Optimizing treatment for soil-transmitted helminthiasis

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Table of Contents

Summary

Soil-transmitted helminths (STHs), namely *Ascaris lumbricoides*¸ *Trichuris trichiura* and hookworms, are intestinal parasites infecting a quarter of the people living in the world today. Highest at risk for infection are children in subtropical and tropical climates with limited access to improved drinking water sources, inadequate sanitation and living in poverty. Infections with helminths tend to be asymptomatic, though more intense infections can cause abdominal discomfort, anemia and wasting. Preventive chemotherapy, or treatment without diagnosis, to at-risk populations is the current recommendation by the World Health Organization (WHO) for the control of STHs. The strategy of preventive chemotherapy is carried out through mass drug administration of either single dose albendazole or mebendazole to reduce the morbidity of infections in the target population. Though there has been a reduction in the prevalence of STHs in the last 15 years, the low efficacy of both treatment options against *T. trichiura*, and to a lesser extent hookworm infections, is a drawback to control strategies. Optimized therapies for STH control are needed if elimination is to be reached and maintained.

The objectives of this thesis are to assess regimens and dosages of current available treatment options that are potentially available for mass drug administration. The first and second objectives of this thesis are to identify an optimal dose of the currently used albendazole in preschool-aged children (PSAC), school-aged children (SAC) and adults infected with either *T. trichiura* or hookworms in Côte d'Ivoire. In the first objective of this PhD thesis, results show albendazole, regardless of dose, has low efficacy against *T. trichiura*, though findings need to be interpreted carefully as recruitment goals were not met for PSAC and adults. In the second objective, moderate efficacy of albendazole was shown against hookworm; specifically, an 800 mg dose (twice the current standard dose) provides superior efficacy in adults.

The third objective of this PhD research was to design and conduct a multi-country trial assessing the efficacy of combination therapy of albendazole and ivermectin against *T.*

trichiura. The trial was conducted in three settings: Pemba Island, Tanzania, Lao PDR and Côte d'Ivoire using the same standardized protocol. Though the combination therapy was proved to be efficacious in Pemba Island, Tanzania and Lao PDR, albendazole combined with ivermectin was not found to be superior than monotherapy of albendazole in Côte d'Ivoire. Potential reasons for this discrepancy range from emerging resistance to population-based differences, which are being further investigated.

The last objective of this thesis aimed to identify potential gut morbidity markers in participants included in the efficacy trial of the third objective. Identifying two potential morbidity markers: fecal calprotectin and fecal occult blood, the study assessed the correlation between the markers and STH infection. No association was found between either marker and STH infection, making them poor markers for STH gut morbidity.

Based on the findings of the research conducted, it is recommended that alternative combination therapies be used for the control of STHs. Drugs such as oxantel pamoate or moxidectin combined with a higher dose of albendazole could be highly efficacious against all three STHs, especially in settings such as Côte d'Ivoire. Furthermore, a thorough evaluation of control programs are needed to assess the benefits and costs of mass drug administration in reducing the burden of disease. Finally, research in the fields of STH and neglected tropical diseases need to expand and diversify the study designs used in assessing drug efficacy and effectiveness.

1. Introduction

1.1. Epidemiology

Soil-transmitted helminths (STHs), namely *Trichiura trichiura*, *Ascaris lumbricoides*, and hookworms (*Ancyclostoma duodenale* and *Necator americanus*), affect mostly individuals living in poverty in tropical and subtropical environments. Prevalence estimates indicate that approximately 1.45 billion people were infected with at least one STH species in 2010 [1]. Though there has been a reduction (32.3% from 2007-2017) in the disability-adjusted life-years (DALYs) attributed by intestinal nematode infections, STHs continue to cause considerable disease burden (1.9 million DALYs as of 2017) primarily in low-income and lower-middle-income countries [2].

A. lumbricoides (roundworm), the most prevalent of all the STHs, caused over 447,000 infections in 2017 with over 32,000 (~ 7%) of those causing complications, defined as heavy infestations, mild abdominal problems and severe wasting [3]. Human infection of whipworm (*T. trichiura*) has a prevalence rate of 3,790 per 100,000 as of 2017 with approximately 10,700 (~ 4%) trichuriasis complications. Though the least prevalent of the STHs, hookworm infections (estimated number of 229,000 in 2017) caused a considerable amount anemia (mild, moderate and severe) and complications (9,500 and 28,900 cases, respectively) [3].

Although STHs are most common in warmer climates, global distribution varies by STH species as seen in Figure 1. *A. lumbricoides* has a higher endemicity South America, Asia, and west and southwest parts of Africa comparatively to trichuriasis and hookworm infections, which are more prevalent in Southeast Asia and the eastern and southern countries of Africa.

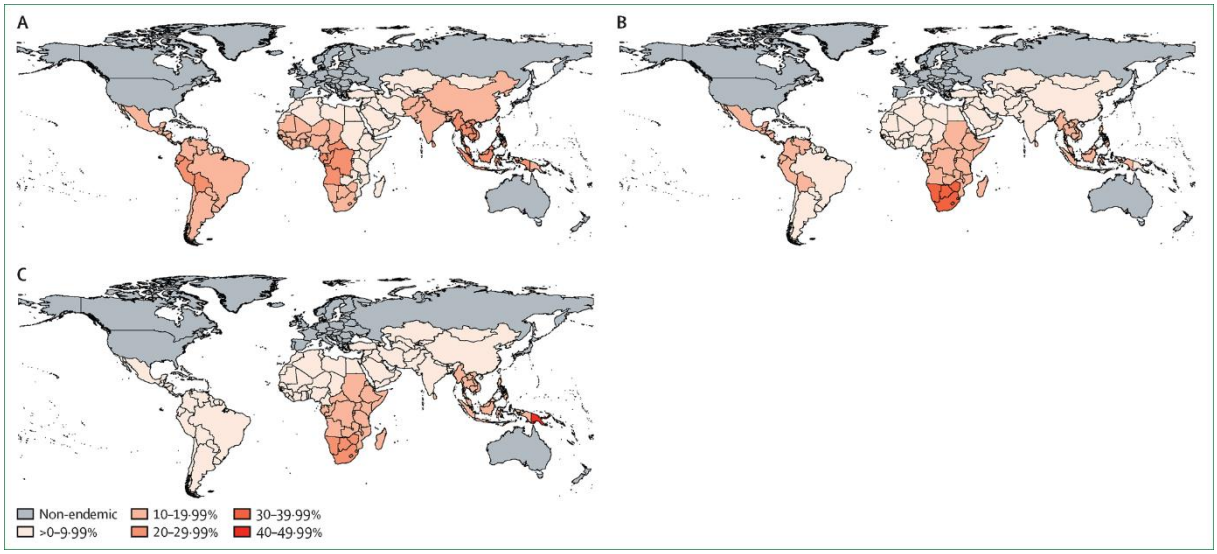


Figure 1: Global prevalence of (A) *Ascaris lumbricoides*, (B) *Trichuris trichiura* and (C) *Ancyclostoma duodenale* and *Necator americanus* infections as of 2010. Adapted from Jourdan and colleagues [4]; data from Pullan and colleagues [1].[1]

Prevalence of each STH also varies by age as seen in Figure 2. Children aged 5-9 years have the highest prevalence rate of ascariasis, while individuals aged 15-24 years have the highest prevalence rates of *T. trichiura* and hookworm infections. While hookworm prevalence rates level off into adulthood, drastic decreases are seen in the prevalence rates after their peaks for ascariasis and trichuriasis [5]. Mortality due to STH infection is uncommon with only ascariasis attributable deaths being reported at 3205 deaths in 2017.

[1] Reprinted from The Lancet, 391, Jourdan, P.M., et al., *Soil-transmitted helminth infections*. 2018, with persmission from Elsevier.

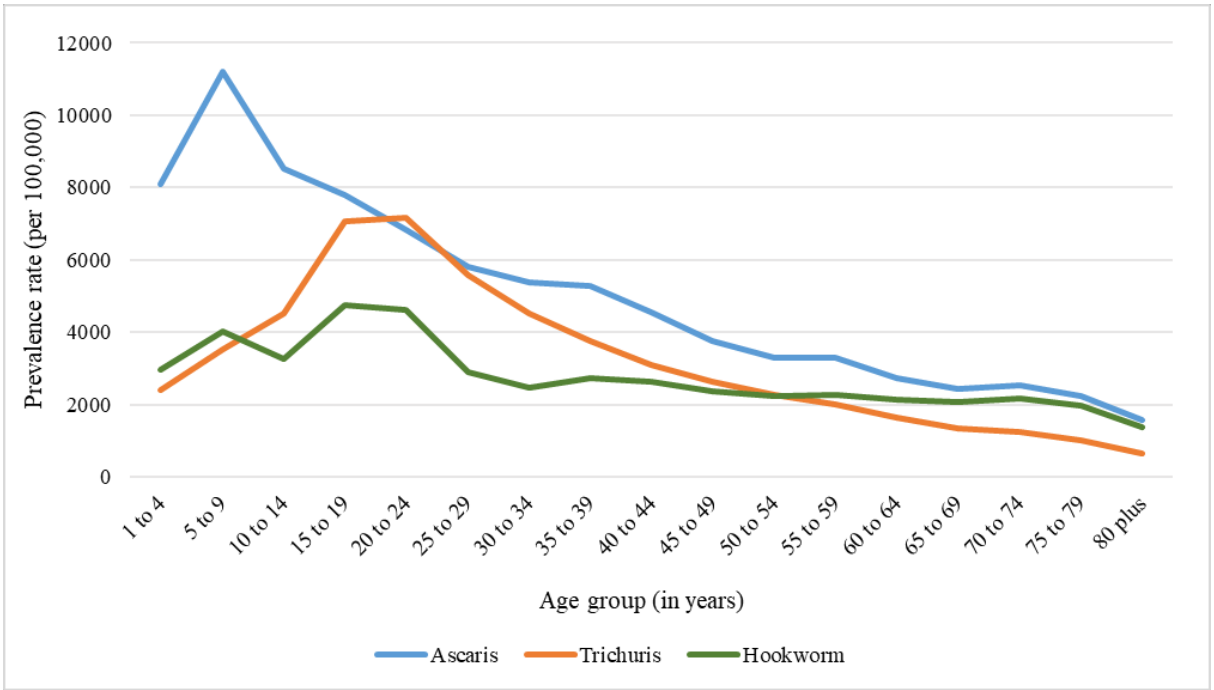


Figure 2: Global prevalence rate of *Ascaris lumbricoides* (blue), *Trichuris trichiura* (orange) and hookworm (green) infections by age group in 2017. Data from Global Health Data Exchange [5].

1.2. Life Cycle

As suggested by the name of infections, soil plays an important role in the life cycle of STHs. For *A. lumbricoides* and *T. trichiura*, eggs mature in warm, moist soils before being ingested orally by human hosts. In the case of *A. lumbricoides*, after maturation into the second-stage, larvae penetrate the mucosa of the small intestine and travel through the vascular system to the pulmonary circulation (Figure 3) [6, 7]. From here, third-stage larvae are able to travel up the trachea to the pharynx, where they are swallowed and return to the small intestine to develop into adults [6, 7]. The larger adult females (20-25 cm) can produce tens to hundreds of thousands of fertilized eggs daily, which are then excreted in the stool. While the time from infection to maturation to adult worm takes 10-12 weeks, adults have a lifespan of 1-2 years [8]. Depending on environmental factors, such as soil temperature and humidity, fertilized eggs can lay dormant for years or become infectious in a matter of weeks [9].

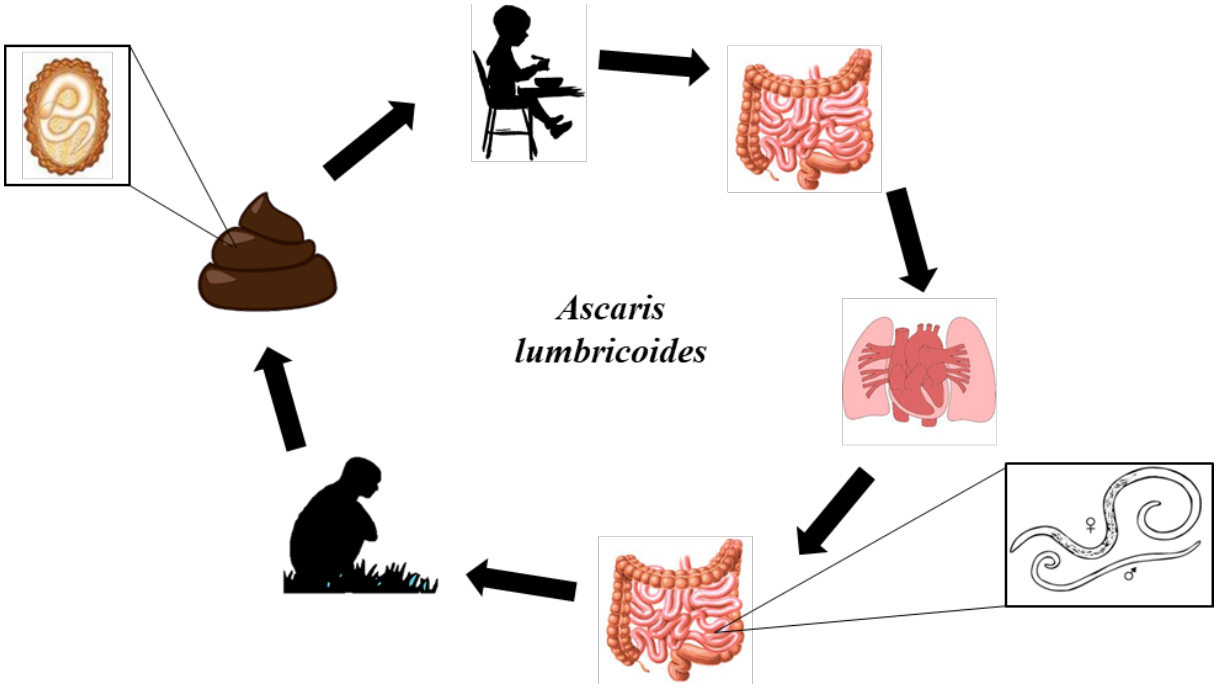


Figure 3: Life cycle of *Ascaris lumbricoides*. Images from http://clipart-library.com/ for non-commercial use excepting the image of adult worms (bottom right), which is taken from Centers for Disease Control and Prevention (CDC) at http://www.cdc.gov.

Meanwhile after ingestion, *T. trichiura* eggs hatch and molt in the intestine, as seen in Figure 4 [4]. First stage larvae (L1) penetrate the epithelium of the large intestine and mature to adulthood [10]. In the final two larval stage (L3 and L4), the thicker, posterior end of the worm extends beyond the epithelial tissue into the gut lumen, while the thinner end remains embedded in the epithelial cells, hence the moniker whipworm [10]. Adult females (3-5 cm in length) may lay thousands of eggs every days, which are excreted by the human host [11]. Patent infection takes 2-3 months to develop and adults can survive several years in the gut [12]. Excreted in a non-infectious state, eggs can become infectious in 2-4 weeks but may survive in harsh conditions for months [11].

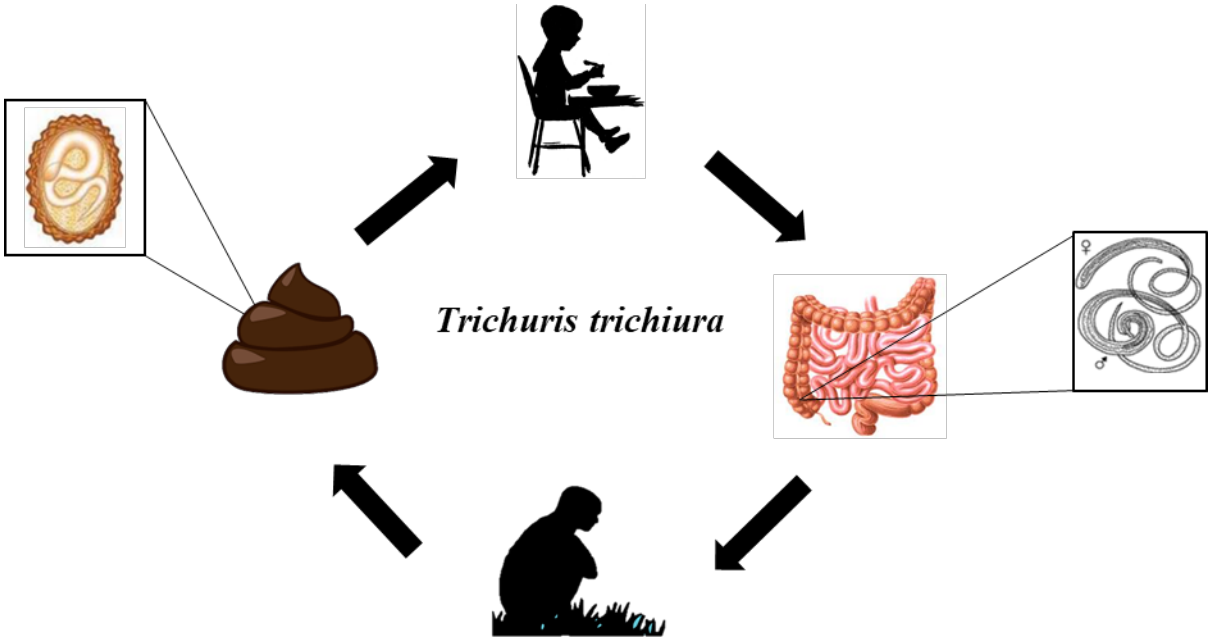


Figure 4: Life cycle of *Trichuris trichiura*. Images from http://clipart-library.com/ for non-commercial use excepting the image of adult worms (right), which is taken from Centers for Disease Control and Prevention (CDC) at http://www.cdc.gov.

A. duodenale and *N. americanus* eggs hatch in soil within 48 hours and mature to third stage larvae (L3) before infecting hosts (Figure 5) [13]. Though *A. duodenale* can be ingested, the primary route of both hookworm species infection is penetration of the skin, usually through bare feet [13, 14]. The larvae migrate to the lungs and then into the gastrointestinal tract in a similar manner as *A. lumbricoides*. Once in the small intestine, L3 larvae mature to fifth stage larvae (L5), which attach to the mucosal lining using teeth (*A. duodenale*) or cutting plates (*N. americanus*) . Once adulthood (about 1 cm in length) is reached, females can produce up to tens of thousands of eggs, depending on species, which are excreted in the stool of hosts [13]. The two species have several major differences: *A. duodenale* is 8-13 cm in length, produces up to 20,000 eggs daily and has a life expectancy of 1-3 years, while *N. americanus* is 7-11 cm long, can lay up 6000 eggs daily and live up to 10 years [15]. The time to patency is about 1-2 months and survival of L3 larvae is dependent on temperature and moisture of the environment, which also varied by species [13, 15].

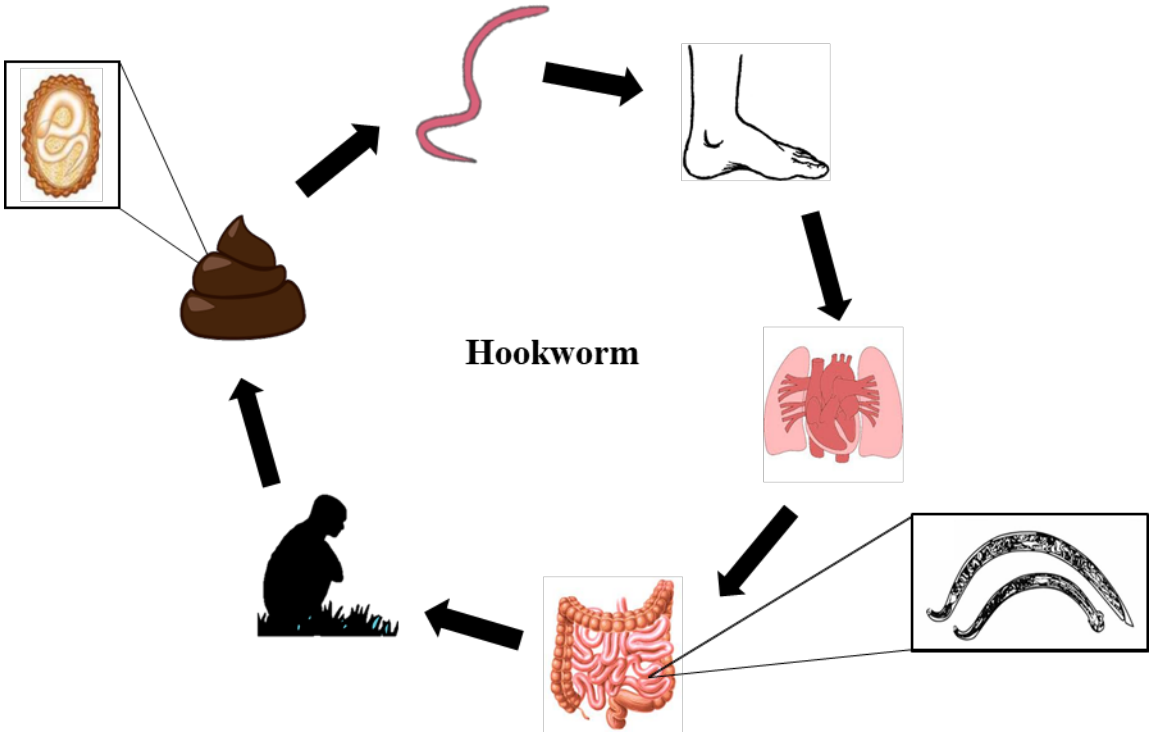


Figure 5: Life cycle of hookworm. Images from http://clipart-library.com/ for non-commercial use excepting the image of adult worms (bottom right), which is taken from Centers for Disease Control and Prevention (CDC) at http://www.cdc.gov.

1.3. Clinical presentation

STHs, while found worldwide, are highly overdispersed, meaning a small minority of the infected population carries the most worms [16]. These highly infected individuals are generally more likely to present with symptoms, while individuals with light of moderate infections can be asymptomatic or have non-specific symptoms [4, 10, 13]. Symptoms also depend on the life cycle of the parasite. A localized, pruritus and rash at the site of entry can occur when hookworm larvae penetrate the skin, known as ground itch [14]. In *A. lumbricoides* and hookworm infections, pulmonary complications (e.g. Loeffler's syndrome, pneumonitis) can occur when larvae migrate through the circulatory system [15, 17].

Adult worms can cause gastrointestinal and abdominal distress, especially with increased worm burden. Ascariasis can cause diarrhea, intestinal bleeding and bowel obstruction; moreover, systemic eosinophilia, anemia, weight loss, malnutrition and hepatobiliary complications can

occur if infections remain untreated [4, 8]. Symptoms of *T. trichiura* and hookworm infections are similar; however, as these worms attach themselves directly to the host, inflammation of the intestinal mucosa is common [10, 14]. Hookworm infection, in particular, can cause severe anemia (especially in small children and women of childbearing age) resultant from both leaking of blood where the worm is attached and the active feeding on the host's blood [15]. Blood loss is estimated 0.15 ml for *A. duodenale* and 0.03 ml for *N. americanus* [15]. Long-term impacts from STH infections also include stunted growth, lower physical fitness and reduced working capacity [11, 18, 19].

1.4. Morbidity

STH infections tend to be chronic in nature lasting years with recurring infections. STH attributable morbidity is difficult to measure and has been thought to be underestimated in terms of DALYs, which are based on prevalence estimates and limited symptoms such as mild abdominal pain, heavy infestation, severe wasting and anemia (only in the case of hookworm infections) [20, 21]. Lacking among these symptoms are long-term effects such as impaired cognition, reduced physical fitness and/or working capacity, and stunting, along with more common (albeit less specific to only STHs) general malaise and diarrhea. Compounding to limitations of DALYs, the economic burden has not been clearly defined for STHs, as is the case for many other NTDs [20].

Furthermore, the burden in an infected person is difficult to assess. Besides patient reported symptoms, the most common and non-invasive method of evaluating the worm burden in an individual is through examination of the stool for eggs. The more eggs in a standardized sample of stool indicates a higher worm burden. The largest drawback of this method is the rate of egg shedding is not constant and egg shedding does not quantify the number of larvae or adult worms in the gut. There is a need for adequate markers of morbidity, which is an objective of this PhD thesis.

1.5. Diagnosis

Since the shedding of eggs occurs in stool, coprological diagnosis methods are most common. The gold standard method for diagnosis of STHs recommended by the World Health Organization (WHO) is the microscopic examination of the Kato-Katz thick smear for eggs after fresh homogenized stool is sieved and stain is applied to the eggs [22, 23]. Egg counts are multiplied by a constant factor to estimate the eggs per gram (EPG) of stool, which defines infection intensity and worm burden. Because the method is low cost, easily performed with a light microscope and does not take much time (several minutes) to assess several parasites, it is the most widely used method of helminth diagnostics [24]. However, there are several drawbacks. Once slides are prepared, there is a window of time in which they should be read (especially for hookworm), which can be constraining to the accurate diagnosis [25]. Moreover, the sensitivity of the technique is reduced in low intensity infections (though this improves with multiple examinations from multiple stool samples) [26-30].

The formol-ether technique, similar to the Kato-Katz in terms of ease and speed, can be used on both fresh and preserved stool samples [31]. However, a drawback to this technique is that is not quantitative. Several other methods base their technique on the flotation of eggs. The McMaster technique uses a saturated saline solution allowing eggs to float to the top of chambers of a slide [32]. The Kato-Katz (two slides) and the McMaster technique have similar sensitivities for each of the STHs, which are greater than the formol-ether technique [29]. Another egg flotation technique is the FLOTAC and mini-FLOTAC, which can be used on preserved samples after several weeks [33, 34]. These techniques have drawbacks in that the protocol is more time-consuming and complex, as well as the resources needed (i.e. centrifuge) are more costly and may not be available in resource-limited settings [35]. The FLOTAC technique is more sensitive in the diagnosis of each STH than the Kato-Katz and McMaster

methods as a large amount of stool is analyzed, while the mini-FLOTAC has similar sensitivities to that Kato-Katz and McMaster techniques [29].

The last microscopic method to mention in my thesis is the FECPACK^{G2}, which is also based on egg flotation [36]. The approach includes capturing an image of a slide that can be stored on a computer and read at any time in any place, though the sensitivity was found to be lower than two slides of the Kato-Katz [37]. Research is underway to improve sensitivity and scalability of this technique.

As strategies for STH control move from reducing transmission to elimination and surveillance, increased sensitivity of diagnostic tools is needed. Molecular diagnostic techniques include polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), which detect DNA from STH in the stool [38]. These molecular methods are more sensitive than microscopic methods; however, they are more resource-straining and have not been fully developed in a standard protocol for STHs [38].

Given the epidemiology of helminths, a cost-effective diagnostic technique is needed, which is scalable and sustainable to control programs in resource-limited settings.

1.6. Clinical management

Administered therapy for the first-line treatment of helminths include the use of benzimidazoles: albendazole and mebendazole (Figure 6). For *A. lumbricoides, T. trichiura* and hookworm, a single dose of either 400 mg of albendazole or 500 mg of mebendazole (preventive chemotherapy) or 100 mg of mebendazole (individual patient management) can be given twice daily for three days [39].

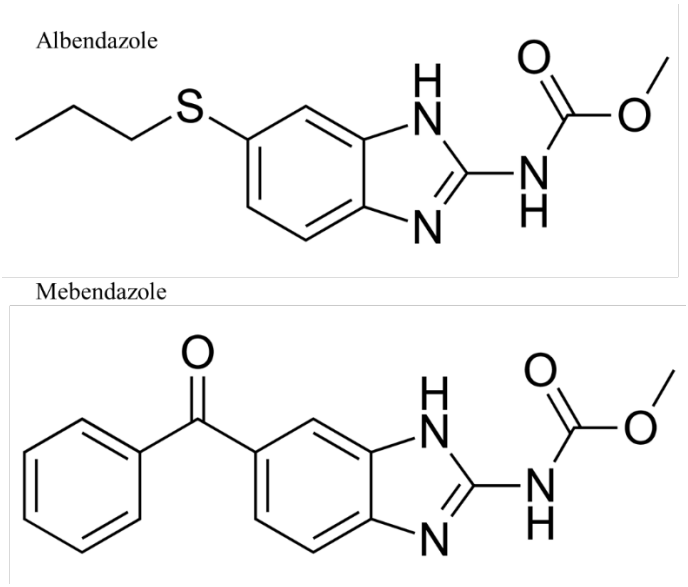


Figure 6: Structures of albendazole and mebendazole.

Albendazole was discovered in 1975 and approved for human use in 1982 [40], while mebendazole was introduced in 1977 as a veterinary anthelmintic [41]. Albendazole is metabolized to albendazole sulphoxide, the active form of the drug, in the liver [40]. This active form binds to the helminth tubulin, which disrupts the formation of cytoplasmic microtubules and inhibits cell division and energy production [41, 42]. Not only are adult worms, but also larvae are affected and hatching of eggs is also prevented [43, 44]. While the metabolite of albendazole prove to be the active form against STHs, mebendazole itself is active against helminths in a similar manner to albendazole [40].

Another class of compounds that has anthelmintic properties are the nicotinic acetylcholine receptor (nAChR) agonists (Figure 7), which include levamisole and pyrantel pamoate [45]. Both drugs bind to the nAChR and cause overstimulation of the muscles of the worm leading to paralysis sand eventual death [46]. Levamisole is given at 80 mg or 2.5 mg/kg in a single dose, while pyrantel pamoate is given as a single dose of 10 mg/kg [47]. Two other nAChR agonists that have shown anthelmintic effects are oxantel pamoate and tribendimidine, though they are not currently on the WHO list of Essential Medicines for use against STHs [45, 48,

49]. While oxantel pamoate has a weight dependent dose (20 mg/kg), tribendimine is given weight independently at a dose of 400 mg [50-52].

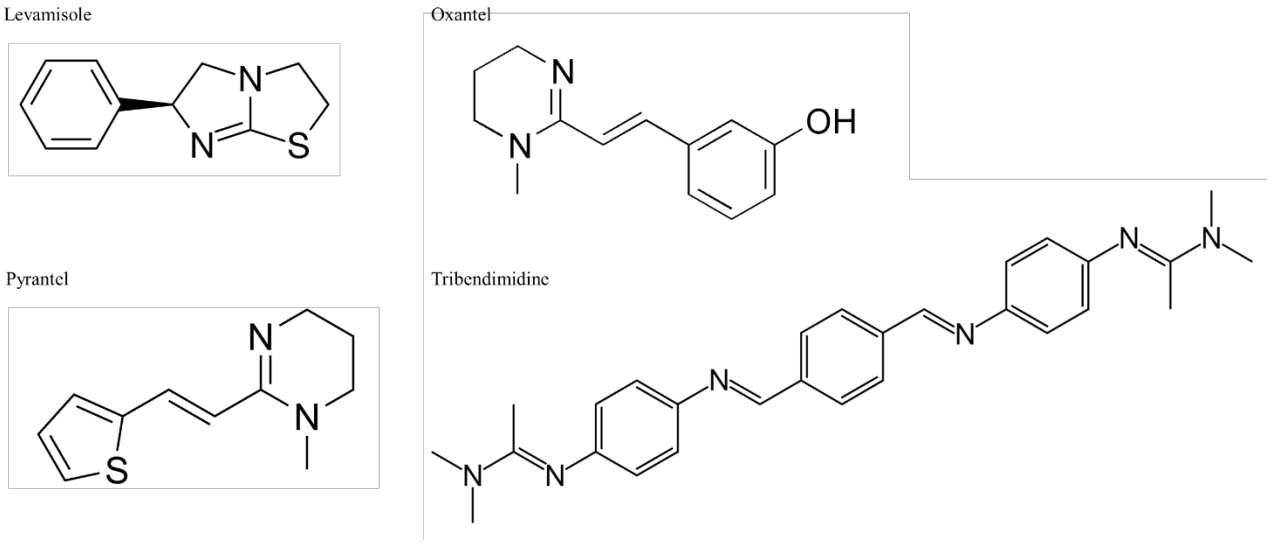
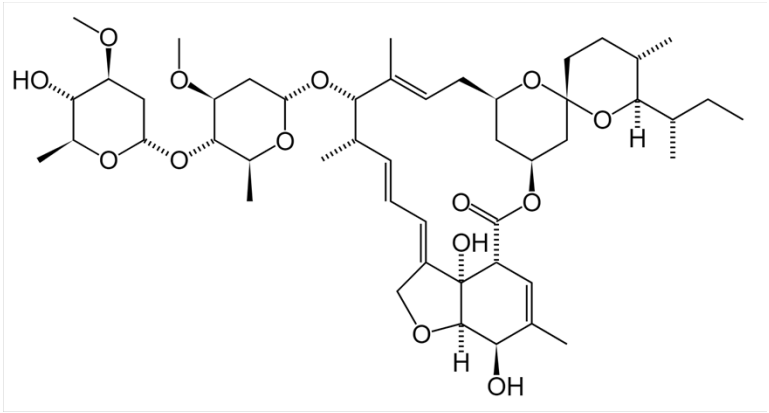


Figure 7: Structures of nicotinic acetylcholine receptor agonists.

Another common anthelmintic is ivermectin, which has been an antiparasitic in veterinary medicine since 1981 [53]. As a macrocyclic lactone, ivermectin binds to glutamate-gated chloride channels (GluCls) on muscle cells (particularly those of the pharynx), which causes a flood of chloride ions leading to paralysis and death [54]. In 2018, another macrocyclic lactone named moxidectin was approved for use against onchocerciasis, or river blindness, by the United States Food and Drug Administration (FDA) [55]. It works in a similar (though not identical) manner as ivermectin binding to GluCls. The drug is part of the milbemycin sub-family, while ivermectin belongs to the sub-family of avermectins (Figure 8) [56]. A weight dependent dose (200 µg/kg) is used for ivermectin , while an 8 mg weight independent dose of moxidectin is used for most helminth infections [57].

Ivermectin



Moxidectin

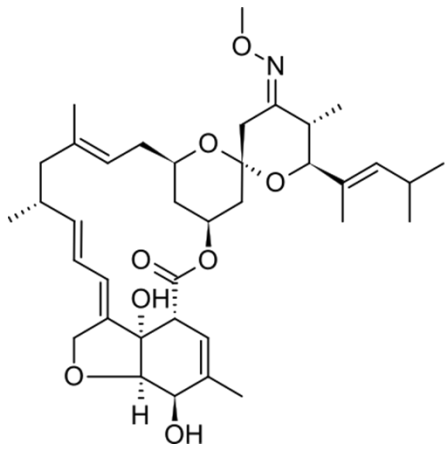


Figure 8: Structures of ivermectin and moxidecitn.

1.7. Public health control

1.7.1. History of STH control

Formal global control of STHs began with resolution of WHA54.19 by the World Health Assembly in 2001 [58]. A goal was set to achieve 75% coverage of deworming therapy in school-aged children at risk for STHs and schistosomiasis by 2010 [58]. Though millions of school-aged children were treated for STHs, the goal was not achieved by 2010, but did provide the momentum needed for development of formal global strategies for the control and elimination of STHs. In 2012, the WHO published *Accelerating work to overcome the global impact of neglected tropical diseases* and *Eliminating soil-transmitted helminthiases as a public health problem in children* [59, 60]. The former details the roadmap to elimination of the 17 neglected tropical diseases by 2020, while the latter outlines the specific strategies for

STHs [59, 60]. Both call for an integrated NTD plan at the country level with 75% coverage in 100% of affected countries by 2020 [59, 60]. Inspired by the WHO roadmap, a group of industry partners, donors, non-government organizations and NTD stakeholders gathered and committed to the control of 10 NTDs, including STHs, in the London Declaration on Neglected Tropical Diseases [61]. Then in 2015, the elimination of NTDs was included as part of Sustainable Development Goal 3 [62]. Following a series of guidelines and updates based on annual progress reports, the WHO recommended expanding annual or bi-annual deworming to preschool-aged children, women or reproductive age and pregnant women after the first trimester of pregnancy [63].

Simultaneous to deworming, Water, Sanitation and Hygiene (WASH) interventions and improvements became imperative to combat STH transmission. In early 2010, the European Region of the WHO made the Parma Declaration on Environment and Health, which pledged to improve WASH conditions, especially for the well-being of children by 2020 [64]. Following the Parma Declaration, resolution WHA64.24 recommends an integrated approach for proper WASH practices for primary prevention [65]. Furthermore, Sustainable Development Goal 6 focuses on providing adequate access to water and sanitation services by 2030 [62].

While targets for 75% deworming coverage are well on track in the 103 countries that remain endemic for STHs, new targets were developed for 2030 [66]. These include STH elimination in preschool- and school-aged children and expanding control programs to adolescents, women of reproductive age and pregnant/lactating women, as well as universal access to basic WASH [66].

1.7.2. WASH interventions

Approaches for WASH control of soil-transmitted helminths focus on the prevention of contamination of soil with eggs/larvae and prevention of contact with contaminated substances,

such as food and water. Health education and behavior change play a large part in preventing open defecation, poor hygiene practices during self-care and food preparation and walking barefoot. Prevention of contaminated soil can be achieved through appropriate waste management. Usually the installation of improved latrines with adequate maintenance that are easily accessible to community members lowers the odds of STH infection [67]. Ingestion of or contact with contaminated substances can be prevented with access to clean water and improved hygiene practices (i.e. handwashing with soap before eating). The use of treated water and access to piped water have both been shown to reduce the odds of STH infection [67]. Handwashing (before and after eating and after defecation) lower the odds of STH infection with a significant lowering of infection when using soap [67]. Particularly in the case of hookworm, the wearing of shoes also lowered the odds of infection [67]. WASH continues to play an important role, not only in the control and elimination of STHs, but also in the prevention of many other NTDs and infectious diseases, which is why integrated approaches that include WASH and deworming therapy are parts of successful and comprehensive control programs [68].

1.7.3. Preventive chemotherapy

Another way to control for STHs is preventive chemotherapy (PC), or treatment of STHs without diagnosis. The use of PC not only interrupts transmission by reducing soil contamination, but also reduces the morbidity of STHs. Routine distribution of PC to either entire populations or targeted populations (i.e. school-aged children) is called mass drug administration (MDA). Compared to WASH interventions, PC remains the fastest and lowest costing form of STH control.

Current WHO guidelines recommend one of the benzimidazoles (albendazole or mebendazole) annually for endemic countries with prevalence of STHs ≥20% and <50% or biannually in

countries with prevalence ≥50% [63]. As seen in Figure 9, the frequency of PC depends on the prevalence within the endemic country and should be evaluated and tailored periodically [66].

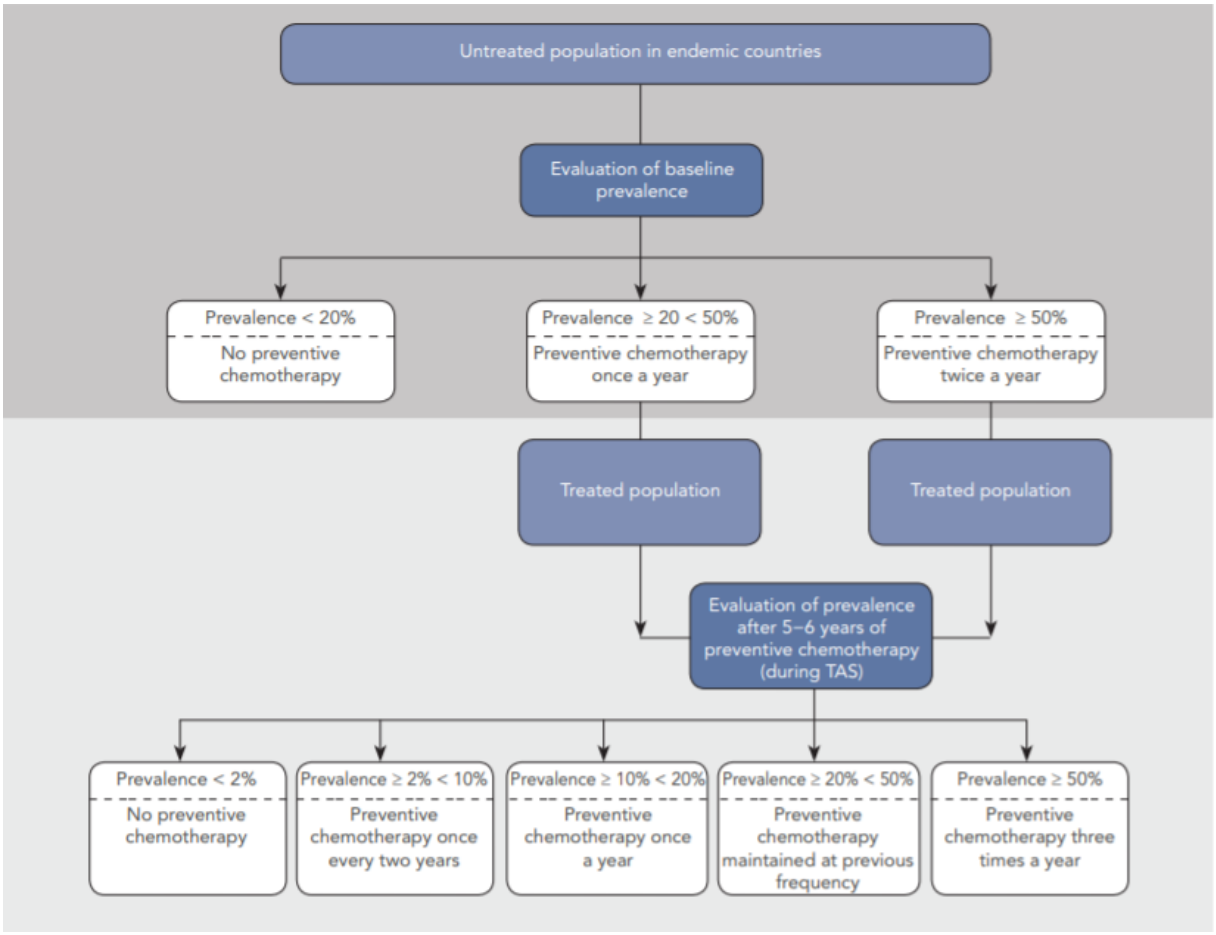


Figure 9: Decision tree for preventive chemotherapy. From WHO's 2030 targets [66].

