.7%, 68.7-100.0) or ivermectin (85.4%, 68.0-100.0) were slightly lower.

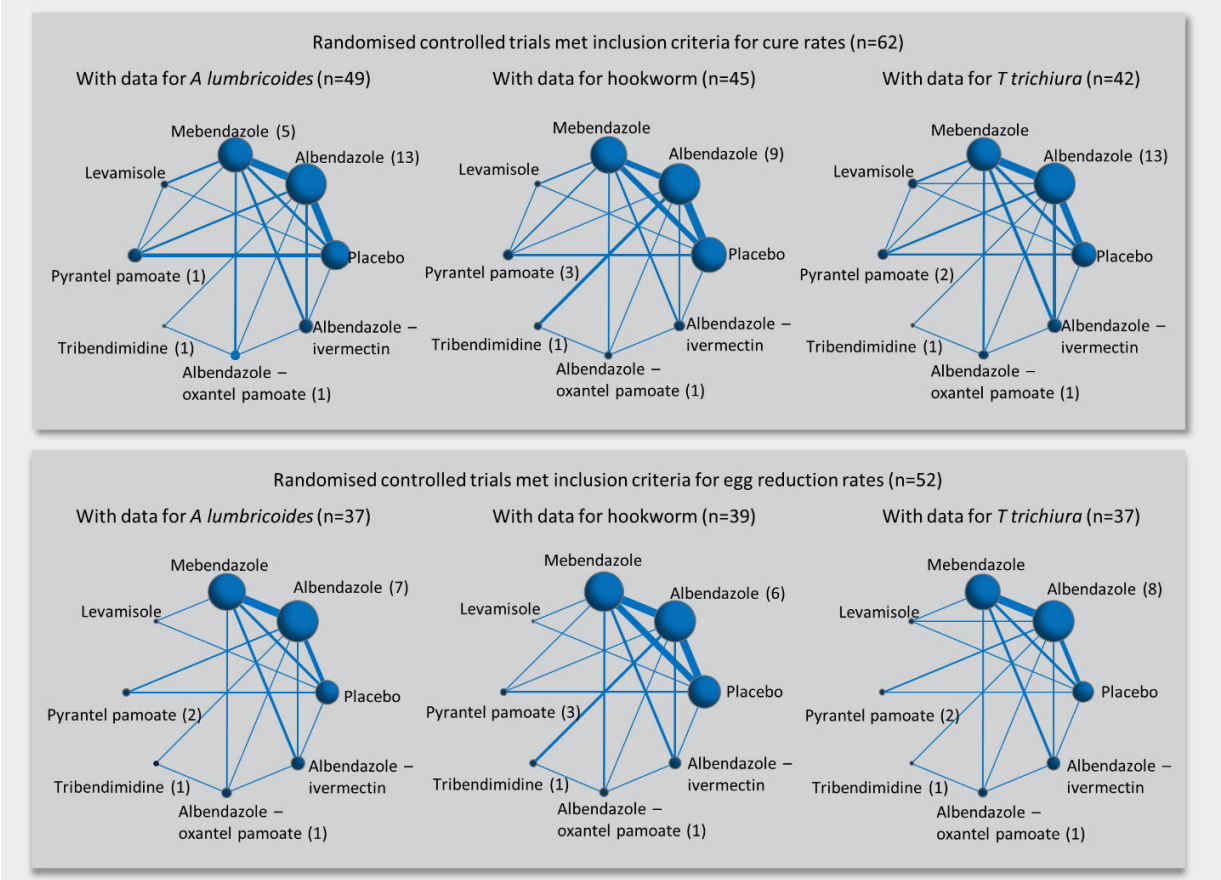


Figure 12. Updated flow chart and network.

Tribendimidine had no effect against *T. trichiura* and the CR (3.2%, 0.5-18.2) and ERR (12.4%, -8.3-33.0) were even lower than for the placebo. The CR of albendazole-oxantel pamoate (75.0%, 44.8-91.7) against *T. trichiura* was significantly higher compared to albendazole (p=0.004), mebendazole (p=0.045), pyrantel pamoate (p=0.003), tribendimidine (p<0.001) and not significantly higher to albendazole-ivermectin (57.1%, 37.6-74.6). Albendazole-ivermectin showed significantly higher CRs against *T. trichiura* compared to albendazole (p=0.004), pyrantel pamoate (p=0.009) and tribendimidine (p<0.001). The ERRs of the combination albendazole plus oxantel pamoate (93.6%, 79.3-100.0) or ivermectin (91.0%, 78.3.-100.0) were significantly higher against *T. trichiura* compared to any mono-therapy (p<0.01).

Since the anthelminthic drug efficacy contains two different measures, i.e. CRs and ERRs, the drugs could not be ranked by the network meta-analysis [14]. Therefore, a simpler ranking of

Treatment	Cure rates				Egg reduction rates			
	No. studies	No. subjects	Rate	(95%CI)	No. studies	No. subjects	Rate	(95%CI)
Ascaris lumbricoides								
PLC	14	842	12.3	6.6-21.7	9	525	20.8	15.3-26.3
ALB	35	3410	95.9	93.6-97.3	27	2904	98.5	95.3-100.0
MEB	13	1548	96.1	92.4-98.0	13	1529	98.0	94.3-100.0
LEV	2	149	97.2	84.7-99.5	1	125	96.6	83.7-100.0
PYP	11	1374	92.5	85.8-96.2	6	284	94.2	88.8-99.7
TRB	3	125	96.2	80.6-99.4	4	125	99.8	87.5-100.0
ALB-OXP	4	312	95.5	84.9-98.8	4	312	98.9	91.6-100.0
ALB-IVR	4	242	95.9	84.6-99.0	4	242	99.3	92.2-100.0
Hookworm								
PLC	18	1309	14.7	9.3-22.4	14	1046	15.8	5.6-26.0
ALB	34	3392	77.7	70.5-83.6	28	3024	89.9	81.7-98.1
MEB	14	2305	33.1	22.2-46.1	14	2263	60.4	50.4-70.3
LEV	2	230	10.6	2.8-33.3	1	202	61.6	26.5-96.7
PYP	7	230	48.8	29.8-68.2	5	144	70.5	51.6-89.3
TRB	6	465	80.8	63.3-91.1	5	363	92.4	75.2-100.0
ALB-OXP	4	395	66.1	38.6-85.6	4	395	85.7	68.7-100.0
ALB-IVR	4	279	88.5	70.0-96.2	4	279	85.4	68.0-100.0
Trichuris trichiura								
PLC	11	1417	9.8	5.3-17.7	8	1049	19.7	7.9-31.4
ALB	32	4492	30.5	51.4-41.4	29	3467	50.6	40.7-60.6
MEB	13	2514	42.6	27.7-59.0	14	2507	70.1	59.4-80.7
LEV	2	203	28.7	6.8-68.9	1	197	37.4	16.7-58.1
PYP	6	275	19.3	7.5-41.1	4	158	54.8	31.9-77.6
TRB	3	158	3.2	0.5-18.2	3	158	12.4	-8.3-33.0
ALB-OXP	4	519	75.0	44.8-91.7	4	519	93.6	79.3-100.0
ALB-IVR	5	508	57.1	37.6-74.6	4	491	91.0	78.3-100.0

Table 7. Updated network meta-analysis and including placebo (PLC), albendazole (ALB), mebendazole (MEB), levamisole (LEV), pyrantel pamoate (PYP), tribendimidine (TRB), albendazole-oxantel pamoate (ALB-OXP) and albendazole-ivermectin (ALB-IVR).

the CRs and ERRs was done as presented in Table 8. Since the ERRs are a more important indicator, the weight was doubled when taking the average of both (CR and ERR). A ranking was done for each helminth species and the final ranking including all three species. For *A. lumbricoides* all drugs and combinations reached similar high efficacy with only slight differences. Tribendimidine ranked first among all treatments against hookworm, followed by albendazole in combination and monotherapy. Against *T. trichiura* the best treatment was albendazole-oxantel pamoate, followed by albendazole-ivermectin and mebendazole, while the other single-drug regimens had much lower efficacies.

In the final combined ranking, the two combinations, albendazole plus oxantel pamoate or ivermectin, outreached all monotherapies and shared the first place. Considering only hookworm and *T. trichiura*, albendazole-oxantel pamoate would slightly outcompete albendazole-ivermectin. The two most widely used drugs albendazole and mebendazole, followed on rank 3 and 4, respectively. Despite tribendimidine reaching the first place for

treating *A. lumbricoides* and hookworm, it has no effect against *T. trichiura* and was therefore ranked only on 6th place in the final ranking.

Treatment	*A. lumbricoides*			Hookworm			*T. trichiura*			Final Rank
	CR	ERR	Rank	CR	ERR	Rank	CR	ERR	Rank	
Placebo	12.0	20.0	8	14.7	15.8	8	9.8	19.7	7	**8**
Albendazole	95.9	98.5	4	77.7	89.9	3	30.5	50.6	4	**3**
Mebendazole	96.1	98.0	5	33.1	60.4	6	42.6	70.1	3	**4**
Levamisole	97.2	96.6	6	10.6	61.6	7	28.7	37.4	6	**7**
Pyrantel pamoate	92.5	94.2	7	48.8	70.5	5	19.3	54.8	5	**5**
Tribendimidine	96.2	99.8	1	80.8	92.4	1	3.2	12.4	8	**6**
Albendazole-oxantel pamoate	95.5	98.9	3	66.1	85.7	4	75.0	93.6	1	**1**
Albendazole-ivermectin	95.9	99.3	2	88.5	85.4	2	57.1	91.0	2	**1**

Table 8. Extended review of the current anthelminthic drugs, a ranking attempt. Traffic light colours are used to indicate the best (green) up to worst (red) treatments.

Whereas the review published during this PhD thesis [17] highlighted the low efficacy of albendazole and mebendazole against *T. trichiura* and decreased efficacy over time, the extended review of this chapter demonstrated the increased efficacy of drug combinations. The two most promising drug combinations albendazole plus oxantel pamoate or ivermectin, revealed superior efficacy over single-dose regimens for the simultaneous treatment of the three STH species. Therefore, drug combinations instead of single-drugs should be used in PC programs.

6.5. Efficacy of the tested drugs

The main goal for the three clinical trials of this PhD thesis was further development of oxantel pamoate. First, a weight-independent oxantel pamoate regimen was determined through a dose-finding trial. In the second trial, oxantel pamoate was combined with another new drug, tribendimidine. In the third clinical trial, the improved efficacy of the TDT albendazole-pyrantel-oxantel pamoate was validated. The subordinate goal was to assess the new Chinese drug tribendimidine, which was for the first time evaluated in combination with other drugs and in an African setting.