However, PC is usually limited to SAC due to the high prevalence of STHs among the population and the ease of access to the target population through a structured school system. Though the global coverage rates are reaching 75% as outlined in the 2020 targets, coverage rates vary substantially by region, as seen in Table 1. Furthermore, over one billion preschool- and school-aged children require PC globally, as of 2019 [69].

Table 1: Coverage rates by region, 2017 [70].

Region	**Coverage of preventive chemotherapy (%)**	
	Preschool-aged children	**School-aged children**
Africa	71.73	63.97
Americas	40.14	50.93
Eastern Mediterranean	76.27	23.39

European	0*	69.57
South-East Asia	76.69	90.3
Western Pacific	50.27	51.34
Global	69.4	70.18

*No preventive chemotherapy required and/or no data available

One of the drawbacks of traditional PC approaches has been limiting the target population to children. Particularly, hookworm infections are hard to control in children, since adolescents and adults contribute predominantly to disease transmission [71, 72]. Though recent recommendations have included expansion of PC to adolescents, women of reproductive age and pregnant women, many countries have yet to implement strategies within control programs targeting these populations.

Compounded to the limitations in targeted populations is the limitations of the therapies themselves. Though pyrantel, levamisole and ivermectin are also included on the WHO's Model List of Essential Medicines, only albendazole and mebendazole are included as part of the recommendations for mass drug administration of chemotherapy [45]. While periodic evaluation of the prevalence of endemic countries aims to reduce drug pressure and the risk of growing resistance, the fact remains that the efficacy of both albendazole and mebendazole has decreased in the last two decades [47]. Though there is very little evidence of human resistance to date, the frequent and expansive use of anthelminthics in veterinary medicine shows the development of resistance [73-76]. Moreover, cure rate (CRs) and egg reduction rates (ERR) remain low and moderate against *T. trichiura* and hookworm infections, respectively, as seen in Table 2 [47].

Table 2: Cure rates and egg reduction rates of benzimidazoles. Data from Moser et al. 2017 [47].

Soil-transmitted helminth	**Albendazole (400 mg)**		**Mebendazole (500 mg)**	
	Cure rate (%)	**Egg reduction rate (%)**	**Cure rate (%)**	**Egg reduction rate (%)**
A. lumbricoides	96	99	96	98
T. trichiura	31	50	42	66
hookworm	80	90	33	61

Though current standard doses of the benzimidazoles are not highly efficacious against *T. trichiura* and hookworm infections, there is an opportunity to optimize the existing therapies, which is the focus of this PhD research. Evidence of efficacy of higher doses of albendazole against both trichuriasis and hookworm infections have been alluded to in the literature; however, a formal dose-response relationship against these two indications has not been assessed [42]. The first and second objectives of my PhD were to determine the optimal dose of albendazole by conducting phase two clinical trials with those infected with *T. trichiura* and hookworm. Since limited evidence is available for pre-school aged children and adults, these two target populations were added to the trial designs along with school-aged children.

Another way to improve standard treatment is to add another therapy, which has long been used in the field of other infectious diseases to prevent resistance and improve efficacy [77, 78]. Recent work has also shown that combination therapy of ivermectin and albendazole may be effective in reducing the risk of STH infections (risk ratio (RR) =0.44) [79]. However, very few trials have shown the impact of combination of standard doses of ivermectin (200 μg/kg) and albendazole (400 mg) [80-83]. To this effect, the third objective of my PhD was to aid in the design and conduct of a multi-country trial evaluating the efficacy and extended effects of co-administered ivermectin and albendazole compared to the monotherapy of albendazole in community-wide framework.

1.8. Study settings

1.8.1. Côte d'Ivoire

The bulk of the PhD research comprising this thesis was conducted in the Republic of Côte d'Ivoire. Côte d'Ivoire is a country on the coast of West Africa and has a tropical climate in which STHs thrive. The three trials conducted in Côte d'Ivoire took place in the southeast region of the country in four different settings: Azaguié, Rubino, Dabou and Jacqueville, as seen in Figure 10. In collaboration with the Centre Suisse de Recherches Scientifiques (CSRS),

located in Abidjan, and local Ministry of Health officials, the recruitment of the first trial begin in October 2018 and the final sample for final trial was collected in February 2020.

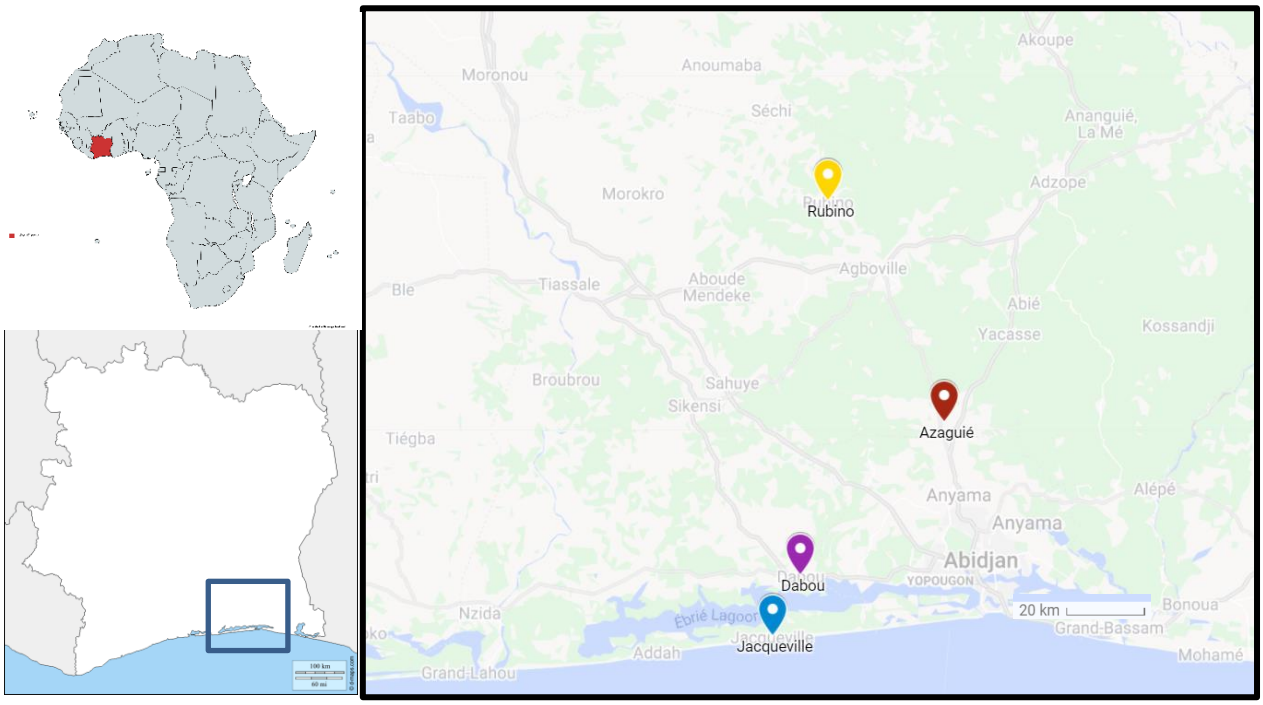


Figure 10: Counter-clockwise from top left: map of Africa highlighting Côte d'Ivoire in red; map of Côte d'Ivoire with reference box of southeast region; map of study sites.

Côte d'Ivoire, having an STH prevalence ≥20%, provides annual MDA to preschool- and school-aged children [70]. Coverage with ivermectin for onchocerciasis begin in the early 2000s and expanded to include albendazole for the treatment of lymphatic filariasis in 2010 as NTD programs took a more integrated approach [84]. Formal school-based mass drug administration of albendazole for STH began in 2012. However, interruption due to civil unrest in the early 2010s left gaps in coverage. Coverage for STH remains high with ≥75% coverage within the last 5 years, though combination ivermectin and albendazole was discontinued in 2019 at the close of the national lymphatic filariasis program [66]. However, STH prevalence varies not only geographically, but also by species [84]. *T. trichiura* and hookworm prevalences vary village to village within a district and single infections (without multiparatism) are more often the case than not.

1.8.2. Other study settings

Some of the research that took place during this PhD was done outside of Côte d'Ivoire. The third and fourth objectives of this thesis took place in the framework of a multi-country clinical trial conducted in Chake Chake district, Pemba Island, Tanzania, Nambak district, Lao People's Democratic Repiblic (PDR) and Dabou and Jacqueville districts of Côte d'Ivoire. Pemba Island is off the east coast of Africa in the Indian Ocean. While the island is relatively small in size and mass drug administration with PC is conducted biannually (since 2003), STH prevalence remains high [85]. There has also been a history of combination ivermectin and albendazole used for the control of lymphatic filariasis from the early 2000s to the mid-2010s. Nambak district is located within the province of Luang Prabang in northern Lao PDR, which has high rate of STH infections [86]. Since 2006, there has been biannual mass drug administration with mebendazole to both preschool- and school-aged children. Besides the bezimidazoles, there has been no other mass drug administration of anthelminthics, such as ivermectin, through control programs in Lao PDR.

In addition to the collaboration with the CSRS in Abidjan, Côte d'Ivoire, specific partner institutes were identified to work with in both other trial settings. Namely, the Public Health Laboratory Ivo de Carneri in Pemba Island and the Lao Tropical and Public Health Institute in Lao PDR. The importance of choosing three diverse settings where community-based research can take place is an integral part of my third and fourth objectives, which delve into the differences of efficacy and morbidity within and between countries.

1.9. Research aim and objectives

The overall goal of this research is to evaluate the efficacy and safety of optimized treatment regimens and dosages against STH infections, and to assess potential association of morbidity markers with infection status and intensity. The overall aim of this research is to better inform

decisions and guide ongoing helminth control programs on best treatment and monitoring options by achieving the following objectives:

1. To assess the safety and efficacy of albendazole against *T. trichiura* among preschool-aged children, school-aged children and adults in Côte d'Ivoire by conducting a dose-finding (DF) randomized controlled trial
2. To assess the safety and efficacy of ascending doses of albendazole against hookworm infection in preschool-aged children, school-aged children and adults in Côte d'Ivoire by conducting a dose-finding (DF) randomized controlled trial
3. To design and conduct a multi-country trial evaluation on the efficacy and safety of co-administered albendazole and ivermectin versus albendazole monotherapy.
4. To evaluate gut morbidity markers for STH infections within the framework of a multi-country trial of albendazole-ivermectin.

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2. Efficacy and safety of ascending dosages of albendazole against *Trichuris trichiura* in preschool-aged children, school-aged children and adults: A multi-cohort randomized controlled trial

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Research Paper

Efficacy and safety of ascending dosages of albendazole against *Trichuris trichiura* in preschool-aged children, school-aged children and adults: A multi-cohort randomized controlled trial

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ABSTRACT

Background: The efficacy of the widely used albendazole against the soil-transmitted helminth *Trichuris trichiura* is limited; yet optimal doses, which may provide increased efficacy, have not been thoroughly investigated to date.
Methods: A randomized-controlled trial was conducted in Côte d'Ivoire with preschool-aged children (PSAC), school-aged children (SAC), and adults infected with *T. trichiura*. Participants were randomly assigned (1:1:1:1) using computer-generated randomization. PSAC were randomized to 200 mg, 400 mg, 600 mg of albendazole or placebo. SAC and adults were randomized to 400 mg, 600 mg, 800 mg of albendazole or placebo. The primary outcome was cure rates (CRs) against trichuriasis. Secondary outcomes were *T. trichiura* egg reduction rates (ERRs), safety, CRs and ERRs against other soil-transmitted helminths. Outcome assessors and the trial statistician were blinded. Trial registration at ClinicalTrial.gov: NCT03527745.
Findings: 111 PSAC, 180 SAC, and 42 adults were randomized and 86, 172, and 35 provided follow-up stool samples, respectively. The highest observed CR among PSAC was 27·8% (95% CI: 9·7%–53·5%) in the 600 mg albendazole treatment arm. The most efficacious arm for SAC was 600 mg of albendazole showing a CR of 25·6% (95% CI: 13·5%–41·2%), and for adults it was 400 mg of albendazole with a CR of 55·6% (95% CI: 21·2%–86·3%). CRs and ERRs did not differ significantly among treatment arms and flat dose-responses were observed. 17·9% and 0·4% of participants reported any adverse event at 3 and 24 h follow-up, respectively.
Interpretation: Albendazole shows low efficacy against *T. trichiura* in all populations and doses studied, though findings for PSAC and adults should be carefully interpreted as recruitment targets were not met. New drugs, treatment regimens, and combinations are needed in the management of *T. trichiura* infections.
Funding: Bill and Melinda Gates Foundation.

1. Introduction

Almost a quarter of the world's population is infected with soil-transmitted helminths (STHs): *Trichuris trichiura* (whipworm), *Ascaris lumbricoides* (roundworm), and *Ancylostoma duodenale* or *Necator americanus* (hookworms) [1]. STH infections account for a burden of over 1·9 million disability-adjusted life-years (DALYs) per year with trichuriasis accounting for 213 thousand DALYs [2]. Greatest at risk for infection are those living without access to potable water and living with inadequate sanitation in tropical climates [3–5]. Heavy intensity infections may lead to diarrhea, abdominal pain, inflammation, obstruction and, if untreated, nutrition and immune system impairment [3,6].

Preventive chemotherapy (PC), recommended by the World Health Organization (WHO) is the periodic administration of anthelmintic drugs through mass drug administration (MDA) campaigns [1]. PC has been successful in reducing the number of STH infections and reducing the burden of disease (especially moderate and heavy infections) by averting an estimated 61 thousand DALYs from 2010 to 2015 [7,8]. Current recommendations for MDA of first-line treatment include monotherapy of 400 mg of albendazole or 500 mg of

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Research in context

Evidence before this study

A literature review was conducted in PubMed searching for "Trichuris" and "albendazole" from inception to January 1, 2020. Different dosages and regimens of albendazole have been tested against *T. trichiura*; however, a thorough dose-finding study has not been done to date for preschool-aged children, school-aged children, and adults.

Added value of this study

This is the first multi-cohort, randomized-controlled trial conducted to determine the optimal dose of albendazole in several age groups targeted by treatment recommendations of the WHO. Based on the results of this trial in Côte d'Ivoire, it can be concluded that the current recommended dose of 400 mg of albendazole is not efficacious and higher doses do not provide any added benefit.

Implications of all the available evidence

Treatment with a single dose of the benzimidazoles annually or biannually in areas of STH prevalence ≥20% is the current key recommendations for the control of soil-transmitted helminths; however, efficacy of the drugs against trichuriasis is very low. Evidence from this trial confirms that albendazole, even at higher doses, is insufficient for treating *T. trichiura* infections; and, therefore novel treatments or combination therapy should be considered as part of control efforts to control and ultimately eliminate STHs.

mebendazole once or twice a year targeting children, girls and women of reproductive age, and women after the first trimester of pregnancy in settings where STH prevalence is ≥20% [1,9]. However, albendazole and mebendazole show limited efficacy against *T. trichiura* (cure rates of 30% and 42%, respectively) [9,10].

To date, the optimal dose for albendazole has not been determined and 400 mg is the standard dose regardless of age and/or weight [1,11]. Though a driver of efficacy has not been identified, hypothetically, a higher dose may be needed for school-aged children (SAC) and adults. Different doses of albendazole have been tested for efficacy against *T. trichiura*, but a formal dose-response relationship has not been conducted [12]. The objective of this trial was for the first time to determine the efficacy and safety of ascending doses of albendazole against *T. trichiura* in three population cohorts (preschool-aged children (PSAC), SAC, and adults).

2. Methods

2.1. Study design

A phase two, parallel, randomized, placebo-controlled, dose-finding trial was conducted in seven villages near the town of Azaguié in the Agboville department of southern Côte d'Ivoire from October 23, 2018 to January 12, 2019. PSAC 2–5 years of age, SAC 6–12 years of age, and adults over the age of 21 years were invited to participate in the trial. It was decided not to include community members aged 13–20 years for two reasons: no differences between teenagers and adults were expected and teenage community members are the most transient population in remote villages based on our own previous experience. Ethical clearance was obtained from the Comité National d'Éthique des Sciences de la Vie et de la Santé (July 3, 2018, reference No. 089-18//MSHP/CNESVS-km), and the Ethics Committee of Northwestern and Central Switzerland (July 20, 2018, Nr 2018-00545).

2.2. Participants

Prior to enrolment, an information session explaining the purpose, procedure, benefits, and risks of the trial was conducted in each of the seven villages for all community members. Written informed consent was obtained from all participants and/or parents and guardians of the children after they had attended the information session. Additionally, SAC provided written assent.

PSAC, SAC, and adults were eligible if they provided two stool samples and were positive for *T. trichiura*. Only PSAC with *T. trichiura* infection intensities ≥60 eggs per gram of stool (EPG) and SAC and adults with *T. trichiura* infection intensities ≥100 EPG were included in the trial. Excluded from the trial were those with acute or uncontrolled systemic illnesses (e.g., severe anemia defined as hemoglobin <8.0 g/dl, infection, clinical malaria) as assessed by a medical doctor upon initial clinical assessment and liver function tests, those who had received anthelmintic within the previous 4 weeks, those who were allergic to benzimidazoles, and pregnant or breastfeeding women.

2.3. Randomization and masking

Study participants eligible for treatment were randomly assigned to one of the treatment arms using a computer-generated, stratified block randomization code. A random allocation sequence with varying random blocks of 4 and 8 stratified by 2 levels of baseline infection intensity (light and moderate/heavy *T. trichiura*) according to WHO guidelines was provided by a statistician not involved in recruitment or data collection [13]. Since so few moderate and high infections are present in the setting, it was decided to combine these two levels of intensity into one stratum. PSAC were 1:1:1:1 randomized to placebo or albendazole at 200 mg, 400 mg, or 600 mg. SAC and adults were 1:1:1:1 randomized to placebo or albendazole at 400 mg, 600 mg, or 800 mg. Study-site investigators were aware of the study group assignment, whereas outcome assessors and the trial statistician were masked to group assignment. Participants might have recognized the study group assignment due to the number of tablets administered.

2.4. Procedures

Children and adults provided two stool samples collected on two different days after providing name, age, and sex in a village-wide census. Duplicate Kato-Katz thick smears (41·7 mg each) were prepared from each sample and examined for *T. trichiura, Ascaris lumbricoides*, and hookworm eggs [14]. Egg counts were recorded by experienced technicians and classified as light, moderate, or heavy [14]. An independent quality control was conducted for 10% of the slides prepared with results considered correct if: (i) no difference in presence/absence of *T. trichiura, A. lumbricoides,* and hookworm; (ii) egg counts were +/−10 eggs for counts ≤100 eggs or +/−20% for counts >100 eggs (for each species separately) [15].

Before treatment, eligible children and adults were examined physically and questioned for clinical symptoms by a trial physician. Height using a stadiometer, weight using a scale, and temperature using an ear thermometer were collected by trial nurses. In addition, venous blood samples were taken at baseline to assess organ toxicity, complete blood count, and hepatic/renal function, while capillary blood samples using the finger-prick method were used to measure hemoglobin levels (HemoCue 301) and diagnose malaria through a rapid test (ICT Malaria P.f. Antigen Test, ICT Diagnostics).

On the day of treatment, enrolled participants were given potable water and a high-fat breakfast before receiving either albendazole or

placebo orally with water. Active adverse event assessment using a questionnaire was conducted at 3 h, 24 h, and 14–21 days after treatment. Participants were asked about the occurrence and intensity of headache, abdominal pain, nausea, vomiting, diarrhea, itching, allergic reactions, and any other symptom; moreover, temperature was taken to assess fever. Between 14 and 21 days post-treatment, treatment efficacy was assessed by collecting and examining two additional stool samples as described above. At the end of the trial, all participants and excluded children/adults remaining positive for any helminth infection were treated with 400 mg of albendazole in accordance with WHO guidelines [1].

2.5. Outcomes

The primary outcome is cure rate (CR) against *T. trichiura* at 14–21 days post-treatment. Secondary outcomes include egg reduction rate (ERR) against *T. trichiura*, CRs and ERRs against *A. lumbricoides* and hookworm, and drug safety. Drug safety was assessed at 3 h, 24 h, and 14–21 days after treatment.

2.6. Sample size

The main aim of this trial is to determine the dose-response relationship of albendazole against *T. trichiura*. A series of simulations were carried out to determine the required sample size. We assumed a true CR of 5%, 10%, 20%, 30%, and 40% against *T. trichiura* and a loss to follow-up of 5%. We estimated that enrolling 40 participants per arm will be sufficient to predict the dose response curve with a precision of about 10% points [16]. See supplementary material for details in sample size determination.

2.7. Statistical analyses

All data were collected on paper forms and entered twice into a database (Access 2010, Microsoft), cross-checked using the Data Compare utility of EpiInfo, version 3.5.4 (Centers for Disease Control and Prevention, Atlanta, GA, SA), and any discrepancies corrected by consulting the hard copy. Data management and descriptive results were done in Stata, version 15 (StataCorp, College Station, TX, USA), and the estimation of the dose-response curve was performed in R, version 3.5.1 (www.r-project.org).

An available-case analysis was performed according to the intention-to-treat principle using a full analysis set of all randomized participants providing any follow-up data. A per protocol analysis was planned according to the protocol. Since there were no protocol deviations the per protocol population was identical to the available case population. CRs were calculated as the percentage of egg-positive participants at baseline who become egg-

a

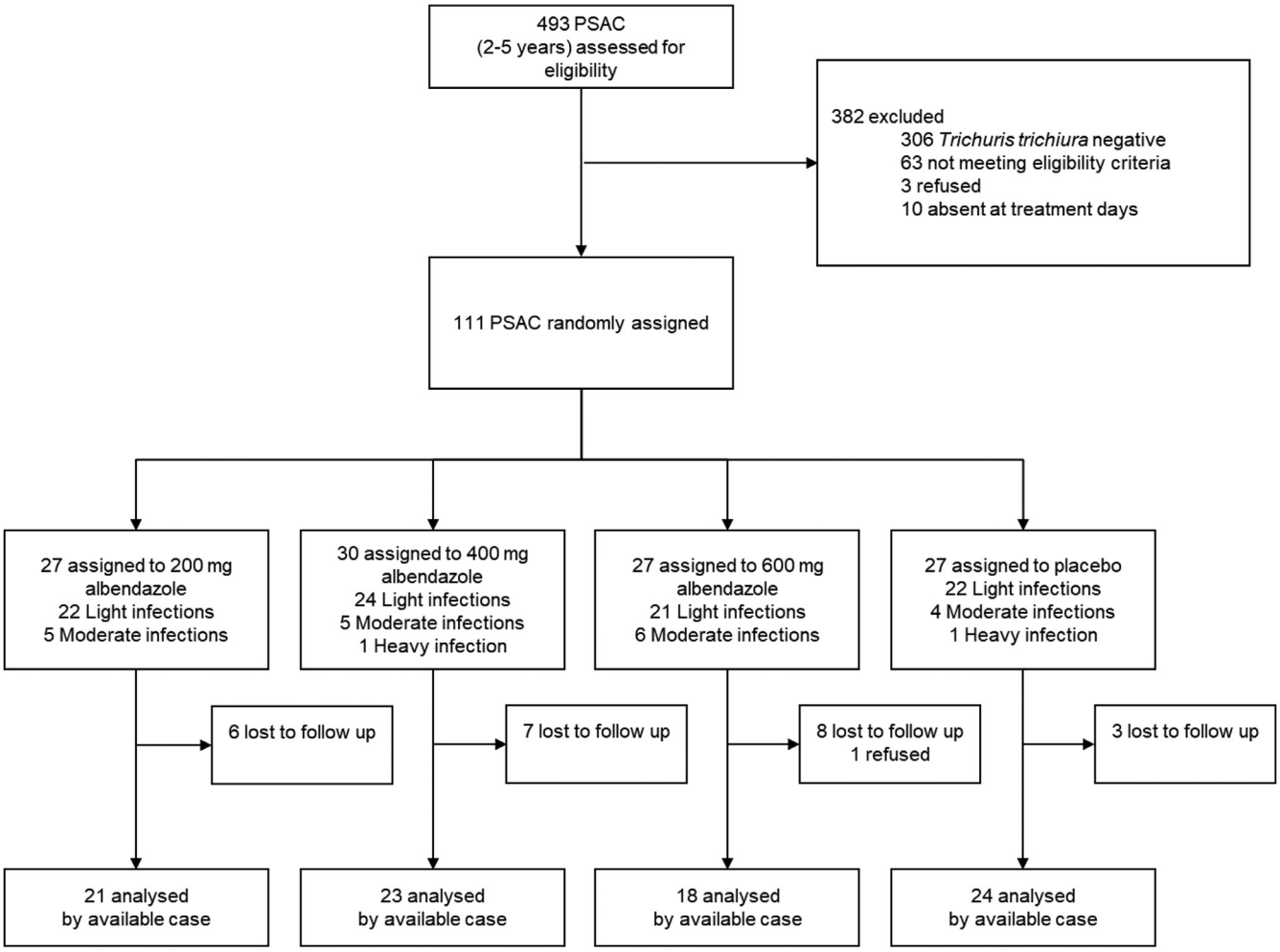

Fig. 1. a: PSAC participant flow-chart. Abbreviations: EPG, eggs per gram; PSAC, preschool-aged children. b: SAC participant flow-chart. Abbreviations: EPG, eggs per gram; SAC, preschool-aged children. c: Adults participant flow-chart. Abbreviations: EPG, eggs per gram.

b

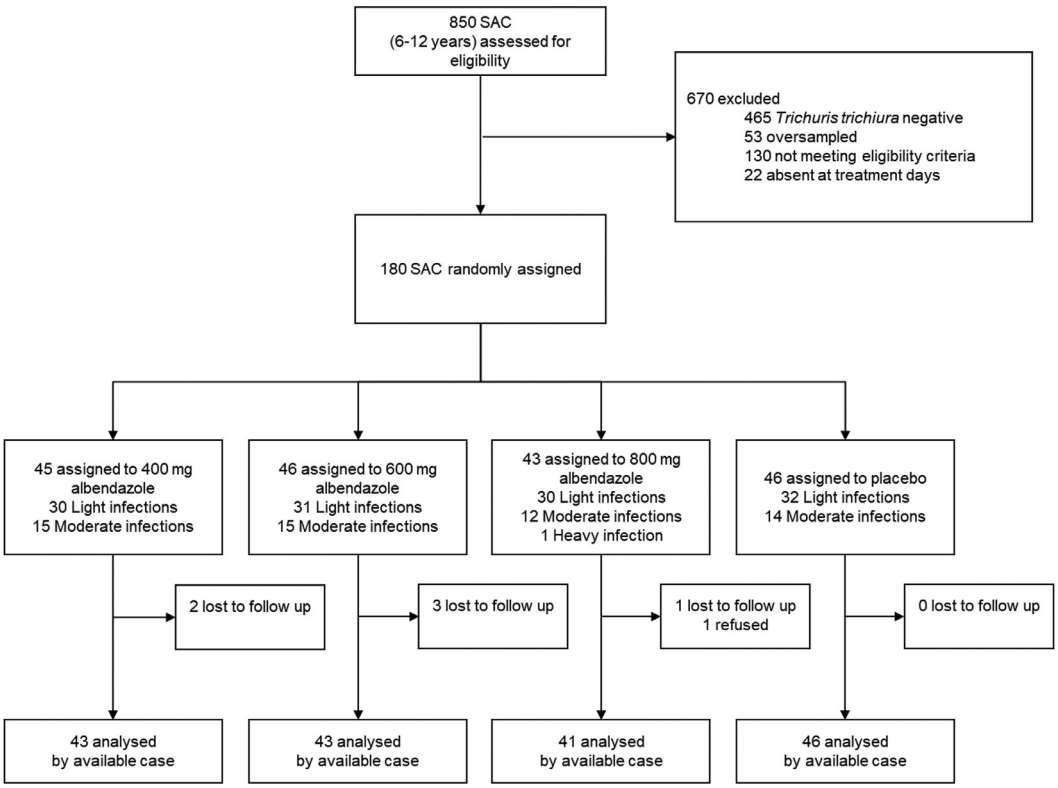

Fig. 1 Continued.

negative after treatment. EPG was assessed by calculating the mean egg counts from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of 24. The ERR was calculated with the following formula:

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

Geometric mean egg counts were calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5000 replicates was used to calculate 95% confidence intervals (CIs) for ERRs point estimates. ERRs were not calculated when too few infections were observed; moreover, 95% CIs were not calculated in cases of too small sample sizes for other helminth infections.

The hyperbolic E_{max} dose-response model was fitted using the DoseRange packages version 0.9–17 with the coefficients and the variance-covariance matrix estimates from a logistic regression. The analysis code is similar to the one used for the sample size simulations (Supplementary Material). The half–maximal–effect dose (ED_{50}) was estimated as half of the maximal placebo-adjusted effect on logit scale and afterwards back-transformed to probability (i.e., cure rate) scale.

Adverse events were descriptively evaluated as the number and proportion of participants reporting adverse events before and after treatment. The trial is registered at ClinicalTrials.gov (NCT03527745).

2.8. Role of the funding source

The funder of the study had no role in the 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.

3. Results

3.1. Baseline characteristics

Participants were recruited for the trial between October 23, 2018 and December 1, 2018. Despite an intensive screening effort, the targeted sample size (160 participants in each age cohort) was not reached for PSAC (69%) and adults (26%). A total of 2330 (493 PSAC, 850 SAC, and 987 adults) were screened for eligibility. Of those screened, 137 PSAC, 273 SAC, and 56 adults were invited for clinical examination, while 1607 were negative for *T. trichiura* and 50 PSAC, 112 SAC, and 95 adults had too low infection intensities. Of those invited to clinical examination, 26 PSAC, 40 SAC, and 14 adults either refused participation, were excluded based on eligibility criteria, or absent on treatment day. 53 eligible SAC children were oversampled and not enrolled in the trial. A total of 111 PSAC, 180 SAC, and 42 adults were randomly assigned to one of four treatment arms (Fig. 1a–c). At follow-up, 25 PSAC (22·5%), 7 SAC (3·9%), and 7 adults (16·7%) did not provide stool samples or were absent.

Baseline demographic and parasitological characteristics of PSAC, SAC, and adults included in the trial are presented in Table 1. Age, weight, height, and infection intensity were balanced across arms within the PSAC and SAC cohorts. In PSAC and adult cohorts, there were more female than male participants across all arms. Among SAC, there were more females in the placebo group in comparison to those receiving 400 mg of albendazole (25 vs 15); all other arms were balanced in terms of sex. The majority of infections were of light intensity (ranging 67–81% across arms) in all three age cohorts. In total, 22 PSAC, 57 SAC, and 3 adults had moderate or heavy intensity infections. There were very few co-infections with *A. lumbricoides* and/or hookworm: 20 PSAC, 54 SAC, and 6 adults were infected with *A. lumbricoides* and 1 PSAC, 7 SAC, and 2 adults were infected with hookworm.

c

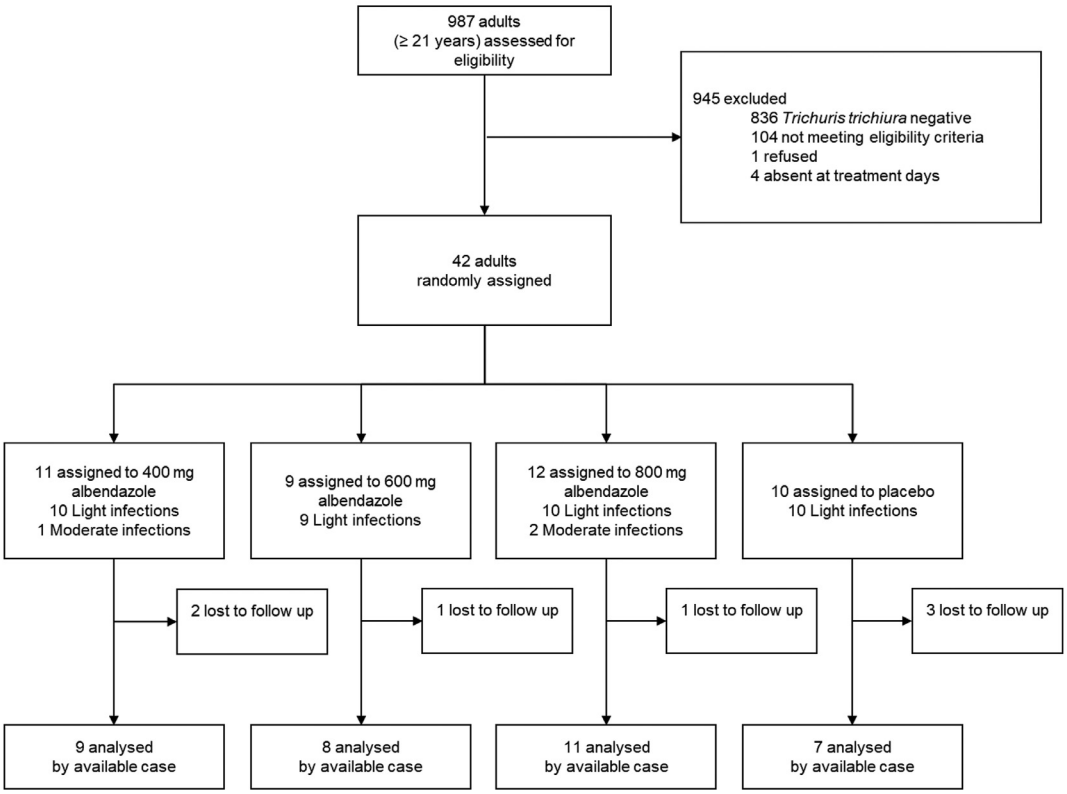

Fig. 1 Continued.

3.2. *Efficacy against* T. trichiura

CRs and ERRs of each arm against *T. trichiura* are shown in Table 2 for SAC, while results for PSAC and adults can be found in Supplementary Table S1. The E_{max} model predicted a maximal CR (E_{max}) of 24·7% and an ED_{50} at 118 mg. This predicted dose–response curve showed a plateau at approximately 500 mg (Fig. 2) with predicted CRs for the investigated doses of 6·5% (95% CI: 2·1%–18·3%) in the placebo group and 18·7% (95% CI: 9·7%–32·9%), 20·2% (95% CI: 13·9%–28·4%), and 21·1% (95% CI: 12·0%–34·6%) at 400 mg, 600 mg, and 800 mg, respectively. The observed CRs were close to the predicted values, except at 600 mg where the observed CR was 5.4%–points higher (25·6%, 95% CI: 13·5%–41·2%). Observed ERRs plateaued already at the first investigated dose (400 mg, ERR: 82·0%, 95% CI: 67·8%–90·5%).

Observed CRs among PSAC were similar across all arms ranging from 9·5% (95% CI: 1·2%–30·4%) to 27·8% (95% CI: 9·7%–53·5%). The corresponding geometric ERRs found were also similar across treatment arms ranging from 63·8% in the 200 mg albendazole arm to 88·5% in the 600 mg albendazole arm. Observed CRs for adults were 55·6% (95% CI: 21·2%–86·3%), 50·0% (95% CI: 15·7%–84·3%), 27·3% (95% CI: 6·0%–61·0%), 14·3(95% CI: 0·4%–57·9%) for albendazole at 400 mg, 600 mg, 800 mg, and placebo, respectively. The geometric ERRs ranged from 85·2% (placebo arm) to 97·7% (400 mg of albendazole arm).

Supplementary Table S2 presents the proportions of participants cured within each treatment arm by sex.

3.3. *Efficacy against hookworm and* A. lumbricoides

Data on CRs and ERRs against other helminth infections for PSAC, SAC, and adults are presented in Supplementary Table S3. For hookworm, 7 out of the 10 infections with follow-up data were cured with any dose of albendazole across all treatment arms in all cohorts.

For roundworm, CRs ranged between 66·7%–100% for PSAC given albendazole and 0% for PSAC given placebo. For SAC, CRs ranged from 84·6%–100% in active treatment arms and 38·5% in the placebo arm. All adults with *A. lumbricoides* infections were cured receiving either 400 mg or 800 mg of albendazole. Geometric and arithmetic ERRs had similarly large ranges for PSAC and SAC for all active treatment groups (placebo groups were considerably lower), and 100% for the adults cured by 400 mg or 800 mg of albendazole.

3.4. *Safety*

At baseline 102 PSAC, 172 SAC, and 42 adults were questioned for symptoms. A total of 84 (26·6%) participants reported mild symptoms at baseline, such as abdominal pain (11·4%), headache (11·1%), itching (5·4%), and diarrhea (4·4%).

104 PSAC, 175 SAC, and 41 adults were interviewed at 3 h post-treatment for adverse events and 86 PSAC, 154 SAC, and 32 adults were interviewed at 24 h post-treatment for adverse events. After treatment the proportion of reporting any adverse events was 17·9% and 0·4% at 3 and 24 h follow-up, respectively. Numbers of participants reporting each adverse event is reported in Table 3.

After treatment of 200 mg of albendazole, one participant (PSAC) reported a case of clinical malaria requiring inpatient hospitalization due to a serious adverse event. The participant received anti-malaria treatment and was released from the hospital within 24 h. No allergic reaction to any treatment arm was observed.

4. Discussion

The WHO has approved five different drugs and combinations against STH infections, of which albendazole is the most widely

Table 1
Baseline characteristics of participants. Numbers represent N (%) unless otherwise stated. Abbreviations: ALB, albendazole; EPG, eggs per gram; IQR, interquartile range; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children; SD, standard deviation.

	PSAC				SAC				Adults			
	ALB 200 mg (*n* = 27)	ALB 400 mg (*n* = 30)	ALB 600 mg (*n* = 27)	PLAC (*n* = 27)	ALB 400 mg (*n* = 45)	ALB 600 mg (*n* = 46)	ALB 800 mg (*n* = 43)	PLAC (*n* = 46)	ALB 400 mg (*n* = 11)	ALB 600 mg (*n* = 9)	ALB 800 mg (*n* = 12)	PLAC (*n* = 10)
Mean (SD) age (years)	4·0 (0·9)	3·8 (1·1)	4·0 (0·9)	4·1 (0·9)	8·9 (1·9)	8·7 (2·0)	8·8 (1·8)	8·9 (1·9)	40·5 (11·9)	36·6 (12·2)	44·8 (15·9)	44·4 (12·3)
Females	18 (67%)	16 (53%)	18 (67%)	16 (59%)	15 (33%)	19 (41%)	17 (40%)	25 (54%)	9 (82%)	7 (78%)	8 (67%)	6 (60%)
Mean (SD) weight (kg)	15·1 (2·9)	14·8 (2·2)	15·1 (2·3)	14·6 (2·5)	26·2 (6·5)	24·1 (5·7)	24·3 (4·1)	25·3 (6·6)	54·0 (7·6)	60·5 (8·9)	57·0 (6·0)	57·4 (9·7)
Mean (SD) height (cm)	97·2 (9·5)	99·2 (10·4)	97·6 (10·1)	97·5 (10·7)	126·8 (10·5)	123·7 (11·4)	125·0 (8·8)	125·8 (12·2)	157·5 (5·3)	158·8 (5·8)	158·5 (8·3)	160·1 (8·5)
Trichuris trichiura												
Median EPG [IQR]	264 [96–558]	210 [144–510]	228 [150–726]	216 [120–438]	426 [192–1266]	507 [186–1134]	414 [162–1116]	387 [222–1068]	168 [114–504]	156 [114–288]	195 [150–402]	138 [114–270]
EPG geometric mean	303·4	303·9	328·5	371·7	538·6	510·3	501·1	521·4	257·4	204·8	272·8	173·0
Infection intensity												
Light (1–999 EPG)	22 (81%)	24 (80%)	21 (78%)	22 (81%)	30 (67%)	31 (67%)	30 (70%)	32 (70%)	10 (91%)	9 (100%)	10 (83%)	10 (100%)
Moderate (1000–9999 EPG)	5 (19%)	5 (17%)	6 (22%)	4 (15%)	15 (33%)	15 (33%)	12 (28%)	14 (30%)	1 (9%)	0 (0%)	2 (17%)	0 (0%)
Heavy (≥10,000 EPG)	0 (0%)	1 (3%)	0 (0%)	1 (4%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Hookworm												
Infected children	0	1	0	0	1	2	3	1	1	0	0	1
Ascaris lumbricoides												
Infected children	8	5	3	4	18	10	13	13	3	0	3	0
Median EPG [IQR]	7929 [3123–10,395]	2118 [1428–6144]	1938 [30–10,530]	6216 [723–12,987]	5526 [690–17,160]	14,823 [1962–25,050]	7668 [2520–14,952]	528 [210–13,122]	13,500 [3906–18,954]		1068 [168–12,600]	
EPG geometric mean	3190·4	2163·3	857·6	2606·3	2752·7	4418·1	3241·1	1078·3	9998·5		1314·5	
Infection intensity												
Light (1–4999 EPG)	2 (25%)	3 (60%)	2 (67%)	2 (50%)	8 (44%)	4 (40%)	6 (46%)	8 (62%)	1	0	2	0
Moderate (5000–49,999 EPG)	6 (75%)	2 (40%)	1 (33%)	2 (50%)	9 (50%)	6 (60%)	7 (54%)	5 (38%)	2	0	1	0
Heavy (≥50,000 EPG)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (6%)	0 (0%)	0 (0%)	0 (0%)	0	0	0	0

Table 2
Observed and predicted cure rates and egg reduction rates against *Trichuris trichiura* of SAC at 3 weeks follow-up. Abbreviations: ALB, albendazole; CI, confidence interval; CR, cure rate; EPG, eggs per gram; ERR, egg reduction rate; PLAC, placebo; SAC, school-aged children.