6.5.1. Comparing the drug efficacy from three clinical trials

In 1974, oxantel pamoate was introduced on the market [51], examined in combination with pyrantel pamoate in multiple clinical trials [52] and approved in several countries (Quantrel; Pfizer). Because of the depletion of the anthelminthic drug pipeline, oxantel pamoate had its 'comeback' in the laboratory of the Group from Prof Jennifer Keiser [53,54] and later on, in two clinical trials led by Speich and colleagues [7,8]. In this PhD-thesis, the development of oxantel pamoate was carried further with a dose-finding study and combination trials. Our dose-finding trial included regimens from 5 to 30 mg/kg in order to investigate a weight-independent dose. Considering oxantel pamoate is used for PC in the future and millions of doses would be administrated every year, a weight-independent tablet is pivotal for optimizing the delivery process.

While oxantel pamoate has a high trichuricidal and no activity against *A. lumbricoides* and hookworm, tribendimidine offers high activity against the two latter helminth species. The objective of the second trial was to assess tribendimidine in African settings and to evaluate the potential of tribendimidine in combination with other drugs against all three STH. Considering the possibility of anthelminthic resistance against albendazole and mebendazole, tribendimidine could complement albendazole in PC programs or replace it in the case of resistance. The advantage of combination chemotherapy presents the increased efficacy over single-dose treatments and the mitigates drug pressure of the current anthelminthics [19]. The last clinical trial of this PhD-thesis had a similar objective, for example, evaluating the increased efficacy against hookworm by adding pyrantel pamoate to the currently most efficacious combination albendazole-oxantel pamoate.

The CRs and ERRs (i.e. arithmetic and geometric) of the three clinical trials are summarized in Table 9. For oxantel pamoate the dose-finding results of only two doses are presented (20 mg/kg and 25 mg/kg). The final ranking included placebo, oxantel pamoate and tribendimidine as single drug regimens and six different drug combinations. For estimating the most efficacious treatment among the three clinical trials, the average of the CRs and the arithmetic and geometric ERRs was ranked from 1 to 11. To increase the weight of the ERRs, the results

based on the arithmetic and geometric mean were included (table 9). Of note, the three trials were conducted in different settings and age groups with varying infection intensities. Hence, for a final conclusion, which treatment to include in PC programs, the multicontinental head to head comparisons might be considered (Table 9).

All single-drugs, co-administrations and triple drug therapies reached a high efficacy against *A. lumbricoides*, except oxantel pamoate, which had no effect. Thus, it is not surprising that oxantel pamoate and placebo are found at the bottom of the ranking. In contrast, pyrantel-oxantel pamoate and the mebendazole TDT cured all participants and therewith shared the first rank. For the remaining treatments ERRs of 99.9% or slightly below were observed and the ranking between palace 1 and 8 was defined by only slight differences.

For treating hookworm infections, the albendazole TDT outreached any other treatment. Despite a four times higher infection intensity (geometric EPG: 671 versus 166), higher geometric (99.9%) and arithmetic ERRs (98.4%) were reached compared to the second ranking co-administration, tribendimidine plus ivermectin (geometric 99.5%, arithmetic 96.3%). The TDT with mebendazole ranked third and pyrantel-oxantel pamoate fourth. Whereas the CRs of the mebendazole TDT were clearly higher (69.6% *versus* 52.2%), the ERRs were lower based on the arithmetic (96.3% *versus* 95.9%), and higher according to the geometric mean (99.2% *versus* 99.6%). Surprisingly, albendazole-oxantel pamoate reached higher efficacy against hookworm in the Asian setting (rank 5) compared to the African setting (rank 6) regardless of the more than three times higher baseline infection intensity (geometric EPG: 707 *versus* 195). Tribendimidine plus oxantel pamoate or as single-drug, followed on the rank 7 and 8, respectively. Nonetheless, the tribendimidine trial (chapter 4a) showed non-inferiority for tribendimidine-oxantel pamoate (rank 7) and albendazole-oxantel pamoate (rank 6).

Against *T. trichiura*, all oxantel pamoate co-administrations ranked first, followed by the mono-therapies, whereas placebo ranked before tribendimidine. In the clinical trial in Laos, all children receiving albendazole-oxantel pamoate were cured and three children remained positive after treatment with albendazole or mebendazole TDT. Nevertheless, the comparison between the

	Treatments	*A. lumbricoides*				Hookworm				*T. trichiura*				Total Rank
		CR	ERR		Rank	CR	ERR		Rank	CR	ERR		Rank	
			AM	GM			AM	GM			AM	GM		
Moser 2016 (Chapter 3)	Placebo	15.0	22.4	50.8	10	21.0	-42.3	42.7	11	0.0	49.5	34.5	10	11
	Oxantel pamoate[1]	22.0	27.7	84.8	9	33.0	51.5	77.3	9	50.0	75.9	97.7	7	9
	Oxantel pamoate[2]	24.0	-12.7	72.2	11	28.0	40.6	64.8	10	60.0	62.0	97.5	8	10
Moser 2017 (Chapter 4a)	Tribendimidine[3]	98.6	99.9	99.9	3	53.6	77.5	96.7	8	8.2	15.0	53.1	11	8
	Tribendimidine[3]-ivermectin[4]	98.7	99.9	99.9	3	84.4	96.3	99.5	2	33.7	88.6	96.4	9	5
	Tribendimidine[3]-oxantel pamoate[2]	94.3	97.9	99.9	6	52.0	81.5	96.5	7	66.3	94.1	99.5	5	7
	Albendazole[3]-oxantel pamoate[2]	93.6	98.7	99.9	6	48.0	87.2	96.0	6	82.8	97.0	99.8	4	4
Moser 2018 (Chapter 4b)	Albendazole[3]-oxantel pamoate[1]	95.8	99.9	99.9	5	52.9	91.0	99.0	5	100.0	100.0	100.0	1	3
	Pyrantel pamoate[1]-oxantel pamoate[1]	100.0	100.0	100.0	1	52.2	96.3	99.2	4	74.2	75.4	97.9	6	6
	Albendazole[3]-pyrantel pamoate[1] -oxantel pamoate[1]	90.9	99.9	99.9	8	84.1	98.4	99.9	1	90.7	94.4	99.6	2	1
	Mebendazole[5]-pyrantel pamoate[1] -oxantel pamoate[1]	100.0	100.0	100.0	1	69.6	95.9	99.6	3	88.5	92.9	98.8	3	2