	ALB 400 mg	ALB 600 mg	ALB 800 mg	PLAC
Positive before treatment (n)	43	43	41	46
Cured after treatment (n)	7	11	7	3
Observed CR [95% CI]	16·3 [6·8, 30·7]	25·6 [13·5, 41·2]	17·1 [7·2, 32·1]	6·5 [1·4, 17·9]
Predicted CR [95% CI]	18·7 [9·7, 32·9]	20·2 [13·9, 28·4]	21·1 [12·0, 34·6]	6·5 [2·1, 18·3]
EPG geometric mean				
Baseline	542·3	517·3	507·9	521·9
3 weeks follow-up	97·4	59·9	108·1	230·1
Observed ERR [95% CI]	82·0 [67·8, 90·5]	88·4 [74·8, 94·9]	78·7 [60·5, 89·0]	55·9 [26·1, 75·2]
EPG arithmetic mean				
Baseline	1094·4	955·3	1247·4	1094·0
3 weeks follow-up	545·2	687·1	893·6	1009·8
Observed ERR [95% CI]	50·2 [21·5, 67·9]	28·1 [−49·5, 69·6]	28·4 [2·6, 54·3]	7·7 [−46·0, 43·1]

used [11]. Although albendazole has been licensed for human use since 1982, there have been limited studies of its efficacy against *T. trichiura* in PSAC and adults; and, the optimal dosages of the drug have not been determined in humans [3,10,17]. There are many studies on the single dose of 400 mg recommended by the WHO, which reveal albendazole has low efficacy against *T. trichiura* [6,9]. Despite the lack of effective alternative treatment against trichuriasis, to date only a handful of studies have been conducted on higher dosages and dose-finding studies in different age-groups have not been carried out [12,18,19]. As national control programs move towards elimination, treatment of all age groups becomes increasingly important to prevent spread of infection; therefore, optimal doses for PSAC, SAC, and adults are necessary [6].

This trial revealed that there is no remarkable dose response of albendazole against *T. trichiura* in PSAC, SAC, and adults within the observed range. The current recommended dose of 400 mg for SAC is not efficacious against *T. trichiura* and higher doses of 600 mg or 800 mg do not increase CRs or provide a greater reduction in infection intensity. These results are confirmed by the predictions of the E_{max} model, which show no visual difference between CRs between doses of albendazole for SAC. Furthermore, only the 400 mg dose of albendazole has an arithmetic ERR slightly surpassing the proposed ≥50% arithmetic ERR by the WHO [20]. Though recruitment was limited for PSAC and adults, a similar conclusion is plausible as CRs for albendazole were low in all treatment arms. Though this study was conducted in a single country only, we are convinced that the findings of this trial are generalizable to wider populations as possible confounding factors were limited (e.g., a rigorous diagnostic procedure was used). Moreover, strain differences resulting in varying albendazole susceptibility are not to be expected. In one of our recent trials, an albendazole combination with oxantel pamoate found similar CRs against STHs in both Laos and a nearby setting in Côte d'Ivoire [21].

It is impossible to conclude if 400 mg is the optimal dose for SAC and adults, since a 200 mg dose of albendazole was not tested. A flat dose response was not anticipated for any cohort, so the dose of 200 mg of albendazole was not included as a treatment arm when the trial was designed. In PSAC, however, a dose 200 mg of albendazole provided a similar efficacy and ERR as albendazole at 400 mg, hinting that the recommended higher dose does not provide any

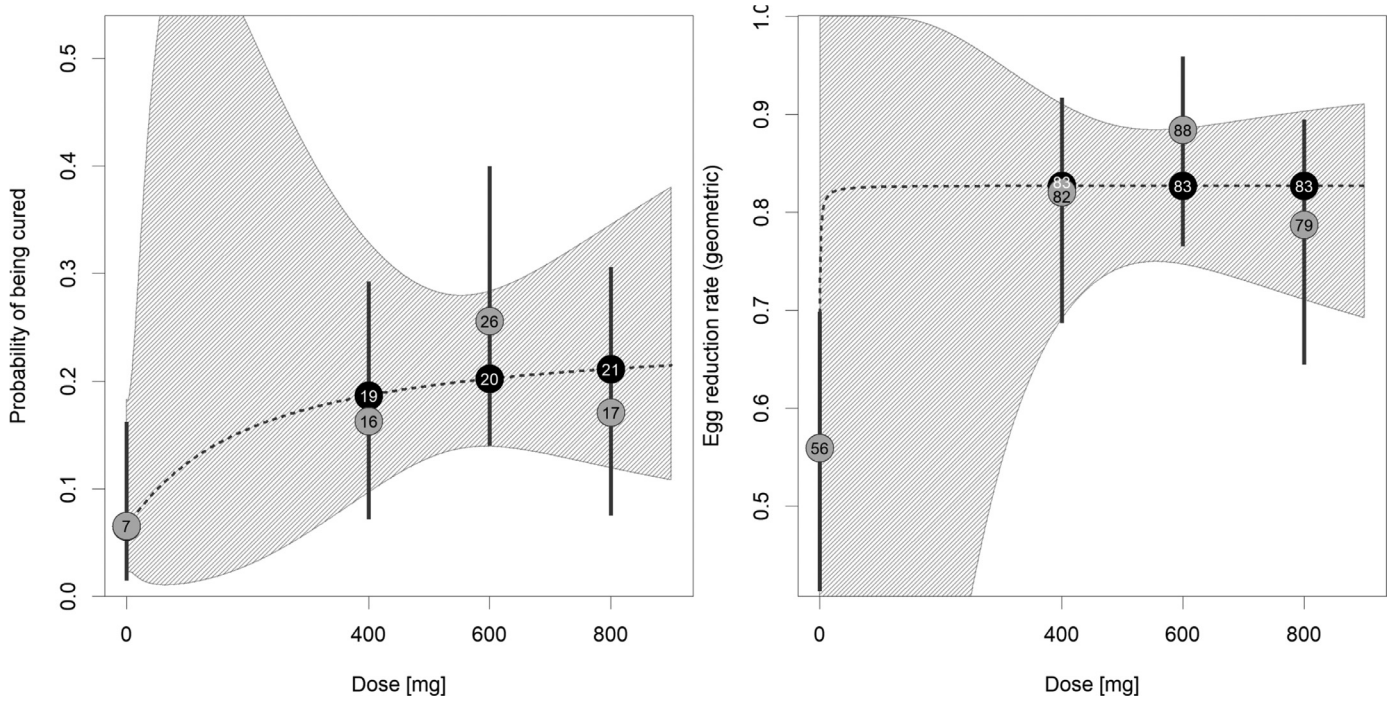

Fig. 2. CRs and ERRs predicted by the hyperbolic E_{max} model. Dotted lines represent the dose-response curve and the hatched area corresponds to the 95% confidence band. White numbers present the predicted CRs and ERRs for the investigated doses. Gray circles with black numbers represent the observed dose group CRs and the gray vertical lines correspond to the 95% confidence intervals. Predicted and observed estimates are similar in the placebo group; therefore, only one number is provided.

Table 3
Number of participants experiencing adverse events. Abbreviations: ALB, albendazole; CI, confidence interval; CR, cure rate; EPG_s, eggs per gram; ERR, egg reduction rate; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

	PSAC				SAC				Adults			
Adverse Event	ALB 200 mg	ALB 400 mg	ALB 600 mg	PLAC	ALB 400 mg	ALB 600 mg	ALB 800 mg	PLAC	ALB 400 mg	ALB 600 mg	ALB 800 mg	PLAC
Before treatment												
Headache	0/24 (0·0)	2/26 (7·7)	0/26 (0·0)	1/26 (3·9)	5/41 (12·2)	3/45 (6·7)	5/42 (11·9)	6/44 (13·6)	6/11 (54·6)	2/9 (22·2)	3/12 (25·0)	2/10 (20·0)
Abdominal pain	0/24 (0·0)	1/26 (3·9)	2/26 (7·7)	1/26 (3·9)	8/41 (19·5)	5/45 (11·1)	10/42 (23·8)	4/44 (9·1)	0/11 (0·0)	1/9 (1·1)	1/12 (8·3)	3/10 (30·0)
Nausea	0/24 (0·0)	0/26 (0·0)	0/26 (0·0)	0/26 (0·0)	1/41 (2·4)	0/45 (0·0)	0/42 (0·0)	1/44 (2·3)	1/11 (9·1)	1/9 (1·1)	1/12 (8·3)	0/10 (0·0)
Vomiting	0/24 (0·0)	0/26 (0·0)	0/26 (0·0)	0/26 (0·0)	0/41 (0·0)	0/45 (0·0)	2/42 (4·8)	1/44 (2·3)	0/11 (0·0)	0/9 (0·0)	0/12 (0·0)	0/10 (0·0)
Diarrhea	2/24 (8·3)	1/26 (3·9)	2/26 (7·7)	1/26 (3·9)	0/41 (0·0)	2/45 (4·4)	2/42 (4·8)	4/44 (4·6)	0/11 (0·0)	0/9 (0·0)	0/12 (0·0)	0/10 (0·0)
Itching	0/24 (0·0)	0/26 (0·0)	1/26 (3·9)	1/26 (3·9)	4/41 (9·8)	1/45 (2·2)	3/42 (7·1)	0/44 (0·0)	2/11 (18·2)	1/9 (1·1)	3/12 (25·0)	1/10 (10·0)
3 hr post treatment												
Headache	2/25 (8·0)	3/27 (11·1)	0/26 (0·0)	1/26 (3·9)	1/42 (2·4)	3/45 (6·7)	5/42 (11·9)	3/46 (6·5)	3/11 (27·3)	2/9 (22·2)	2/11 (18·2)	1/10 (10·0)
Abdominal pain	0/25 (0·0)	1/27 (3·7)	2/26 (7·7)	1/26 (3·9)	4/43 (9·3)	3/45 (6·7)	7/42 (16·7)	4/46 (8·7)	3/11 (27·3)	1/9 (11·1)	0/11 (0·0)	2/10 (20·0)
Nausea	0/25 (0·0)	0/27 (0·0)	0/26 (0·0)	0/26 (0·0)	0/43 (0·0)	0/45 (0·0)	0/41 (0·0)	0/46 (0·0)	1/11 (9·1)	0/9 (0·0)	0/11 (0·0)	0/10 (0·0)
Vomiting	0/25 (0·0)	0/27 (0·0)	0/26 (0·0)	0/26 (0·0)	0/43 (0·0)	0/45 (0·0)	0/42 (0·0)	1/46 (2·2)	0/11 (0·0)	0/9 (0·0)	0/11 (0·0)	0/10 (0·0)
Diarrhea	0/25 (0·0)	2/27 (7·4)	1/26 (3·9)	1/26 (3·9)	3/43 (7·0)	1/45 (2·2)	0/42 (0·0)	2/46 (4·4)	0/11 (0·0)	0/9 (0·0)	0/11 (0·0)	0/10 (0·0)
Itching	0/25 (0·0)	0/27 (0·0)	0/26 (0·0)	0/26 (0·0)	1/43 (2·3)	1/45 (2·2)	0/42 (0·0)	0/46 (0·0)	0/11 (0·0)	0/9 (0·0)	1/9 (11·1)	0/10 (0·0)
Serious adverse event	1/25 (4·0)	0/27 (0·0)	0/26 (0·0)	0/26 (0·0)	0/43 (0·0)	0/45 (0·0)	0/42 (0·0)	0/46 (0·0)	0/11 (0·0)	0/9 (0·0)	0/11 (0·0)	0/10 (0·0)
24 hr post treatment												
Headache	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)
Abdominal pain	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)
Nausea	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)
Vomiting	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)
Diarrhea	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)
Itching	0/21 (0·0)	0/25 (0·0)	0/21 (0·0)	0/19 (0·0)	0/37 (0·0)	0/41 (0·0)	0/39 (0·0)	0/37 (0·0)	0/10 (0·0)	0/7 (0·0)	0/9 (0·0)	0/6 (0·0)

benefit in this age group for *T. trichiura* infection. However, this finding would need to be confirmed in a follow-up study as our recruitment targets for this age group were not met.

More females were recruited into the trial than males. Additional analysis stratified by sex showed a difference in CRs between females and males. Females and males had similar baseline infection intensities (Table 1) and it was confirmed through pharmacokinetic analyses that drug exposure of albendazole was similar across sex regardless of treatment arm (data not shown). Further studies will need to assess whether this difference may be due to chance or to evaluate the underlying reasons for this finding.

The findings of this trial are comparable to others on albendazole in the literature. In a recent network meta-analysis, Moser and colleagues found that 400 mg of albendazole had a limited efficacy against *T. trichiura* with CRs having decreased from 38·6% in 1999 to 16.4% in 2015 [10]. However, most of the trials included in the analysis limited their study populations to SAC [10]. In comparison, mebendazole was found to have a slightly higher CR (42·1%) with a single dose and higher CRs when given at 100 mg twice/day for 3 days (CR=63%) [10,19].

In 2000, Horton et al. summarized in a literature review that increasing the single dosage and using repeated doses improves the efficacy of albendazole against *T. trichiura* [12]. In contrast, in our study increased single dosages did not provide improved efficacy against *T. trichiura*. Differences could be attributed to decreasing efficacy over the last two decades, differing study design, or geographic area [12]. The scope of this trial did not include evaluation of repeated doses of albendazole, which shows mixed results in humans and would be more difficult to administer through control programs [19,22].

A low number of study participants due to low prevalence in adults, and to a lesser extent, in PSAC, is a main limitation of our trial. Only 4·3% of adults screened were included in the trial as the majority (84·7%) of adults screened were found to be *T. trichiura* negative. The limited sample size of adults (n = 35 across four treatment arms) might have triggered higher CRs for the 400 mg and 600 mg treatment arms (55·6% and 50·0%, respectively). For PSAC, 62·1% of screened PSAC were negative for *T. trichiura* infection and only 22·5% of PSAC participated in the trial.

In conclusion, albendazole is not an effective treatment of *T. trichiura* even at the highest doses administered. Recently, a few trials have shown that combination therapy of albendazole and ivermectin is more efficacious against trichuriasis compared to monotherapy with the added benefit of *Strongyloides stercoralis* control [23]. This trial confirms to reduce the burden of STHs, new first-line treatments are needed, or combination therapy should be administered.

Data sharing

Deidentified individual participant data reported in this research article and the study protocol are available upon request from the corresponding author after all findings are published. Data will be shared after the approval of a proposal by the authors for legitimate scientific purposes.

Declaration of Competing Interest

We declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2020.100335.

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Supplementary Material

Sample size calculations

Sample size determination for Patel et al. 'Efficacy and safety of ascending dosages of albendazole against Trichuris trichiura in preschool-aged children, school-aged children and adults: a multi-cohort randomized controlled trial'. The true apparent cure rates used in the simulations are shown in table 1.

Table 1: Assumed true apparent cure rates (in percent) used in the simulations

Doses [mg]	T.t. 1	T.t. 2	T.t. 3	T.t. 4	Hook 1	Hook 2	Hook 3	Hook 4
0	5	5	5	5	5	5	5	5
400	20	15	25	25	20	50	50	50
600	30	30	35	35	30	70	70	75
800	40	40	45	45	35	80	80	85

Simulation code

```
library(DoseFinding)
library(dplyr)
set.seed(11031103)

# True cure rates against Trichuris (S1-S3) and Hookworm (S4-S6)
Tcrs <- list(T1 = c(0.05, 0.20, 0.30, 0.40),
             T2 = c(0.05, 0.15, 0.30, 0.40),
             T3 = c(0.05, 0.25, 0.35, 0.45),
             T4 = c(0.05, 0.20, 0.30, 0.35),
             H1 = c(0.05, 0.50, 0.70, 0.80),
             H2 = c(0.05, 0.50, 0.75, 0.85),
             H3 = c(0.05, 0.40, 0.65, 0.75),
             H4 = c(0.05, 0.60, 0.75, 0.85))
Nseq <- seq(30, 50, 5)      # Samples sizes per arm to evaluate
lossFU <- 0.05              # 5% loss to follow-up
doses <- c(0, 400, 600, 800) # Doses
pred.range <- 0:800         # predict dose response curve from 0 to 800
loops <- 200                # simulation runs per combination
counter <- 0
# data frame to store the simulation results
res <- data.frame(matrix(NA, loops * length(Nseq) * length(Tcrs), 5))
colnames(res) <- c("Seq","N","nfu","Median_0_800","Median_400_800")

for(k in 1:length(Tcrs)){
  Tcr <- Tcrs[[k]]
  for(j in Nseq){
    for(i in 1:loops){
      counter <- counter +  1
      res[counter, 1] <- k
      res[counter, 2] <- j
      n <- rbinom(4, j, 1-lossFU)
      res[counter, 3] <- sum(n)
```

```
      cured <- rbinom(4, n, Tcr)
      # ensure variation in placebo arm
      if(cured[1] == 0) cured[1] <- 1

      # fit logit model
      df <- data.frame(cbind(dose=doses, cured=cured, n=n))
      Crate <- df$cured/df$n
      fitBin <- glm(Crate ~ factor(doses) - 1, family = binomial, weights = df$n)
      drEst <- coef(fitBin)
      vCov <- vcov(fitBin)

      # fit dose range model
      gfit <- fitMod(doses, drEst, S=vCov, model = "emax", type = "general")
      logitPred <- predict(gfit, predType ="ls-means", doseSeq = pred.range, se.fit=TRUE)

      # confidence band on logit scale
      LB <- logitPred$fit-qnorm(0.975)*logitPred$se.fit
      UB <- logitPred$fit+qnorm(0.975)*logitPred$se.fit
      # confidence band on probability (cure rate) scale
      LB <- 1/(1+exp(-LB))
      UB <- 1/(1+exp(-UB))
      # Range 0 - 800 mg
      res[counter, 4] <- median(UB-LB)
      # Range 400 - 800 mg
      res[counter, 5] <- median(UB[401:801]-LB[401:801])
    }
  }
}

res$para <- ifelse(res$Seq <= 3, "Tri", "Hook")
sum.res <-res %>% group_by(para, N) %>%
  summarise(`median 0-800mg`=median(Median_0_800)*100/2,
            `median 400-800mg`=median(Median_400_800)*100/2,
            nFU =mean(nfu/4))
```

Table 2: One half length of the 95CI (percentage-pts)

para	N	median 0-800mg	median 400-800mg	nFU
Hook	30	12.9	11.6	28.5
Hook	35	11.9	10.7	33.3
Hook	40	11.0	10.0	38.0
Hook	45	10.5	9.4	42.7
Hook	50	9.9	8.9	47.5
Tri	30	12.3	12.1	28.5
Tri	35	11.3	11.2	33.2
Tri	40	10.5	10.4	38.0
Tri	45	9.9	9.9	42.7
Tri	50	9.3	9.4	47.5

Results Tables

Table S1: Cure rates and egg reduction rates against *Trichuris trichiura* of PSAC and adults at 3 weeks follow-up.

	PSAC				Adults			
	ALB 200 mg	**ALB 400 mg**	**ALB 600 mg**	**PLAC**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**	**PLAC**
Positive before treatment	21	23	18	24	9	8	11	7
Cured after treatment	2	4	5	4	5	4	3	1
Observed CR [95% CI]	9·5 [1·2, 30·4]	17·4 [5·0, 38·8]	27·8 [9·7, 53·5]	16·7 [4·7, 37·4]	55·6 [21·2, 86·3]	50·0 [15·7, 84·3]	27·3 [6·0, 61·0]	14·3 [0·4, 57·9]
Predicted CR [95% CI]	17·2 [6·5, 38·3]	20·1 [10·3, 35·6]	22·5 [9·8, 43·8]	13·6 [5·1, 31·3]				
EPG geometric mean								
Baseline	273·3	327·4	324·7	285·2	257·2	223·4	291·4	198·6
3 weeks follow-up	99·1	42·4	37·4	59·6	6·0	11·8	21·6	29·4
Observed ERR [95% CI]	63·8 [33·1, 83·1]	87·1 [67·3, 95·3]	88·5 [70·4, 95·7]	79·1 [53·5, 91·2]	97·7 [86·7, 99·8]	94·7 [61·0, 99·6]	92·6 [71·9, 98·5]	85·2 [65·9, 95·3]
EPG arithmetic mean								
Baseline	491·4	914·1	1126·0	952·5	444·7	291·8	448·4	229·7
3 weeks follow-up	456·9	273·7	816·3	206·5	298·0	184·5	228·5	87·4
Observed ERR [95% CI]	7·0 [-25·3, 50·7]	70·1 [33·2, 86·1]	27·5 [-6·4, 76·5]	78·3 [32·6, 89·6]	33·0 [-18·9, 99·4]	36·8 [-105·0, 96·0]	49·0 [7·6, 94·4]	61·9 [36·1, 86·1]

Abbreviations: ALB, albendazole; CI, confidence interval; CR, cure rate; EPG, eggs per gram; ERR, egg reduction rate; PLAC, placebo; PSAC, preschool-aged children.

Table S2: Proportion of participants cured by treatment arm and sex in PSAC, SAC and adults.

		PSAC	SAC	Adults
ALB 200 mg	**F**	2/14 (14·3%)	na	na
	M	0/7 (0%)	na	na
ALB 400 mg	**F**	2/10 (20·0%)	3/14 (21·4%)	4/7 (57·1%)
	M	2/13 (15·4%)	4/29 (13·8%)	1/2 (50·0%)
ALB 600 mg	**F**	5/12 (41·7%)	5/18 (27·8%)	3/7 (42·9%)
	M	0/6 (0%)	6/25 (24·0%)	1/1 (100·0%)
ALB 800 mg	**F**	na	3/16 (18·8%)	1/7 (14·3%)
	M	na	4/25 (16·0%)	2/4 (50·0%)
PLAC	**F**	3/15 (20·0%)	1/25 (4·0%)	1/4 (25·0%)
	M	1/9 (11·1%)	2/21 (9·5%)	0/3 (0%)

Abbreviations: ALB, albendazole; F, female; M, male; na, not applicable; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

Table S3: Cure rates and egg reduction rates against hookworm and *Ascaris lumbricoides* of participants at 3 weeks follow-up.

	PSAC				SAC				Adults			
	ALB 200 mg	**ALB 400 mg**	**ALB 600 mg**	**PLAC**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**	**PLAC**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**	**PLAC**
Hookworm												
Positive before treatment	0	1	0	0	1	2	3	1	1	0	0	1
Cured after treatment	na	1	na	na	0	2	3	0	1	na	na	0
CR	na	100	na	na	0·0	100	100	0·0	100	na	na	0·0
Ascaris lumbricoides												
Positive before treatment	6	3	1	2	18	9	13	13	2	0	3	0
Cured after treatment	5	2	1	0	16	9	11	5	2	na	3	na
CR	83·3	66·7	100	0	88·9	100	84·6	38·5	100	na	100	na
EPG geometric mean												
Baseline	3436·1	3958·5	30·0	4240·9	2781·0	3770·7	3262·2	1087·5	7261·8	na	1314·5	na
3 weeks follow-up	0·9	14·3	0	1711·4	0·9	0	0·9	190·8	0	na	0	na
ERR	99·9	99·6	100	59·7	99·9	100	99·9	82·5	100	na	100	na
EPG arithmetic mean												
Baseline	7091·0	4880·0	30·0	7989·0	12519·0	15535·3	8971·4	9067·7	8703·0	na	4612·0	na
3 weeks follow-up	7·0	1194·0	0	2436·0	125·0	0	51·2	7525·4	0	na	0	na
ERR	99·9	75·5	100	69·5	99·0	100	99·4	17·0	100	na	100	na

Abbreviations: ALB, albendazole; CR, cure rate; na, not applicable; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

3. Efficacy and safety of albendazole in hookworm-infected preschool-aged children, school-aged children and adults in Côte d'Ivoire: a phase II randomized controlled dose-finding trial

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MAJOR ARTICLE

Efficacy and Safety of Albendazole in Hookworm-infected Preschool-aged Children, School-aged Children, and Adults in Côte d'Ivoire: A Phase 2 Randomized, Controlled Dose-finding Trial

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Background. Infections with hookworms affect about half a billion people worldwide. Recommended therapy includes 400 mg of albendazole, which is moderately efficacious. Higher doses have been rarely assessed.

Methods. A randomized, controlled dose-finding trial was conducted in Côte d'Ivoire with the aim of recruiting 120 preschool-aged children (PSAC), 200 school-aged children (SAC), and 200 adults. Eligible PSAC were randomized 1:1:1 to 200 mg, 400 mg, or 600 mg of albendazole; the other age groups were randomized 1:1:1:1:1 to placebo or 200 mg, 400 mg, 600 mg, or 800 mg. The primary outcome was cure rates (CRs) assessed 14–21 days post-treatment by quadruplicate Kato-Katz thick smears. Hyperbolic E_{max} models were used to determine dose-response.

Results. 38 PSAC, 133 SAC, and 196 adults were enrolled. In adults, predicted CRs increased with ascending doses of albendazole, with a CR of 74.9% (95% confidence interval [CI], 55.6%–87.7%) in the 800-mg arm. Observed CRs increased with ascending doses of albendazole reaching a maximum of 94.1% (95% CI, 80.3%–99.3%). In SAC, the predicted dose-response curve increased marginally, with CRs ranging from 64.0% in the 200-mg arm to 76.0% in the 800-mg arm. Sample size in PSAC was considered too small to derive meaningful conclusions. 10.7% and 5.1% of participants reported any adverse event at 3 hours and 24 hours post-treatment, respectively.

Conclusions. A single 800-mg albendazole dose provides higher efficacy against hookworm and is well tolerated in adults and should be considered for community-based strategies targeting adults. For PSAC and SAC, current recommendations suffice.

Clinical Trials Registration. NCT03527745.

Keywords. hookworm; Côte d'Ivoire; albendazole; soil-transmitted helminthiasis; drug safety.

Necator americanus or *Ancylostoma duodenale*, two types of hookworm, affect approximately 500 million individuals worldwide [1, 2]. As a soil-transmitted helminth (STH), hookworm is most common in tropical and subtropical settings where larvae are able to survive in moist, warm soil.

Hookworm accounted for approximately 845 000 disability-adjusted life-years lost in 2017, with an estimated economic loss of more than $100 billion [3, 4]. The hookworm can cause skin irritation when it enters through the skin, acute lung inflammation and difficulty breathing when larvae migrate through the pulmonary system, and gastrointestinal issues such as blood loss, diarrhea, and abdominal pain [5, 6]. Morbidity is highly correlated with infection intensity, and more serious infections can cause anemia from blood loss [3, 7].

Albendazole is one of two benzimidazoles currently on the World Health Organization (WHO) Model List of Essential Medicines as a anthelmintic against intestinal STHs [8]. Developed in 1975 and licensed less than a decade later for human use, albendazole is part of current global control strategies [9, 10]. The mainstay of control is based on targeted preventative chemotherapy (PC) in preschool-aged children (PSAC), school-aged children (SAC), and women of reproductive age (WRA) given annually/biannually using either 400 mg of albendazole or 500 mg of mebendazole [10]. In 2012, the WHO published targets for 75% of coverage with PC in PSAC and SAC in all endemic countries and regular treatment of about 600 million PSAC and SAC who are in need of treatment by 2020 [11]. In 2018, additional targets were developed

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with the aim of STH control by 2030, including achieving and maintaining elimination in PSAC and SAC and expanding control programs to WRA [12].

Though PC with benzimidazoles has been successfully used as a cornerstone of control programs, efficacy against hookworm remains moderate (cure rates [CRs] of 79.5% for 400 mg albendazole and 32.5% for 500 mg of mebendazole) [13]. Several studies have shown that multiple doses of albendazole over an increased number of days have higher efficacy; however, this strategy could prove difficult during mass drug administration (MDA) campaigns [14–18]. A single dose that is greater than 400 mg may be more efficacious and effective at curing hookworm infection, though an optimal dose has not been identified, and limited evidence on the efficacy and safety for PSAC and adults is available. The novel aim of this trial is to characterize the dose-response relationship for the efficacy and safety of albendazole against hookworm in PSAC, SAC, and adults.

METHODS

Study Design, Area, and Population

A phase 2, parallel, randomized, controlled trial was conducted in 21 villages surrounding the town of Rubino in southeastern Côte d'Ivoire from 5 January 2019 to 17 April 2019. PSAC aged 2–5 years, SAC aged 6–12 years, and adults aged ≥21 years were invited to participate in the trial.

Ethical Considerations

Ethical permission from the Comité National d'Éthique des Sciences de la Vie et de la Santé in Côte d'Ivoire was issued on 3 July 2018, while the Northwestern and Central Switzerland Ethics Committee provided ethical clearance on 20 July 2018. Written informed consent was obtained from adult participants and from the parents/guardians of participating children after they had attended a community-wide information meeting. Additionally, SAC provided written consent.

Eligibility Criteria

Participants were eligible for the trial if they were willing to provide informed consent and two stool samples. Only participants with hookworm infection intensity of ≥24 eggs per gram (EPG) of stool were included. Those with acute or uncontrolled systemic illness (eg, severe anemia defined as hemoglobin <8.0 g/dL, infection, clinical malaria with fever) as assessed by a medical doctor, those who had received anthelmintics within the previous four weeks, those with allergies to benzimidazoles, and women who were pregnant or breastfeeding were excluded.

Randomization and Treatment

A computer-generated, stratified block randomization code was generated by the trial statistician who was not involved in participation recruitment, treatment administration, or outcome assessment. The randomization list with varying blocks of three and six for PSAC or five and ten for SAC and adults was stratified by baseline hookworm infection intensity (either light or moderate/heavy) per WHO guidelines [19]. After a lengthy period of recruitment, it was decided to eliminate the placebo group for the PSAC cohort. PSAC were randomized 1:1:1 to albendazole at 200 mg, 400 mg, or 600 mg. SAC and adults were randomized 1:1:1:1:1 to albendazole at 200 mg, 400 mg, 600 mg, or 800 mg or placebo. Outcome assessors were blinded to treatment allocation. Participants were potentially aware of the treatment arm that they were assigned due to the different number of tablets provided by unblinded study investigators.

Field and Laboratory Procedures

After a census in each community was conducted, children and adults in the appropriate age groups were asked to provide two stool samples. From each sample, duplicate Kato-Katz thick smears (each 41.7 mg) were prepared and examined for hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* eggs by two independent, trained technicians using light microscopes [20]. A third technician conducted an independent quality control for 10% of the slides. Results were considered correct if no difference in presence/absence of each helminth or egg counts were ±10 eggs for counts ≤100 eggs or ±20% for counts >100 eggs (for each species) [21]. Slides with incorrect results were re-read until a consensus was met.

Participants were invited for a clinical examination where individuals were physically examined, questioned for clinical symptoms, and underwent a series of rapid tests for malaria and pregnancy. Additionally, individuals were asked to provide a venous blood sample to assess complete blood count and hepatic/renal function.

Active adverse event (AE) reporting was conducted 3 hours, 24 hours, and 14–21 days post-treatment. At 14–21 days post-treatment, enrolled participants were asked to provide two stool samples for quadruplicate Kato-Katz smears, which were collected and analyzed as described above. At the end of the trial, all individuals who provided any stool sample and remained positive for any STH infection were treated with 400 mg of albendazole per WHO recommendations [10].

Outcome Measures

CR against hookworm infection at 14–21 days post-treatment was the primary outcome. Secondary outcomes were egg reduction rates (ERRs) against hookworm, CRs and ERRs against other STHs, and drug safety.

Sample Size

Multiple simulations were conducted to justify the required sample size. Assumed CRs against hookworm for placebo and albendazole at 200 mg, 400 mg, 600 mg, and 800 mg were 2.5%, 30%, 50%, 70%, and 80%, respectively, while loss to follow-up

was estimated at 10%. Simulations indicated that 40 participants per arm would be sufficient to predict the dose-response curve with a median precision, defined as one-half the length of the corresponding confidence band of approximately 10 percentage points [22].

Statistical Analyses

Data were collected on paper forms by trained personnel and entered twice into a database (Microsoft Access 2010, Wallisellen, Switzerland). Data were cross-checked using Beyond Compare 4 (Scooter Software, Madison, WI), and discrepancies were corrected using the original data. Analysis was performed using Stata, version 15 (StataCorp, College Station, TX) and R software, version 3.5.1 (www.r-project.org).

An analysis set of all randomized participants who provided any follow-up data was used to perform an available-case analysis. CRs were calculated as the percentage of egg-positive participants at baseline who become egg-negative post-treatment. EPG was calculated using the mean egg counts from the quadruplicate Kato-Katz thick smears and multiplying by a factor of 24. ERRs were calculated using geometric mean egg counts with the following formula:

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

The 95% confidence intervals (CIs) for ERR point estimates were calculated using a bootstrap resampling method with 5000 replicates. ERRs were not calculated for infections of *T. trichiura* and *A. lumbricoides* because too few infections were observed. To predict dose-response curves in terms of CRs and ERRs, hyperbolic E_{max} models were fitted using the R software DoseFinding package.

The number and proportion of AEs and participants reporting AEs were descriptively summarized before and after treatment.

RESULTS

Baseline Characteristics

Figure 1 shows participant flow charts for each age cohort. A total of 642 PSAC, 947 SAC, and 1691 adults were screened for eligibility. Of those screened, 57 PSAC, 173 SAC, and 289 adults were positive for hookworm infection. Hence, despite exhaustive screening efforts, target sample sizes for PSAC (120) and SAC (200) were not reached. Based on eligibility criteria of ≥24 EPG, 50 PSAC, 164 SAC, and 256 adults were invited to participate in clinical examination. There were 12 PSAC, 31 SAC, and 60 adults who refused to participate, were excluded based on eligibility criteria on the day of treatment, or were absent.

On treatment day, 38 PSAC, 133 SAC, and 196 adults were randomized to either three or five treatment arms (Figure 1). At 14–21 days post-treatment, 3 SAC (2.3%) and 23 adults (11.7%) did not provide any stool samples or were absent; all PSAC provided stool samples at follow-up.

Baseline demographic and parasitological characteristics of PSAC, SAC, and adults included are presented in Table 1. The proportions of females were lower in the 200-mg group in PSAC and higher in the 200-mg arm in SAC and adults. Other baseline characteristics were balanced. The majority of infections were of mild intensity (ranging from 92% to 100% across arms) in all three age cohorts; only 3 PSAC, 5 SAC, and 4 adults had moderate-intensity infections, and there were no high-intensity infections. Only 1 PSAC, 6 SAC, and 2 adults were coinfected with *T. trichiura*, and 2 PSAC, 3 SAC, and 1 adult were coinfected with *A. lumbricoides*.

Efficacy

For adults, CRs and ERRs of each arm against hookworm infection are presented in Table 2; results for PSAC and SAC can be found in Supplementary Table 1. The E_{max} model predicted a maximum CR of 97.2% (placebo adjusted, 80.4%) and a half maximal-effect dose (ED_{50}) on logit scale at 724 mg in the adult cohort (314 mg on probability scale). The dose-response curve showed no plateau within the observed dose range. The predicted CRs were 16.8% (95% CI, 7.4%–33.8%), 37.9% (95% CI, 26.2%–51.3%), 55.6% (95% CI, 44.7%–66.0%), 67.3% (95% CI, 55.6%–77.2%), and 74.9% (95% CI, 55.6%–87.7%) for placebo, 200 mg, 400 mg, 600 mg, and 800 mg, respectively (Figure 2). Likewise, observed CRs increased with ascending doses of albendazole and reached a maximum of 94.1% (95% CI, 80.3%–99.3%). In terms of ERRs, the E_{max} predicted ERR at the first investigated dose of 200 mg was 93.5% (95% CI, 79.1%–98.2%) and increased to 97.5% (95% CI, 86.0%–99.6%) in the 800-mg albendazole arm (Figure 2). Observed and predicted ERRs differed only slightly across all treatment arms.

In SAC, observed CRs and ERRs against hookworm showed a less pronounced dose-response effect compared with adults. The increase in terms of CR after 400 mg was small. Observed CRs ranged from 63.0% (95% CI, 42.4%–80.6%) in the 200-mg arm to 76.0% (95% CI, 54.9%–90.6%) in the 800-mg arm. The observed CR in the placebo group was 43.5% (95% CI, 23.2%–65.5%). Geometric ERRs were high in all arms. The number of eligible PSAC was far below the anticipated sample size, and results are only descriptively analyzed. Observed CRs were 69.2%, 61.5%, and 83.3% and geometric mean–based ERRs were 99.0%, 96.2%, and 99.6% for 200 mg, 400 mg, and 600 mg of albendazole, respectively.

Supplementary Table 2 presents the proportions of participants cured of hookworm infection within each treatment arm by sex.

There were very few coinfections with *T. trichiura* and/or *A. lumbricoides* (9 and 5 participants, respectively). Only two participants remained coinfected with mild infection at three

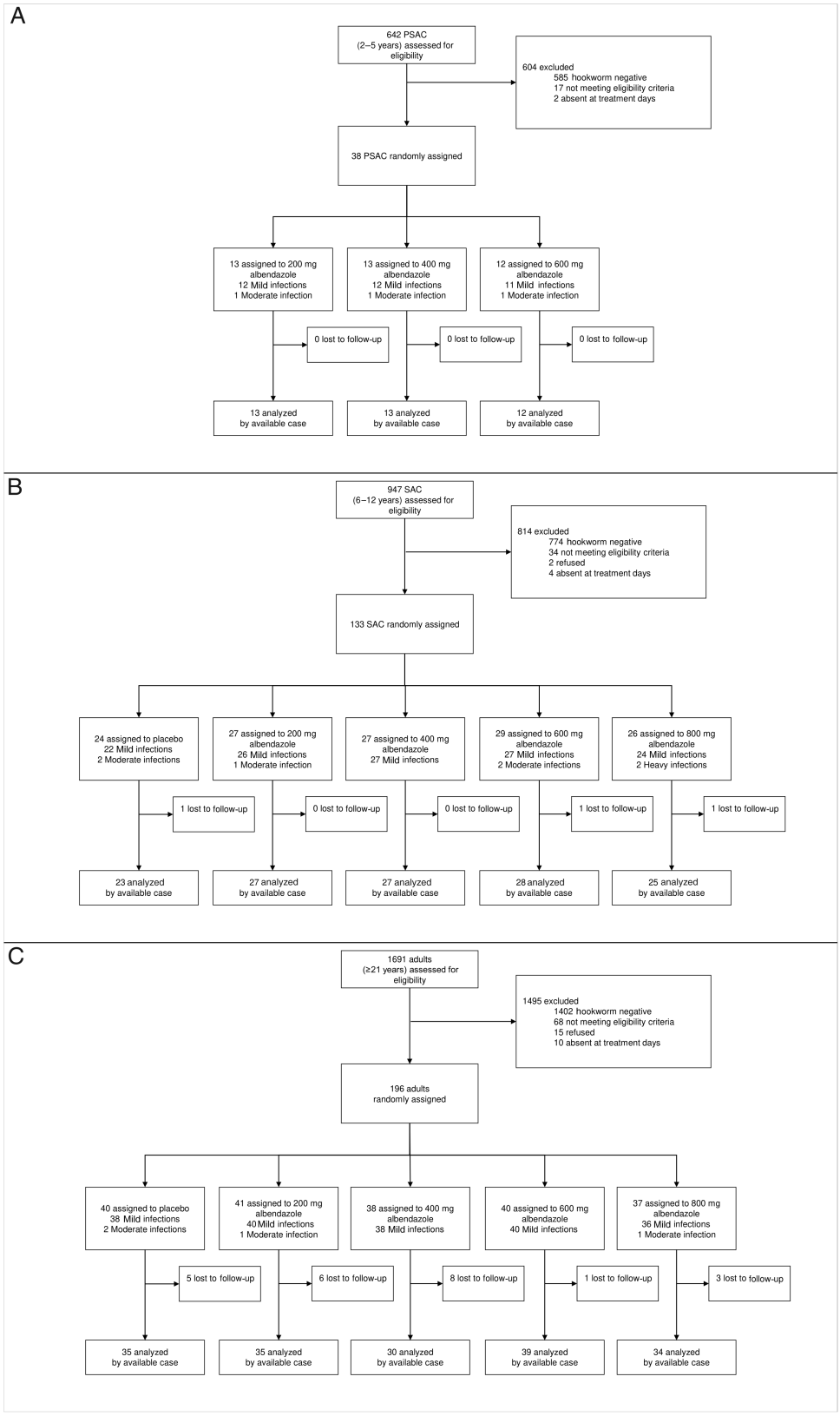

Figure 1. Trial flow charts. *A*, PSAC participant flow chart. *B*, SAC participant flow chart. *C*, Adults participant flow chart. Abbreviations: PSAC, preschool-aged children; SAC, preschool-aged children.

Table 1. Baseline Characteristics of Participants

Characteristic	Preschool-aged Children			School-aged Children					Adults				
	Albendazole, 200 mg (n = 13)	Albendazole, 400 mg (n = 13)	Albendazole, 600 mg (n = 12)	Placebo (n = 24)	Albendazole, 200 mg (n = 27)	Albendazole, 400 mg (n = 27)	Albendazole, 600 mg (n = 29)	Albendazole, 800 mg (n = 26)	Placebo (n = 40)	Albendazole, 200 mg (n = 41)	Albendazole, 400 mg (n = 38)	Albendazole, 600 mg (n = 40)	Albendazole, 800 mg (n = 37)
Age, mean (SD), y	3.8 (1.2)	3.8 (1.3)	3.8 (1.1)	9.1 (2.2)	9.1 (1.9)	9.8 (1.9)	9.2 (2.2)	9.4 (2.3)	35.4 (10.3)	39.8 (12.2)	36.3 (12.2)	38.2 (11.5)	35.7 (12.0)
Females	5 (38%)	9 (69%)	8 (67%)	8 (33%)	13 (48%)	8 (30%)	5 (17%)	7 (27%)	7 (18%)	16 (39%)[a]	7 (18%)	7 (18%)	9 (24%)
Weight, mean (SD), kg	15.5 (3.6)	13.8 (3.8)	14.4 (2.9)	26.0 (5.4)	25.7 (5.5)	27.7 (6.8)	24.8 (7.0)	27.9 (10.3)	59.7 (9.9)	61.3 (9.5)	61.6 (9.3)	62.7 (8.0)[b]	59.7 (8.8)
Height, mean (SD), cm	98.8 (12.3)	98.1 (9.5)	96.1 (9.5)	132.6 (13.2)	127.2 (12.7)	129.8 (13.1)	123.9 (18.2)	137.5 (39.7)	165.8 (10.7)[a]	166.6 (10.7)	168.1 (7.5)	168.3 (10.0)[a]	165.2 (9.3)[a]
Hookworm													
Median EPG (interquartile range)	216 (150–618)	126 (60–540)	252 (111–423)	183 (78–450)	126 (72–336)	138 (78–246)	156 (84–528)	153 (66–366)	57 (96–318)	126 (54–444)	96 (54–264)	189 (63–570)	114 (84–216)
EPG geometric mean	251.1	178.4	238.8	209.1	166.7	162.1	219.5	226.4	136.5	147.9	116.3	198.4	151.8
Infection intensity													
Mild (1–1999 EPG)	12 (92%)	12 (92%)	22 (92%)	22 (92%)	26 (96%)	27 (100%)	27 (93%)	24 (92%)	38 (95%)	40 (98%)	38 (100%)	40 (100%)	36 (97%)
Moderate (2000–3999 EPG)	1 (8%)	1 (8%)	2 (8%)	2 (8%)	1 (4%)	0 (0%)	2 (7%)	0 (0%)	2 (5%)	1 (2%)	0 (0%)	0 (0%)	1 (3%)
Heavy (≥4000 EPG)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Trichuris trichiura	0	0	1	2	2	0	0	2	1	0	0	0	1
Ascaris lumbricoides	1	0	1	1	1	0	1	0	0	0	0	1	0

Abbreviations: EPG, eggs per gram; SD, standard deviation.
[a]Missing data from 1 participant.
[b]Missing data from 2 participants.

Table 2. Observed and Predicted Cure Rates and Egg Reduction Rates Against Hookworm of Adults at 3 Weeks Follow-up

Result	Placebo	Albendazole, 200 mg	Albendazole, 400 mg	Albendazole, 600 mg	Albendazole, 800 mg
Positive before treatment	35	35	30	39	34
Cured after treatment	5	16	16	22	32
Observed CR (95% CI)	14.3 (4.8 to 30.3)	45.7 (28.8 to 63.4)	53.3 (34.3 to 71.7)	56.4 (39.6 to 72.2)	94.1 (80.3 to 99.3)
Predicted CR (95% CI)	16.8 (7.4 to 33.8)	37.9 (26.2 to 51.3)	55.6 (44.7 to 66.0)	67.3 (55.6 to 77.2)	74.9 (55.6 to 87.7)
EPG geometric mean					
Baseline	135.9	132.3	118.6	204.1	139.6
3 weeks follow-up	91.1	8.2	5.0	6.2	0.2
Observed ERR (95% CI)	33.0 (−27.1 to 67.3)	93.8 (87.0 to 97.3)	95.8 (90.2 to 98.3)	97.0 (92.5 to 98.9)	99.8 (99.5 to 100.0)
Predicted ERR (95% CI)	33.0 (19.6 to 49.9)	93.5 (79.1 to 98.2)	96.4 (90.4 to 98.7)	97.1 (88.4 to 99.3)	97.5 (86.0 to 99.6)
EPG arithmetic mean					
Baseline	348.3	325.0	242.0	414.9	275.6
3 weeks follow-up	478.6	63.9	32.2	69.5	2.1
Observed ERR (95% CI)	−37.4 (−137.1 to 44.6)	80.3 (58.1 to 91.8)	86.7 (75.3 to 94.0)	83.3 (68.0 to 92.7)	99.2 (97.6 to 100.0)

Abbreviations: CI, confidence interval; CR, cure rate; EPG, eggs per gram; ERR, egg reduction rate.

weeks post-treatment, and one participant with trichuriasis was lost to follow-up.

Safety

At baseline, 38 PSAC, 130 SAC, and 173 adults were questioned for symptoms. Among these, 34 participants (10.0%) reported a total of 43 mild symptoms at baseline, the majority of which were reported by adults. The most common reported symptoms at baseline were abdominal pain (9.2%), headache (6.4%), and itching (4.7%) in adults.

At three hours post-treatment, 34 PSAC, 118 SAC, and 137 adults were interviewed. From these, 2 PSAC, 11 SAC, and 18 adults reported at least one AE (10.7%). The most common AEs reported at 3 hours follow-up were abdominal pain (10.1% of SAC and 5.8% of adults) and headache (10.0% in adults, 5.9% in PSAC, and 2.2% in SAC).

A total of 28 PSAC, 89 SAC, and 120 adults were interviewed at 24 hours follow-up; 12 reported experiencing any AE (5.1%). Abdominal pain was the most common reported AE at the 24-hour examination, reported by 2.2% of SAC and 5.0% of adults.

The number of AEs reported and number of participants reporting any AE by each treatment arm can be found in Table 3. A more detailed report of symptom-specific AEs can be found in Supplementary Table 3.

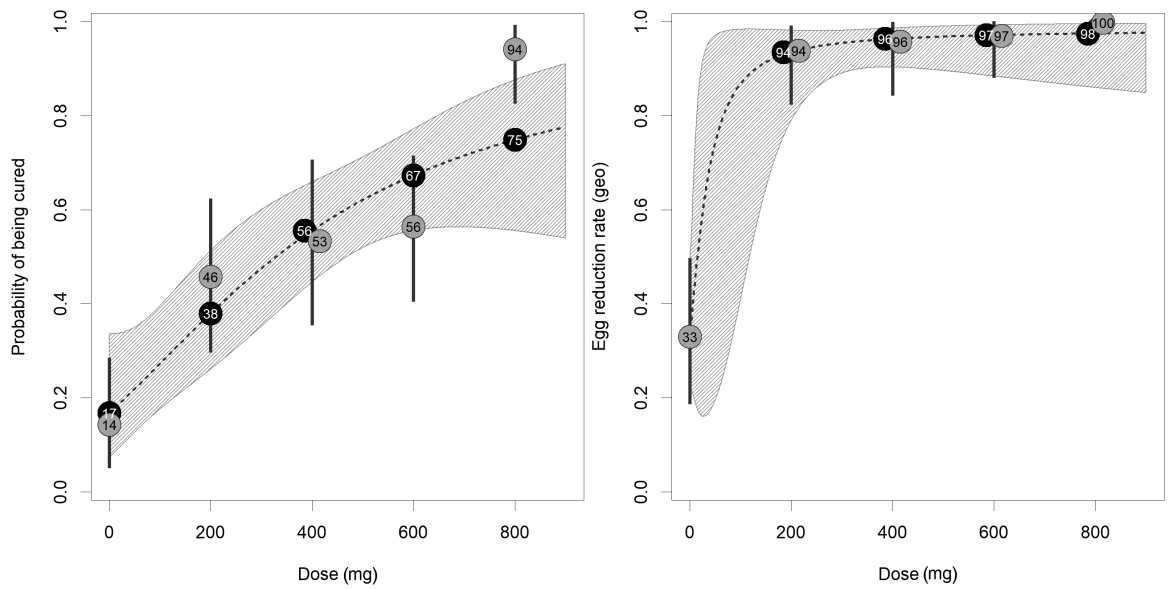

Figure 2. Cure rates (CRs) and egg reduction rates (ERRs) of albendazole in adults predicted by the hyperbolic E_{max} model. Dotted lines represent the dose-response curve, and the hatched area corresponds to the 95% confidence band. White numbers present the predicted CRs and ERRs for the investigated doses. Gray circles with black numbers represent the observed dose group CRs, and the gray vertical lines correspond to the 95% confidence intervals. Abbreviation: geo, geometric.

Table 3. Number of Adverse Events and Number of Participants Reporting Adverse Events

Time Point	Number of	Preschool-aged Children					School-aged Children					Adults				
		Albendazole, 200 mg	Albendazole, 400 mg	Albendazole, 600 mg	Total	Placebo	Albendazole, 200 mg	Albendazole, 400 mg	Albendazole, 600 mg	Albendazole, 800 mg	Total	Placebo	Albendazole, 200 mg	Albendazole, 400 mg	Albendazole, 600 mg	Albendazole, 800 mg	Total
Before treatment	Symptoms	0	0	1	1	1	0	0	1	1	3	6	10	11	5	7	39
	Participants	0	0	1	1	1	0	0	1	1	3	5	8	8	4	5	30
3 hours after treatment	Adverse events	1	0	1	2	2	3	2	1	3	11	2	4	5	4	5	20
	Participants	1	0	1	2	2	3	2	1	3	11	2	4	5	3	4	18
24 hours after treatment	Adverse events	0	0	0	0	0	0	2	1	0	3	1	2	2	5	1	11
	Participants	0	0	0	0	0	0	2	1	0	3	1	2	2	3	1	9

DISCUSSION

Currently, there are several drugs approved by the WHO for the treatment/control of STH infections, though albendazole and mebendazole are the most common due to their ease of administration (single dose regardless of weight) and low cost [8, 23]. Albendazole, being more efficacious than mebendazole against hookworm, is a cornerstone of PC programs in both moderate/high and low STH transmission settings; however, the standard dose of 400 mg varies in efficacy [13]. Factors such as infection intensity, coinfection with other STHs, and species type have all been proposed as determinants of the variability of albendazole efficacy [13, 15, 23–25].

The results of this trial confirm that at a recommended 400-mg dose, albendazole is moderately efficacious against hookworm infection in PSAC, SAC, and adults (CRs of 61.5%, 74.1%, and 53.3%, respectively). In a recent systematic review, a comparable CR of 400 mg of albendazole against hookworm was found to be 70.5% (95% CI, 54.5%–82.6%) in trials conducted between 2000 and 2016 [13]. A published review of the literature until 1999 showed that efficacy of a single dose of 400 mg of albendazole was 77.7%, specifically 91.8% against *A. duodenale* and 75.0% against *N. americanus* [15]. In the same review, the efficacy of 200 mg of albendazole was found to be significantly lower (70.0%) in older children and adults, which is clearly confirmed in this trial for adults [15].

Remarkably, a higher dose of albendazole has increased efficacy, with a single dose of 800 mg of albendazole having a CR of 94.1% in adults. In PSAC and SAC, efficacy of albendazole remained moderate in all intervention arms; moreover, a slight increase in efficacy was seen in PSAC with ascending doses, though results should be interpreted carefully due to the small number of PSAC in each arm. No well-controlled trials were found specifically for PSAC or SAC; however, CRs for 200 mg, 600 mg, and 800 mg that were reported in a systematic review were within the ranges reported in this trial for the respective doses [15].

The greatest limitation of this trial was the poor recruitment of children. Only 24% and 67% of needed PSAC and SAC, respectively, were recruited into the trial. Hence, as mentioned before, it was difficult to identify the optimal dose of albendazole in these cohorts. The generalizability of the results are also limited to settings where infection intensity and coinfection with other STHs are low; evidence suggests that albendazole's efficacy is affected by these factors [13, 23]. Since the diagnostic method used did not differentiate between the two common hookworm species, efficacy results may not be applicable to settings where *A. duodenale* is the predominant species, as *N. americanus* is most prevalent in sub-Saharan Africa [6, 16, 26, 27].

Current recommendations of a single dose of a benzimidazole administered annually or biannually to SAC may not be enough to eliminate helminthiasis. Recent models have shown that PC that targets only SAC will not eliminate hookworm infections

and that to interrupt hookworm transmission, community-based PC that also targets adults who are the main reservoir of hookworm transmission, which was confirmed in this trial, is needed [25, 28, 29]. Community-based MDA was shown to reduce the infection rate of hookworm by 91% in a study carried out from 2012 to 2013 in the Republic of Congo [30]. A recent clinical trial conducted in Kenya showed community-based treatment strategies to be more effective than school-based treatment in reducing hookworm prevalence; moreover, the costs were equitable [31]. Currently, a series of trials are being conducted in one Asian and two African settings to evaluate the feasibility of hookworm and other STH transmission interruption with community-based MDA [32].

Community-based MDA aligns with the 2030 targets of the NTD Roadmap, which plans to expand control programs to WRA, who are at risk of iron-deficiency anemia [12, 23]. Evidence from this trial can have a direct impact on the program strategies for the control and elimination of hookworm infection. Given the remarkable higher efficacy observed, increasing the dose of albendazole for adults targeted by community-based MDA may aid in the interruption of transmission.

The estimated cost of one albendazole tablet is $0.018 with an additional 10% for overhead costs [33]. Recent estimates have shown that the average per-person cost of annual treatment at the community level is $0.68, which could be reduced during scale-up to $0.33 if coverage remains high [31]. These estimates are within the reported range of costs ($0.33–$0.70) for treating an infected individual reported by the WHO in 2017 [10]. Integrating an additional albendazole tablet into program strategies for treatment of adults would not drive costs outside of current ranges, though more evidence on the cost-effectiveness of two tablets of 400 mg albendazole for adults is needed.

In conclusion, this trial provides direct evidence on the optimal dose of albendazole for treatment of hookworm infection in low-transmission settings. Though sample sizes in SAC and PSAC were lower than anticipated, our findings support use of the currently recommended albendazole doses since increasing doses revealed no benefit in these age groups. However, we observed a considerably higher efficacy with administration of 800 mg of albendazole in the adult cohort. Strategies to integrate an 800-mg dose of albendazole to adults during community-based MDA should therefore be explored.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. C. P., J. T. C., J. H., and J. K. designed the study. C. P., J. T. C., D. H., and J. K. performed the study. C. P., J. H., and J. K. analyzed and interpreted the data. C. P. and J. K. wrote the initial draft of the manuscript. J. T. C., D. H., and J. H. revised, read, and approved the final version of the manuscript.

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Supplementary Table 1: Cure rates and egg reduction rates against hookworm of PSAC and SAC at 3 weeks follow-up. Abbreviations: ALB, albendazole; CI, confidence interval; CR, cure rate; EPG, eggs per gram; ERR, egg reduction rate; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

	PSAC			**SAC**				
	ALB 200 mg	**ALB 400 mg**	**ALB 600 mg**	**PLAC**	**ALB 200 mg**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**
Positive before treatment	13	13	12	23	27	27	28	25
Cured after treatment	9	8	10	10	17	20	18	19
Observed CR	69.2	61.5	83.3	43.5	63.0	74.1	64.3	76.0
[95% CI]	[38.6, 90.9]	[31.6, 86.1]	[51.6, 97.9]	[23.2, 65.5]	[42.4, 80.6]	[53.7, 88.9]	[44.1, 81.4]	[54.9, 90.6]
Predicted CR				43.4	64.0	68.9	71.0	72.1
[95% CI]				[25.2, 63.6]	[46.4, 78.5]	[59.1, 77.2]	[59.8, 80.1]	[57.7, 83.1]
EPG geometric mean								
Baseline	251.1	178.4	238.8	229.5	166.7	162.1	200.1	234.9
3 weeks follow-up	2.6	6.8	0.9	21.5	3.9	1.6	3.4	3.8
Observed ERR	99.0	96.2	99.6	90.6	97.7	99.0	98.3	98.4
[95% CI]	[94.7, 99.9]	[83.5, 99.4]	[98.5, 100]	[72.3, 97.3]	[93.5, 99.3]	[97.4, 99.7]	[95.4, 99.5]	[95.9, 99.6]
Predicted ERR [95% CI]				90.6 [70.4, 97.5]	97.9 [80.3, 99.8]	98.3 [92.9, 99.6]	98.5 [91.1, 99.7]	98.5 [88.2, 99.8]
EPG arithmetic mean								
Baseline	528.5	422.8	398.0	500.1	343.3	262.7	398.8	1102.6
3 weeks follow-up	42.8	275.5	7.2	347.8	150.7	13.6	30.2	524.9
Observed ERR	91.9	34.8	98.2	30.4	56.1	94.8	92.4	52.4
[95% CI]	[72.2, 99.8]	[-1.5, 94.1]	[93.6, 100]	[-6.4, 68.0]	[19.0, 96.3]	[89.5, 98.6]	[83.2, 97.0]	[32.9, 91.6]

Supplementary Table S2: Proportion of participants cured by treatment arm and sex in PSAC, SAC and adults. Abbreviations: ALB, albendazole; F, female; M, male; na, not applicable; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

		PSAC	SAC	Adults
PLAC	**F**	na	3/8 (37.5%)	1/7 (14.3%)
	M	na	7/15 (46.7%)	5/35 (14.3%)
ALB 200 mg	**F**	3/5 (60.0%)	8/13 (61.5%)	9/14 (64.3%)
	M	6/8 (75.0%)	9/14 (64.3%)	14/21 (33.3%)
ALB 400 mg	**F**	5/9 (55.6%)	5/8 (62.5%)	2/4 (50.0%)
	M	3/4 (75.0%)	15/19 (79.0%)	14/26 (53.9%)
ALB 600 mg	**F**	7/8 (87.5%)	2/5 (40.0%)	4/7 (57.1%)
	M	3/4 (75.0%)	16/23 (69.6%)	18/32 (56.3%)
ALB 800 mg	**F**	na	6/7 (85.7%)	9/9 (100.0%)
	M	na	13/18 (72.2%)	23/25 (92.0%)

Supplementary Table S3: Reported number of participants experiencing adverse events by treatment arm among PSAC, SAC, and adults questioned. Abbreviations: ALB, albendazole; PLAC, placebo; PSAC, preschool-aged children; SAC, school-aged children.

	PSAC			SAC					Adults				
Adverse event	**ALB 200 mg**	**ALB 400 mg**	**ALB 600 mg**	**PLAC**	**ALB 200 mg**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**	**PLAC**	**ALB 200 mg**	**ALB 400 mg**	**ALB 600 mg**	**ALB 800 mg**
Before treatment													
Headache	0/13 (0.0)	0/13 (0.0)	0/12 (0.0)	0/23 (0.0)	0/27 (0.0)	0/27 (0.0)	0/28 (0.0)	0/25 (0.0)	2/35 (5.7)	2/35 (5.7)	2/30 (6.7)	3/39 (7.7)	2/34 (5.9)
Abdominal pain	0/13 (0.0)	0/13 (0.0)	0/12 (0.0)	0/23 (0.0)	0/27 (0.0)	0/27 (0.0)	1/28 (3.6)	0/25 (0.0)	3/35 (8.6)	6/35 (17.1)	4/30 (13.3)	1/39 (2.6)	2/34 (5.9)
Nausea	0/13 (0.0)	0/13 (0.0)	0/12 (0.0)	0/23 (0.0)	0/27 (0.0)	0/27 (0.0)	0/28 (0.0)	0/25 (0.0)	0/35 (0.0)	0/35 (0.0)	0/30 (0.0)	0/39 (0.0)	0/34 (0.0)
Vomiting	0/13 (0.0)	0/13 (0.0)	0/12 (0.0)	0/23 (0.0)	0/27 (0.0)	0/27 (0.0)	0/28 (0.0)	0/25 (0.0)	0/35 (0.0)	0/35 (0.0)	0/30 (0.0)	0/39 (0.0)	0/34 (0.0)
Diarrhea	0/13 (0.0)	0/13 (0.0)	1/12 (8.3)	1/23 (4.4)	0/27 (0.0)	0/27 (0.0)	0/28 (0.0)	0/25 (0.0)	0/35 (0.0)	1/35 (2.86)	2/30 (6.7)	0/39 (0.0)	1/34 (2.9)
Itching	0/13 (0.0)	0/13 (0.0)	0/12 (0.0)	0/23 (0.0)	0/26 (0.0)	0/27 (0.0)	0/27 (0.0)	1/24 (4.2)	1/34 (3.0)	1/35 (2.86)	3/30 (10.0)	1/39 (2.6)	2/33 (6.1)
3 hr after treatment													
Headache	1/12 (8.3)	0/10 (0.0)	1/12 (8.3)	1/21 (4.8)	1/24 (4.2)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	2/31 (6.5)	3/28 (10.7)	4/21 (19.1)	2/31 (6.5)	1/26 (3.9)
Abdominal pain	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	1/21 (4.8)	2/24 (8.3)	2/25 (8.0)	1/26 (3.9)	3/22 (13.6)	0/31 (0.0)	1/28 (3.6)	1/21 (4.7)	2/31 (6.5)	3/26 (11.5)
Nausea	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	0/21 (0.0)	0/24 (0.0)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	0/31 (0.0)	0/28 (0.0)	0/21 (0.0)	0/31 (0.0)	1/26 (3.9)
Vomiting	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	0/21 (0.0)	0/24 (0.0)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	0/31 (0.0)	0/28 (0.0)	0/21 (0.0)	0/31 (0.0)	0/26 (0.0)
Diarrhea	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	0/21 (0.0)	0/24 (0.0)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	0/31 (0.0)	0/28 (0.0)	0/21 (0.0)	0/31 (0.0)	0/26 (0.0)
Itching	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	0/21 (0.0)	0/24 (0.0)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	0/31 (0.0)	0/28 (0.0)	0/21 (0.0)	0/31 (0.0)	0/26 (0.0)
Serious adverse event	0/12 (0.0)	0/10 (0.0)	0/12 (0.0)	0/21 (0.0)	0/24 (0.0)	0/25 (0.0)	0/26 (0.0)	0/22 (0.0)	0/31 (0.0)	0/28 (0.0)	0/21 (0.0)	0/31 (0.0)	0/26 (0.0)
24 hr after treatment													
Headache	0/8 (0.0)	0/9 (0.0)	0/11 (0.0)	0/15 (0.0)	0/18 (0.0)	0/23 (0.0)	0/17 (0.0)	0/16 (0.0)	0/24 (0.0)	0/24 (0.0)	0/21 (0.0)	1/27 (3.7)	0/24 (0.0)

Abdominal pain	0/8 (0.0)	0/9 (0.0)	0/11 (0.0)	0/15 (0.0)	0/18 (0.0)	2/23 (8.7)	0/17 (0.0)	0/16 (0.0)	0/24 (0.0)	1/24 (4.2)	2/21 (9.5)	3/27 (11.1)	0/24 (0.0)
Nausea	0/7 (0.0)	0/6 (0.0)	0/8 (0.0)	0/11 (0.0)	0/14 (0.0)	0/17 (0.0)	0/14 (0.0)	0/12 (0.0)	0/21 (0.0)	1/24 (4.2)	0/16 (0.0)	1/25 (4.0)	1/21 (4.8)
Vomiting	0/7 (0.0)	0/6 (0.0)	0/8 (0.0)	0/11 (0.0)	0/14 (0.0)	0/17 (0.0)	1/14 (7.1)	0/12 (0.0)	0/21 (0.0)	0/22 (0.0)	0/16 (0.0)	0/25 (0.0)	0/21 (0.0)
Diarrhea	0/7 (0.0)	0/6 (0.0)	0/8 (0.0)	0/11 (0.0)	0/14 (0.0)	0/17 (0.0)	0/14 (0.0)	0/12 (0.0)	0/21 (0.0)	0/22 (0.0)	0/16 (0.0)	0/25 (0.0)	0/21 (0.0)
Itching	0/8 (0.0)	0/9 (0.0)	0/11 (0.0)	0/15 (0.0)	0/18 (0.0)	0/23 (0.0)	0/17 (0.0)	0/16 (0.0)	1/24 (4.2)	0/24 (0.0)	0/21 (0.0)	0/27 (0.0)	0/24 (0.0)
Serious adverse event	0/7 (0.0)	0/6 (0.0)	0/8 (0.0)	0/11 (0.0)	0/18 (0.0)	0/17 (0.0)	0/14 (0.0)	0/12 (0.0)	0/21 (0.0)	0/22 (0.0)	0/16 (0.0)	0/25 (0.0)	0/21 (0.0)

4. Design and conduct of a randomized controlled multi-country trial of co-administered ivermectin and albendazole

4.1. Efficacy and safety of ivermectin and albendazole co-administration in school-aged children and adults infected with *Trichuris trichiura*: study protocol for a multi-country randomized controlled double-blind trial

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Efficacy and safety of ivermectin and albendazole co-administration in school-aged children and adults infected with *Trichuris trichiura*: study protocol for a multi-country randomized controlled double-blind trial

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Abstract

Background: Soil-transmitted helminthiasis affects almost 2 billion people worldwide in tropical climates. Preventive chemotherapy, using the benzimidazoles (albendazole and mebendazole) is the current main recommended control strategy. Nevertheless, there is limited efficacy of these drugs against hookworm infection and, to a greater extent, against trichuriasis. We describe a protocol for a trial investigating the efficacy and safety of the co-administration of ivermectin and albendazole against trichuriasis.

Methods: A double-blind, placebo-controlled randomized controlled trial will be conducted in three countries (Côte d'Ivoire, Tanzania and Lao PDR) with the aim to determine the efficacy, safety and extended effects of co-administered ivermectin and albendazole compared to standard albendazole monotherapy. We will enroll 600 participants aged 6–60 years in each setting. The primary outcome is cure rate (CR) against *Trichuris trichiura* infection as assessed by Kato-Katz 14–21 days after treatment. Secondary outcomes include CRs against concomitant soil-transmitted helminth (STH) infections (*Ascaris lumbricoides*, hookworm and *Strongyloides stercoralis*) and egg reduction rates (ERRs) against STH at 14–21 days, 180 days and 360 days. Tolerability of treatment, infection status assessed by polymerase chain reaction (PCR), and potential benefits of deworming on nutritional and morbidity indicators will be assessed. The primary analysis will include an available-case set and use logistic regression models adjusted for age, sex and weight.

Discussion: This trial will provide robust results on the efficacy and safety of co-administration of ivermectin and albendazole with the aim to better inform WHO recommendations on control of STHs. Furthermore, secondary and explanatory outcomes will provide direct evidence on the extended effects of combination therapy and insight on the relationship between nutrition and morbidity parameters and infection status and intensity.

Trial registration: NCT03527732 (date assigned: 17 May 2018).

Keywords: *Trichuris trichiura*, Côte d'Ivoire, Lao PDR, Tanzania, Drug efficacy, Ivermectin, Albendazole, Soil-transmitted helminthiasis, Drug safety

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Background

Almost 2 billion people are infected with soil-transmitted helminths (STHs), the majority being preschool and school-aged children living in Asia and Africa [1, 2]. *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Necator americanus* or *Ancylostoma duodenale* (hookworms), the most common STHs, account for an annual burden of 1.9 million disability-adjusted life-years (DALYs) related to infection [1, 3]. Infection can be the result of the ingestion of *T. trichiura* or *A. lumbricoides* eggs or the penetration of skin by hookworm larvae [4]. Inadequate access to clean water, poor hygiene and unimproved sanitation lead to an increase in risk of STH infection, thus particularly affecting populations in low- and middle-income countries [4]. Morbidity due to STHs are related mostly to high intensity infections and may include acute symptoms such as diarrhea, dysentery, abdominal pain or obstruction; if left untreated, STH infections can lead to inflammation, nutrition and immune system impairment and, finally, can cause physical and mental development retardation in children and limited working capacity in adults [1, 4].

To date, preventive chemotherapy (PC) that is the periodic administration of anthelminthic drugs to at-risk populations without prior diagnosis, is the cornerstone of helminth control put forth by the World Health Organization (WHO) to reduce the burden of STHs. PC is implemented in the form of annual mass drug administration (MDA) campaigns and the recommended target populations have been expanded from only school-aged children to include younger children (1–5 years of age), adolescent (10–19 years) girls, women of reproductive age (15–49 years) and pregnant women after the first trimester in areas with an STH prevalence of ≥20% [5]. A biannual frequency of MDA is recommended in case of high prevalence (> 50%). Albendazole, one of the two main drugs used for MDA, is considered safe and well tolerated; however, it is not efficient in clearing infection and reducing worm loads in all three types of STHs [2, 6]. While it shows satisfyingly high cure rates (CRs) (96%) and egg reduction rates (ERRs) (> 98%) against *A. lumbricoides*, efficacy against hookworm is lower (CR = 80% and ERR = 90%) and against *Trichuris trichiura* is disturbingly low with CRs of 31% and ERRs of 50% [6]. Furthermore, this drug has been used for more than three decades and comparison of efficacy measures over time indicates a decreasing trend over time; although resistance has so far not been documented in its use in humans [5]. In view of insufficient efficacy, especially against *T. trichiura*, coupled with the potential for resistance emergence from long-term use, there is not only a pressing need for the development of new treatments against STH infections, but also a need to optimize current treatment schemes [7].

The co-administration of standard drugs together with other anthelminthics, such as ivermectin, could be a way to achieve universal impact on all STH species [8]. Moreover, the combined use of ivermectin and albendazole against STH infection has been added recently to the WHO Model Lists of Essential Medicines paving the way for application in control programs [9]. A recent meta-analysis indeed shows a lower risk (risk ratio (RR) =0.44) of still being positive for *T. trichiura* post-treatment for co-administered ivermectin and albendazole when compared to albendazole alone; however, these findings are based on a very limited amount of qualifying studies ($n = 3$) conducted in various settings, limited to school-aged children and/or adolescents with considerable variation among the reported efficacy measures [10]. Interestingly, two studies also highlight potential extended effects on follow-up infection status or intensity (18 weeks and 1 year) compared to single or other combined drug regimens [11, 12]. To better inform and guide ongoing helminth control programs on optimization and implementation of ivermectin-albendazole integrated treatment schemes, a deeper understanding of its impact across a range of different transmission settings, including a broader age range and a prolonged monitoring period, is needed.

To the best of our knowledge, this is the first large, multi-country trial assessing the safety and efficacy of the combination of ivermectin and albendazole and the extended effects of treatment against *T. trichiura*.

Methods/design

Trial design

This is a multi-country, parallel group, double-blind, placebo-controlled, randomized controlled trial (RCT). It will be implemented as a community-based study targeting children, adolescents and adults aged 6 to 60 years. In each of the three countries, 600 *T. trichiura*-positive community members will be randomly assigned to either receive the current standard treatment (albendazole/placebo) or the combination therapy (ivermectin/albendazole). All participating communities will be followed-up over a period of one year including four assessment time points at baseline, 3 weeks, 6 months and 12 months after treatment (see Fig. 1). The WHO Trial Registration Data Set summarizes the most important trial information and is given in Additional file 1. The study adheres to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement [13] and a checklist is provided as supplementary file (see Additional file 2).

Study area and participants

The trial will be conducted in two African settings, namely Côte d'Ivoire and Pemba Island, Tanzania, and one Asian setting, Lao People's Democratic Republic

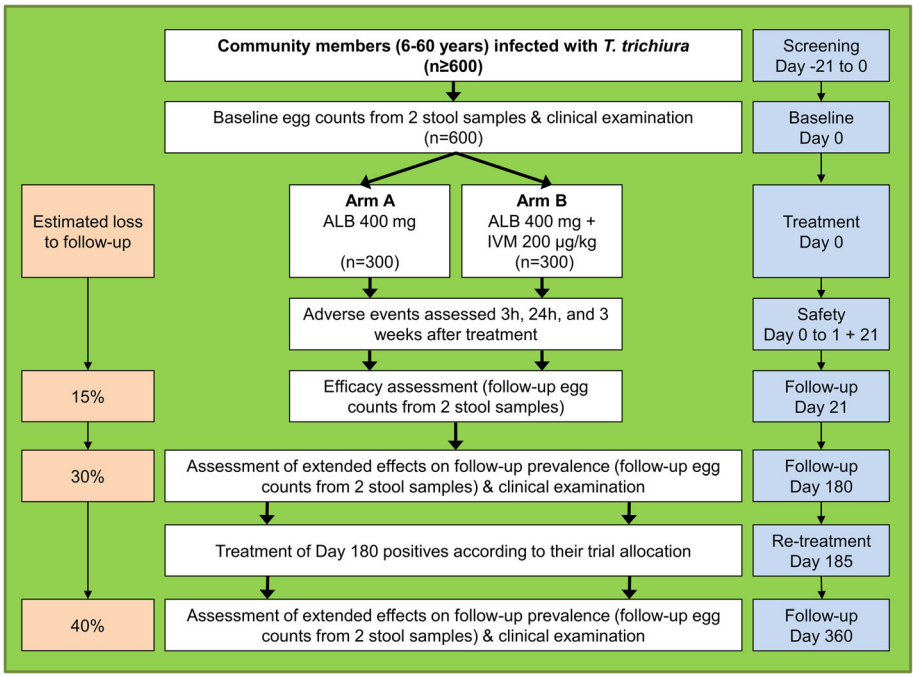

Fig. 1 Design and timeline of the randomized controlled trial to be implemented in each of three settings. The study is designed as a two-armed trial including one arm with a single drug administration (arm A; albendazole) and one arm with combined treatment through co-administration of separate tablets (arm B; ivermectin and albendazole). The trial will be conducted as a multi-country study with two settings in Africa and one in Asia, namely Côte d'Ivoire, Pemba (Zanzibar, Tanzania) and Lao PDR

(PDR). Potential study areas will be selected based on earlier findings and insights from local collaborators on *T. trichiura* endemicity. In Côte d'Ivoire, the Agnéby-Tiassa region in the southeast of the country has been identified with high STH infection prevalence (particularly for *T. trichiura*) in previous community-based studies or RCTs [14, 15]. Pemba Island is still highly endemic to STH infections [16]. Potential study communities on Pemba will be selected based on accessibility besides *T. trichiura* endemicity. An area fulfilling these criteria is considered the Shehia of Pujini, 9 km south-east of Chake Chake, Pemba. In Lao PDR recent data suggests highest infection rates for *T. trichiura* to be found in the northern zone of the country including the province of Luang Prabang [17]. In each study location community members from 6 to 60 years of age will be invited for participation.

Recruitment

In order to recruit participants from a broad age range (6–60 years), entire communities with less than 1000 inhabitants (smaller communities are easier to be mobilized and monitored) will be pre-screened and a census conducted to identify *T. trichiura* cases and eligible individuals in each household, respectively.

All adult community members will be invited to participate in an informational meeting explaining the purposes and procedures of the study, including potential benefits and risks. In this open discussion forum, parents/caregivers/potential participants will be encouraged to ask questions and be informed of actions to prevent acquiring STH infections in the future.

Individuals (including parents/caregivers of children) interested in participating in the trial will be invited to complete the process of informed consent; thereafter, individuals will be assessed for study eligibility during screening procedures.

Eligibility criteria

Participants will be eligible to be included in the trial if they fulfill all of the following criteria:

1. Provide written informed consent signed by either the participant him/herself (≥18 years of age in Lao PDR and Pemba, Tanzania or ≥ 21 years of age in Côte d'Ivoire) or by parents and/or caregivers for children/adolescents; and oral assent by child/adolescent (aged 6–17 years Lao PDR and Pemba, Tanzania or aged 6–20 years in Côte d'Ivoire).
2. Agree to comply with study procedures, including provision of two stool samples at the beginning (baseline) and on three follow-up assessments (approximately 3 weeks, 6 months, and 12 months later).
3. Aged ≥6 to ≤60 years and weighing at least 15 kg.

4. Positive for *T. trichiura* infection in at least two slides of the quadruple Kato-Katz thick smears and infection intensity of at least 100 eggs per gram (EPG) of stool.

Participants will be ineligible to be included in the trial if they fulfill any of the following criteria:

1. Presence of major systemic illnesses, e.g. severe anemia (below 80 g/l Hemoglobin (Hb) according to WHO [18]), clinical malaria as assessed by a medical doctor (positive *Plasmodium* rapid diagnostic test (RDT) and ≥ 38 °C ear temperature), upon initial clinical assessment.
2. History of acute or severe chronic disease (e.g. cancer, diabetes, chronic heart, liver or renal disease).
3. Recent use of anthelmintic drug (within past 4 weeks).
4. Attendance in other clinical trials.
5. Known allergy to study medications (i.e. ivermectin and albendazole).
6. Pregnancy or lactating in the 1st week after birth (according to WHO guidelines within lymphatic filariasis control programs [19]).
7. Current use of medication with known interaction (e.g. for ivermectin: warfarin; for albendazole: cimetidine, praziquantel and dexamethasone).

Intervention

All *T. trichiura*-infected, consenting, and participating community members will be treated with the respective single or combination treatment regimen at day 0. Re-treatment with the intervention assigned at randomization will occur at 6 months in all participants found to be positive for any STH. 400 mg albendazole tablets will be the product of Glaxo Smith Kline, UK (Zentel®) and a single tablet will be administered. 3 mg tablets of ivermectin (Stromectol®) will be obtained from Merck, France and administered at a dose of 200 μg/kg body weight recorded for each participant. Matching ivermectin placebo tablets (in terms of appearance) will be produced and a certificate of manufacture and analysis be provided by the University of Basel. Since ivermectin and albendazole are known to be better absorbed in humans after a high-fat meal is consumed, participants will receive a local high-fat breakfast prior to treatment [20, 21]. After ingestion of the medication, the subjects will be observed for 3 h to ensure retention of the drug. Vomiting within 1-h post-dosing will require re-dosing. The subjects will not be allowed more than one repeated dose. No re-administration will be needed for subjects vomiting after one hour.

Outcomes

Primary outcome

The primary outcome is *T. trichiura* infection status as assessed by Kato-Katz 14–21 days after treatment measured as CR, calculated as the percent of infected individuals at baseline free from infection after treatment.

Secondary outcomes

Secondary outcomes include the ERR against *T. trichiura*, CRs and ERRs against other concomitant STH infections (*A. lumbricoides*, hookworm and *Strongyloides stercoralis*), reinfection rates, tolerability of treatment and infection status assessed by polymerase chain reaction (PCR). Outcomes will be assessed at 14–21 days, 180 days and 360 days post-treatment.

Exploratory outcomes

Exploratory outcomes include the molecular characterization of *T. trichiura* strains from different settings and investigation of potential resistance markers through deep sequencing as well as the evaluation of the potential benefits of deworming on nutrition status and morbidity indicators.

Sample size calculation

Based on a recent systematic review and the published literature, we assume that the CR of albendazole against *T. trichiura* is 30% compared to 50% in the ivermectin-albendazole treatment regimen [6]. To achieve a power of 90% at a significance level of 5%, 121 participants per study arm are needed to detect a statistically significant difference. With an estimated loss to follow-up of 15%, 143 participants will be required in each study arm. Furthermore, we assume the same treatment efficacy and a reinfection risk of 10% at 6 months. Consequently we expect a proportion of negative patients after 12 months of 44% in the albendazole arm and of 65% in the ivermectin-albendazole arm resulting in a required sample size of 111 participants per arm. To account for a loss to follow-up of 30% after 6 months and 40% at final assessment (12 months), we aim to recruit 300 participants in each treatment group (600 in total) in each country for a total of 1800 participants.

Randomisation

Study participants eligible for treatment will be randomly assigned in a 1:1 allocation to one of the treatment arms using sealed, opaque sequentially-numbered envelopes prepared by persons independent of the trial. Since treatment success is influenced by infection intensity, stratified block randomization will be used (baseline infection intensity: light infections and moderate/heavy infections) to ensure balanced treatment groups in terms of infection intensity. The computer-generated stratified randomization sequence, provided by a statistician, will vary randomly in blocks of four, six and eight and will be stratified by 2 levels of baseline infection intensity (light: < 1000 EPG, and moderate and heavy: ≥1000 EPG *T. trichiura* infections).

Blinding

The trial will be double-blinded (i.e. study participants and the trial team/researchers conducting the treatment and assessing the outcomes will be blinded). One 400 mg albendazole tablet will be given to each participant. The ivermectin (or corresponding placebo) tablets used for each treatment arm will be repacked into neutral separate plastic bags each containing the maximum number of ivermectin tablets with regard to weight and dose or the corresponding number of appearance-matched placebo tablets produced by the University of Basel. If at any point during the trial an unanticipated need to unblind a participant's treatment allocation arises for reasons of safety, the principal investigator, site investigators, and ethics committee will be notified and the instance will be documented.

Trial timeline

The trial will last for fourteen months. The screening for the baseline will start three weeks prior to the treatment. Follow-up screening will take place 14–21 days, 180 days and 360 days post-treatment and each will last for about three weeks. Schedules of visits are summarized in Table 1.