Table 9. Efficacy of the tested drugs against *A. lumbricoides*, hookworm and *T. trichiura* in terms of cure rates (CR, %) and egg reduction rates (ERR %, arithmetic and geometric mean). Traffic light colours are used to indicate the best (dark green) up to worst (dark red) treatments [1] 20mg/kg, [2] 25mg/kg, [3] 400mg, [4] 200µg/kg, [5] 500mg

two combination trials (chapter 4a and 4b) is restricted by the more than 7 times lower infection intensity in the Laos trial. The results from two oxantel-pamoate studies of Speich and colleagues [7,8] already indicated an improved efficacy with lower infection intensity, which might be driven by the increased efficacy of albendazole for lower infection intensities [30]. Comparison of pyrantel-oxantel pamoate (rank 6) with both oxantel pamoate dose regimens (rank 7 and 8), indicates the only low efficacy of pyrantel pamoate against *T. trichiura*, which was supported by the results of the review [17].

The review indicated the lack of the current drugs against *T. trichiura*. However, the results of the three trials conducted during this PhD thesis highlighted that; i) oxantel pamoate has high activity against *T. trichiura* and could be given at a dose of 500 mg, ii) tribendimidine and pyrantel pamoate are valuable candidates against hookworm, iii) co-administrations reach high efficacy against any STH species and iv) TDTs have highest CRs and ERRs among all tested treatments.

Since the current goal of WHO is to control STH infections by reducing moderate and heavy infection intensities an ideal treatment requires a high ERRs and a fast administration (weight-independent dose). Two combinations fulfil the required criteria; albendazole (400 mg) plus oxantel pamoate (500 mg) and tribendimidine (200/400 mg) and oxantel pamoate (500 mg). After a thorough dose-finding study of ivermectin (currently in progress) and pyrantel pamoate, two additional co-administrations would fulfil the above-mentioned criteria: tribendimidine plus ivermectin and pyrantel plus oxantel pamoate. However, before adding tribendimidine and oxantel pamoate on the WHO Model List of Essential Medicine, an FDA approval is needed.

Once the control of STH infections is reached and the strategy shifts towards STH elimination, the higher CRs of the two evaluated TDTs (albendazole-pyrantel-oxantel pamoate and mebendazole-pyrantel-oxantel pamoate) could become valuable. A similar strategy is currently applied for the elimination of lymphatic filariasis, i.e. the use of a TDT (albendazole-ivermectin-diethylcarbamazine) which is included in the latest WHO guidelines [55] and promising outcomes have been modelled [56].

6.6. Preventive chemotherapy – a debate and outlook

This PhD thesis could have a considerable impact on PC recommendations. First, the results from the review (chapter 2a) and the recommendations of the measure of central tendency (Hölder or geometric mean) compel the need for adaption of the current WHO guidelines. Second, the clinical trials (chapter 3, 4a and 4b) demonstrated the high efficacy of the drugs, oxantel pamoate, pyrantel pamoate and tribendimidine and highlighted the improved efficacy of drug combinations. All these facts underline the necessity of including new drugs on the Essential List of Medicine against STH and the change to combination chemotherapy. In this sub-chapter, the advantages and disadvantages of PC are discussed and the next steps for the successful continuation of STH control programs are presented.

6.6.1. Does preventive chemotherapy have a benefit?

Mass drug administration has been the main strategy against STH since the endorsement by the World Health Assembly in 2001 [57]. The term “preventive chemotherapy” was introduced by WHO for mass drug administration against helminth infections [58]. However, the outcome of PC is controversially discussed, with no less than eight different reviews on this topic within the three last years (Table 11).

Two Cochrane reviews from 2015 revealed no improvement in anaemia, birth weight etc. in pregnant women (after the second and third trimester) [59] and a weight and height gain in children with a confirmed infection (only children diagnose with an infection) and no gain for children with an unknown infection (infected and uninfected children) after PC [60]. However, several concerns about the methodology were raised and addressed in two further reviews [61,63]. The first review published by the World Bank showed a weight gain for infected children in endemic settings (prevalence ≥20%) and calculated that PC is 35 times more cost-effective than school feeding programs [61]. In contrast, a second review from the Campbell Collaboration [63] demonstrated no improvement in weight, height, stunting, cognition, school attendance and mortality for children after PC. The impact of anthelminthics on HIV co-

infections showed a short-term suppression of the viral load, while the evidence about a long-term suppression is lacking [62]. PC was able to reduce the STH prevalence in school-aged children [63] and non-pregnant adolescent girls and women (Welch et al., unpublished, data shown in WHO guidelines [18]). Additionally, the prevalence of *A. lumbricoides* and hookworm significantly decreased after community-wide PC compared to targeted PC (only children) [64] and more importantly, a high reduction of moderate and heavy infections for any STH was demonstrated by Marocco and colleagues [65].

Reference	Objective	Pro	Contra
Salam 2015 [59][1]	Impact of anthelminthics in pregnant women (2&3 trimester) on anaemia and pregnancy		- Single dose has no impact on maternal anaemia, low birth weight, preterm birth and perinatal mortality
Taylor-Robinson 2015 [60][1]	Impact of PC on children's weight, height, haemoglobin, cognition, physical well-being, school attendance/ performance mortality	- Confirmed infection in children: weight and height gain	- Confirmed infection in children: no difference in haemoglobin, body mass index etc. - Unknown infection in children: no effect on weight, height, haemoglobin, average cognition for children
Croke 2016 World bank [61][3]	impact of PC on child growth	- Confirmed and unknown infection in children: weight gain in endemic settings (prevalence >20%) - PC 35 times more effective on weight gain than school feeding programs	
Means 2016 [62][1]	Impact of anthelminthics on HIV disease progression	-Confirmed infection: short-term suppressive effect on mean viral load - no additional risk of anthelminthics on HIV-population	- Unknown infection: maybe little short and long-term effect on viral load
Welch 2017 [63][2]	Impact of PC on health and performance outcome of school-aged children	- only minimal adverse effects due to PC - PC was able to reduce prevalence of all STH	- No evidence of improved weight, height, stunting, cognition (attention, general intelligence and mathematical achievements), school attendance and mortality after PC
Clarke 2017 [64][4]	Impact of targeted (school-children) versus community-wide PC on prevalence	- Prevalence reduction is significant higher after PC compared to targeted treatment for *A. lumbricoides* and hookworm in school-aged children	- No effect was seen for *T. trichiura,* maybe because of low efficacy of the available drugs
Marocco 2017 [65][4]	Impact of PC on moderate and heavy infected	- The reduction of moderate and heavy infection was 80%, 57% and 75% after 1 year and 90%, 100% and 78% after 5 years *for A. lumbricoides*, hookworm and *T. trichiura*, respectively	
Welch unpublished [18][2]	Impact of PC on non-pregnant adolescent girls and women	- Lower STH prevalence after treatment - All drugs are safe	- No difference in anaemia, iron-deficiency anaemia and morbidity

Table 11. Most recent systematic reviews and meta-analysis about the advantages and disadvantages of PC against soil-transmitted helminths. [1] Cochrane review, [2] Campbell Collaboration, [3] World Bank, [4] Other

The Cochrane reviews [60,66] have been profoundly criticized; among other reasons, because they included only randomised controlled trials (RCT) which evaluate the effect over a short period (12 month) [60], long term studies were excluded and pooled data of infected and

uninfected children diluted the effect [67]. Only a few outcomes were investigated and improvement of immediate (diarrhoea and abdominal pain) and long-term symptoms (e.g. cancer portal hypertension and infertility) were neglected [68]. The aim of Welch and colleagues [63] was to address the criticism of the previous reviews but they obtained a similar results. However, they failed to correctly address all the points mentioned above and therefore, the debate about the benefits of PC persists [68].

The essential question about PC is: do we accept to treat all children, while only moderate and heavy infected children profit directly from PC? This question can be answered asking two additional questions first: i) does the drug harm uninfected children and ii) is the cost of mass treatment larger than screening and treating individuals. Since the answer to both questions is no [68,69], PC programs were implemented with the goal of reducing moderate and heavy infections, which are known to cause the highest disease burden [2,70]. Hence, the Cochrane and Campbell reviews failed to address the main question, whether PC was able to reduce moderate and heavy infections. Only one review answered this question adequately and reported a large reduction of any moderate and heavy STH infections by 73% after one, 80% after five and 95% after 10 years [65]. Subsequently, more specified sub-questions should include, for example, the impact of PC on parameters like weight, height, haemoglobin, cognition etc., which was turned into the main question by in the Cochrane and Campbell reviews. Moreover, the effect of PC on specific parameters is obviously diluted, first, by pooling uninfected and infected children regardless of the intensity and second, by considering only short-term studies [67]. In conclusion, to correctly address the sub-questions the next review should focus on aforementioned parameters from moderate and heavy infected children after one, two or more years of PC for drawing final conclusion about the benefit of PC.

6.6.2. Next steps for STH control

Coming to the end of my PhD, I would like to look forward and describe the next steps for PC. Nevertheless, first it is time to look back by “learning from history” as stated by John Horton [71].

Despite high prevalence and non-existence of appropriate drugs, J.D. Rockefeller Jr was able to control hookworm infections with treatment and sanitation [71]. Moreover, after World War II Japan experienced high STH prevalence of over 50% for *A. lumbricoides* and *T. trichiura* [72]. STH control gained national priority and through improved sanitation, water supply, education, improved economic status and deworming, STHs were eliminated within 15-20 years [71,72]. A similar success story was reported from South Korea, in 1969 the STH prevalence reached 77% and compelled the government to set the focus on STH control. Bi-annual mass treatment of school-aged children, economic development, social infrastructure and improved sanitation over 25 years led to a massive decrease in prevalence to 0.2% in 1995 [73].