Data collection

All data besides a household questionnaire will be collected during scheduled visits and recorded on paper case report forms (CRFs), laboratory reporting forms or logs. Subsequently, data will be double-entered into a database using EpiInfo (v3.5.4). Access will be limited to study investigators and study personnel entering data; both working independently from the project funder. Data reported in the household questionnaire will be collected using paper forms in Pemba Island and using Open Data Kit (ODK) on mobile tablet computer devices in Côte d'Ivoire and Lao PDR where electronic data collection has already been applied in earlier studies. Data entered via ODK collect will be uploaded to a server hosted by the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland). For quality assurance in-built error, range and consistency checks will be programmed for the data entry masks (i.e., in EpiInfo and ODK). The obtained data will be handled strictly confidentially. Personal data will be coded for data analysis. No names will be published at any time, and published reports will not allow for identification of single subjects.

Clinical assessment

A clinical examination of the study participants assessing general health (blood pressure, pulse rate, symptoms, medical history, etc.), anthropometric parameters including height, weight, mid-upper arm circumference (MUAC) and skinfold thickness (i.e. tricep and subscapular skinfolds) as well as tympanic temperature using an ear thermometer (Braun Thermoscan 5, Braun GmbH, Kronberg, Germany) will precede the treatment and will be repeated on two

Table 1 Schedule of visits in trial

	Screening	Baseline/Treatment/Safety			Follow-up			
	-21 to -1 days	Hours			Days			
		0	3	24	21	180	185	360
Diagnosis (stool and urine examination)	X				X	X		X
Gut morbidity (stool RDTs)	X				X	X		X
Informed consent	X							
Demographics	X							
Medical history		X						
Clinical examination		X				X		X
Pregnancy testing		X				X		X
Hemoglobin measurement		X				X		X
Plasmodium co-infection (in Côte d'Ivoire/ Lao PDR only)		X				X		X
W. bancrofti co-infection (in Africa only)		X				X		X
Venous blood examination (in Côte d'Ivoire/ Lao PDR only)		X			X	X		X
Physical functioning		X				X		X
Randomization and treatment		X						
Selective re-treatment							X	
Capturing AEs			X	X	X			
Capturing SAEs			X	X	X			
Treatment satisfaction			X		X	X		

follow-up assessments (days 180 and 360) to evaluate potential benefits from deworming. Blood pressure will be measured using a sphygmomanometer (OMRON M6, Omron Healthcare CO., LTD, Kyoto, Japan). Body weight will be measured using a mobile, digital scale (SECA Model 803, Seca Gmbh Co, Hamburg, Germany) with a precision to the nearest 0.1 kg, while height will be measured using a measuring stick in centimetres. Mid-upper arm circumference will be measured to the nearest 0.1 cm using a MUAC tape; and a caliper (Harpenden Skinfold Caliper, HaB International Ltd., Warwickshire, England) will be used to measure tricep and subscapular skinfold thicknesses to the nearest 0.2 mm. All anthropometric measurements will be taken twice, recorded and the average value then used. A licensed physician will conduct a physical examination on each participant before treatment at baseline, 6 months and 12 months.

Biospecimen collection and testing

Stool samples

Community members providing informed consent will be asked to provide two stool samples of at least 15 g each within a maximum of 5 days at baseline. From every stool specimen, duplicate Kato-Katz thick smears (41.7 mg each) will be prepared and read under a microscope for eggs of *T. trichiura*, *A. lumbricoides* and hookworm by experienced technicians [22]. A subsequent independent quality control of sample results (approximately 10%) will be conducted. All microscopically analyzed quadruplicate Kato-Katz thick smears will be destroyed within one day (after passing the quality control).

A portion of 2–3 g of stool from each specimen will be preserved in 70% ethanol and shipped to the Swiss TPH, Basel, Switzerland for PCR analysis which will allow further classification of hookworm infection into the three species *N. americanus*, *A. duodenale* and *A. ceylanicum* [23]. A subsample of 10 participants with high intensity infections following treatment from each of the settings (30 in total) will be subjected to deep sequencing for characterization of *T. trichiura* strains and investigation of potential resistance markers [24].

Additionally, a small amount of feces (less than 1 mg) from the second stool sample of participants identified as positive for *T. trichiura* in the first sample will further be tested for fecal occult blood and fecal calprotectin (Actim Fecal Blood and Calprotectin, Oy Medix Biochemica Ab, Espoo, Finland) as proxies for gut morbidity and inflammation using a rapid diagnostic immunoassay test [25]. In Lao PDR, the remains of each stool sample (ideally 10 to 20 g) will be processed by the Baermann technique for identification of *S. stercoralis* infections and be recorded qualitatively as larva-positive or negative [26].

Blood samples

Enrolled participants undergoing a clinical examination at baseline, 6 months and 12 months will be asked to provide a finger-prick blood sample to evaluate Hb levels using a HemoCue analyzer (Hemocue Hb 301 system; Angelholm, Sweden). Additionally, participants in Côte d'Ivoire and Lao PDR will be asked to provide a finger-prick blood sample for an RDT (i.e. Humasis Pf/Pan and Humasis Pf/Pv) for *Plasmodium* spp. infection, while participants in Côte d'Ivoire and Pemba will be asked to provide a finger-prick blood sample for an RDT (i.e. ENCODE Filariasis IgG/IgM) detecting antibodies in the blood to identify potential co-infection with *Wuchereria bancrofti*, as patients with filariasis have shown a significantly higher frequency of adverse events after combined treatment with ivermectin and albendazole [27, 28].

In one African (i.e., Côte d'Ivoire) and the Asian setting (i.e., Lao PDR), participants will be asked to give approximately 8 ml of venous blood at baseline, 6 months and 12 months to assess potential improvement on nutritional indicators for micronutrient (i.e. (pro-) vitamins, inflammation markers, and iron/ferritin) and macronutrient (i.e. albumin) deficiencies. Blood will be collected in EDTA and Serum Vacutainers (BD, Franklin Lakes, NJ, USA). In Côte d'Ivoire and Lao PDR we will undertake analysis of biochemical and hematological parameters as a proxy for functioning of vital organs. These parameters may include urea, creatinine, bilirubin, azotemia, Alanine Amino Transferase (ALAT), Aspartate Amino Transferase (ASAT), hematocrit, erythrocytes and platelets. Serum separated from collected blood in serum blood collection tubes after centrifugation will be aliquoted at local laboratories in Côte d'Ivoire and Lao PDR and then sent on dry ice to accredited reference laboratories within Switzerland and Germany. Samples will be kept at − 20 °C in field labs and in transport and stored at − 80 °C in Switzerland and in Germany. Ferritin, soluble transferrin receptor, retinol-binding protein, α1-acid glycoprotein and C-reactive protein will be measured using Sandwich enzyme-linked immunosorbent assay (ELISA) techniques [29]. Transferrin will be measured using immunoturbidimetry; iron will be measured using spectrophotometry; hepcidin will be measured using a solid phase ELISA; and vitamin A will be measured using high performance liquid chromatography.

Urine samples

Female participants over the age of ten years will be asked to give a urine sample of at least 10 ml for a pregnancy RDT at baseline, 6 months and 12 months before (re-)treatment is administered to avoid accidental treatment of pregnant girls/women.

Questionnaire

Household questionnaire

A household questionnaire will be administered to all participating households between screening and 3 weeks follow-up to assess socioeconomic factors (e.g. structure, condition, amenities), presence of sanitation/water facilities (e.g. shower, latrine/toilet, water sources), and hygiene attitudes/practices (e.g. defecation, hand-washing, water use). Collected information will be used to assess the relationship between reinfection rates and household characteristics/behaviors; moreover, an evaluation of the potential associations of high intensity and persistent infections with sociodemographic characteristics will be conducted.

Treatment satisfaction

Subjective treatment satisfaction will be assessed at 3 h, 3 weeks and 6 months after treatment to investigate relationship with treatment compliance and observed efficacy in reducing egg output and morbidity. Participants will be asked to provide short-term treatment satisfaction (e.g. convenience of treatment) at 3 h and 3 weeks post-treatment, while long-term treatment satisfaction (e.g. effectiveness in reducing symptoms) will be asked at 6 months follow-up.

Physical functioning and well-being

Children 6 to 16 years of age will be administered a patient-rated physical functioning and well-being questionnaire during clinical examination before treatment and at 6 months and 12 months follow-up using tools already applied and evaluated in school-aged children from rural settings in Côte d'Ivoire and pre-tested in a comparable school-aged population not otherwise involved in this trial [30].

Adverse events

Very few adverse events (AEs) are expected after ivermectin-albendazole co-administration in STH-infected individuals. The most common AEs reported were abdominal cramps, headache, fatigue, nausea, diarrhea, fever and vertigo [31–33].

Subjects will be kept for observation for at least 3 h following treatment for any acute AEs. In addition, patients will also be interviewed 3 h, 24 h, and 3 weeks after treatment about the occurrence of AEs. If there is any abnormal finding, the local study physician will perform a full clinical, physical and biochemical examination and findings will be recorded. An emergency kit will be available on site to treat any medical conditions that warrant urgent medical intervention. Information on all AEs (onset, duration, intensity, seriousness and causality) will be immediately entered in the appropriate AE module of the CRF that serves as source document. For all AEs and serious adverse events (SAEs), sufficient information will be pursued and/or obtained so as to be graded on severity, relatedness and expectedness. These data will be recorded on the appropriate CRF sections, regardless of whether they are thought to be associated with the study or the drug under investigation. Any study-related unanticipated problem posing risk of harm to subjects or others (including all unexpected adverse drug reactions) and any type of SAE will be immediately (within a maximum of 24 h after becoming aware of the event) notified to the study sponsor-investigator and co-PIs.

Statistical methods

The primary available-case analysis will include all participants with primary endpoint data. In addition, an intention-to-treat analysis for the primary endpoint assessed at 3 weeks will be conducted considering all participants with missing endpoint data as treatment failure or all as treatment success to ensure that the results are not sensitive to potential loss to follow-up bias. CRs will be calculated as the percentage of egg-positive participants at baseline who become egg-negative after treatment. Infection intensity expressed as the arithmetic and geometric mean EPG will be calculated for each treatment arm. EPG will be assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears and multiplying this number by a factor of six. The ERR will be calculated as:

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

In the primary model we estimate the difference among CRs by using unadjusted logistic regressions. In a subsequent analysis an adjusted logistic regression (adjustment for age, sex and weight) will be performed.

Geometric mean egg counts will be calculated for the different treatment arms before and after treatment to assess the corresponding ERRs. Bootstrap resampling method with 5000 replicates will be used to calculate 95% confidence intervals for ERRs and the difference between the ERRs.

Results from the stool RDT for fecal occult blood will be categorized as negative, trace and positive. For calprotectin, the semiquantitative RDT allows classifying individuals by concentration into negative (levels below 50 μg/g of feces), low (50–200 μg/g) or high (≥ 200 μg/g) intensities.

Anthropometric measurements such as height and weight of school-aged children will be translated into weight-for-age, height-for-age and weight-for-height related z-scores using readily available Stata commands

calculating growth indicators for children/adolescents 5–19 years [34]. Body mass index and indicators for muscle and fat tissue, such as MUAC and skinfold thickness, will serve as additional indicators of nutritional status for adults [35].

Questionnaires on physical functioning and treatment satisfaction will be evaluated by creating summary scores by summing up and transforming the single question scores according to the following formula: [(actual raw score-lowest possible raw score)/(possible raw score range)]*100 [30].

Nutritional and morbidity indicators will be analyzed using logistic and linear regression as appropriate. To compare individual's changes in nutrition/morbidity categories as an effect from treatment, McNemar's test will be applied. The analysis after 6 and 12 months of follow-up will be complemented by generalized estimating equation models with independent correlation structure and empirical estimators to account for missing data.

AEs will be evaluated descriptively as the difference of proportion reporting AEs before and after treatment.

Discussion

Soil-transmitted helminthiasis remains a public health burden in many low- and middle-income countries [4]. Since the passing of the resolution WHA 54.19 by the World Health Assembly in 2001, great strides have been taken to reduce the morbidity and mortality of STH infections [36]. With the changing epidemiological landscape of soil-transmitted helminthiasis moving from control to elimination, MDA implementation is shifting from school-based to community-based [37, 38]. This trial marks the first multi-country, longitudinal, randomized double-blind controlled trial assessing the safety and efficacy of a combination therapy at the community level.

With the scaling up of PC programs, mounting drug pressure increases the risk for drug resistance against the benzimidazoles in populations infected by STHs. Nonetheless, combination treatment of two or more drugs can provide heightened efficacy and protection against drug resistance [39]. Our data will provide robust evidence on the possible increased efficacy and extended effects of combined albendazole and ivermectin treatment when compared to albendazole alone to pave the way of the former as recommended treatment for soil-transmitted helminthiasis for use in control programs.

We will report not only on efficacy and safety outcomes of combination ivermectin and albendazole therapy, but also the effects of drug administration on morbidity in a broad age range. In the midst of controversy on the impact of PC through mass deworming campaigns, this trial provides direct evidence in determining the relationship between deworming campaigns and clinical morbidity and nutritional indicators [5, 40–44]. The role of vitamin A as a protective factor against re-infection will be assessed, as well as the long-term (6 and 12 months) effects of STH therapy on anemia. Moreover, serum hepcidin levels will be quantified to determine the dynamics between iron-deficiency anemia, anemia of chronic disease and infection status/intensity. The use of fecal rapid diagnostics tests as surrogates for gut morbidity will provide a novel proof of concept between STH infection status intensity. These nutritional and gut parameters will be measured in various settings (Asia and Africa) using standardized methods at 6 and 12 months in the hopes of providing clarity to the potential impact of mass deworming in communities.

We will also report on the differences in CRs and sensitivity between the current diagnostic method recommended by the WHO (Kato-Katz smear) and DNA-based methods (qPCR). A major drawback of the Kato-Katz method is the low sensitivity for infections of low intensity [45]. As countries shift from STH control to elimination, more sensitive methods of diagnosis are needed as infection intensities lower. PCR methods offer the advantage that only single samples need to be taken and multiple infections can be detected in one reading; however, estimates of sensitivity vary and standardization of technique is needed [46, 47]. This trial will provide an opportunity to collect samples from various settings where co-infections may vary and refine the current qPCR technique.

In conclusion, this trial will aim to generate evidence to inform future WHO guidelines of STH therapy and its impact on morbidity. Currently, the combination of ivermectin and albendazole is a safe and effective treatment that is currently given to more than 500 million individuals yearly as part of the Global Programme to Eliminate Lymphatic Filariasis; and there may be opportunities to transition the program to control and elimination of soil-transmitted helminthiasis [48, 49]. However, evidence on the efficacy of the co-administration of ivermectin and albendazole against STHs from high quality RCTs is needed to confirm results and scale up combination therapy in endemic areas.

Additional files

Additional file 1: World Health Organization Trial Registration Data Set for the efficacy and safety of IVM-ALB co-administration trial summarizing the most important trial information. (DOCX 20 kb)

Additional file 2: SPIRIT checklist for the efficacy and safety of IVM-ALB co-administration trial referring to study protocol elements within manuscript sections. (DOC 122 kb)

Abbreviations

AE: Adverse event; CR: Cure rate; CRF: Case report form; CSRS: Centre Suisse de Recherches Scientifiques; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; EPG: Eggs per gram; ERR: Egg reduction rate; Hb: Hemoglobin; MDA: Mass drug administration; MUAC: Mid-upper arm circumference; NIOPH: National Institute of Public

Health; PC: Preventive chemotherapy; PCR: Polymerase chain reaction; PHL-IdC: Public Health Laboratory Ivo de Carneri; RDT: Rapid diagnostic test; SAE: Serious adverse event; SPIRIT: Standard protocol items: recommendations for interventional trials; STH: Soil-transmitted helminth; Swiss TPH: Swiss Tropical and Public Health Institute; WHO: World Health Organization

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Availability of data and materials
The datasets used and/or analyzed as well as data collection forms and model consent forms of the current study are available from the corresponding author on reasonable request.

Authors' contributions
JK is the principal investigator of the trial. EH, JH, SS, SAI, SAm, JTC and JK contributed to the original protocol and CP and LK assisted in revisions of the protocol versions. CP, EH and JK drafted the manuscript. All authors are significantly involved in the trial preparation and conduct. All authors read and approved the final manuscript.

Ethics approval and consent to participate
This study protocol has been approved by the institutional research commissions of the Centre Suisse des Recherches Scientifiques (CSRS) in Abidjan, Public Health Laboratory Ivo de Carneri (PHL-IdC) in Pemba, National Institute of Public Health (NIOPH) in Vientiane, and Swiss TPH in Basel. Ethical approval was obtained from the ethics committees in Switzerland: "Ethikkommission Nordwest- und Zentralschweiz" (BASEC Nr Req-2018-00494; date of approval 05 July 2018); in Côte d'Ivoire: Comité National d'Éthique et de la Recherche, Ministère de la Santé et de Lutte contre le SIDA (reference no. 088–18/MSHP/CNESVS-km; date of approval 24 January 2019) and Direction de la Pharmacie, du Médicament et des Laboratoires (reference no. ECCI00918; date of approval *to be issued*); in Pemba: Zanzibar Medical Research and Ethics Committee, Ministry of Health (protocol no. ZAMREC/0003/Feb/2018; date of approval 23 May 2018); and in Lao PDR: National Ethics Committee for Health Research, Ministry of Health (reference no. 093/NECHR; date of approval 23 October 2018). Written informed consent will be sought from adults and parents or legal guardians of children below the age of adulthood (21 years in Côte d'Ivoire and 18 years in Pemba and Lao PDR) prior to study enrollment. Children aged below the age of adulthood will sign an informed assent form. The informed consent is provided by trained field staff in the local language and the participant will receive a copy of the consent form in written format. Participation is voluntary and study participants have the right to withdraw from the study at any given point in time with no further obligations. Participants will be treated free of charge at the end of the trial with albendazole. A general liability insurance of the Swiss TPH is in place (Winterthur Police Nr. 4,746,321) and patient liability insurances will be issued in the respective trial countries to cover any potential study-related harm.

Consent for publication
Not applicable.

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

Publisher's Note

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Additional file 1. World Health Organization Trial Registration Data Set for the IVM-ALB co-administration multi-country trial.

Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT03527732
Date of registration in primary registry	17 May 2018
Secondary identifying numbers	Ethikkommission Nordwest- und Zentralschweiz: BASEC Nr Req-2018-00494; Ministère de la santé et de l'hygiène publique, comité national d'éthique des sciences de la vie et de la santé: 088-18/MSHP/CNESVS-km; Ministry of Health, National Ethics Committee for Health Research, Lao PDR: 093/NECHR; Zanzibar Medical Research and Ethics committee: ZAMREC/0003/Feb/2018
Source(s) of monetary or material support	Bill and Melinda Gates Foundation, the United States of America
Primary sponsor	Swiss Tropical and Public Health Institute
Secondary sponsor(s)	None
Contact for public queries	Jennifer Keiser, jennifer.keiser@swisstph.ch, +41612848218 Swiss Tropical and Public Health Institute, Basel, Switzerland
Contact for scientific queries	Jennifer Keiser, jennifer.keiser@swisstph.ch, +41612848218 Swiss Tropical and Public Health Institute, Basel, Switzerland
Public title	Efficacy and safety of IVM/ALB co-administration
Scientific title	Efficacy and safety of ivermectin and albendazole co-administration in school-aged children and adults infected with *Trichuris trichiura*: a multi-country randomized controlled trial
Countries of recruitment	Côte d'Ivoire, Lao PDR, Pemba Island (Tanzania)
Health condition(s) or problem(s) studied	Trichuriasis
Intervention(s)	• Comparator drug: 400 mg albendazole single tablet (Zentel®) and tablets of placebo at day 0 administered orally • Experimental drug: 400 mg albendazole single tablet (Zentel®) and 200µg/kg using 3mg tablets of ivermectin (Stromectol®) at day 0 administered orally
Key inclusion and exclusion criteria	Inclusion criteria: community members (6-60 years, minimum weight: 15 kg) infected with *T. trichiura* (at least 2/4 Kato-Katz thick smears positive and infection intensities of at least 100 eggs per gram of stool) providing written informed consent signed by the adult participant or caregiver and assent by child/adolescent. Exclusion criteria: presence of major systemic

	illnesses (*e.g.* severe anaemia, clinical malaria), history of acute or severe chronic disease (*e.g.* cancer, diabetes, chronic heart, liver or renal disease), recent use of anthelmintic drug (within past 4 weeks), attending other clinical trials, known allergy to study medications (*i.e.* ivermectin and albendazole), pregnancy or lactating in the 1st week after birth, currently taking medication with known interaction (*e.g.* for ivermectin: warfarin; for albendazole: cimetidine, praziquantel and dexamethasone)
Study type	Multi-country, parallel group, double-blind, placebo-controlled, randomized controlled trial. Phase III
Date of first enrolment	24 September 2018
Target sample size	1800
Recruitment status	Recruiting: participants are currently being recruited and enrolled
Primary outcome(s)	Cure rate (CR) against *Trichuris trichiura* 14-21 days post-treatment
Key secondary outcomes	• Egg reduction rate (ERR) against *T. trichiura* • CRs and ERRs against other concomitant soil-transmitted helminth infections (i.e., *Ascaris lumbricoides*, hookworm and *Strongyloides stercoralis*) • Reinfection rates • Tolerability of treatment • Infection status assessed by polymerase chain reaction

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	_____1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_____4_____
	2b	All items from the World Health Organization Trial Registration Data Set	Additional file 1
Protocol version	3	Date and version identifier	See manuscript publication date
Funding	4	Sources and types of financial, material, and other support	____23_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	____1, 23_____
	5b	Name and contact information for the trial sponsor	Additional file 1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_____23_____
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____23-24____

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5-7
	6b	Explanation for choice of comparators	6-7
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7-8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10-11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	12, 28, Figure 1

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8

Methods: Assignment of interventions (for controlled trials)

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	12
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	12

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-17
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	17

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17-19
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18-19
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	12
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	17
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	22
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	22
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	13
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	23
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	23
	31b	Authorship eligibility guidelines and any intended use of professional writers	23
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	23
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	23
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

4.2. Efficacy and safety of co-administered ivermectin and albendazole in school-aged children and adults infected with *Trichuris trichiura* in Côte d'Ivoire, Laos, and Pemba Island, Tanzania: a double-blind, parallel-group, phase 3, randomised controlled trial

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Efficacy and safety of co-administered ivermectin and albendazole in school-aged children and adults infected with *Trichuris trichiura* in Côte d'Ivoire, Laos, and Pemba Island, Tanzania: a double-blind, parallel-group, phase 3, randomised controlled trial

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Summary

Background Preventive chemotherapy with albendazole or mebendazole remains one of the cornerstones of soil-transmitted helminth control. However, these drugs are less effective against *Trichuris trichiura*. Combined ivermectin–albendazole is a promising treatment alternative, yet robust evidence is lacking. We aimed to demonstrate superiority of co-administered ivermectin–albendazole over albendazole monotherapy in three distinct epidemiological settings.

Methods We conducted a double-blind, parallel-group, phase 3, randomised controlled trial in community members aged 6–60 years infected with *T trichiura* in Côte d'Ivoire, Laos, and Pemba Island, Tanzania, between Sept 26, 2018, and June 29, 2020. Participants with at least 100 *T trichiura* eggs per g of stool at baseline were randomly assigned (1:1) using computer-generated randomisation sequences in varying blocks of four, six, and eight, stratified by baseline *T trichiura* infection intensity, to orally receive either a single dose of ivermectin (200 µg/kg) plus albendazole (400 mg) or albendazole (400 mg) plus placebo. Patients, field staff, and outcome assessors were masked to treatment assignment. The primary outcome was cure rate against *T trichiura*, defined as the proportion of participants with no eggs in their faeces 14–21 days after treatment, assessed by Kato-Katz thick smears, and analysed in the available-case population according to intention-to-treat principles. Safety was a secondary outcome and was assessed 3 h and 24 h after drug administration. The trial is registered at ClinicalTrials.gov, NCT03527732.

Findings Between Sept 13 and Dec 18, 2019, Jan 12 and April 5, 2019, and Sept 26 and Nov 5, 2018, 3737, 3694, and 1435 community members were screened for trial eligibility in Côte d'Ivoire, Laos, and Pemba Island, respectively. In Côte d'Ivoire, Laos, and Pemba Island, 256, 274, and 305 participants, respectively, were randomly assigned to the albendazole group, and 255, 275, and 308, respectively, to the ivermectin–albendazole group. Primary outcome data were available for 722 participants treated with albendazole and 733 treated with ivermectin–albendazole. Ivermectin–albendazole showed significantly higher cure rates against *T trichiura* than albendazole in Laos (66% [140 of 213] *vs* 8% [16 of 194]; difference 58 percentage points, 95% CI 50 to 65, p<0·0001) and Pemba Island (49% [140 of 288] *vs* 6% [18 of 293], 43 percentage points, 36 to 49, p<0·0001) but had similar efficacy in Côte d'Ivoire (14% [32 of 232] *vs* 10% [24 of 235], 4 percentage points, –2 to 10, p=0·24). No serious adverse events were reported; observed events were mostly classified as mild (95% [266 of 279] in the albendazole group and 91% [288 of 317] in the ivermectin–albendazole group), and all were transient in nature.

Interpretation Treatment with ivermectin–albendazole resulted in higher efficacy against trichuriasis than albendazole alone in Laos and Pemba Island but not in Côte d'Ivoire. We recommend implementation of this combination therapy for soil-transmitted helminth control in countries with high *T trichiura* prevalence and proven enhanced efficacy of this treatment, particularly where ivermectin is beneficial against other endemic helminthiases.

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See Comment page 10

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Introduction

Soil-transmitted helminthiasis affects approximately one in five people in the world and disproportionally affects the most neglected populations.[1] It is caused by infections with the nematodes *Trichuris trichiura*, *Ascaris lumbricoides*, and hookworm.[2] The mainstay of soil-transmitted helminth (STH) control is preventive chemotherapy as periodic mass drug administration with a single dose of either albendazole or mebendazole to at-risk populations without prior diagnosis, besides

Research in context

Evidence before this study

We searched PubMed, ISI Web of Science, ScienceDirect, Cochrane Central Register of Controlled Trials (CENTRAL), and ClinicalTrials.gov from Jan 1, 1960, to April 15, 2021. We used "ivermect* [AND] albendaz* [AND] (hookworm [OR] trichuri* [OR] ascari* [OR] soil-transmitted helminth*) [AND] (cure* [OR] trial)" as search terms for PubMed, ISI Web of Science, and ScienceDirect, and the keywords "ivermectin" and "albendazole" for CENTRAL and ClinicalTrials.gov. The latest evidence on the efficacy and safety of co-administered ivermectin plus albendazole against soil-transmitted helminths is presented in our systematic review, meta-analysis, and individual-patient data analysis, which was published in 2018. In that study, we identified four randomised controlled trials that investigated the standard dose of combined ivermectin (200 µg/kg) and albendazole (400 mg). All four trials exclusively assessed efficacy and safety in school-aged children and adolescents (aged 6–20 years), yet adult populations are also targeted in preventive chemotherapy interventions against soil-transmitted helminths. Generalisability of the four trials to other endemic settings is limited. Two studies were done on the Zanzibar archipelago (Pemba and Unguja Islands), Tanzania; one in the Philippines; and one in Sri Lanka. The trials in the Philippines and Sri Lanka were done before 2000 and used a weak diagnostic approach, with only one stool sample analysed before and after treatment.

Added value of this study

We conducted a randomised controlled trial in three settings with distinct epidemiological profiles and treatment history with anthelmintic drugs. We targeted whole communities and recruited approximately 600 participants aged 6–60 years in each of the three settings to provide robust data and to allow for subgroup analysis. Our study showed higher efficacy against *Trichuris trichiura* if ivermectin–albendazole was used for treatment than if albendazole monotherapy was used in two settings. Both treatment regimens showed excellent safety profiles with mostly mild and transient adverse events. The trial results from Côte d'Ivoire highlight the existence of settings in which combination treatment does not show enhanced efficacy over albendazole alone, warranting the need for further investigation of emerging drug resistance, parasite genetics, and host–parasite interactions affecting drug efficacy.

Implications of all the available evidence

The availability of efficacious drugs to treat all types of soil-transmitted helminth infections is a requirement for progress in morbidity control. Our findings support the integration of combination therapy as recommended treatment in preventive chemotherapy schemes against soil-transmitted helminthiasis, although the efficacy needs to be confirmed in a pilot study.

other strategies tackling exposure (eg, by improving sanitation and providing access to safe water).[3] The main goal of preventive chemotherapy is to reduce morbidity by decreasing infection intensities and to ultimately eliminate soil-transmitted helminthiasis as a public health problem. Elimination as a public health problem is defined as the prevalence reduction of moderate and heavy intensities to below 2%, as assessed in preschool-aged and school-aged children, by 2030.[4]

Single-dose albendazole treatment shows high and moderate efficacy against *A lumbricoides* and hookworm infections, respectively.[5] Yet, performance against *T trichiura* is unsatisfactory, with egg reduction rates (ERRs) and cure rates below 50%.[5] Furthermore, albendazole has been used for many decades, and several hundred million doses are donated yearly to endemic areas for STH control.[5] Its consistent use makes it prone to drug resistance, a phenomenon common in veterinary medicine but not yet proven for human soil-transmitted helminthiases.[5,6]

In view of the empty pipeline of new potent anthelmintic drugs, the evaluation of drug combinations of already registered products has been identified as a research priority.[7] The use of drug combinations with different mechanisms of action might not only enhance efficacy but also delay the onset of resistance.[8] Ivermectin–albendazole is one of the most promising drug combination candidates and was recently added to the WHO List of Essential Medicines, which paves the road for its use in deworming programmes.[9] Since 2000, ivermectin–albendazole has been successfully used for the control of lymphatic filariasis, showing an excellent safety profile.[10] However, robust evidence on the potentially enhanced efficacy from co-administered ivermectin–albendazole against STHs in varying age groups and endemicity settings is lacking.[11–15] The primary objective of this trial was to assess the efficacy and safety of co-administered ivermectin–albendazole 14–21 days after treatment in community members aged 6–60 years infected with *T trichiura* in different transmission settings. This trial has the ultimate goal to inform STH preventive chemotherapy schemes put forth by WHO, and hence includes long-term follow-up outcomes that are presented elsewhere.[16]

Methods

Study design and participants

We did a phase 3, randomised, controlled, double-blind, parallel group, superiority trial in Côte d'Ivoire, Laos, and Pemba Island, Tanzania, between Sept 26, 2018, and June 29, 2020. Details on trial design and methodology were published elsewhere.[17] All community members aged 6–60 years who were positive for *T trichiura* in at least two slides of quadruplicate Kato-Katz smears, with an infection intensity of at least 100 eggs per g of stool (EPG), were eligible for trial inclusion. Excluded from

the trial were individuals presenting with severe chronic or acute systemic illness (eg, severe anaemia with haemoglobin levels below 80 g/L, clinical malaria [positive rapid diagnostic test plus body temperature ≥38°C], reported or suspected diabetes, HIV, AIDS, or tuberculosis), pregnant women, lactating women in the first week after birth, and children who weighed less than 15 kg, as assessed upon baseline clinical examination.

This trial was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The protocol was approved by independent ethics committees in Côte d'Ivoire (reference numbers 088–18/MSHP/CNESVS-km and ECCI00918), Laos (reference number 093/NECHR), Pemba (Zanzibar, reference number ZAMREC/0003/Feb/2018), and Switzerland (reference number BASEC Nr Req-2018-00494). Participating adults, or parents or guardians of participating children, provided written informed consent. Children provided written (Côte d'Ivoire) or oral (Laos and Pemba) assent. All authors take responsibility for the accuracy and completeness of the data and the fidelity of the trial to the protocol, which is available together with the statistical analysis plan in appendix 1.

Randomisation and masking

Computer-generated randomisation sequences in varying blocks of four, six, and eight participants, stratified by two levels of baseline *T trichiura* infection intensity (light=<1000 EPG; moderate to heavy=≥1000 EPG), were provided by the trial statistician. Enrolled participants were randomly assigned in a 1:1 ratio to receive either a single dose of albendazole (400 mg; Zentel; GlaxoSmithKline, London, UK) plus appearance-matched placebo (produced by the University of Basel) or albendazole (400 mg) plus ivermectin (200 µg/kg; Stromectol; Merck Sharp & Dohme, Readington, NJ, USA) using sealed, opaque, sequentially numbered envelopes containing the treatment assignment (A or B) and six ivermectin or corresponding placebo tablets (sufficient to dose participants weighing up to 97 kg). The envelopes were prepared in Basel, Switzerland, by people who were independent of the trial. Participants were randomly assigned in the order they presented to the administrating investigators on the day of treatment. Investigators verified whether participants had baseline light or moderate-to-heavy infection intensity, chose the treatment envelope from the respective pile based on treatment allocation, and opened the sealed envelopes. Albendazole tablets were kept in bottles of 100 pieces as provided by WHO, and a single tablet was administered to every patient together with the necessary number of ivermectin or placebo tablets according to the patient's weight. Through the use of pre-packed treatment group labels, together with ivermectin appearance-matched placebos, field staff, including investigators, participants, and outcome assessors, were masked to treatment assignment.

Procedures

At baseline, participants were asked to provide two stool samples from different days (ideally within a 5 day interval), from which duplicate Kato-Katz thick smears using 41·7 mg of stool were prepared and assessed under a light microscope for the identification of *T trichiura*, *A lumbricoides*, and hookworm ova by laboratory technicians.[18] In Laos, both stool samples of *T trichiura*-infected participants were also assessed for *Strongyloides stercoralis* larvae using the Baermann technique.[19] For participants who were identified as *T trichiura* positive after the second sample only, one *S stercoralis* examination of that sample was done. 10% of all Kato-Katz slides were randomly chosen for subsequent quality control by picking every tenth slide of all slides read by each laboratory technician on the respective day.

Before treatment, participants underwent a physical examination to ensure recruitment criteria were met. These examinations included rapid diagnostic testing for haemoglobin levels and malaria (in Côte d'Ivoire and Laos) and lymphatic filariasis (in Côte d'Ivoire and Pemba Island) parasites, as well as pregnancy tests in girls and women aged 10 years and older in Côte d'Ivoire and Pemba Island and aged 12 years and older in Laos. Participants were further examined physically, and a medical anamnesis was done by trial physicians to assess baseline conditions. On Pemba Island, treatment was administered on the same day as the clinical examination, whereas in Côte d'Ivoire and Laos, treatment took place approximately 1 week after baseline due to the preceding analysis of blood parameters to evaluate liver and kidney functions as a required safety measure put forth by the responsible ethical or drug safety boards.

See Online for appendix 1

Adverse events were assessed actively at 3 h and 24 h after treatment and graded into light, moderate, and severe using adapted Common Toxicity Criteria version 2.0 put forth by the Cancer Therapy Evaluation Program. Study physicians were asked to evaluate relatedness of the most common adverse events to drug administration. If causality could not be ruled out by other conditions or reasons, the adverse events were considered as possibly related. Newly emerging, as well as persistent, adverse events after 24 h and within 14–21 days of treatment were followed up using a passive monitoring scheme, as specified in the protocol. Treatment efficacy was determined 14–21 days after treatment by collecting another two stool samples per participant. At study termination, all participants positive for STH were offered the combination therapy, if found to be more efficacious, or standard therapy.

Outcomes

The primary outcome was the cure rate of *T trichiura*, defined as the proportion of participants with no eggs in their faeces 14–21 days after treatment. Secondary outcomes were the ERR against *T trichiura*; cure rates and ERRs against *A lumbricoides*, hookworm, and

S stercoralis; and infection status assessed by qPCR. Safety outcomes included adverse events assessed 3 h and 24 h after treatment, serious adverse events, and adverse events potentially related to treatment. In this manuscript we do not present the cure rates determined by qPCR analysis or related methods and results. At the time of writing, this analysis had been done only for samples from Pemba Island, and thus qPCR-based cure rates cannot be compared between countries. Furthermore, detailed results of this analysis have been published elsewhere.[20]

Statistical analysis

For the sample size calculation, we used the formula $n = (Z_{\alpha/2} + Z_{\beta})^2 \times [\pi_1 \times (1-\pi_1) + \pi_2 \times (1-\pi_2)] / (\pi_2 - \pi_1)^2$, where $Z_{\alpha/2}$ and Z_{β} denote the critical values of the normal distribution for significance level and type 2 error, respectively, and π_1 and π_2 denote the proportion of cured individuals in the two treatment groups. For each location, the trial was powered to detect a significant difference in cure rates between the two treatment groups with 90% power at a two-sided 5% significance level, assuming that the cure rate of albendazole for *T trichiura* was 30% and of ivermectin–albendazole was 50%.[5,15] We calculated that 121 participants per group would be sufficient to test the primary hypothesis that ivermectin–albendazole has a higher efficacy against *T trichiura* infection than albendazole alone. Taking a potential loss to follow-up of 15% into account, we anticipated that we needed to enrol 143 participants per treatment group. In view of the prolonged follow-up period to assess the long-term secondary outcomes (up to 12 months), we aimed to include 600 community members in each trial setting. For subgroup analysis, the data from all locations were pooled to ensure sufficient power to assess a potential difference in efficacy by baseline infection intensity (ie, light *vs* moderate to heavy) and age group (ie, 6–12 years *vs* 13–60 years).

The primary analysis estimated the cure rate of *T trichiura* using the available-case population according to intention-to-treat principles. The available-case population was defined as all randomly assigned participants, excluding those who entered the study despite not satisfying the entry criterion of positive at baseline, with at least one follow-up stool sample at 14–21 days post-treatment. To check the robustness of the primary analysis, the results were confirmed using the per-protocol population. Exact definitions of the different analysis populations are provided in the statistical analysis plan in appendix 1. As outlined in the statistical analysis plan, the available-case analysis was further complemented by an intention-to-treat analysis using multiple imputation if the proportion of missing data exceeded 10%. Major protocol deviations, leading to exclusion from the per-protocol analysis, included negative infection status for *T trichiura* at baseline, not satisfying the enrolment criteria, meeting withdrawal criteria, or receiving no treatment, the wrong treatment, or concomitant treatment with anthelmintics in the past 4 weeks or drugs with known interaction with the study drugs.

The mean egg counts from the quadruplicate Kato-Katz thick smears were multiplied by 24 to be expressed as EPG. Helminth infection intensity was classified as light or moderate to heavy depending on the species following cutoffs put forth by WHO.[21] We calculated the proportion of individuals with moderate-to-heavy infection intensities at baseline who had light infection intensities or no infection at follow-up. This indicator is used to assess progress within control programmes to eliminate morbidity due to STHs.[21]

A melded binomial test with mid-p correction was used to calculate differences in cure rates between the two treatment groups and to estimate the corresponding confidence intervals. The prespecified analytical code is provided in appendix 1 (p 171).

Geometric mean and arithmetic mean egg counts were calculated for the two treatment groups before and after treatment to assess the corresponding ERRs. Geometric mean-based and arithmetic mean-based ERRs were calculated as:

$$ERR_{GM} = 1 - \frac{\frac{1}{n} e^{\sum \log(\text{EPGfollow-up} + 1)} - 1}{\frac{1}{n} e^{\sum \log(\text{EPGbaseline} + 1)} - 1}$$

$$ERR_{AM} = 1 - \frac{\frac{1}{n} \sum(\text{EPG}_{\text{follow-up}})}{\frac{1}{n} \sum(\text{EPG}_{\text{baseline}})}$$

A bootstrap resampling method with 5000 replicates was used to calculate 95% CIs for geometric mean-based ERRs and the difference between them. The code for implementing the bootstrap routine is provided in the statistical analysis plan (appendix 1 p 171). All estimates presented are unadjusted estimates. Multiple imputations were done using the mi package in R. The missing outcomes were predicted using the variables sex, age, height, weight, treatment group, and baseline infection intensity. The imputation used six chains with 30 iterations each. Imputation performance was assessed via the R-hat statistics, and convergence was visually inspected.

No statistical testing was done on safety outcomes; adverse events are presented in frequency tables. Data were analysed using R software version 4.0.3 and Stata 16.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, interpretation of the findings, writing of the report, or the decision to submit for publication.

Results

Between Sept 13 and Dec 18, 2019, Jan 12 and April 5, 2019, and Sept 26 and Nov 5, 2018, 3737, 3694, and 1435 community members were screened for trial eligibility in Côte d'Ivoire, Laos, and Pemba Island, respectively (figure 1). The recruitment phase to assess the eligibility of community members finished after 133 days in Côte d'Ivoire, 83 days in Laos, and 40 days in Pemba Island. In Côte d'Ivoire, Laos, and Pemba Island, 256, 274, and 305 participants, respectively, were randomly assigned to and received albendazole co-administered with placebo, whereas 255, 275, and 308 participants, respectively, were randomly assigned to and received ivermectin–albendazole.