STH control is a product of behavioural changes, public consent and PC. As witnessed from history, STH control is achievable only through a combined effort. Behavioural change in the light of water and sanitation is a complex interaction between psychological factors and corresponding interventions [74]. The change embraces the in-depth understanding of the individual up to the community level and is a long-standing process with many pitfalls [75]. In 2015, WHO encouraged the integration of water sanitation and hygiene (WASH) for targeting neglected tropical diseases, which include STH [76]. Much time, effort and research is needed for a successful implementation of a combined control approach [77]. Equally, the public consent is a slow process and demands the social agreement for allocating the required resources for helminth control [73]. However, resources in STH endemic countries are limited and priority is given to greater public health problems (i.e. malaria, HIV-Aids, tuberculosis etc.). Whereas behavioural change and public consent are complex and a long-standing process, PC is less convoluted. This PhD thesis showed that the current drugs are insufficient in successfully treating all three STH, while we have demonstrated the high efficacy of two potential drug candidates against *A. lumbricoides* and hookworm (tribendimidine) and *T. trichiura* (oxantel pamoate) or in combination against all three STH species. Drug combinations have an improved efficacy against any STH species, as highlighted in this discussion. Hence, research has provided new tools and it's time for global health authorities to move forwards by adapting the guidelines and extending PC. For example, tribendimidine is currently under assessment

towards the approval by the FDA [78]. Once the approval is granted and WHO has added tribendimidine on the Essential List of Medicine against STH, an agreement with the producing pharmaceutical company Shandong Xinhua (Zibo, Shandong, China) could lead to tribendimidine donations for PC. The development of oxantel pamoate trails behind, since Pfizer stopped the production. For completing the same process, a pharmaceutical company willing to produce the tablets at very low cost has to be identified. History has demonstrated that with an integrated approach combining health education together with improved WASH and new drugs for PC, control of STH is feasible.

6.7. Further steps for diagnostics

The foundation of STH research, e.g. the collection of data on distribution, prevalence, intensity or drug efficacy builds on accurate diagnostic tools. Through different steps from the initial mapping over morbidity and transmission control up to the elimination of STH, different diagnostic attributes are required [79]. However, in helminth diagnostics there is a trade-off between sensitivity and time or cost-effectiveness, meaning cheap and fast tools have a low sensitivity compared to expensive and time-intensive methods with high sensitivity. Diagnostic methods with lower sensitivity are acceptable for large scale mapping in order to identify priority areas. Moving from transmission control to surveillance or even elimination, the correct identification of light or even micro-infections gain importance and hence, higher sensitivity are required.

6.7.1. Helminth diagnostics: available tools, limitations and further steps

Among well-established diagnostic tools (Table 12), Kato-Katz remains the recommended technique by the WHO for analysing STH in human stool [5]. Kato-Katz is used since almost half a century because of its simplicity, low cost and short preparation time and quantification of eggs in stool [11]. Within a half an hour a duplicate Kato-Katz is performed and it costs only about 0.04 US$ [80]. The major disadvantage of Kato-Katz is the low sensitivity for low infection

intensity, i.e. the correct identification of positive individuals with a very light infection. In low intensity settings, the sensitivity can be increased by analysing a second slide or second sample [81], which also accounts for the day-to-day variation in egg output [82]. Further limitations involve the disappearance of hookworm eggs after one hour [83], the incapability of storing stool samples [84] and the desiccation of the prepared Kato-Katz slides.

Method	Advantages	Limitations	Sensitivity
Copro-microscopic diagnostic methods			
Kato-Katz (2 slides)	- little equipment and fast preparation, field deployable -relatively inexpensive -good sensitivity for moderate and heavy infections	- low sensitivity for light infections - time sensitive (i.e. for hookworm only 60 min) - not accounting for day-to-day variation	**A** 55.2-97.0[1] **H** 52.6-74.0[1] **T** 79.8-95.3[1]
Direct Micro.	- little equipment - very fast analysis - field deployable	- only qualitative - low sensitivity - not accounting for day-to-day variation	**A** 39.2[1] **H** 16.3-53.7[1] **T** 14.9[1]
Ether-concentration	-good sensitivity for moderate and heavy infections - diagnosis of STH and intestinal protozoa -preservation of samples	- only qualitative - requires centrifugation, sedimentation and formalin solutions	**A** 51.3[1] **H** 38.9[1] **T** 21.5[1]
FLOTAC	- little equipment, field deployable - relatively inexpensive -good sensitivity for moderate and heavy infections - preservation possible, not time sensitive	- requires centrifugation, flotation and formalin solution - low sensitivity for light infections (better than the others) - long preparation time - not accounting for day-to-day variation	**A** 81.8-97.1[1] **H** 68.8-98.1[1] **T** 85.7-99.6[1]
Mini-FLOTAC	- little equipment, field deployable - relatively inexpensive -good sensitivity for moderate and heavy infections - preservation possible, not time sensitive	- low sensitivity for light infections - not accounting for day-to-day variation - requires flotation and formalin solution - lower egg counts are underestimates the true burden and influence the ERRs	**A** 47.1[1] **H** 44.3-97.3[1] **T** 58.3[1]
McMaster	- little equipment, field deployable - very fast analysis, inexpensive -good sensitivity for moderate and heavy infections - easier reading compared to Kato-Katz	- low sensitivity for light infections - not accounting for day-to-day variation - requires flotation solution	**A** 48.9-94.7[1] **H** 34.5-69.7[1] **T** 75.5-90.4[1]
FECPAK^{G2}	- storage of the result in form of an image (jpeg) - mark-up at later stage possible	- by now, low sensitivity - depends on quality of captured images - not accounting for day-to-day variation - (needs further improvement)	**A** 75.5[2] **H** 68.0[2] **T** 65.7[2]
Copro-molecular diagnostic methods			
qPCR	- frozen material can be used - Good sensitivity for light infection - Analysing different parasites (multiplex qPCR) - High throughput	- semi-quantitative - correlation between DNA level and number of worms or eggs not yet established - DNA from nonviable might give signal - requires infrastructure and thermocycler	**A** 85.7-100.0[3] **H** 75.7-100.0[3] **T** 100.0[3]
LAMP	- frozen material can be used - field deployable - relatively fast, little equipment	- Semi-quantitative - Uses a minimum of 4 specific primers - not yet developed for *T. trichiura*	**A** 96.3[4] **H** 97.0[4] **T** -

Table 12. The different diagnostic methods, their advantage and disadvantage and sensitivity (A, *Ascaris lumbricoides*; H, Hookworm; T, *Trichuris trichiura*). [1]Nikolay et al. 2014 (review) [81], [2]Moser et al. 2017, unpublished, [3]O'Connel and Nutman (review) [85], [4]Shiraho et al. 2016 [86]

Among all copro-microscopic methods direct microscopy has the lowest sensitivity, followed by the ether-concentration technique [81,87]. Both techniques are only qualitative; hence they are unemployable for assessing infection intensities or drug efficacy. McMaster has shown similar sensitivity and egg recovery rates compared to Kato-Katz [29,88]FLOTAC has highest sensitivity amongst the copro-microscopic methods and is less affected by infection intensity [81], indicating its potential for transmission control [79]. Yet, FLOTAC requires a centrifuge, longer time for sample preparation, expensive equipment and an additional ether washing step [89]. Consequently, mini-FLOTAC was developed by the same team, which is a simplified method of FLOTAC [90]. On the downside, the simplification came along with a lower sensitivity, which is no longer superior to Kato-Katz [81,91].

Within this PhD thesis, FECPAK^{G2}, a new remote-location, online diagnostic tool was assessed for qualitative and quantitative parameters within a clinical trial. Although lower sensitivity and number of recovered eggs was shown for FECPAK^{G2} compared to Kato-Katz (Chapter 5a), the sensitivity was high for moderate infection intensity and the estimated ERRs were indiscernible from Kato-Katz. Since WHO aims to reduce the burden attributed to moderate and heavy infection by 2020 [2], FECPAK^{G2} is a suitable tool to correctly identify these infections. Moreover, FECPAK^{G2} offers several advantages including capturing of an image, which can be stored on a computer and uploaded onto a cloud once an internet connection is established.

In comparison, stool samples can be preserved with mini-FLOTAC for 15 days [92], but not with Kato-Katz [84]. For Kato-Katz, the stool samples have to be prepared within three hours after collection to avoid a substantial sensitivity drop [84] and analysed within one hour to evade the disappearance of hookworm eggs [83], leaving only a short time window for quality control [93]. By applying FECPAK^{G2} under field conditions, a larger number of samples can be prepared, stored, uploaded and analysed at a later time point to shorten the study duration. Anytime, anywhere, the uploaded image can be accessed and processed with computer software. For quality control or in case of discrepancies, specialists around the world can be consulted. Efforts are being made to develop an image-analyse algorithm for the automatic counting of STH eggs, which would lead to many grateful researchers spending endless hours in front of a microscope

and counting thousands of eggs. In the upcoming studies, the cost-effectiveness and the sample preparation time should be compared to current established diagnostic methods.

Apart from new field deployable methods, the future of STH diagnostics are highly sensitive molecular methods, for example, quantitative polymerase chain reaction (qPCR) and loop mediated isothermal amplification (LAMP). In settings with declining infection intensities, moving from transmission control to surveillance or elimination, qPCR is able to detect micro-infections without the constraint of the day-to-day variation. Moreover, multiplex qPCRs are able to estimate infection intensities [94] and result in the concomitant diagnosis of multiple parasites, which is of particular interest for routine screening in hospitals and for research in settings with different hookworm species [94,95]. Whereas the sensitivity of qPCR is higher compared with any copro-microscopic methods [85], the major drawbacks are the required laboratory equipment, inhibition of PCR product amplification by stool substances, decreased sensitivity due to preservation solvents [85], residual DNA [96] and a higher cost. The expenses for one qPCR, including the analysis of eight parasites at once, is less than 1$ [97] compared to 0.04$ for one Kato-Katz [80], given all equipment is present. Another molecular method is LAMP, an inexpensive, field deployable, highly sensitive method, which produces a visible result and requires only a heat block or water bath [98]. LAMP still needs further development, for example, infection intensities are not yet accurately reflected and LAMP was only developed for *A. lumbricoides* [86] and *N. americanus* [98] in laboratory and not yet evaluated under field conditions.

Conclusion

Every fifth person in the world is infected with at least one soil-transmitted helminths species; nonetheless, they remain neglected by health and policy makers in most of the endemic countries. Preventive chemotherapy – the administration of anthelminthic drugs to at risk populations is the current strategy and was the general topic of this PhD thesis. First, the currently available drugs used for preventive chemotherapy were reviewed and their efficacy meta-analysed. Our findings indicate the shortcomings of the two mainly used drugs albendazole and mebendazole, in particular against *T. trichiura* and their decreased efficacy over time. Moreover, we presented the first-time summary estimates of the egg reduction rates, the key measure of anthelminthic drug efficacy and recommended use of geometric mean based on expert's opinion. Both results call for an adaption of the current guidelines, first, for monitoring anthelminthic drug resistance and second, for the calculation of egg reduction rates.