Demographic, baseline anthropometric, and helminth infection characteristics of the 1673 randomised participants are summarised in table 1. Co-infections with other STH species were common and found in 951 (57%) of 1673 participants, but endemicity profiles differed between countries. In Laos, hookworm was the predominant co-infection, whereas *A lumbricoides* co-infection was most common in Côte d'Ivoire and

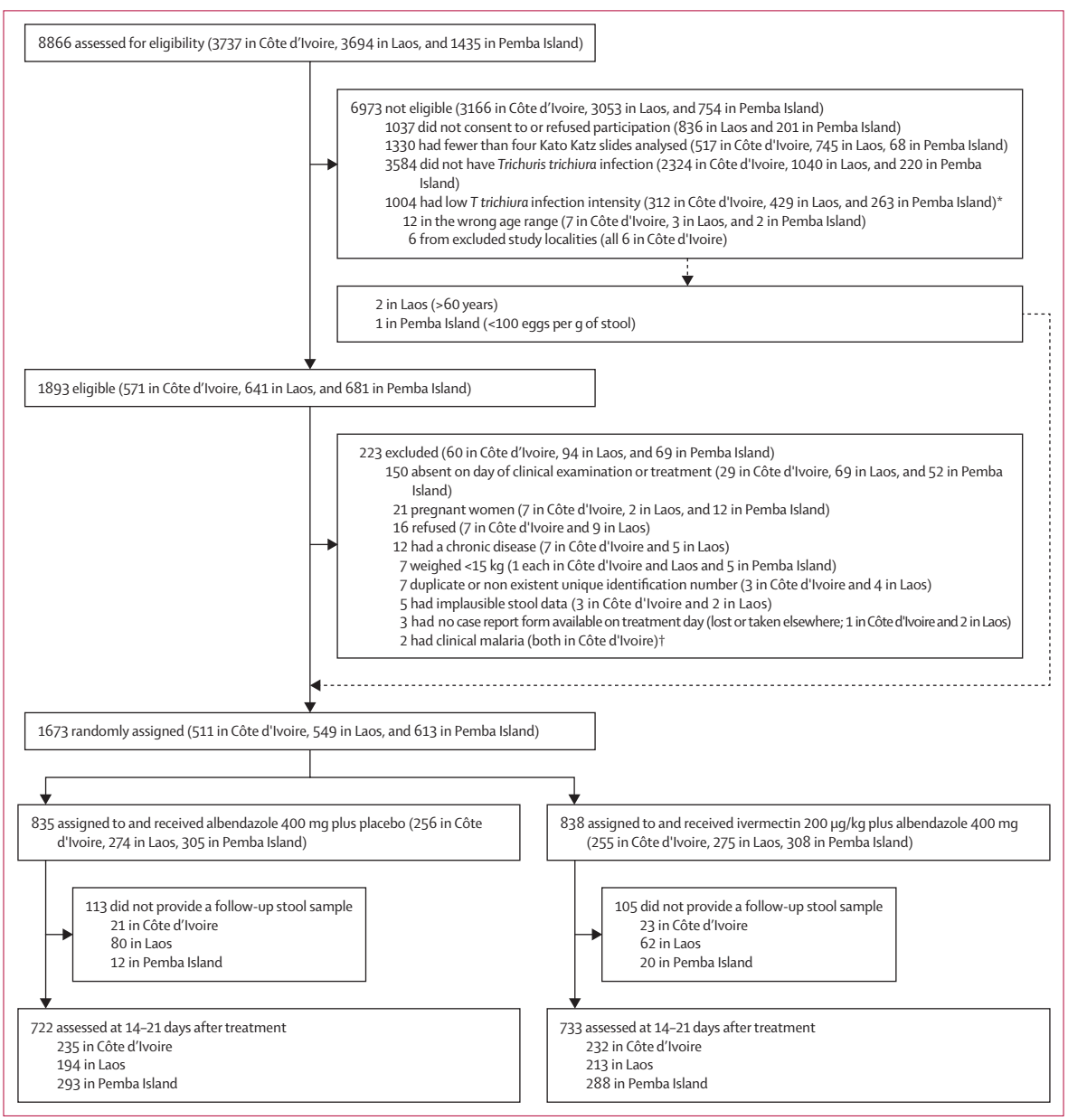

Figure 1: **Trial profile**

*Low *T trichiura* infection intensities not qualifying for trial inclusion were egg counts below 100 eggs per g of stool, fewer than two of four Kato-Katz slides found to be positive, or both. †Clinical malaria was defined as a rapid diagnostic test positive for *Plasmodium* spp, together with a body temperature ≥38°C.

Pemba Island. *S stercoralis* infection was only tested for in Laos and was found in 59 (11%) of 549 participants. *T trichiura* infection intensities were well balanced between treatment groups. Between study countries, differences were seen with respect to not only helminth co-infections but also demographic variables. The Laos study cohort was older (mean age 26·8 years [SD 17·4]) than the two African cohorts (mean age 16·3 years [13·7] in Côte d'Ivoire and 14·0 years [10·0] in Pemba Island).

	Côte d'Ivoire		Laos*		Pemba Island†	
	Albendazole (n=256)	Ivermectin–albendazole (n=255)	Albendazole (n=274)	Ivermectin–albendazole (n=275)	Albendazole (n=305)	Ivermectin–albendazole (n=308)
Age, years	16·5 (14·1)	16·0 (13·4)	27·7 (17·3)	25·9 (17·4)	14·0 (10·5)	13·9 (9·6)
Age group‡						
School-aged (6–12 years)	171 (67%)	168 (66%)	92 (34%)	113 (41%)	191 (63%)	184 (60%)
Youth or young people (13–24 years)	30 (12%)	36 (14%)	37 (14%)	30 (11%)	81 (27%)	90 (29%)
Adults (25–60 years)	55 (21%)	51 (20%)	145 (53%)	132 (48%)	33 (11%)	34 (11%)
Sex						
Female	120 (47%)	129 (51%)	144 (53%)	147 (53%)	171 (56%)	169 (55%)
Male	136 (53%)	126 (49%)	130 (47%)	128 (47%)	134 (44%)	139 (45%)
Weight, kg	37·5 (20·1)	37·2 (19·7)	41·1 (14·5)	39·7 (15·7)	34·2 (15·5)	34·0 (14·8)
Height, cm	136·7 (20·3)	135·8 (19·4)	144·3 (16·6)§	142·3 (16·7)	137·3 (18·6)	137·7 (18·9)
Trichuris trichiura infection						
Geometric mean EPG	480·5	469·9	365·9	349·3	466·9	460·9
Arithmetic mean EPG	1036·0	1079·9	607·2	636·7	846·7	923·9
Infection intensity¶						
Light	190 (74%)	192 (75%)	232 (85%)	232 (84%)	231 (76%)	234 (76%)
Moderate	64 (25%)	60 (24%)	42 (15%)	42 (15%)	74 (24%)	71 (23%)
Heavy	2 (1%)	3 (1%)	0	1 (<1%)	0	3 (1%)
Ascaris lumbricoides infection						
Infected	91 (36%)	91 (36%)	112 (41%)	96 (35%)	74 (24%)	90 (29%)
Geometric mean EPG	5499·1	4129·9	3991·2	3635·0	4515·3	2979·5
Arithmetic mean EPG	22 597·0	19 266·7	13 444·7	11 931·3	10 050·3	7593·1
Infection intensity‖						
Light	38 (42%)	42 (46%)	58 (52%)	48 (50%)	35 (47%)	52 (58%)
Moderate	40 (44%)	39 (43%)	47 (42%)	44 (46%)	38 (51%)	37 (41%)
Heavy	13 (14%)	10 (11%)	7 (6%)	4 (4%)	1 (1%)	1 (1%)
Hookworm infection						
Infected	31 (12%)	18 (7%)	250 (91%)	253 (92%)	53 (17%)	42 (14%)
Geometric mean EPG	82·1	91·3	804·8	840·3	100·5	79·9
Arithmetic mean EPG	214·5	830·0	1820·3	1743·4	236·8	205·1
Infection intensity**						
Light	31 (100%)	16 (89%)	182 (73%)	184 (73%)	53 (100%)	42 (100%)
Moderate	0	0	37 (15%)	43 (17%)	0	0
Heavy	0	2 (11%)	31 (12%)	26 (10%)	0	0
Strongyloides stercoralis infection††	ND	ND	29 (11%)	30 (11%)	ND	ND

Data are mean (SD) or n (%), unless otherwise stated. EPG=eggs per g of stool. ND=not determined. *549 randomised; two did not fulfill eligibility criteria (age >60 years) but were included in the available-case analysis. †613 randomised; one did not fulfill eligibility criteria (<100 EPG) but was included in the available-case analysis. ‡Age groups were classified and adapted into school-aged children according to WHO definitions (originally 5–12 years, but 6–12 years in this study) and youth or young people and adults according to UN definitions (originally 15–24 years for youth or younger people and >25 years for adults, but redefined as 13–24 years for youth or young people and >25 years for adults in this study). §One individual value omitted due to irrational value (479 cm). ¶*T trichiura* infection intensity was classified according to mean EPG into light (1–999 EPG), moderate (1000–9999 EPG), and heavy (≥10 000 EPG). ‖*A lumbricoides* infection intensity was classified according to mean EPG into light (1–4999 EPG), moderate (5000–49 999 EPG), and heavy (≥50 000 EPG). **Hookworm infection intensity was classified according to mean EPG into light (1–1999 EPG), moderate (2000–3999 EPG), and heavy (≥4000 EPG). ††The Baermann technique to detect *S stercoralis* infection was applied only to stool samples collected in Laos; two participants (one in each group) had no *S stercoralis* result.

Table 1: **Baseline characteristics of trial participants by country**

44 (9%), 142 (26%), and 32 (5%) participants were lost to follow-up in Côte d'Ivoire, Laos, and Pemba Island, respectively, with similar numbers in each group (figure 1). Data from 467, 407, and 581 trial participants in Côte d'Ivoire, Laos, and Pemba Island, respectively, were used for the final analysis. Of note, two participants in

	Côte d'Ivoire		Laos		Pemba Island	
	Albendazole (n=235)	Ivermectin–albendazole (n=232)	Albendazole (n=194)	Ivermectin–albendazole (n=213)	Albendazole (n=293)	Ivermectin–albendazole (n=288)
Trichuris trichiura						
Participants positive for infection after treatment	211	200	178	73	275	148
Cure rate, % (95% CI)	10% (7 to 15)	14% (10 to 19)	8% (5 to 13)	66% (59 to 72)	6% (4 to 10)	49% (43 to 55)
Difference in cure rate, percentage points (95% CI)*	..	4 (–2 to 10)	..	58 (50 to 65)	..	43 (36 to 49)
Cure rate by infection intensity, % (n/N)						
Light	13% (22/175)	15% (25/172)	9% (15/164)	74% (131/176)	8% (17/222)	53% (116/219)
Moderate	3% (2/58)	12% (7/57)	3% (1/30)	25% (9/36)	1% (1/71)	36% (24/66)
Heavy	0% (0/2)	0% (0/3)	..	0% (0/1)	..	0% (0/3)
Geometric mean EPG						
Baseline	488·8	475·4	369·8	361·0	462·8	463·2
After treatment	175·7	141·6	115·5	3·0	198·5	8·0
Geometric mean ERR, % (95% CI)	64% (54 to 72)	70% (61 to 77)	69% (61 to 75)	99% (99 to 99)	57% (48 to 65)	98% (98 to 99)
Difference in geometric mean ERR, percentage points (95% CI)*	..	6 (–6 to 18)	..	30 (24 to 38)	..	41 (34 to 50)
Arithmetic mean EPG						
Baseline	1048·8	1110·9	626·1	688·7	848·5	938·0
After treatment	874·6	866·8	403·2	70·8	698·9	105·1
Arithmetic mean ERR, % (95% CI)	17% (–14 to 39)	22% (–14 to 46)	36% (19 to 50)	90% (81 to 96)	18% (4 to 30)	89% (83 to 93)
Moderately or heavily infected participants with no or light infection after treatment, % (95% CI; n/N)	40% (27 to 53; 31/60)	52% (39 to 65; 24/60)	63% (45 to 82; 19/30)	97% (92 to 103; 34/71)	48% (36 to 60; 36/37)	94% (89 to 100; 65/69)
Ascaris lumbricoides						
Participants positive for infection						
Baseline	82	81	77	70	70	86
After treatment	4	5	0	0	2	1
Cure rate, % (95% CI)	95% (90 to 100)	94% (89 to 99)	100% (100 to 100)	100% (100 to 100)	97% (93 to 101)	99% (97 to 101)
Cure rate by infection intensity, % (n/N)						
Light	100% (34/34)	100% (36/36)	100% (44/44)	100% (38/38)	97% (33/34)	100% (50/50)
Moderate	92% (33/36)	86% (30/35)	100% (28/28)	100% (31/31)	97% (34/35)	97% (34/35)
Heavy	92% (11/12)	100% (10/10)	100% (5/5)	100% (1/1)	100% (1/1)	100% (1/1)
Geometric mean EPG						
Baseline	5315·6	4748·2	3458·5	3374·7	4378·8	3173·9
After treatment	0·1	0·2	0	0	0·3	0·1
Geometric mean ERR, % (95% CI)	100% (100 to 100)	100% (100 to 100)	100% (100 to 100)	100% (100 to 100)	100% (100 to 100)	100% (100 to 100)

(Table 2 continues on next page)

	Côte d'Ivoire		Laos		Pemba Island	
	Albendazole (n=235)	Ivermectin–albendazole (n=232)	Albendazole (n=194)	Ivermectin–albendazole (n=213)	Albendazole (n=293)	Ivermectin–albendazole (n=288)
(Continued from previous page)						
Arithmetic mean EPG						
Baseline	22 874·2	19 907·6	12 977·7	9801·6	9525·3	7782·0
After treatment	0·9	5·1	0·0	0·0	101·1	24·4
Arithmetic mean ERR, % (95% CI)	100% (100 to 100)	100% (99·9 to 100)	100% (100 to 100)	100% (100 to 100)	99% (97 to 99)	100% (99 to 100)
Moderately or heavily infected participants with no or light infection after treatment, % (95% CI; n/N)	100% (100 to 100; 48/48)	100% (100 to 100; 45/45)	100% (100 to 100; 32/32)	100% (100 to 100; 33/33)	100% (100 to 100; 36/36)	100% (100 to 100; 36/36)
Hookworm						
Participants positive for infection						
Baseline	28	17	180	194	49	39
After treatment	1	2	80	79	9	11
Cure rate, % (95% CI)	96% (89 to 104)	88% (71 to 105)	56% (48 to 63)	59% (52 to 66)	82% (70 to 93)	72% (57 to 87)
Cure rate by infection intensity, % (n/N)						
Light	96% (27/28)	93% (14/15)	63% (85/136)	67% (95/142)	82% (40/49)	72% (28/39)
Moderate	..	..	36% (10/28)	41% (14/34)	..	..
Heavy	..	50% (1/2)	31% (5/16)	33% (6/18)	..	..
Geometric mean EPG						
Baseline	94·4	108·6	731·4	861·8	100·6	80·5
After treatment	0·1	0·9	7·9	6·5	1·3	2·5
Geometric mean ERR, % (95% CI)	100% (100 to 100)	99% (97 to 100)	99% (98 to 99)	99% (99 to 100)	99% (97 to 100)	97% (93 to 99)
Arithmetic mean EPG						
Baseline	232·9	878·5	1620·6	1678·7	223·0	214·0
After treatment	0·4	89·3	146·6	129·5	30·6	48·0
Arithmetic mean ERR, % (95% CI)	100% (99 to 100)	90% (75 to 100)	91% (87 to 94)	92% (88 to 95)	86% (77 to 95)	78% (64 to 90)
Moderately or heavily infected participants with no or light infection after treatment, % (95% CI; n/N)	ND	100% (100 to 100; 2/2)	95% (89 to 102; 42/44)	98% (94 to 102; 51/52)	ND	ND
***Strongyloides stercoralis*†**						
Participants positive for infection						
Baseline	ND	ND	22	22	ND	ND
After treatment	ND	ND	4	1	ND	ND
Cure rate, % (95% CI)	ND	ND	82% (64 to 99)	96% (86 to 105)	ND	ND

EPG=eggs per g of stool. ERR=egg reduction rate. ND=not determined. *Significant differences are highlighted in bold; for cure rates, significance was defined when the p-value was <0·05 according to the melded binomial test for difference (mid-p version), whereas for ERRs, significance was defined when the 95% CI did not include 0. †*S stercoralis* infection was assessed qualitatively only (positive *vs* negative) in stool samples collected in Laos; 406 participants (193 in the albendazole group and 213 in the ivermectin–albendazole group) in Laos had at least one stool sample examined for *S stercoralis* at baseline and follow-up.

Table 2: **Efficacy against *T trichiura* and co-infecting soil-transmitted helminths by trial country (available-case analysis)**

Laos (aged >60 years) and one in Pemba Island (*T trichiura* intensity <100 EPG) were randomised erroneously. These participants were included in the available-case analysis.

Cure rates and ERRs of the available-case population are summarised in table 2. Treatment with ivermectin–albendazole resulted in significantly higher cure rates than albendazole alone in Laos (66% [140 of 213] *vs* 8%

[16 of 194], difference 58 percentage points, 95% CI 50 to 65, p<0·0001) and Pemba Island (49% [140 of 288] *vs* 6% [18 of 293], 43 percentage points, 36 to 49, p<0·0001). Similarly, ERRs were significantly higher in the combination therapy group than in the monotherapy group in Laos (geometric mean ERR 99% *vs* 69%, difference 30 percentage points, 95% CI 24 to 38) and Pemba Island (98% *vs* 57%, 41 percentage points, 34 to 50). In Côte d'Ivoire, ivermectin–albendazole showed similarly low efficacy to albendazole in terms of cure rates (14% [32 of 232] *vs* 10% [24 of 235], difference 4 percentage points, 95% CI –2 to 10, p=0·24) and ERRs (geometric mean ERR 70% *vs* 64%, difference 6 percentage points, 95% CI –6 to 18). Adjusting for age category, sex, and weight did not notably change point or interval estimates (data not shown). *T trichiura* egg distributions before and after treatment by treatment group, for each country, are shown in a violin plot (figure 2A–C). Of participants who received ivermectin–albendazole with moderate-intensity or heavy-intensity infections, 52% (95% CI 39 to 65; 31 of 60), 97% (92 to 103; 36 of 37), and 94% (89 to 100; 65 of 69) showed no or only light intensity infections after treatment in Côte d'Ivoire, Laos, and Pemba Island, respectively (table 2).

In Côte d'Ivoire, no major protocol deviations were observed and therefore the per-protocol population is identical to the available-case population. On Pemba Island, one participant in the ivermectin–albendazole group with a baseline infection intensity of 18 EPG was inappropriately randomised. Excluding this participant from the per-protocol analysis did not notably change the estimates (cure rate 48% in the ivermectin–albendazole group *vs* 6% in the albendazole group, difference 42 percentage points, 95% CI 36–49, p<0·0001). In Laos, one of the two participants older than 60 years who was erroneously randomised to the albendazole group provided a follow-up stool sample and was included in the available-case population. The per-protocol analysis excluding this participant showed similar estimates to the available-case analysis (66% *vs* 8%, 57 percentage points, 50–64, p<0·0001). The proportion of missing data in Laos was higher than expected. Missing data for the intention-to-treat analysis were imputed by multiple imputation but neither point nor interval estimates changed substantially from the available-case analysis (appendix 2 p 2).

Subgroup analysis showed higher cure rates against *T trichiura* in participants with light infection intensities (45% [156 of 348] in the ivermectin–albendazole group and 11% [37 of 339] in the albendazole group) than in participants with moderate or heavy infection intensities (16% [16 of 97] and 3% [three of 90]) at baseline, but the conclusions with respect to superiority of the ivermectin–albendazole combination did not change (appendix 2 p 3). The cure rate for ivermectin–albendazole in the 6–12 years age group (26%; 64 of 243) was lower than the cure rate in the 13–60 years age group (53%; 108 of 202),

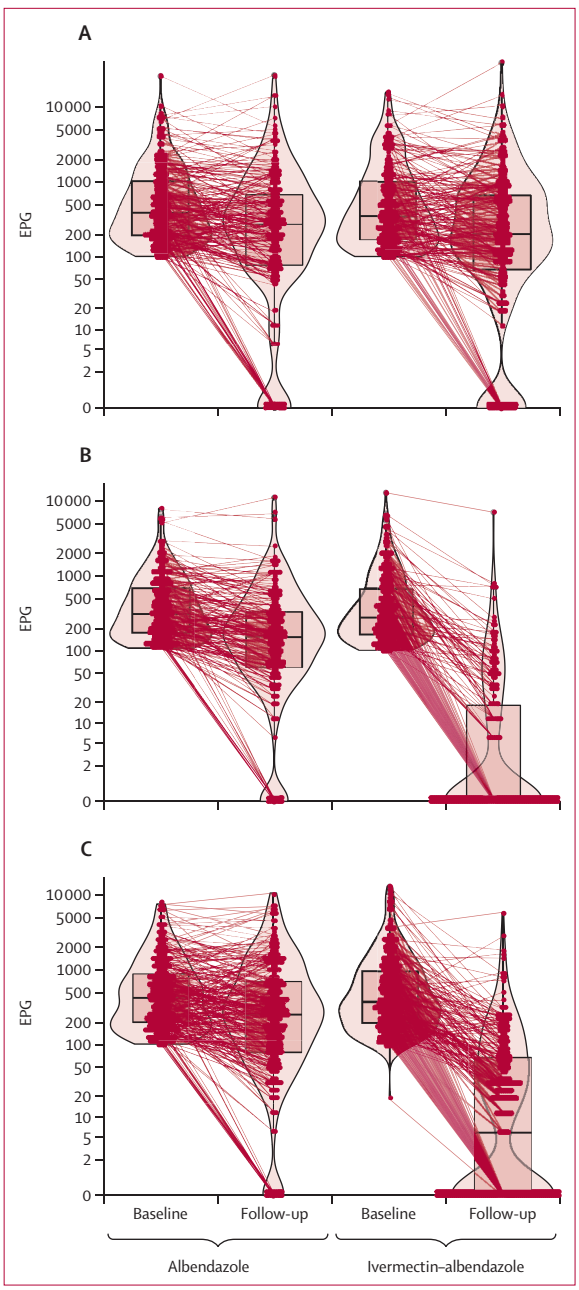

***Figure 2*: Violin plots illustrating *Trichuris trichiura* egg reduction in Côte d'Ivoire (A), Laos (B), and Pemba Island (C), by treatment group**
Violins represent egg densities at baseline and follow-up; boxes represent IQRs and the dots connected by lines the individual participants. EPG=eggs per g of stool.

See Online for appendix 2

but we also observed higher baseline infection intensities irrespective of treatment group in the younger than in the older age group (median EPG at baseline was 444 [IQR 204–1236] *vs* 264 [159–525]; appendix 2 p 3).

Both treatment regimens showed high efficacy against *A lumbricoides*, with cure rates above 93% and ERRs of 99–100% in all trial settings. Cure rates in hookworm-infected participants differed between settings but not

	Albendazole				Ivermectin–albendazole			
	Number of participants assessed	Participants with adverse event (%)	Number of adverse events	Number of possibly related adverse events/number assessed for relatedness (%)*	Number of participants assessed	Participants with adverse event (%)	Number of adverse events	Number of possibly related adverse events/number assessed for relatedness (%)*
Baseline	830	324 (39%)	644	..	831	351 (42%)	699	..
Côte d'Ivoire	254	194 (76%)	467	..	254	198 (78%)	498	..
Laos	273	83 (30%)	110	..	273	94 (34%)	117	..
Pemba Island	303	47 (16%)	67	..	304	59 (19%)	84	..
3 h after treatment	829	111 (13%)	139	43/90 (48%)	834	121 (15%)	152	43/109 (40%)
Côte d'Ivoire	253	47 (19%)	61	29/54 (54%)	253	54 (21%)	77	22/61 (36%)
Laos	273	41 (15%)	50	14/36 (39%)	273	51 (19%)	59	21/48 (44%)
Pemba Island	303	23 (8%)	28	ND	308	16 (5%)	16	ND
24 h after treatment	779	104 (13%)	140	82/121 (68%)	786	109 (14%)	165	103/130 (79%)
Côte d'Ivoire	227	50 (22%)	77	59/65 (91%)	231	62 (27%)	110	78/86 (91%)
Laos	247	48 (19%)	57	23/56 (41%)	247	42 (17%)	49	25/44 (57%)
Pemba Island	305	6 (2%)	6	ND	308	5 (2%)	6	ND
2–21 days after treatment	356	15 (4%)	15	ND	361	14 (4%)	17	ND
Côte d'Ivoire†	15	0	0	ND	23	0	0	ND
Laos‡	42	8 (19%)	8	ND	39	4 (10%)	5	ND
Pemba Island§	299	7 (2%)	7	ND	299	10 (3%)	12	ND

ND=not determined. *On Pemba Island, only four out of 50 events were assessed for causality; relatedness was thus considered as ND. †Safety monitoring after the 24 h post-treatment period was done actively for participants showing moderate or severe adverse events or needing medical intervention at 24 h and passively for all remaining participants. ‡Mainly includes participants who were missed out at, or had persisting symptoms from, the 24-h assessment and symptoms occurring after 24 h but within 14–21 days after treatment that were mentioned by participants during monitoring visits. §We aimed to visit all trial participants to ask about symptoms occurring after 24 h; symptoms mentioned after 24 h could include persisting symptoms from the 24-h assessment or symptoms occurring after 24 h but within 14–21 days after treatment that were possibly related to drug administration.

Table 3: **Baseline symptoms and adverse events after treatment**

between treatment groups. We found the highest cure rate against hookworm in Côte d'Ivoire (96% [27 of 28] for albendazole and 88% [15 of 17] for ivermectin–albendazole), followed by Pemba Island (82% [40 of 49] and 72% [28 of 39]), where infections were primarily of light intensity. In Laos, we found moderate efficacy in terms of cure rates for both treatment groups (56% [100 of 180] and 59% [115 of 194]). Hookworm infections were well reduced in terms of ERRs (geometric mean ERR ≥97%) in all settings. Of 22 participants in Laos infected with *S stercoralis* in each treatment group, four were still infected after treatment in the albendazole group (cure rate 82%) and one in the ivermectin-albendazole group (96%).

Safety was assessed in 508, 546, and 613 trial participants in Côte d'Ivoire, Laos, and Pemba Island, respectively. Not all participants were available at each timepoint (table 3). We did not observe any serious adverse events in any of the three countries. Adverse event reporting was similar between treatment groups. Before treatment, any symptom or condition was reported by 392 (77%) of 508, 177 (32%) of 546, and 106 (18%) of 607 examined participants from Côte d'Ivoire, Laos, and Pemba Island, respectively. All reported baseline symptoms and adverse events assessed 3 h and 24 h after treatment are illustrated in figure 3. Details on country-specific adverse events are provided in appendix 2 (pp 4–6). 232 (14%) of 1663 participants reported any adverse event 3 h after treatment, and 213 (14%) of 1565 reported any adverse event 24 h after treatment. The most frequently reported adverse events in both groups were headache, abdominal pain, and itching. Adverse events were mostly transient and resolved within 24 h. Few participants experienced moderate (23 of 1663, 1%) and severe (six of 1663, <1%) adverse events. 61% (146 of 239) and 59% (125 of 211) of all assessed adverse events were classified as possibly treatment-related in the ivermectin–albendazole and albendazole groups, respectively. More moderate-to-severe adverse events were observed 24 h than 3 h after treatment (27 *vs* 15 events), with higher numbers in Côte d'Ivoire (21 events) than in Laos (18 events) and Pemba Island (three events). At 24 h after treatment, more moderate-to-severe adverse events were reported in the ivermectin–albendazole group (21 events) than in the albendazole group (six events). These mostly included allergy-like symptoms involving itching, rash, and disorders of the digestive tract, but also diarrhoea as a separate clinical symptom, that were resolved without intervention or with antihistamines and glucocorticoid treatment. Adverse events as a result of ivermectin acting on lymphatic filariasis parasites were negligible. None of 607 participants in Pemba Island and four (1%) of 510 participants in Côte d'Ivoire tested positive for IgG or

IgM antibodies against *Wuchereria bancrofti* before treatment, only one of whom reported mild abdominal pain 3 h after treatment.

Discussion

We did a randomised controlled trial in three distinct epidemiological settings in people aged 6–60 years with the overarching goal to inform STH control guidelines and programmes on the potential benefit of using ivermectin in combination with albendazole against trichuriasis. Our findings revealed superiority in terms of cure rate and ERR of the combination therapy over albendazole monotherapy against *T trichiura* infections in Laos and Pemba Island. Yet, in Côte d'Ivoire, ivermectin–albendazole showed unsatisfactory efficacy. The trial results further highlight the need for a change in treatment for mass drug administration campaigns, since we found low efficacy of albendazole according to the WHO reference efficacy (arithmetic mean-based ERR <50%) in all three settings.[22] The observed cure rates were even lower than the initially assumed cure rate for albendazole of 30%, which might be explained by a more rigid eligibility criterion (minimal egg count of 100 EPG) and diagnostic approach (four Kato-Katz slides analysed per timepoint) than used in earlier studies. Ivermectin–albendazole showed a good safety profile, with mostly mild and transient adverse events. More moderate and severe adverse events were observed 24 h after treatment than 3 h after treatment and in the ivermectin–albendazole group than in the albendazole monotherapy group. This finding might in part be explained by the half-life of ivermectin (ie, approximately 18–28 h in humans).[23] Nevertheless, trials like this one are not powered to assess statistical differences in safety outcomes.

The efficacy of ivermectin–albendazole on Pemba Island was slightly higher in our study (cure rate 49%) than in earlier studies in Zanzibari school-aged children (cure rates 38% and 28%);[13,14] however, in a subgroup analysis in which we considered only school-aged children (6–12 years), the cure rate was similar to the earlier studies (26%). Previous trials done in Asian settings showed similar or higher cure rates for combination therapy in *T trichiura*-infected children from the Philippines (65%) and Sri Lanka (79%) compared with children from Laos (52%),[11,12] yet these data were collected around 20 years ago and the diagnostic approaches used in those studies were less rigid than the diagnostic approach we applied. While the two African trial cohorts were relatively comparable with regard to age composition and baseline infection intensity of *T trichiura*, the mean age in Laos was much older and in turn baseline EPGs were lower. Subgroup analysis has shown that baseline infection intensities have an important role in treatment efficacy and thus might in part explain why the highest cure rates for ivermectin–albendazole were found in Laos.

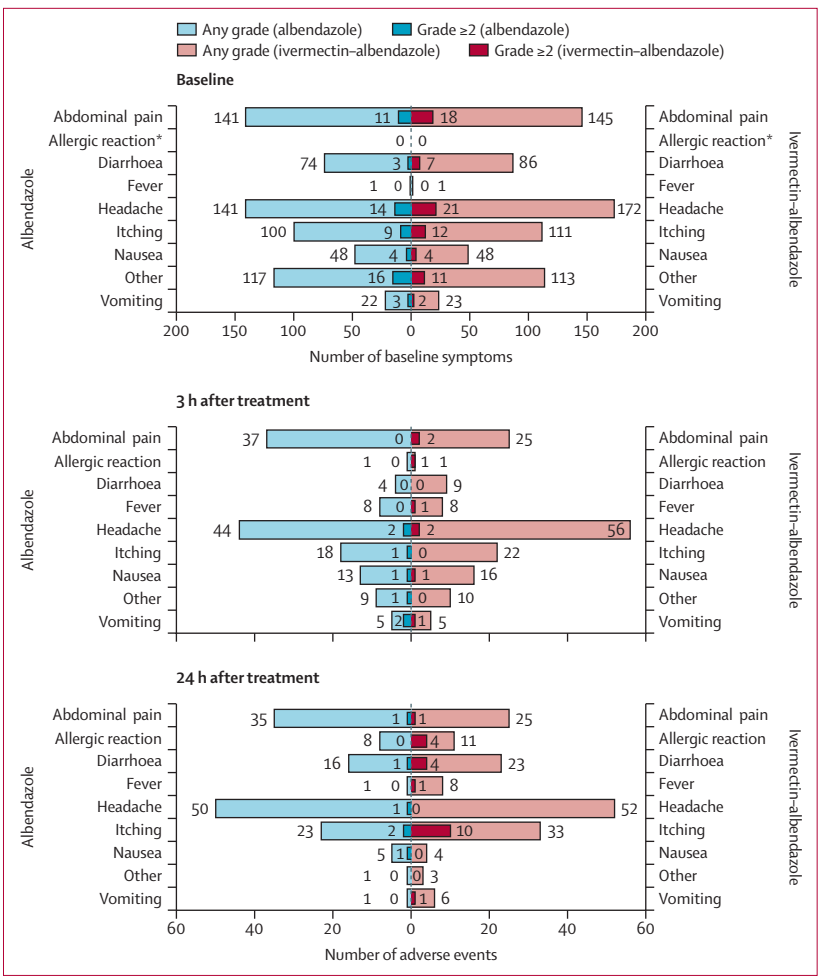

Figure 3: **Baseline symptoms and adverse events at each timepoint by group and grade**
*Not applicable at this timepoint.

The low efficacy of ivermectin–albendazole against *T trichiura* in Côte d'Ivoire warrants further investigation. Several patient factors might influence treatment outcome. Metabolisation and drug absorption are universally accepted to play a role in drug efficacy, but in the case of intestinal parasites, direct drug exposure in the lumen might be as important to kill them. To date, the influence of changes in drug disposition of albendazole and ivermectin on efficacy have not been described. Moreover, the nutritional and immunological status, as well as the intestinal microbiome, of patients are believed to have a potential impact on pharmacokinetics or how effectively parasitic infections are cleared.[24,25]

Acquired drug resistance might be another explanation for treatment failure. However, there is no evidence for resistance in human STH infections, and potential mechanisms of ivermectin resistance in *T trichiura* are yet to be fully understood.[23] Nonetheless, drug pressure might trigger anthelmintic resistance and should not be

underestimated. Côte d'Ivoire has the longest history of community-wide use of ivermectin to fight filarial diseases, compared with Laos, where to date ivermectin has not been used in any programme.[26,27] In Laos, mainly mebendazole is provided for STH control. On Pemba Island, mass drug administration against lymphatic filariasis was stopped in 2015 after six rounds.[28] We observed no typical patterns of acquired resistance in the data, such as geographical heterogeneity in efficacy, as efficacy was similarly low in villages situated within a perimeter of up to 40 km distance. Moreover, a surprisingly low efficacy of ivermectin was observed in dose-finding studies in another study area in Côte d'Ivoire.[29]

Differences in parasite genetics, causing variance in parasite defense systems (eg, drug efflux pumps and detoxification enzymes), among *T trichiura* strains might have a role in reduced treatment efficacy.[7,30] Whole-genome and amplicon sequencing of local *T trichiura* parasites or eggs might provide further insight into potential adaptations of the Côte d'Ivoire strain.[31]

The biggest limitation of our study was the reduced sensitivity of the Kato-Katz diagnosis technique compared with PCR-based diagnosis. However, to minimise potential overestimation of efficacy measures, we applied rigorous inclusion criteria for positive individuals (ie, 100 EPG and two out of four Kato-Katz slides positive). Furthermore, we performed qPCR diagnosis on samples from Pemba Island and compared efficacy measures. These findings revealed lower cure rates in PCR-based diagnosis than in diagnosis with the Kato-Katz technique, but ivermectin–albendazole still showed significantly higher cure rates against *T trichiura* than albendazole.[8] Our safety outcomes were descriptive and did not explore for multiple events nor account for differential follow-up between settings. Comparison of safety outcomes between settings should thus be interpreted with caution.

Countries affected by trichuriasis, strongyloidiasis, and scabies would clearly benefit from introduction or broader application of ivermectin–albendazole, which is currently exclusively used for onchocerciasis and lymphatic filariasis.[28] These efforts would go hand in hand with the recommendations put forth by WHO in the 2021–30 roadmap for neglected tropical diseases.[4] A current obstacle for use of ivermectin–albendazole in STH programmes is the prohibitive costs of good-quality ivermectin and the absence of donations for this purpose; the Mectizan Donation Program covers only lymphatic filariasis and onchocerciasis.[4] Challenges for community-wide ivermectin–albendazole implementation include administration to identified at-risk groups (preschool-aged children and pregnant and lactating women).[3] Ivermectin is not recommended for children shorter than 90 cm or weighing less than 15 kg, pregnant women, or lactating women in the first week after birth.[9] New evidence on ivermectin safety and field-applicable solutions for dosing are needed. Simultaneously, efforts should be made to identify and test alternative treatment options to effectively treat infections in areas with low ivermectin–albendazole efficacy against *T trichiura*. Alternative drug combination candidates could be oxantel pamoate or moxidectin with albendazole against *T trichiura* infections.[8]

For more on the **Mectizan Donation Program** see https://mectizan.org/

In conclusion, ivermectin–albendazole, the only approved and available combination anthelmintic therapy, is a valuable and safe alternative treatment to albendazole monotherapy, which showed low efficacy against *T trichiura* in all settings. Future trials might look at effectiveness rather than efficacy using cluster-randomised designs, allowing for inclusion of bigger sample sizes and more conclusive safety comparisons. Current diagnosis to assess STH efficacy in trials should be complemented by molecular techniques (eg, qPCR) in at least a subsample from each trial site to better depict the real-life performance.

Contributors

EH, JH, and JK designed the study. EH, LK, CP, SW, SMAl, SMAm, SS, JTC, and JK planned the study. EH, LK, CP, SW, SMAl, SMAm, SS, JTC, and JK implemented the study. EH, JH, and JK analysed and interpreted the trial data and wrote the first draft of the manuscript. LK, CP, SW, SMAl, SMAm, SS, and JTC revised the manuscript. All authors read and approved the final version of the manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit for publication. EH, LK, CP, SW, and JH accessed and verified the data.

Declaration of interests

We declare no competing interests.

Data sharing

The study protocol is available on ClinicalTrials.gov, NCT03527732, and in appendix 1, together with the statistical analysis plan, which also includes key elements of the analysis code. Individual deidentified participant data that underlie the results reported in this Article will be available upon request directly after publication, with no end date. Supporting clinical documents, including approval of the proposals and the informed consent form plan, will be made available upon request immediately following publication for at least 1 year. Access will be granted to researchers who provide a methodologically sound proposal. The sponsor, investigators, and collaborators will approve the proposals on the basis of scientific merit. Requests should be directed to the corresponding author (jennifer.keiser@swisstph.ch). Researchers who request data will need to sign a data access agreement before they are granted access.

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Supplementary appendix

Tables of contents

Multiple imputation in Laos

In Laos, the proportion of missing data exceeded the threshold of 10%. Therefore, the available case analysis was complemented by an intention to treat analysis using multiple imputation (as implemented in R's mi package).

Participants' sex, age, height, weight, *T. trichiura* egg counts at baseline and treatment arm were used to impute the infection status (cured) at follow-up. Except the variable infection status at follow-up (142 values missing) none of the other variables had missing values.

The imputation algorithm generated 20 imputed datasets (30 iterations, 6 chains). Imputation performance was assessed via the R-hat statistics and convergence was visually inspected. Imputation performance was assessed via the R-hat statistics and convergence was visually inspected. Imputation diagnostics indicated convergence after 30 iterations ($\widehat{R} = 1.006$).

Below point and interval estimates are provided for the available case on the imputed intention to treat analysis (unadjusted logistic regression).

Table S1. Comparison of odds ratios from unadjusted logistic regression analysis of available case and intention to treat population samples in Laos

Analysis population	N ALB	N IVM-ALB	Odds ratio	95% CI
Available case	194	213	21.3	12.2 – 39.5
Intention to treat (multiple imputation)	274	275	21.1	12.0 – 37.3

Table S2. Sub group analysis

Subgroup	**N**	**Baseline EPG Median (Q1-Q3)**	**CR ALB % (N_{cured}/N_{total})**	**CR IVM-ALB % (N_{cured}/N_{total})**	**Difference %-points**	**95% CI**
Age 6 to 12 years	467	444 (204-1236)	8% (17/224)	26% (64/243)	18.7	12 - 25
Age 13 years and older	407	264 (159-525)	11% (23/205)	53% (108/202)	42.2	34 – 50
Light baseline infection intensity	687	246 (156-441)	11% (37/339)	45% (156/348)	33.9	28 - 40
Moderate/heavy baseline infection intensity	187	1908 (1293-3330)	3% (3/90)	16% (16/97)	13.2	5 – 22

ALB, albendazole; CI,: confidence interval; CR, cure rate; IVM-ALB, ivermectin-albendazole; Q1-Q3, interquartile range.

Table S3. Specific adverse events by treatment arm and time point in Côte d'Ivoire

	Baseline						3h post-treatment						24h post-treatment					
	ALB (n=254)		IVM-ALB (n=254)		TOTAL (n=508)		ALB (n=253)		IVM-ALB (n=253)		TOTAL (n=506)		ALB (n=227)		IVM-ALB (n=231)		TOTAL (n=458)	
Adverse event	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2
Abdominal pain	102	11	96	17	198	28	15	0	17	1	32	1	20	0	15	1	35	1
Allergic reaction	NA	NA	NA	NA	NA	NA	1	0	0	0	1	0	8	0	11	4	19	4
Diarrhea	51	3	66	7	117	10	3	0	9	0	12	0	10	1	20	3	30	4
Fever[a]	0	0	0	0	0	0	0	0	3	0	3	0	0	0	5	1	5	1
Headache	76	7	88	13	164	20	14	0	16	1	30	1	15	0	20	0	35	0
Itching	84	5	96	8	180	13	18	1	19	0	37	1	19	1	28	6	47	7
Nausea	37	2	32	3	69	5	7	0	7	0	14	0	3	0	4	0	7	0
Other[b]	97	6	101	7	198	13	1	0	2	0	3	0	1	0	1	0	2	0
Vomiting	20	1	19	2	39	3	2	0	4	0	6	0	1	0	6	1	7	1
Total adverse events	467	35	498	57	965	92	61	1	77	2	138	3	77	2	110	16	187	18

ALB, albendazole; IVM-ALB, ivermectin-albendazole; NA, not applicable.