The review highlighted the need for new drugs with high efficacy against *T. trichiura* and against any soil-transmitted helminths in case of drug resistance. In the three clinical trials, we investigated two new drugs: oxantel pamoate and tribendimidine. First, a weight-independent dose of oxantel pamoate against *T. trichiura* was evaluated, for accelerating the process of drug administration in preventive chemotherapy. Second, tribendimidine in combination (with ivermectin or oxantel pamoate) was compared to albendazole-oxantel pamoate against hookworm. Third, the improved efficacy for treating hookworm infections by adding pyrantel pamoate to the combination albendazole-oxantel pamoate was studied. The results highlight the high efficacy of oxantel pamoate against *T. trichiura* and tribendimidine and pyrantel pamoate against *A. lumbricoides* and hookworm. Moreover, the combinations were able to successfully treat all three soil-transmitted helminth species at once, which call for an adaption of the WHO guidelines towards the use of combination for preventive chemotherapy.

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Curriculum Vitae

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Epidemiologist

Date of birth	22 October 1987
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PhD Study

06/2014-12/2017	PhD in Epidemiology
Thesis title	**Soil-transmitted helminthiasis: the efficacy of recommended drugs, new drugs and combinations**
Supervisor	Prof Dr Jennifer Keiser
Collaborations	- Public Health Laboratory- Ivo de Carneri (Pemba Island, Pemba) - Centre Suisse de Recherches Scientifiques, Abidjan, Côte d'Ivoire - Seboche Hospital, Butha-Buthe, Lesotho and SolidarMed Lesotho, Butha-Buthe, Lesotho - National Institute of Public Health, Vientiane, Laos People's Democratic Republic

Presentations

October 2017	Research Seminar, Basel, Switzerland: "Controlling soil-transmitted helminth infections: reviewing current drugs, clinical trials using new drugs and new diagnostic tools" (oral presentation)
June 2017	2017 Australian Society for Parasitology Conference (ASP): Leura, Blue Mountains, Australia "Tribendimidine combinations against hookworm and concomitant soil-transmitted helminth infections in Tanzania and Côte d'Ivoire: a randomized, controlled, single blinded, clinical trial" (oral presentation)
May 2016	Medical Parasitology Infection Biology Research Seminar, Swiss Tropical and Public Health Institute, Basel, Switzerland: "Development

	of oxantel pamoate against *Trichuris trichiura* infection in school-aged children" (oral presentation)
February 2016	Starworms, Principal Investigator Meeting, Ghent, Belgium: "Which is the appropriate measure of central tendency for helminth egg burden estimations?" (oral presentation)

Lectures

December 2016	Master Program in Infection Biology and Epidemiology Drug development (1 lecture)
December 2015	Master Program in Infection Biology and Epidemiology Drug development (1 lecture)

Education

09/2012–02/2014	Master of Science in Epidemiology Swiss Tropical and Public Health Institute, Basel (Switzerland) Research in the field of Neglected Tropical Diseases, with a focus on schistosomiasis and fascioliasis Field studies in Chad and Côte d'Ivoire
09/2008–01/2012	Bachelor of Science in Biology University of Basel, Basel (Switzerland) Major in Organismic Biology. Participating in field research in Zambia.

Work experience

03/2015–05/2015	Clinical Trial Consultant Janssen (Johnson & Johnson), Beerse (Belgium) Monitoring diagnostic quality of a clinical trial in Ethiopia and Rwanda.
02/2012–06/2012	Research Assistant Swiss Tropical and Public Health Institute, Basel (Switzerland) Data entry, management and analysis.

Additional Skills

Language	German (native speaker) English (fluent) French (fluent)
Trainings	Certificate in Good Clinical Practice for Principal Investigators.

Publications

1. Schulz **& Moser** et al. Preventive chemotherapy in the fight against soil-transmitted helminthiasis: achievements and limitations. Trends in Parasit 2018;34;7;590-602
2. **Moser** et al. Diagnostic comparison between FECPAK^{G2} and the Kato-Katz method for analysing soil-transmitted helminth eggs in stool. PLOS NTD 2018; June 4
3. **Moser** et al. Efficacy and tolerability of triple drug therapy with albendazole, pyrantel pamoate, and oxantel pamoate compared with albendazole plus oxantel pamoate, pyrantel pamoate plus oxantel pamoate, and mebendazole plus pyrantel pamoate and oxantel pamoate against hookworm infections in school-aged children in Laos: a randomised, single-blind trial. Lancet Infect Dis. 2018; April 16
4. **Moser** et al. Efficacy of recommended drugs against soil-transmitted helminths: systematic review and network meta-analysis. BMJ 2017;358:j4307
5. **Moser** et al. Efficacy and safety of tribendimidine, tribendimidine-ivermectin, tribendimidine-oxantel pamoate and albendazole-oxantel pamoate against hookworm and concomitant soil-transmitted helminth infections in Tanzania and Côte d'Ivoire: a randomized, controlled, single blinded, noninferiority trial. Lancet Infect Dis. 2017;17:1162-71
6. **Moser** et al. Unexpected low soil-transmitted helminth prevalence in the Butha-Buthe district in Lesotho, results from a cross-sectional survey. Parasit Vectors 2017;10
7. Speich **& Moser** et al. Efficacy and reinfection with soil-transmitted helminths 18-weeks posttreatment with albendazole-ivermectin, albendazole-mebendazole, albendazole-oxantel pamoate and mebendazole. Parasit Vectors. 2016 Mar 2;9(1):123
8. **Moser** et al. 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. 2016 Jan;16(1):53-60
9. **Moser** et al. The spatial and seasonal distribution of *Bulinus truncatus, Bulinus forskalii* and *Biomphalaria pfeifferi*, the intermediate host snails of schistosomiasis, in N'Djamena, Chad. Geospat. Heath. 2014 Nov;9(1):109-18