[a] Fever; defined as body temperature ≥38°C. Assessed in 508, 503 and 422 participants at baseline, 3h and 24h time points, respectively. Fever was graded in grade1=38-39°C, grade2=39-40°C, grade3=>40°C (<24h) and grade4=>40°C (>24h).

[b] Other baseline symptoms include: reported fever (n=57), cough (n=55), rhinorrhea (n=33), body pain (n=17; including myalgia, arthralgia, lumbago, thoracic pain, limbs, scrotum), vertigo (n=11), ear/eye/throat problems (n=4; including: otalgia, ophthalmalgia, dysphagia, drooling), heartburn (n=4), asthenia/fatigue (n=3), anorexia (n=2), sneezing (n=2), dental problems (n=2; including tooth pain and decay), cardiovascular symptoms (n=2; including high BP and palpitations), psychomotor retardation (n=2), bronchitis (n=1), blood in stool (n=1), insomnia (n=1) and malaria (n=1). Other 3h symptoms: lumbago (n=1), myalgia (n=1), vertigo (n=1). Other 24h symptoms: liquid or foul-smelling stool (n=2).

Table S4. Specific adverse events by treatment arm and time point in Laos

	Baseline						3h post-treatment						24h post-treatment					
	ALB (n=273)		IVM-ALB (n=273)		TOTAL (n=546)		ALB (n=273)		IVM-ALB (n=273)		TOTAL (n=546)		ALB (n=247)		IVM-ALB (n=247)		TOTAL (n=494)	
Adverse event	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2
Abdominal pain	14	0	19	0	33	0	**10**	**0**	**2**	**1**	12	1	12	1	8	0	20	1
Allergic reaction	NA	NA	NA	NA	NA	NA	0	0	0	0	0	0	0	0	0	0	0	0
Diarrhea	14	0	10	0	24	0	0	0	0	0	0	0	6	0	3	1	9	1
Fever[a]	1	0	1	0	2	0	8	0	5	1	13	1	1	0	3	0	4	0
Headache	51	7	67	8	118	15	23	1	35	1	58	2	34	1	28	0	62	1
Itching	7	2	10	4	17	6	0	0	2	0	2	0	2	1	5	4	7	5
Nausea	3	2	2	0	5	2	2	1	8	1	10	2	2	1	0	0	2	1
Other[b]	18	10	8	4	26	14	5	1	6	0	11	1	0	0	2	0	2	0
Vomiting	2	2	0	0	2	2	2	1	1	1	3	2	0	0	0	0	0	0
Total adverse events	110	23	117	16	227	39	50	4	59	5	109	9	57	4	49	5	106	9

ALB, albendazole; IVM-ALB, ivermectin-albendazole; NA, not applicable.

[a] Fever; defined as measured body temperature ≥38°C. Assessed in 545, 542 and 494 participants at baseline, 3h and 24h time points, respectively. Fever was graded in grade1=38-39°C, grade2=39-40°C, grade3=>40°C (<24h) and grade4=>40°C (>24h).

[b] Other baseline symptoms: migraine (n=6), reported fever (n=5), cough (n=3), body pain (including lumbago, thoracic pain) (n=4), vertigo (n=3), common cold (n=2), anorexia (n=1), gonorrhea (n=1), skin rash on limbs (n=1). Other 3h symptoms: vertigo (n=9), fatigue (n=1), hot sensation of face (n=1). Other 24h symptoms: heart burn (n=1), vertigo (n=1).

Table S5. Specific adverse events by treatment arm and time point on Pemba Island

	Baseline						3h post-treatment						24h post-treatment					
	ALB (n=303)		IVM-ALB (n=304)		TOTAL (n=607)		ALB (n=303)		IVM-ALB (n=308)		TOTAL (n=611)		ALB (n=305)		IVM-ALB (n=308)		TOTAL (n=613)	
Adverse event	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2	No., Any grade	No., Grade ≥2
Abdominal pain	25	0	30	1	55	1	12	0	6	0	18	0	3	0	2	0	5	0
Allergic reaction	NA	NA	NA	NA	NA	NA	0	0	1	1	1	1	0	0	0	0	0	0
Diarrhea	9	0	10	0	19	0	1	0	0	0	1	0	0	0	0	0	0	0
Fever[a]	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache	14	0	17	0	31	0	7	1	5	0	12	1	1	0	4	0	5	0
Itching	9	2	5	0	14	2	0	0	1	0	1	0	2	0	0	0	2	0
Nausea	8	0	14	1	22	1	4	0	1	0	5	0	0	0	0	0	0	0
Other[b]	2	0	4	0	6	0	3	0	2	0	5	0	0	0	0	0	0	0
Vomiting	0	0	4	0	4	0	1	1	0	0	1	1	0	0	0	0	0	0
Total adverse events	67	2	84	2	151	4	28	2	16	1	44	3	6	0	6	0	12	0

ALB, albendazole; IVM-ALB, ivermectin-albendazole; NA, not applicable.

[a] Fever; defined as body temperature ≥38° C. Assessed in 613, 610 and 612 participants at baseline, 3h and 24h time points, respectively.

[b] Other symptoms include: dizziness (n=2), cough (n=1), hemoptysis (n=1), feeling feverish (n=1), wrist pain (n=1) at baseline; dizziness (n=3), abdominal discomfort (n=1), feeling feverish (n=1) 3h post-treatment.

5. Assessment of fecal calprotectin and fecal occult blood as point-of-care markers for soil-transmitted helminth attributable intestinal morbidity in a case-control substudy conducted in Côte d'Ivoire, Lao PDR and Pemba Island, Tanzania

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Research Paper

Assessment of fecal calprotectin and fecal occult blood as point-of-care markers for soil-transmitted helminth attributable intestinal morbidity in a case-control substudy conducted in Côte d'Ivoire, Lao PDR and Pemba Island, Tanzania

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ABSTRACT

Background: Infections with soil-transmitted helminths (STHs) may result in chronic inflammatory disorders affecting the human host. The objective of this study was to evaluate Fecal Calprotectin (FC) and Fecal Occult Blood (FOB) in individuals infected and non-infected with STHs to identify potential intestinal morbidity markers.
Methods: Stool from participants diagnosed positive for *Trichuris trichiura* and concomitant STH infections from three countries was used to perform FC and FOB point-of-care assays. Simultaneously, identified STH negative participants underwent FC and FOB testing as controls. Potential associations between test results and determinants were analyzed using multivariable logistic regression.
Findings: In total, 1034 *T. trichiura* infected cases (mostly light infections) and 157 STH negative controls were tested for FC and FOB. Among all participants tested, 18·5% had $\geq$ 50 μg/g FC concentration, while 14 (1·2%) were positive for FOB. No statistically significant association was found between *T. trichiura* infection or *Ascaris lumbricoides* co-infection and FC concentration, while an inverse association (odds ratio (OR): 0·45, 95% credible intervals (CrI): 0·26, 0·75) was found between hookworm co-infection and FC concentration. In Lao PDR, the proportion of participants in the $\geq$ 50 μg/g FC category was significantly higher in the oldest age category compared to the 5–11 years group (OR: 3·31, 95% CrI: 1·62, 7·24). Too few participants were found positive for FOB to derive any conclusions.
Interpretation: Studies are needed to better understand the relationship between intestinal morbidity and STH infections. Suitable, standardized, low-cost markers of STH attributable morbidity to better monitor the impact of STH control interventions are necessary.
Funding: BMGF (OPP1153928)

1. Introduction

More than 1·5 billion people worldwide are infected with soil-transmitted helminths (STHs), namely *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura* [1]. Helminthiases typically affect the poorest and the most marginalized populations, particularly in tropical and subtropical regions, where access to water, sanitation, and hygiene is inadequate [2,3]. STH infections manifest, if left untreated, as generally asymptomatic chronic infections causing both concurrent and delayed-onset pathologies affecting the human host [4]. Chronic infections and/or infections of heavy intensities can result in malnutrition, malabsorption, reduced growth rate, intestinal obstruction, poor iron status, and iron deficiency

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Research in context

Evidence before this study

Long-term infections with soil-transmitted helminths (STHs) contribute to substantial morbidity; however, evidence on appropriate point-of-care indicators of STH attributable morbidity is scarce. We searched in PubMed for all articles published before Nov 2, 2020 which mentioned "helminth", *"Trichuris trichiura"*, "*Ascaris lumbricoides*", or "hookworm" and "fecal calprotectin" or "fecal occult blood" in the abstract, without language restrictions. Contradictory findings result from a small body of evidence (nine studies) on the association between STH infection status, infection intensity and intestinal morbidity, using Fecal Calprotectin (FC) or Fecal Occult Blood (FOB) as indicators. Only one study used novel rapid diagnostic immunoassay tests for FOB detection, while none had a sufficient sample size to derive meaningful conclusions.

Added value of this study

This is the first large scale study testing FC and FOB as potential proxy markers for STH attributable intestinal morbidity in three different countries. No association between the presence of intestinal inflammation or mucosal bleeding, assessed with FC and FOB, and *T. trichiura* and *A. lumbricoides* infection status was found, while a negative association between FC concentration and hookworm infection was found.

Implications of all the available evidence

Further research should focus on the development and evaluation of potential morbidity markers, as the appropriate monitoring of STH attributable morbidity might become as important as diagnosing the infection itself. Thus, appropriate indicators of helminth attributable morbidity are still lacking.

anemia [4–9], particularly in those on marginal diets [10]. Long-term consequences include subtle effects on cognition [11], educational performance [12], and school absenteeism [13], impacting individuals' workforce potential, and economic progress among affected groups [14].

Embryonated *T. trichiura* eggs hatch in the small intestine and potentially attach to the mucosa in the large intestine, whereas larvae penetrate the epithelial cells for subsequent growing and molting into adult stages [15]. Adult worms embed their head part into a intracellular niche in the large intestine [16] causing petechial lesions, blotchy mucosal hemorrhage, and oozing leading to both mucosal and systemic immune responses [17–19]. Similarly, hookworm larvae use their cutting plates to attach to the mucosa to begin feeding and molting into adult worms, attributing to intestinal morbidity [20]. In contrast, *A. lumbricoides* feeds passively and never attaches directly to the mucosa [4,21].

The present goal of global control programs recommended by the World Health Organization (WHO), is to reduce morbidity with preventive chemotherapy (i.e. administration of albendazole or mebendazole without prior diagnosis) to at-risk populations (i.e. school-aged children and women of reproductive age) with accompanying improvements in access to clean water and sanitation to reduce worm burden associated morbidity [22]. Inherent with such control interventions is the necessity to define and validate indicators of helminth attributable morbidity. Even though in most cases morbidity is associated with infecton intensity [23], assessed by classic microscopic diagnosis detecting eggs in the feces, appropriate morbidity parameters are of pivotal importance as they provide additional information on the degree to which the STH infection affects the patient. This is particularly important after anthelmintic treatment, when intensity of infection has reduced markedly, but morbidity still is present [24]. A better clinical understanding of measurable reductions in STH attributable morbidity in response to anthelmintics is needed to appropriately shape and evaluate ongoing or future STH control programs [25].

Fecal biomarkers are promising non-invasive indicators possibly reflecting mucosal inflammation or damage, as molecules from the intestinal mucosa are transported in passing with the feces [26]. It is a well-known phenomenon that the occurrence of blood in feces can be indicative of pathologic changes, especially in the context of malignancies or inflammation [27].

Fecal Calprotectin (FC), a neutrophil cytoplasmic multimeric complex of the calcium-binding proteins, is abundant in neutrophil granulocytes, monocytes, and early stage macrophages [28,29]. Translocation of these cells into the intestinal mucosa and degranulation inside the intestinal lumen leads to increased secretion of FC as a response to local inflammation [30,31]. Given the fact that FC levels are stable in feces and not influenced by systemic infections, FC is an interesting biomarker for understanding the association between intestinal infection and inflammation by measuring the localized intestinal inflammation [19,32–34]. Therefore, FC is widely used across gastroenterology practices as a non-invasive surrogate marker for disease activity and response to treatment [35,36]. In the past years, its use in enteric infections is increasing, particularly as a correlative marker for clinical severity and in evaluating bacterial and viral pathogens [37].

Fecal blood is a late symptom of inflammatory tissue damage [30]. For example, blood loss due to *T. trichiura* infections has been estimated to be 5 μL per adult worm per day [38]. Thus, fecal occult blood (FOB) might be another candidate for a morbidity marker of helminthiasis. FOB tests have been previously used for identifying blood loss in hookworm infection, trichuriasis, and intestinal schistosomiasis [39–42]. However, most studies used guaiac-based FOB tests that are known to be less sensitive than immunochemical assays [43].

Rapid immunological FC and FOB dipstick tests are non-invasive, easily preserved, and reliable diagnostic tools allowing immediate detection of FC for diagnosing intestinal inflammation and FOB for occult blood in the feces indicating intestinal morbidity [44]. Characteristics and applicability of field-appropriate diagnostic tools using proxy markers for STH morbidity are not well investigated to date [42]. Moreover, previous work on morbidity indicators is mostly limited to research on schistosomiasis causing gastrointestinal morbidity [41–43,45–47]. Although, some case reports suggest mucosal damage as a consequence of STH infections [48,49], evidence on the association between infection and intestinal inflammation is lacking [8].

The objective of the current study was to investigate FC and FOB as potential STH gut morbidity markers as a tool to monitor the impact of community-level deworming. Using FC and FOB as biomarkers, we aimed at assessing the potential association between the presence of intestinal inflammation and STH infection status.

2. Methods

2.1. Study design

The presented data derive from a randomized controlled trial (RCT) assessing efficacies of ivermectin-albendazole and albendazole alone against *T. trichiura* and concomitant STH infections in participants aged 6–60 years. The study was conducted in communities in the Lagunes region in Côte d'Ivoire, in the Luang Prabang Province in Lao PDR, and in South Pemba on Pemba Island, Tanzania during the screening period (Nov 2018 to Dec 2019) of the trial. Prior to study initiation, ethical clearance was granted by the Ethics Committee of Northwestern and Central Switzerland (EKNZ; reference no: BASEC Nr Req-2018–00494), the Zanzibar Medical Research and Ethics Committee (ZAMREC, reference no.: ZAMREC/0003/Feb/2018), the Comité National d'Éthique et de la Recherche, Ministère de la Santé et de Lutte contre le SIDA (reference no.: 088–18/MSHP/CNESVS-

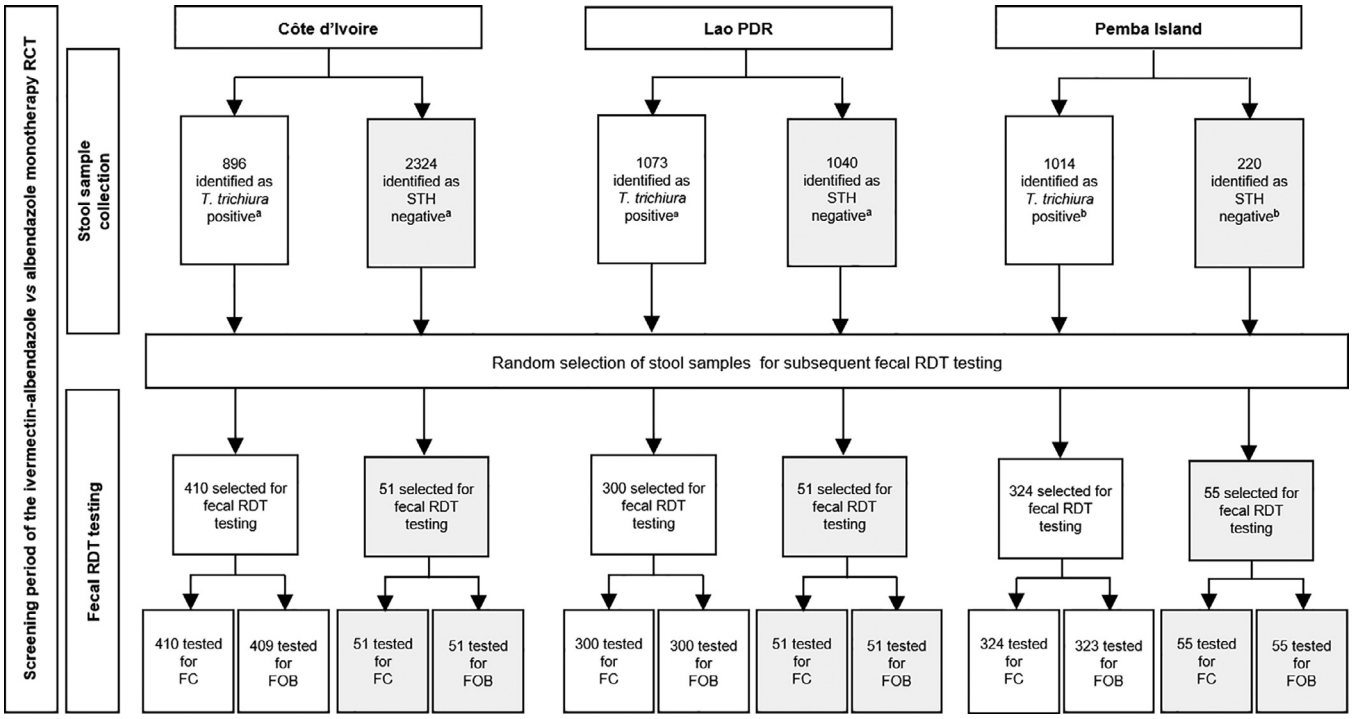

Fig. 1. Study design.

Abbreviations: *T. trichiura, Trichuris trichiura*; FC, Fecal Calprotectin, FOB; Fecal Occult Blood; Lao PDR, Lao People's Democratic Republic; RCT, Randomized Controlled Trial; RDT, Rapid Diagnostic Test; STH, Soil-transmitted helminth

[a] Positive or negative according to the first and second stool sample; second stool sample was used for subsequent fecal RDT

[b] Positive or negative according to the first stool sample, which was used for subsequent fecal RDT on the same day.

km), the Direction de la Pharmacie, du Médicament et des Laboratoires (reference no. ECCI00918) in Côte d'Ivoire, and the National Ethics Committee for Health Research, Ministry of Health in Lao PDR (reference no. 093/NECHR). Trial and study details are summarized in the published trial protocol [50] and in the trial registration (clinicaltrials.gov, reference: NCT03527732, date assigned: 17 May 2018).

Prior to study enrollment, all inhabitants of the chosen villages were invited to information sessions at local places, during which the research staff explained the purpose and procedures of this study, as well as the potential benefits and risks of participation. Written informed consent was obtained from adults and parents or legal guardians of children below the age of adulthood (21 years in Côte d'Ivoire and 18 years in Lao PDR and Pemba Island). Children aged below the age of adulthood gave written assent (Côte d'Ivoire) or oral assent (Lao PDR and Pemba Island).

2.2. Study procedures

A short census was conducted at the start of screening, during which the name, sex, age, and village name were recorded for all participants. Consenting and eligible participants (aged 6–60, as the only inclusion criteria for screening) were asked to provide two fresh morning stool samples (as per inclusion criteria), preferably on consecutive days, in containers labelled with their assigned unique ID. Collected stool samples were kept in a cool box containing ice packs while being transported to the field laboratory.

Duplicate Kato-Katz thick smears ($2 \times 41{\cdot}7$ mg of stool) were prepared and examined under a microscope by experienced laboratory technicians for species-specific diagnosis of STH ova (i.e. *T. trichiura, A. lumbricoides,* and hookworm) within 60 min after preparation to avoid over-clearing of hookworm eggs [51] following the World Health Organization (WHO) standard procedures [52]. Additionally, in Lao PDR, Kato-Katz thick smear slides were examined for *Opisthorchis viverrini* infection and infections of *Strongyloides stercoralis* were classified as larvae-positive or negative using the Baermann technique [53]. To assure high quality of the microscopic evaluation, 10% of all Kato-Katz slides were randomly chosen, re-labeled, and re-examined for *A. lumbricoides* and *T. trichiura*. In brief, microscopic results were considered inconsistent if there was a difference in presence/absence of a specific helminth species, or if differences in egg counts exceeded (i) 10 eggs for Kato-Katz thick smears with ≤ 100 eggs, or (ii) exceeded 20% for Kato-Katz thick smears with > 100 eggs. In case of discrepancies between the original and the quality control read, slides were read by a third independent microscopist and results were discussed until consensus was reached [54].

Of those found positive for *T. trichiura*, a random subsample of unique ID numbers (generated by a co-investigator not involved in the laboratory work) were chosen to undergo FC and FOB testing the same day. At the same time, of those participants found negative for *T. trichiura, A. lumbricoides*, and hookworm, a subsample of approximately 50 participants were chosen at random (using a list generated in the same manner as described above) in each setting to undergo FC and FOB testing. Participants who were found not to be infected with *T. trichiura*, but were infected with *A. lumbricoides* and/or hookworm infections were not included in this study. All included participants donated two fecal samples in order to increase the precision of the STH infection result. On Pemba Island, the first of the two fecal sample was used for subsequent rapid diagnostic testing (RDT). Due to the low prevalence of *T. trichiura* infections in the communities chosen in Lao PDR and Côte d'Ivoire, it was decided to use the second donated stool sample to ensure participants in this study would also be included in the larger trial, which had a minimum infection intensity cut-off of 100 *T. trichiura* eggs per gram of stool.

Cases that were subsequently enrolled in the clinical trial, were clinically examined by a physician and their height, weight, and hemoglobin levels recorded. However, negative controls, whom were not eligible for trial inclusion, did not undergo clinical examination.

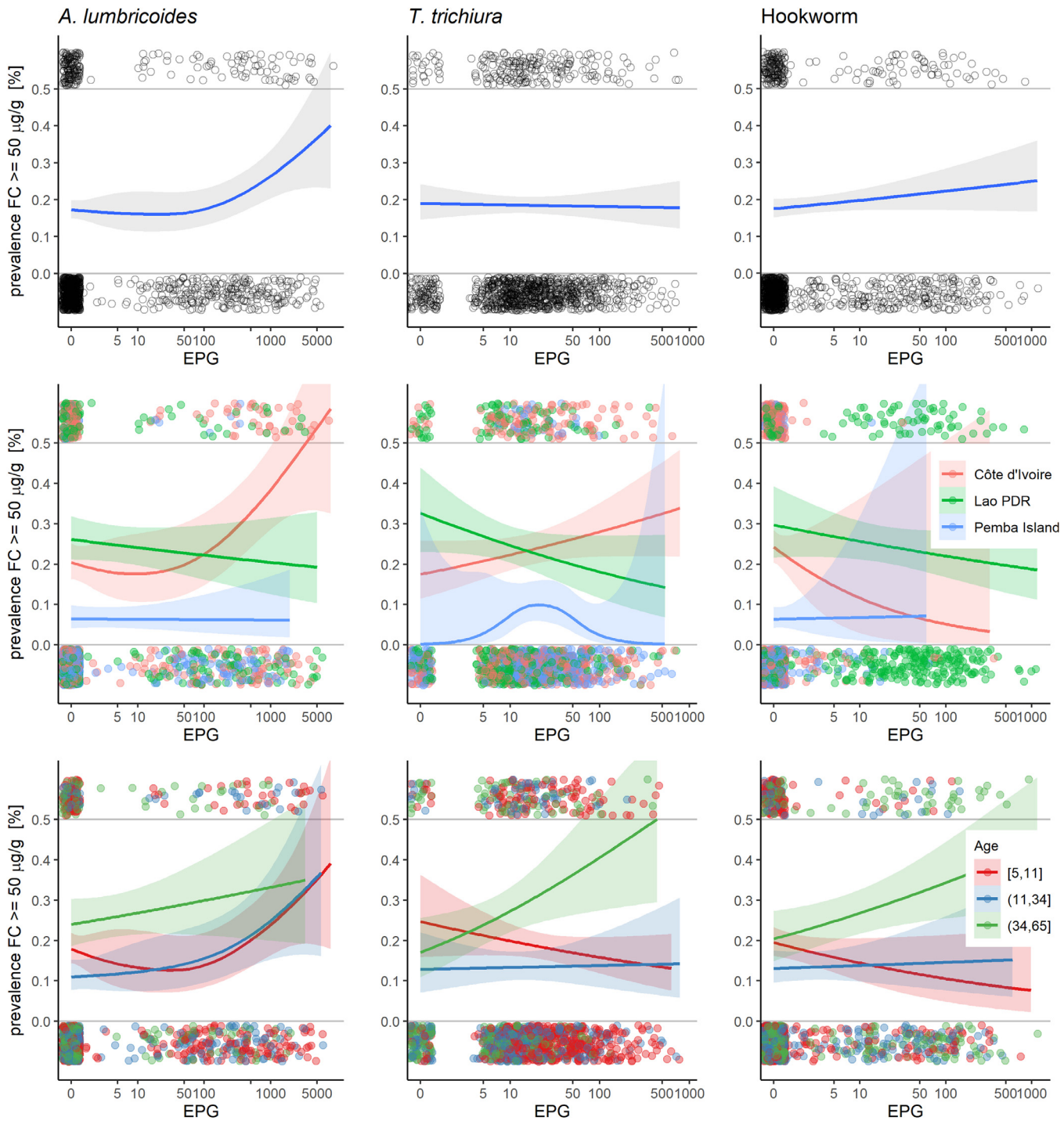

Fig. 2. Prevalence of ≥ 50 μg/g FC concentration by EPG for *Trichiura trichuris* infections, *Ascaris lumbricoides*, and hookworm co-infections, and, in addition, by country (middle row), and age group (bottom row).

Abbreviations: *A. lumbricoides, Ascaris lumbricoides*; EPG, eggs per gram; FC, fecal calprotectin; *T. trichiura, Trichuris trichiura*.

2.3. Point-of-care tests: fecal calprotectin and fecal occult blood

A semi-quantitative chromatographic immunoassay (Actim® Fecal Calprotectin test, Medix Biochemica, Finland) was applied for FC detection according to the manufacturer's instructions. In brief, the participant number was first written on the Specimen Dilution Buffer tube (3 mL) before unscrewing. The sampling stick, attached to the cap, was first twisted into different places of the fecal sample in order to make sure that both slits at the head of the stick contained stool. The sampling stick was put back in the tube and shaken to suspend the feces in the buffer. A dipstick was then inserted into the perforation area of the buffer tube before inverting the tube by 90° for two seconds. The tube was then placed on a flat surface in an upright position. Results were read after ten minutes with one test line indicating a valid test result with a FC level < 50 μg/g, two lines indicating a level of 50–200 μg/g, and three lines indicating a level > 200 μg/g of FC concentration in the test specimen based on reference values provided by the manufacturer. FC levels < 50 μg/g were interpreted as no inflammation, 50–200 μg/g of FC as possible inflammation and > 200 μg/g of FC as likely active inflammation, similar to suggestions by Bressler et al [55].

Table 1
Baseline characteristics of *T. trichiura* positive cases and STH negative controls.

	T. trichiura positive, n (%)	STH negative, n (%)
Total of participants included, N. (%)[a]	1034 (86·8)	157 (13·2)
Females, n (%)	533 (51·5)	104 (66·2)
Mean age, years (SD)	18·7 (15·0)	30·1 (16·2)
Mean BMI, kg/m^2 (SD)[b]	18·1 (4·6)	NA
Mean hemoglobin, g/L (SD)[b]	124·6 (14·5)	NA
T.trichiura infection		NA
Geometric mean EPG	492·0	
Infection intensity[c], n (%)		
Light	784 (75·8)	
Moderate	240 (23·2)	
Heavy	10 (1·0)	
A. lumbricoides co-infection		NA
Infected, n (%)	345 (33·3)	
Geometric mean EPG	4317·9	
Infection intensity[c], n (%)		
Light	167 (48·4)	
Moderate	150 (43·5)	
Heavy	28 (8·1)	
Hookworm co-infection		NA
Infected, n (%)	338 (32·7)	
Geometric mean EPG	559·4	
vInfection intensity[c], n (%)		
Light	253 (74·9)	
Moderate	49 (14·5)	
Heavy	36 (10·7)	
S. stercoralis co-infection[d]		NA
Infected/Surveyed, n (%)	49/277 (17·7)	
O. viverrini co-infection[d]		NA
Infected, n (%)	54/300 (18·0)	

Abbreviations: *A. lumbricoides, Ascaris lumbricoides*; BMI, body mass index; EPG, eggs per gram; NA, not applicable; *O. viverrini, Opisthorchis viverrini*; SD, standard deviation; *S. stercoralis, Strongyloides stercoralis*; *T. trichiura, Trichuris trichiura*.

[a] Baseline characteristics were assessed for 1191 participants surveyed for FC. Additionally, 2 participants missed FOB testing, however, baseline characteristics between FC and FOB are thus very similar.

[b] BMI and hemoglobin was only assessed in participants attending subsequent clinical examination.

[c] Infection intensities were classified according to WHO recommendations, based on guidelines established by Montresor et al. (1998).

[d] *O. viverrini* and *S. stercoralis* co-infections were only assessed in Lao PDR.

A simple immunochemical test was applied for FOB (Actim® Fecal Blood test, Medix Biochemica, Finland) detection, following the manufacturer's instructions. Unlike traditional guaiac tests, this test is based on highly specific monoclonal antibodies that only detect human hemoglobin, thus, food substances containing hemoglobin or peroxidase activity do not influence test results. Test procedure was similar to the aforementioned FC test, however; positive results were readable immediately as soon as two blue lines appeared, negative samples remained with only one blue line after ten minutes. Detailed test characteristics (e.g. sensitivity and specificity) are summarized by the manufacturer and published online [56].

Fecal rapid tests were conducted by laboratory personnel not involved in assessing parasitological data and masked to the identity of participants providing samples. The results from each test were then recorded on a personal log form by the technician involved in the fecal rapid diagnostic testing. If no control line appeared, the result was recorded as invalid and was repeated with a new dipstick.

2.4. Outcomes

The objective of this study was to evaluate FC and FOB as potential STH gut morbidity markers. Adjusted odds ratios of FC and FOB values were calculated for STH infected participants compared to STH negative controls.

2.5. Sample size

We aimed for a total sample size of 1050: 300 *T. trichiura* positive individuals and 50 individuals negative for all STH infections per country. This study was conducted within the framework of a RCT; therefore, no separate sample size calculation has been conducted. Since prevalence of FC and FOB is unlikely very rare and varies drastically by setting, a sample size of above 1000 was deemed to be sufficient. Moreover, experience has shown that the study would be sufficiently powered unless the outcome or the exposure is rare. We estimated we would be able to detect a halving of risk with at least 80% power if the prevalence in the non-exposed group is at least 10% or higher.

2.6. Statistical analysis

Data were double entered into EpiInfo 3.5.4 by two independent data clerks and crosschecked using the data compare tool of EpiInfo 3.5.4 Any discrepancies between the two datasets were resolved by consulting the original hardcopy. Descriptive analysis was done in Stata IC 15 (StataCorp.; College Station, TX), whereas all statistical estimations were performed in R 3.5.1 (RStudio, PBC, Boston, MA). Proportions of infection with *T. trichiura* and co-infections with *A. lumbricoides* and/or hookworm were assessed for all participants in all three settings, while for Lao PDR *O. viverrini* and *S. stercoralis* co-infections were additionally assessed.

Results from the duplicate Kato-Katz smears from each of the two stool samples were summed and multiplied by a factor of six to be expressed as mean egg count per gram (EPG) of feces. Associations between FC with participant's age, sex, country, and parasitic infections was assessed using Bayesian logistic regression with logit link to estimate odds ratios (ORs) with corresponding 95% credible intervals (CrI). The Bayesian framework - as implemented in the R package 'rstanarm' (v 2.19.3) - was preferred to avoid potential quasi separation problems. We used the default weakly informative prior distributions for all parameters, i.e. normal priors with mean 0 and standard deviation 2·5. The Markov chain Monte Carlo alogorithm drew 1000 samples from 4 chains after a warm-up of additional 1000 samples per chain. Convergence was assessed by the R-hat statistic. Smoothing lines in figures were predicted via generalized additive models for binomial data. Due to the low number of individuals (n = 14/1189) testing positive for FOB, it was decided to forego formal statistical testing and present the results descriptively.

2.7. 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.

3. Results

3.1. Participants characteristics

Fig. 1 shows the study design by setting. Out of the 6567 participants screened, 2983 (45·4%) were positive for *T. trichiura* infection and 3584 (54·6%) were negative for all STH infections. From these, 1034 of the 2983 *T. trichiura* positive cases and 157 of the STH negative cases were randomly selected for FC and FOB testing.

Baseline parasitological and demographic characteristics of participants surveyed for FC and FOB are summarized in Table 1. Of those participants infected with *T. trichiura* (n = 1034), 51·5% were female, while the mean age of those was 18·7 (±15·0) years. The identified STH negative participants (n = 157) were slightly older with a mean age of 30·1 (± 16·2), while 66·2% of those participants were female.

Table 2
Test results of fecal calprotectin and fecal occult blood in *T. trichiura* positive and STH negative participants.

	Fecal calprotectin				Fecal occult blood	Total N(%)
	< 50 μg/g	50–200 μg/g	> 200 μg/g	Negative	Positive	
Number of test results, n (%)	971 (81·5)	167 (14·0)	53 (4·5)	1175 (98·8)	14 (1·2)	1191[a] (100)
***T. trichiura* positive, n (%)**	842 (86·7)	149 (12·5)	43 (3·6)	1020 (98·8)	12 (1·2)	1034 (100)
Females, n (%)	436 (81·8)	77 (14·4)	20 (3·8)	520 (97·9)	11 (2·1)	533 (100)
Mean age, years (SD)	18·0 (14·3)	22·1 (17·5)	21·1 (17·4)	18·6 (14·9)	29·6 (18·2)	18·8 (15·0)
Mean BMI, kg/m^2 (SD)[b]	18·0 (4·5)	18·9 (5·1)	18·0 (4·5)	18·1 (4·6)	19·2 (3·7)	18·1 (4·6)
Mean hemoglobin, g/L (SD)[c]	124·6 (14·5)	124·6 (14·3)	126·0 (14·9)	124·6 (14·4)	123·3 (20·6)	124·6 (14·5)
Median EPG (IQR)	408 (216–948)	360 (210–966)	390 (186–720)	405 (210–960)	279 (138–432)	402 (210–948)
Co-infections with *A. lumbricoides*						
Positive (%)	273 (78·9)	54 (15·6)	19 (5·5)	343 (99·1)	2 (0·9)	346 (100)
Median EPG (IQR)	4716 (1212–14,376)	9864 (1830–32,802)	10,716 (4014–25,362)	5508 (1332–18,462)	12,861 (360–25,362)	5508 (1332–18,462)
Co-infections with hookworm						
Positive (%)	271 (79·9)	49 (14·5)	19 (5·6)	330 (97·3)	9 (2·7)	339 (100)
Median EPG (IQR)	714 (186–1974)	954 (492–2064)	522 (210–2556)	729 (198–1968)	1158 (252–6042)	759 (204–2064)
Co-infections of *T. trichiura*						
with 1 other STH	442 (79·0)	87 (15·5)	31 (5·5)	548 (98·2)	10 (1·8)	560 (100)
with 2 other STHs	101 (82·1)	15 (12·2)	7 (5·7)	122 (12·0)	1 (8·3)	123 (100)
with *O. viverrini*[d]	43 (79·6)	8 (14·8)	3 (5·6)	53 (98·1)	1 (1·9)	54 (100)
with *S. stercoralis*[d]	39 (79·6)	7 (14·3)	3 (6·1)	48 (98·0)	1 (2·0)	49 (100)
STH negative, n (%)	**129 (82·2)**	**18 (11·4)**	**10 (6·4)**	**155 (98·7)**	**2 (1·3)**	**157 (100)**
Females (%)	89 (69·0)	9 (50·0)	6 (60·0)	104 (67·1)	0 (0·0)	104 (100)
Mean age, years (SD)	31·3 (15·4)	32·4 (20·5)	20·9 (16·1)	30·1 (16·1)	30·5 (29·0)	21·1 (17·4)

Abbreviations: *A. lumbricoides, Ascaris lumbricoides*; BMI, body mass index; EPG, eggs per gram; IQR, interquartile range; *O. viverrini, Opisthorchis viverrini*; SD, standard deviation; *S. stercoralis, Strongyloides stercoralis*; *T. trichiura, Trichuris trichiura*.

[a] 1191 FC test results and 1189 FOB test results were obtained, as not enough stool was collected from two individuals to conduct FOB tests.

[b] Data was only collected in 954 individuals for FC and 953 individuals for FOB, as STH negative and other possibly eligible participants did not attend subsequent clinical examination.

[c] Data was only collected in 957 individuals for FC and 956 individuals for FOB, as STH negative and other possibly eligible participants did not attend subsequent clinical examination.

[d] *O. viverrini* and *S. stercoralis* infections were only assessed in Lao PDR.

In addition to *T. trichiura* infection, 345 (33·3%) participants were co-infected with *A. lumbricoides*, while 338 (32·7%) participants harbored a hookworm co-infection. Among those participants in Lao PDR, 18·0% were found to be co-infected with *O. viverrini* and 17·7% with *S. stercoralis*. Mean body mass index (BMI) and mean hemoglobin concentration were 18·1 (±4·6) kg/m^2 and 124·6 (±14·5) g/L, respectively.

Median EPGs were 402, 5508, and 759 for *T. trichiura* infections and *A. lumbricoides* and hookworm co-infections, respectively (Table 2). Table 3 shows that the EPG distribution differs among settings with Pemba Island, having a higher median EPG (558, IQR 330–1224) than Côte d'Ivoire (median EPG: 408, IQR 198–1068), and Lao PDR (median EPG: 252, IQR 150–543) for those with *T. trichiura* infection. *A. lumbricoides* co-infection was found in 35·9%, 36·0%, and 27·8% of study participants in Côte d'Ivoire, Lao PDR, and Pemba Island, respectively; though the proportion of *T. trichiura* infected participants with hookworm co-infection was higher in Lao PDR (91·7%) compared to Côte d'Ivoire (7·6%) and Pemba Island (9·9%).

3.2. Fecal rapid diagnostic test results

As shown in Table 2, of those diagnosed with *T. trichiura* (n = 1034), most participants (842 (86·7%)) were found to have a normal FC concentration of < 50 μg/g, while 149 (12·5%) were found to have elevated FC concentrations of 50–200 μg/g. Only a few participants (43 (3·6%)) were identified with high (> 200 μg/g) FC levels. Most participants harboring a co-infection with *A. lumbricoides* (78·9%) or hookworm (79·0%) were found to have a normal FC concentration of < 50 μg/g.

Negative controls showed a similar distribution of FC levels; the majority 129 (82·2%) had a normal FC concentration of < 50 μg/g, while 18 (11·4%) were identified with FC concentrations of 50–200 μg/g and 10 (6·4%) with high FC concentrations of > 200 μg/g. Age, Sex, BMI, and hemoglobin level were similar among the different FC levels.

During the study, 1189 participants were surveyed for FOB, while only a minority of 14 (1·2%) participants were found to be positive. Most of the participants (78·6%) tested positive for FOB were found in the Asian setting. Country specific study results are summarized in Table 3.

Fig. 2 shows the proportion of ≥ 50 μg/g concentration of FC by EPG for each STH and, additionally, by country and age group. For *T. trichiura* infections and hookworm co-infections, FC proportion of ≥ 50 μg/g remains flat as EPG increases; while there is an increase in percentage of ≥ 50 μg/g starting at 100 EPG for *A. lumbricoides* co-infections.

Results of multivariable logistic regression models are summarized in Table 4, while country-specific results are shown in S1 Table (Supplementary Table 1). When results from all three countries were combined, no association was found between FC and infection with *T. trichiura* and co-infection with *A. lumbricoides*, while hookworm co-infection was associated with lower odds (OR: 0·45, 95% CrI: 0·26, 0·75). Country was the greatest overall predictor of FC concentration with Lao PDR having a higher odds (OR: 1·77, 95% CrI: 1·09, 2·97)) of ≥ 50 μg/g FC concentration and Pemba Island having a lower odds (OR: 0·23, 95% CrI: 0·14, 0·37) of ≥ 50 μg/g FC concentration when compared, respectively, to Côte d'Ivoire. Older participants (ages 36–64 years) had a statistically significant higher odds of ≥ 50 μg/g FC concentration when compared to participants ages 5–11 years (OR: 1·49, 95% CrI: 1·00, 2·20)). In Lao PDR, the higher odds of ≥ 50 μg/g FC concentration amongst older participants was also seen (OR: 3·31, 95% CrI: 1·62, 7·24 for 36–64 age group). The raw data suggests a country × *T. trichiura* interaction. Therefore, an additional model including the interaction terms was fitted, which can be found in S2 Table (Supplementary Table 2). However, the estimated odds ratios for age categories, sex, and co-infections changed only slightly.

Table 3
Age, sex, and co-infection for participants surveyed for fecal calprotectin and fecal occult blood by country.

	Côte d'Ivoire		Lao PDR		Pemba Island	
	T. trichiura positive	all STH negative	*T. trichiura* positive	all STH negative	*T. trichiura* positive	all STH negative
Participants, n	410	51[a]	300	51[b]	324	55
Mean age in years (SD)	16·3 (13·6)	31·5 (14·8)	28·3 (17·5)	24·6 (17·5)	13·0 (8·8)	24·9 (14·3)
Age categories						
5–11 years (%)	251 (61·2)	8 (16·0)	92 (30·7)	20 (40·0)	182 (56·2)	2 (3·6)
12–34 years (%)	99 (24·2)	18 (36·0)	91 (30·3)	11 (22·0)	126 (38·9)	22 (40·0)
35–64 years (%)	60 (14·6)	24 (48·0)	117 (39·0)	19 (38·0)	16 (4·9)	31 (56·4)
Females (%)	209 (51·0)	29 (58·0)	157 (52·3)	31 (62·0)	167 (51·5)	43 (78·2)
Median EPG (IQR))	408 (198–1068)	0 (0)	252 (150–543)	0 (0)	558 (330–1224)	0 (0)
A. lumbricoides infection (%)	147 (35·9)	0 (0)	108 (36·0)	1 (2·0)[b]	90 (27·8)	0 (0)
Hookworm infection (%)	31 (7·6)	1 (2·0)[a]	275 (91·7)	0 (0)	32 (9·9)	0 (0)
O. viverrini infection (%)	ND	ND	54 (18·0)	1 (2·0)	ND	ND
S. stercoralis infection (%)	ND	ND	49 (17·7)	0 (0·0)	ND	ND
Fecal calprotectin						
$< 50\ \mu g/g$ (%)	311 (75·9)	40 (80·0)	231 (77·0)	33 (66·0)	300 (92·6)	55 (100·0)
$\geq 50\ \mu g/g$ (%)	99 (24·1)	10 (20·0)	69 (23·0)	17 (34·0)	24 (7·4)	0 (0)
Fecal occult blood[c]						
Positive (%)	2 (0·5)	0 (0)	9 (3·0)	2 (4·0)	1 (0·3)	0 (0)
Negative (%)	407 (99·5)	50 (100·0)	291 (97·0)	48 (96·0)	322 (99·7)	55 (100·0)

Abbreviations: *A. lumbricoides, Ascaris lumbricoides*; ND, not determined; *O. viverrini, Opisthorchis viverrini*; SD, standard deviation; STH, soil-transmitted helminths; *S. stercoralis, Strongyloides stercoralis*; *T. trichiura, Trichuris trichiura*.
[a] 1 of 51 participants was wrongly assigned to a STH negative sample. The hookworm co-infection was of light intensity.
[b] 1 of 51 participants was wrongly assigned to a STH negative sample. The *A. lumbricoides* co-infection was of light intensity.
[c] Only 409 and 323 assessed in Côte d'Ivoire and Pemba Island, respectively.

4. Discussion

We applied FC and FOB tests to assess the relationship between STH infections and intestinal inflammation or mucosal bleeding in three different countries. It is of pivotal importance to find an affordable, standardized and simple point-of-care test to assess STH attributable morbidity to better survey control interventions. Evidence on the association between local inflammation and STH infections is scarce; however, key problems with much of the literature are the generally small sample sizes [40,57,58] and the use of different biomarkers [19] for intestinal inflammation leading to contradictory results.

We found no associations of FC and FOB with *T. trichiura* infection and *A. lumbricoides* co-infection status and intensity in each of the three settings tested, demonstrating that FC and FOB are not good proxy markers for STH attributable gut morbidity. Known immunoregulatory properties of STHs have shown a down-regulation of host response to limit inflammation and tissue damage, which may explain our findings [59,60]. Albeit our results show a slight protective effect of hookworm co-infection, this might be attributed to the hypothesis that hookworm infection causes a dampening effect on FC levels by inhibiting neutrophils, the main calprotectin-producing cell type [61]. Of note, *N. americanus* is expected to be the predominant hookworm species in our settings, ingesting around 0·001 mL blood per day [62–65] Our results suggest age and setting are greater predictors of FC concentration than the presence of helminth infection. Though age has been thoroughly established as a predictor for inflammation in the body [66,67], the root cause of why setting is predictive of high calprotectin is yet to be determined although different diets and exercise might play a role [68].

Our findings add to the small body of evidence currently available on FC concentration and helminth infections. The results we found substantiate on a larger scale the previous findings of de Gier and colleagues, who reported no association between FC concentration and STH infection in 2018 [19]. Additionally, de Gier et al. reported no association between hookworm and FC concentration at baseline or at seven months follow-up [69]. Cepon-Robins et al. similarly observed in a small sample size among the Shuar of Amazonian Ecuador that the relationship between infection and intestinal inflammation were age- and species-specific. These researchers found children singly infected with *T. trichiura* to have significantly lower FC levels, regardless their infection intensity, while no significant relationships were found among adults [57].

Surprisingly, we only found 12 FOB positive results among all STH infected participants. Though we did not formally apply a statistical test for FOB, due to the low number of positive test results, our findings correlate fairly well with Raj et al. as they also did not find a significant difference in the rate of FOB between *T. trichiura* or *A. lumbricoides* positive and negative children [70]. These findings are supported by Wakid who did not detect significant evidence on intestinal parasitic infections, including STHs, and positive FOB tests [71]. In contrast, the findings of Kanzaria et al. and Wanachiwanawin et al. support a relationship between FOB and moderate and heavy *T. trichiura* infections only [40,43].

Different findings were documented for schistosomiasis, a strong association between prevalence and intensity of *Schistosoma mansoni* infection and FOB was observed after repeated treatment over a period of one year in a cohort of young children [41]. These findings seem to be supported by Bustinduy et al. who found a significant correlation between FOB and moderate and heavy egg intensities of *S. mansoni* infection [46]. Moreover, Kanzaria et al. found a positive correlation between FOB and *Schistosoma japonicum* [43]. However, comparison to these studies on schistosomiasis needs to be interpreted with caution, as the host-parasite interaction differs compared to STHs. In the case of intestinal schistosomiasis, the disease is progressed by the chronic and downregulated granulomatous response to entrapped eggs causing polypsosis and pseudopolypsosis leading to rectal bleeding [72].

According to our findings, we suggest that intestinal inflammation and mucosal bleeding caused by STH infections potentially are very low-grade. There might be an age attributable reduction in the rate of parasite establishment, survival, and fecundity due to acquired immunity [73]. Moreover, immune system response to presence of STH ova might differ between the chosen settings. Additionally, blood loss in STH infected individuals might particularly result from the

Table 4
Determinants for fecal calprotectin levels in fecal stool samples. Presented are odds ratios and 95% credible intervals estimated by multivariable logistic regression.[a]

Variable	FC ≥ 50 μg/g	FC < 50 μg/g	OR	95% CrI
All participants	220/1191 (18·5%)	971/1191 (81·5%)		
Country				
Côte d'Ivoire	109 (23·6%)	352 (76·4%)	ref	ref
Lao PDR	86 (24·6%)	264 (75·4%)	1·77	1·09, 2·97
Pemba Island	24 (6·3%)	355 (93·7%)	0·23	0·14, 0·37
Age categories				
5–11 years	102 (18·4%)	453 (81·6%)	ref	ref
12–34 years	49 (13·8%)	305 (86·2%)	0·78	0·52, 1·16
35–64 years	69 (24·5%)	213 (75·5%)	1·49	1·00, 2·20
Sex				
Female	112 (17·6%)	525 (82·4%)	ref	ref
Male	108 (19·5%)	446 (80·5%)	1·12	0·82, 1·50
***T. trichiura* infection**				
Negative	28 (17·8%)	129 (82·2%)	ref	ref
Positive	192 (18·6%)	842 (81·4%)	1·48	0·87, 2·60
***A. lumbricoides* co-infection**				
Negative	147 (17·4%)	698 (82·6%)	ref	ref
Positive	73 (21·1%)	273 (78·9%)	1·19	0·85, 1·66
Hookworm co-infection				
Negative	152 (35·9%)	271 (64·1%)	ref	ref
Positive	68 (8·9%)	700 (91·1%)	0·45	0·26, 0·75

Abbreviations: *A. lumbricoides, Ascaris lumbricoides*; CrI, credible intervals; FC, fecal calprotectin; OR, odds ratio; ref, reference group; *T. trichiura, Trichuris trichiura*.
[a] Regression model includes country, age, sex, *T. trichiura, A. lumbricoides,* and hookworm infection status.

active feeding by adult worms, rather than the leakage around the parasite's attachment site in the gut, leading to decreased levels of hemoglobin instead of blood loss in feces [74]. Therefore, it remains challenging measuring morbidity in the context of STH infection and treatment, which is in agreement with Bogoch et al., who conducted a detailed clinical examination to determine whether STH cause measurable morbidity [75], which was not the case.

It is plausible that a number of limitations might have influenced the results obtained. Some controversy remains on the optimal cut-off values. Historical data on FC levels derive from delevoped countries and evidence on normal FC levels in populations equivalent to our study population is still limited, hampering interpretation of our data. Little evidence suggests to consider FC levels of 50–200 μg/g as normal in people of African-Caribbean descent; however, repeated tests at different time points in several settings would need to be done to strengthen this finding [76]. Similar to most point-of-care RDTs, precision can be limited to ease interpretation and dissemination of results. A meta-analysis by Lin et al. found that in regular FC RDTs, sensitivity is decreasing with higher FC levels, while specificity is increasing [77]. Moreover, the limit of detection of the FC test used in this study was > 200 μg/g, however, especially values > 500 μg/g seem to highly predict pathological findings [76]. As most STH infections of our study population were of light infection intensities, we might have missed an association between heavy infections and intestinal inflammation (FC) or mucosal bleeding (FOB). Furthermore, our study design was restricted to cases of *T. trichiura,* which limits the interpretation for *A. lumbricoides* and hookworm infections to co-infections rather than single infections, which may affect the interpretation of the results. As FC and FOB tests are not disease-specific, we were not able to control for chronic gastrointestinal diseases, malaria, schistosomiasis, and other confounding infective agents, such as bacterial pathogens. In addition, due to monetary constraints, we only analysed one fecal sample for FC/FOB per participant and a sufficient internal quality control method for FC/FOB testing could not be established. Future studies should establish such controls, whenever possible, by partially re-labeling and re-examining results of tests. Moreover, as in many multicountry research, various challenges arose across multiple settings. Differences in diet, culture, and social norms were unable to be accounted for and could potentially bias results. In addition, varying seasonality, host susceptibility, and parasite strains would have an uncontrollable impact on the findings of this study. In spite of these challenges, the results of this study add to the expanding body of literature of STH attributable morbidity. Lastly, the generalizability of our findings are limited to populations in resource-limited settings with recurring and chronic STH infections.

In conclusion, we were not able to detect intestinal morbidity in our study population using FC and FOB as proxy markers. Other potential markers of intestinal inflammation including antitrypsin, eosinophilic protein X, TNFα, or lysozyme have been suggested for diagnosing inflammatory bowel disease, which may be expanded to test for STH attributable intestinal morbidity. However, these test suffer from low diagnostic performance or have not been investigated well enough [78]. Furthermore, markers assessing other facets of STH attributable intestinal morbidity, such as mucosal changes, are needed. Thus, field applicable morbidity markers for STH infections need to be further explored to appropriately monitor and evaluate current global control strategies as monitoring levels of morbidity might become as important as diagnosing the infection itself.

5. Author contributions

EH and JK planned and designed the study; CP, LK, SW, SS, SMA, ShMA, JTC, EH, and JK conducted the study; JH and CP did the formal analyses; LK, CP, and JK interpreted the study data and drafted the manuscript; SW and EH assisted with revising and editing. All authors read and approved the final version of the manuscript and took responsibility to submit this manuscript for publication.

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Data sharing

The datasets used and analysed during the current study are available from the corresponding author upon reasonable request after publishing the manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2021.100724.

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Supplementary Material

S1 Table. Determinants for fecal calprotectin levels in fecal stool samples for Côte d'Ivoire, Lao PDR and Pemba Island. Presented are odds ratios and 95% credible intervals estimated by multivariable logistic regression.[a]

Variable	FC ≥ 50 µg/mg	FC < 50 µg/mg	OR	95% CrI	Sensitivity [b]
Côte d'Ivoire	n: 109	n: 352			
Age					
5-11 years	66 (25·5%)	193 (74·5%)	ref	ref	
12-34 years	22 (18·8%)	95 (81·2%)	0·67	0·36, 1·19	
35-64 years	21 (24·7%)	64 (75·3%)	1·02	0·54, 1·85	
Sex					
Female	52 (21·8%)	187 (78·2%)	ref	ref	
Male	57 (25·7%)	165 (74·3%)	1·20	0·77, 1·82	
***Trichuris trichiura* infection**					
Negative	10 (19·6%)	41 (80·4%)	ref	ref	
Positive	99 (24·1%)	311 (75·9%)	1·13	0·51, 2·60	90·8%
***Ascaris lumbricoides* infection**					
Negative	66 (21·0%)	248 (79·0%)	ref	ref	
Positive	43 (29·3%)	104 (70·7%)	1·52	0·95, 2·39	39·4%
Hookworm infection					
Negative	105 (24·5%)	324 (75·5%)	ref	ref	
Positive	4 (12·5%)	28 (87·5%)	0·45	0·13, 1·19	3·6%
Lao PDR	n: 87	n: 264			
Age					
5-11 years	22 (19·6%)	90 (80·4%)	ref	ref	
12-34 years	18 (17·6%)	84 (82·4%)	1·07	0·47, 2·44	
35-64 years	47 (34·3%)	90 (65·7%)	3·31	1·62, 7·24	
Sex					
Female	48 (25·5%)	140 (74·5%)	ref	ref	
Male	39 (23·9%)	124 (76·1%)	0·97	0·53, 1·76	
***Trichuris trichiura* infection**					
Negative	18 (35·3%)	33 (64·7%)	ref	ref	
Positive	69 (23·0%)	231 (77·0%)	0·22	0·00, 7·09	79·3%
***Ascaris lumbricoides* infection**					
Negative	63 (26·0%)	179 (74·0%)	ref	ref	
Positive	24 (22·0%)	85 (78·0%)	0·88	0·49, 1·60	27·6%
Hookworm infection					
Negative	25 (32·9%)	51 (67·1%)	ref	ref	
Positive	62 (22·5%)	213 (77·5%)	0·51	0·20, 1·37	71·3%
***Opisthorchis viverrini* infection**					
Negative	76 (25·7%)	220 (74·3%)	ref	ref	
Positive	11 (20·0%)	44 (80·0%)	0·66	0·28, 1·48	12·6%
***Strongyloides stercoralis* infection**					
Negative	50/228 (21·9%)	178/228 (78·1%)	ref	ref	
Positive	10/49 (20·4%)	39/49 (79·6%)	0·48	0·12, 1·62	60%
Pemba Island	n: 24	n: 355			
Age					
5-11 years	14 (7·6%)	170 (92·4%)	ref	ref	
12-34 years	9 (6·1%)	139 (93·9%)	0·90	0·36, 2·17	
35-64 years	1 (2·1%)	46 (97·9%)	0·54	0·05, 3·09	
Sex					
Female	12 (5·7%)	198 (94·3%)	ref	ref	
Male	12 (7·1%)	157 (92·9%)	1·10	0·46, 2·65	
***Trichuris trichiura* infection**					
Negative	0 (0·0%)	55 (100·0%)	ref	ref	
Positive	24 (7·4%)	300 (92·6%)	14·39	1·32, 467·34	100%
***Ascaris lumbricoides* infection**					
Negative	18 (6·2%)	271 (93·8%)	ref	ref	
Positive	6 (6·7%)	84 (93·3%)	0·85	0·31, 2·06	25·0%
Hookworm infection					
Negative	22 (6·3%)	325 (93·7%)	ref	ref	
Positive	2 (6·3%)	30 (93·8%)	0·75	0·13, 2·81	8·3%

Abbreviations: CrI, credible interval; FC, fecal calprotectin; OR, odds ratio; ref, reference group

[a] Adjusted for age, sex, *T. trichiura*, *A. lumbricoides* and hookworm infection status

[b] Calculated as positive/(positive + negative) in the participants with FC ≥ 50 µg/mg

S2 Table. Determinants for fecal calprotectin levels in fecal stool samples – Country × *T. trichiura* infection model. Presented are odds ratios and 95% credible intervals estimated by multivariable logistic regression.[a]

Variable	OR	95% CrI
Country [b]		
Côte d'Ivoire	ref	ref
Lao PDR	2·41	0·97, 6·15
Pemba Island	0·01	0·00, 0·16
Age categories		
5-11 years	ref	ref
12-34 years	0·80	0·55, 1·17
35-64 years	1·58	1·07, 2·35
Sex		
Female	ref	ref
Male	1·11	0·81, 1·53
***Ttrichuris trichiura* infection [b]**		
Negative	ref	ref
Positive	1·56	0·71, 3·49
***Ascaris lumbricoides* co-infection**		
Negative	ref	ref
Positive	1·19	0·86, 1·65
Hookworm co-infection		
Negative	ref	ref
Positive	0·56	0·29, 1·01
Interactions		
Lao PDR × *T· trichiura* infection	0·58	0·19, 1·77
Pemba Island × *T· trichiura* infection	30·04	1·56, 8038

Abbreviations: CI, credible interval; FC, fecal calprotectin; OR, odds ratio; ref, reference group

[a] Regression model includes country, age, sex, *T. trichiura, A. lumbricoides,* hookworm infection status, and country × *T. trichiura* interaction term

[b] Main country and *T. trichiura* infection effects have a different interpretation in the presence of an interaction term

6. Discussion

Based on the research conducted during this PhD, several conclusions can be drawn. First and foremost, the current mass drug administration guidelines recommending the standard dose of albendazole are ineffective and unsustainable in the control of *T. trichiura*. Though preventive chemotherapy on a large scale has been the mainstay of successful STH control programs in the past due to its low costs and high coverage of targeted populations, the limited efficacy and mounting drug pressure of the two benzimidazoles hinder progress towards elimination, especially in low transmission settings. Secondly, optimized drug regimens and combinations may be successful in combatting STHs, such as *A. lumbricoides* and hookworm; however, they are limited against *T. trichiura* infections in Côte d'Ivoire and perhaps elsewhere. Lastly, research gaps in the fields of anthelminthics and STH morbidity remain, which is why novel approaches fitting to the current state of helminth control and elimination are needed.

This discussion aims to bring the research conducted in this PhD into the context of what can be done to improve the control of STHs. To begin, current available treatment options and promising new treatments will be discussed, as well as the possibility of emerging resistance and other factors playing a role in anthelminthic efficacy. Then a brief outline of the current gaps in STH research will be provided. Since all the research projects conducted during this PhD were part of clinical trials, I will discuss the roles of study design in STH research. Lastly, recommendations for the future of STH control will be provided.

6.1. Controlling STHs with anthelminthics

As previously discussed, current recommendations of using albendazole or mebendazole as preventive chemotherapy during mass drug administration falls short in controlling *T. trichiura* infections, and to a lesser extent, hookworm infections. The three other drugs that the WHO recommends against helminths on their Essential Medicines List are levamisole, pyrantel pamoate and ivermectin, which also have limitations [1]. Levamisole and pyrantel pamoate have high CRs (>90%) and ERRs (>90%) against *A. lumbricoides*, but low efficacy against *T.*

trichuris and hookworm infections [2]. Levamisole's CR and ERR against trichuriasis are 29.5% and 28.3%, respectively, while pyrantel pamoate's are also low (CR: 20.2%, ERR: 47.5%) [2]. Though pyrantel pamoate has a more promising effect against hookworm (CR: 49.8%, ERR: 71.9%), levamisole has lower CR (10.3%) and an ERR of 61.8% [2]. The varying findings for levamisole may be attributed to the small number of trials included in the meta-analysis [2]. Ivermectin showed varying effects against all three STHs. Recently added to Essential Medicines list in 2017, the known antifilarial had shown to have antihelminthic properties in numerous studies and performed modestly against *T. trichiura* (CRs: <45%; ERRs: <88%) [3-6]. However, when combined with the standard dose of albendazole (a combination already used for lymphatic filariasis control in many NTD control programs), CRs ranged from 28% to 81% and ERRs from 91% to 100%, showing a considerable reduction in *T. trichriura* worm morbidity [7]. The same systematic review found moderate CRs (>50%) and high ERRs (>95%) against hookworm infections, though only based on two trials [7]. These efficacy findings for combination therapy are supplemented by those of this PhD. CRs of combination therapy against *T. trichiura* varied across the three different settings ranging from 13.8% to 65.7%, while ERRs ranged from 70.2% to 99.2%.

6.1.1. Anthelminthic resistance

The low efficacy against *T. trichiura* observed in Côte d'Ivoire could be attributed to any number of factors from resistance to differing absorption rates of the drugs. Other factors that may explain the reduced efficacy of the combination therapy in Côte d'Ivoire include population demographics (i.e. age composition), social/cultural practices (i.e. diet) or the low prevalence/transmission of STHs. Though antihelminthic drug resistance has yet to be proven in human populations, there is much evidence in the veterinary field [8-10]. In veterinary nematodes, resistance to the benzimidazoles in *Haemonchus contortus* was proven to be associated to a single nucleotide polymorphism (SNP) in the nematode's β-tubulin gene at

either codon 200, 167 or 198 [11]. In particular, the SNP at codon 200 has been shown to have increased frequencies in *T. trichiura* after treatment with albendazole [12]. However, no association between increased frequencies of SNP alleles and reduced efficacy of benzimidazoles have been confirmed in the literature to date, partly due to the paucity of evidence available on antihelminthic resistance in humans [12, 13]. Evidence for ivermectin resistance has been shown in ATP-binding cassette (ABC) drug transporter genes of *Onchcerca volvulus* and of Glu/Cl receptor genes in the veterinary nematodes *Cooperia oncophora* and *H. contortus* [14, 15]. However, there is no current evidence available in the literature to support ivermectin resistance in human helminths.

Drug resistance can be triggered by long, repeated monotherapy, an expansion of control populations limiting refugia and administration of suboptimal doses leading to propagation of resistant genes. Though Côte d'Ivoire has a long history of repeated use of combination albendazole and ivermectin against lymphatic filariasis, our findings do not suggest anthelminthic resistance. In previous trials, efficacy of albendazole and ivermectin remained low against *T. trichiura* even in age groups not part of mass drug administration of preventive chemotherapy providing wild-type genes to propagate in the next generation [6, 16]. Moreover, geographic analysis of our results show that there was little change in efficacy of the combination therapy from village to village, indicating no clustering effect or patterns of emerging resistance. To confirm our findings, a subset of samples were sent to a third-party laboratory to undergo testing for resistance markers and results are underway. Of note, differences in the *T. trichiura* strain found in Côte d'Ivoire and their susceptibility to resistance cannot be discounted [17].

6.1.2. Other causes of reduced efficacy

A more likely scenario is that there are other causes related to the host to the reduced efficacy of combination therapy. For example, a complex relationship between the host, parasite and

drug in the context of host gut microbiome has been shown to play a role in host immunity and drug metabolism [18]. This complex relationship is further littered by population-related confounding factors that make it difficult to assess the relationship clearly. Evidence tends to vary geographically, where differences in diet and cultural/social norms may have an effect on the local microbiome of communities. This relationship is further complicated by the effects of consistent deworming on the microbial profiles of the gut. For *T. trichiura*, there is conflicting evidence in the literature for the association between infection and bacteria belonging to the genus *Prevotella* [18]. Studies from Malaysia reported an association, while studies from Ecuador, Liberia and Indonesia did not find evidence of an association [19-23]. Nevertheless, gut microbiota of the host can interact with drugs modulating their absorption and activity [24, 25]. In particular, the combination of tribendimidine and ivermectin was shown to change host gut microbiota significantly in hookworm-infected individuals in Côte d'Ivoire. Further investigations are underway to see the extent at which gut microbiota plays a role in the efficacy of combination therapy in Côte d'Ivoire, Pemba Island and Lao PDR.

6.1.3. Changing drug regimens to improve efficacy

Despite the reduced efficacy of combination albendazole and ivermectin therapy in Côte d'Ivoire, there are other regimens and drugs available for the treatment of STHs. Though current therapy with standard doses of mebendazole and albendazole have low or moderate efficacy against *T. trichiura* and hookworm infections, different regimens of the two most commonly used drugs for mass drug administration have improved efficacies. Several studies have assessed the effects of multiple dose of mebendazole on hookworm infections with CRs ranging from 35%-98% [26-30]. Two of these studies reported CRs against trichuriasis of 100 mg of mebendazole twice daily for three days at 39% and 43% [28, 30]. A randomized controlled trial conducted in Chinese children and adults found 54% and 71% CRs (96% and 97% ERRs) against hookworm and *T. trichiura* infection, respectively, using triple dose (3x500 mg of

mebendazole over 3 days) [31]. The same trial found 400 mg of albendazole once daily for three days had high efficacy against hookworm (CR: 92%; ERR: >99%) and 56% CR and 94% ERR against trichuriasis [31]. A prospective study in Indonesian children found CRs of the same triple regimen of albendazole to be 100% and 61% for hookworm and *T. trichiura* infections, respectively, though only 24 individuals had a hookworm infection [32]. An RCT enrolling children in Gabon comparing single dose albendazole (400 mg) to 2 doses (2x400 mg over 2 days) or 3 doses (3x400 mg over 3 days) found significantly high CRs against hookworm (92% for 2 doses and 93% for 3 doses) and trichuriasis (CRs of 67% and 83% for 2 and 3 doses, respectively) [33]. Though CRs of triple dose of albendazole against *T. trichiura* were limited (56%-83%) when compared to the single dose (34%-40%), ERRs were substantial (>91%) when using triple dose, signifying a significant reduction in the worm burden [31, 33].

For hookworm infections, the findings of this thesis confirm results that a higher dose of current standard therapy provides significant increases in the efficacy. A double dose (800 mg) in adults, regardless of regimen, is effective in hookworm control while maintaining low toxicity levels [33, 34]. However, the same cannot be said in the context of *T. trichiura* control. From the limited evidence available, it is difficult to discern any clear conclusion. There is evidence suggesting that a single dose of either benzimidazole, regardless of how high, is not effective, while a repeated dose has somewhat higher CRs (dependent on the benzimidazole, dose, regimen and population assessed) and significantly higher ERRs [28, 30, 31, 33]. This need for a repeated dose over consecutive days for *T. trichiura* may be attributed to the short half-life of the active component of the benzimidazoles (<12 hours) and possibly the location of the parasite, which resides in the cecum of the large intestine rather than the small intestine (as is the case for *A. lumbricoides* and hookworm).

6.1.4. Alternative treatment options

Other drugs are available for the treatment of STHs, especially *T. trichiura* infections. Promising drugs include oxantel pamoate, tribendimine and moxidectin (briefly discussed in the Introduction). A recent systematic review showed a high CR (75.7%) and ERR (85.0%) for oxantel pamoate against trichuriasis [35]. However, oxantel pamoate is no longer marketed today making it difficult to be part of a mainstay mass drug administration control programs[36-39]. Importantly, a weight-independent dose of 500 mg is effective in treating trichuriasis in children [40, 41]. Though oxantel pamoate shows promising effects against *T. trichiura*; however, evidence shows low efficacy against *A. lumbricoides* (CR: 21.8%; ERR: 35.8%) and hookworm species (CR: 23.8%; ERR: 39.5%) [35]. Though this could be a limitation for its use as part of a monotherapy regimen against STHs, oxantel pamoate is a promising candidate as part of a combination therapy. A combination of oxantel pamoate and albendazole was shown to have a high efficacy against all three STHs with CRs of 95.2%, 88.7% and 72.0% for *A. lumbricoides*, *T. trichiura* and hookworm infections, respectively [35]. Additional curative effects against hookworm species may be achieved when a third drug (either pyrantel pamoate or mebendazole) is added to the combination; however, clinical evidence for triple combination therapy with oxantel pamoate is limited to one trial in one setting [42].

Unlike oxantel pamoate, tribendimidine is more efficacious against *A. lumbricoides* (CR: 83.4%; ERR: 91.6%) and hookworm (CR: 96.5%; 88.1%) infections and has low efficacy against *T. trichiura* (CR: 5.9%; ERR: 41.3%) [35]. Tribendimine, which is only approved for use in China, is also efficacious against other NTDs, such as cestodes and trematodes, which is advantageous in the planning and implementation of integrated NTDs programs [43-45] Regardless, only a handful of studies are available including tribendimidine with only one non-inferiority trial assessing combination therapy with oxantel pamoate or ivermectin [46].

Moxidectin, which has a longer half-life than the more common ivermectin, shows high efficacy against ascariasis (CR: 90.5%; ERR: 97.9%) and low to moderate efficacy against *T. trichiura* (CR: 30.1%; ERR: 81.0%) and hookworm (CR: 37.6%; RR: 76.0%) [35]. Already

approved for oncheriasis, there is also early evidence of its diverse use against lymphatic filariasis, strongyloidiasis and human scabies [47-49]. Since moxidectin trials report low adverse events and can be administered weight-independently, it is a good candidate for combination therapy against multiple NTDs. Furthermore, two trials have presented CRs >50% and ERRs >90% against *T. trichiura* for the standard dose of 8 mg of moxidectin in combination with 400 mg of albendazole.

6.1.5. Emerging drugs in the fight against STHs

While existing drugs offer easy access to the ongoing control of STHs, new drug development, which was stagnant for the final decades of the 20th century, is starting to emerge even amidst economic and regulatory challenges. Emodepside, Cry5B and derquantel are examples of drugs under development. Emodepside is a cyclooctadepsipeptide that binds to neural acetylcholine receptors and to G-protrain coupled latrophilin-like receptors causing paralysis of nematodes [50]. Being from another class of drugs entirely, emodepside has reportedly shown activity against nematodes including those resistant to the macrocyclic lactones and benzimidazoles [51, 52]. Cry5B is a protein made by Gram-positive bacterium *Bacillus thuringiensis* [53]. Working as a pore-forming protein, Cry5B is toxic to nematodes, resulting in eventual death [54]. Currently, only *in vitro* and *in vivo* studies are underway examining the effects of Cry5B, but promising results have been reported for hookworm species in particular [54-57]. Derquantel, which is of the new class of spiroindoles, is marketed combination with a macrocyclic lactone (STARTECT®) in veterinary medicine [58]. Acting as a nAChR antagonist, derquantel causes flaccid paralysis of the worm and eventual death [58]. A UK study reported a high efficacy (>99% worm count reduction) of the combination in multi-drug resistant nematodes in sheep; however, limited evidence is available in animal models for specific STHs [59]. More evidence in clinical human subject research are needed before these potential anthelminthics can be marketed to humans.

6.2. Eliminating the STH research gaps

As an NTD, soil-transmitted helminthiasis has many research areas that need cultivation. Relevant to this PhD thesis, assessment of the impact of mass drug administration and integrated NTD programs is lacking. As the controversy of the impact of deworming continues with the 2019 update of the 2015 published Cochrane review, it is important to consider how the current body of evidence shapes the mass drug administration landscape [60, 61]. Updated evidence from 51 RCTs show deworming has little to no effect on height, hemoglobin concentration, cognition, school performance or mortality (with moderate to low quality of evidence); however, there is still not enough evidence to confirm the effects of deworming on weight [60]. Another review including 52 studies (RCTs and prospective studies), similarly, found no impact on the well-being of children [62]. This may come as a surprise, since the most recent WHO guideline has used weight and height gain to rationalize the use of annual or biannual PC [63]. In 2017, two additional published reviews showed a meaningful impact of deworming on children's health outcomes [64, 65].

6.2.1. Geographic gaps in the evaluation of mass drug administration

Most remarkably, closer examination of these reviews shows very little evidence in West African settings. Taylor-Robinson and colleagues only included three trials taking place in West African countries (Benin, Nigeria and Sierra Leone) in their comprehensive update in 2019. The trials occurring in Benin and Nigeria both assessed albendazole's effects on various health outcomes, while the trial from Sierre Leone was excluded from meta-analyses [66-68]. Furthermore, the included trials from Benin and Nigeria only included preschool-aged children (PSAC), which are not the primary targets of mass deworming efforts in West Africa [66, 67]. Another trial included in the meta-analysis was conducted in the school-aged children (SAC) in nearby Cameroon; however, the trial was conducted in 1972 and assessed the effects of thiabendazole, which is not part of any national control program [69]. Welch *et al.* 2017 included the trial from Benin in PSAC and the 1972 trial in Cameroon using thiabendazole in

its meta-analyses, but also included a cluster RCT evaluating the a large scale nutrition intervention by Linnemayr and colleagues conducted in Senegal [62, 70]. Though the Sengalese trial had over 4200 participants, they were all under the age of five and the exact intervention for deworming is unclearly reported [62, 70].

The evidence is as equally sparse for West Africa on the other side of the controversy. The review by Clarke and colleagues did not include any of the aforementioned trials, but included a trial with both praziquantel and mebendazole for schistosomiasis and hookworm infections in SAC of Sierra Leone [64, 71]. Also included was a prospective study in Ghana analyzing both annual and repeated mass drug administration using ivermectin combined with albendazole (as part of a lymphatic filariasis control program) against parasitic nematode *Oesophagostomum bifurcum* and hookworm infections, but the study did not include any health outcomes besides prevalence of infections [72, 73].

Considering all the evidence (or lack thereof), there is little that can be said about the impact of deworming in West African settings, especially among SAC, the target of WHO recommended PC on a national scale. In light of the paucity of evidence, it is recommended that more trials and prospective studies be dedicated to the evaluation of mass deworming efforts. In children, this extends beyond weight, height and hemoglobin concentration to school attendance and performance, cognition and physical fitness.

6.2.2. Difficulties in morbidity measurements

Another research gap is measuring morbidity attributable to STHs. Currently, morbidity is measured indirectly through coprological egg count-based techniques, which are usually done microscopically. Besides the limited diagnostic sensitivity of these techniques, they only quantify unhatched eggs produced by female parasites producing egg. Though it is known more eggs equates to heavier burden of infection, it is unknown the true number of adult worms (or

larvae) in the human body; moreover, the effect of the worms on the host gut remains unclear. Identifying potential markers of gut morbidity was an objective of this PhD research.

Though most infections are asymptomatic and can be chronic (due to repeated exposure and rare resolution of infection), the presence of worms in the gut has been shown to cause changes in host intestinal mucosa and modulate the host's immune response [74-76]. The findings from this thesis support the framework of a heightened Th2 immune response that reduces harmful inflammation marked by high fecal calprotectin levels. The outlook of these findings is further clouded by a complex relationship between host, parasite and intestine. Nonetheless, the search for alternative markers of gut morbidity remains ongoing. Potential markers, such as C-reactive protein (CRP) and alpha(1)-acid glycoprotein (AGP), better characterize generic host immune response rather than gut morbidity, while more specific markers lack evidence in the field of helminths [74]. Hence, there is a necessity for additional studies identifying potential gut morbidity markers that correlate better to worm burden.

6.3. Study design in the context of STH control

All of the research conducted in this PhD were part a series of clinical trials conducted in Côte d'Ivoire (low endemic setting), so it is important to discuss the role of clinical trials in ongoing STH research. Though the gold standard of all study designs in epidemiology, the drawbacks of clinical trials are apparent. High costs, limited external validity and short follow-up opportunities (especially for safety measures) are a tradeoff for a high level of internal validity, minimal risks of bias and confounding and consistent results. More importantly, clinical trials are necessary in clinical drug development pipelines and are required by regulatory agencies when seeking approval for marketing drugs. In the field of NTD, most drugs currently recommended and available to use have gained regulatory approval in the past for various indications; however, there are new drugs that are in need of regulatory approval. This section will discuss the possibility of new trial designs to ensure high-powered clinical trials continue

to be conducted and how alternative study designs may be appropriate for already approved drugs seeking for new indications.

The classic randomized controlled trial (RCT) compares an experimental group to a control group in parallel. Participants provide consent before being recruited and enrolled in the trial. A randomization procedure is used to allocate participants into either the experimental group or the control group, which will be followed by treatment and follow-up examination and outcome assessment. However, over the past two decades, alternatives from crossover trials to adaptive trials have been used to test the superiority, non-inferiority or equivalence of single or multiple interventions in rare to highly prevalent diseases. Designs of trials are not only affected by the research question, but also by stage in pipeline development (Phase I, II, III or IV), logistics and resources available. In resource-limited settings, additional obstacles such as political instability, varying regulations and poor infrastructure might also influence the choice in trial design. Specific to STHs, the endemicity of a setting, the expansion of mass drug administration to groups outside of school-aged children and high likelihood of reinfection are all factors when designing trials.

6.3.1. Alternative trial designs for evaluation of anthelminthics

For dose-finding (Phase II) trials of anthelminthics, the prevalence of the STH in question along with the population tested is crucial when designing trials. In the two dose-finding trials conducted as part of this PhD thesis, recruitment goals were not met for several of the age cohorts assessed. In the case of the albendazole dose-finding trial against *T. trichiura*, results from adults and PSAC were considered underpowered, while the similar dose-finding trial had recruitment difficulties in SAC and PSAC. Poor trial recruitment is not unique to the field of NTD, but common in many trials and is the leading cause of discontinuation of trials [77, 78]. Underpowered trials increase the possibility of a type two error, or false negative result (a study fails to detect a difference or association that does exist). In rare cases, when recruitment goals

are almost met (power is only slightly reduced) and no difference is found, interpretations of the data may still be possible, because it is unlikely a false negative result would be found. However, in the majority of cases, results are not interpretable because the likelihood of missing an actual finding could double or triple [79]. An underpowered, as well as overpowered, studies should be avoided due to ethical implications for participants and resources.

Though the classic design of an RCT has the lowest sample size for recruitment, it may not be the most advantageous when using drugs that have been used for decades for mass drug administration and are deemed "safe" even at higher doses. Albendazole has been in use as an anthelminthic since the 1980s and millions of tablets are distributed annually to children all over the world. An alternative trial design could help reduce the likelihood for an underpowered dose-finding trial. Adaptive trial designs have been used in dose-finding trials in other fields using response-adaptive randomization that allows for modulating allocation ratios based on favorable outcomes. In short, at predetermined intervals, increases in allocation of participants to an intervention, which is more favorable (in terms of risks and benefits), are planned allowing for a smaller sample size. Drop-the-loser adaptive trial designs follow the same mentality allowing for the complete discontinuation of arms that do not meet threshold requirement at fixed time points. The advantage is the "winning arm(s)" will produce a high statistical power during analyses. Adaptive trials in settings with low transmission of STH, such as Côte d'Ivoire, may be useful if planning and resources can be allocated prior to trial initiation and the complexity and time commitment of the adaptive design are in line with research plans.

For larger Phase III trials evaluating the efficacy of an intervention that is delivered to groups, a cluster RCT would be the best fit. Since evidence is scarce in West Africa on the evaluation of mass drug administration programs, a cluster randomized trial would help evaluate the efficacy of anthelminthics as programs switch to community-wide approach rather than a school-based approach. A variation of the cluster RCT, the cluster randomized stepped wedge trial has the added benefit of reducing sample size by allowing each cluster to be randomized

to the experimental group at a different point until all clusters have received the intervention [80]. Within the cluster stepped wedge design, long term observations on morbidity markers or other secondary outcomes along with multiple comparisons could be made; however, the complex design requires much planning and use of a strict statistical analysis plan.

For newer dugs that are still seeking out regulatory approval, such as moxidectin or tribendimidine, a more cautious approach needs to be taken. Though adaptive trial designs in Phase II trials may be appropriate, a more classic parallel design for the Phase III superiority (or non-inferiority) trials are necessary. However, to mitigate poor recruitment or failed treatment arms, the use of a "rescue" therapy arm or a trial within a cohort might be fruitful in resource-limited settings. Since trials in NTDs are conducted my a very few number of research groups in many of the same settings, an ongoing registry could be created as part of technological and infrastructure improvements. As is the case in many research projects, this requires collaboration of on-site staff, government officials and partner institutes to ensure that best practices are implemented and maintained.

6.3.2. Complimenting trials with non-randomized studies

Along with Phase II and III trials, pragmatic trials have gained momentum to improve generalizability and external validity in real-world settings. Though not directed at measuring efficacy, the pragmatic trial measures effectiveness of an intervention, making it a perfect tool in evaluating large integrated NTD programs across multiple communities. Alternatively, to be more conservative and less cost-prohibitive, prospective studies could be used to test if an association is present before preceding to more costly RCTs. Since case-control and cohort studies are not held to the most stringent rules of RCTs, which usually seek regulatory approval, studies could be done at lower cost and shorter timelines to provide initial evidence to secure funding and aid in planning efforts of larger trials. The combination of ivermectin and albendazole had been used in Côte d'Ivoire for several years for lymphatic filariasis, but its

effects on STHs in the same setting remained unclear. A smaller case-control or cohort study could have shown very little association between the combination therapy and STH cure rate. Of course, the value of evidence produced in prospective studies is not to the gold standard of clinical trials as bias and confounding usually overestimate results; however, there has been an overwhelming push to devalue prospective study designs [81-83]. Current best practices assessing the quality of evidence includes Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach, which classifies the quality of a body of evidence as "low" (only "very low" is worse) if composed of nonrandomized studies and "high" (the highest level) if composed of randomized studies [84, 85]. Though a body of evidence can move up levels in 2-3 other criteria, most evidence of thorough systematic reviews that must include observational studies from before the 1990s will always be downgraded. In certain fields the risk of overestimating the quality of evidence could be life-threatening (such as surgical interventions or radiation therapy); however, in many fields (as in the case of STHs), the penalization of the GRADE system is harsh and always drives the quality of evidence lower or risks the exclusion of vast bodies of literature including observational studies. This is particularly apparent in the previous chapter's discussion on the lack of evidence of the effects of mass drug administration in West Africa. Recently, tools have been created to evaluate evidence from nonrandomized studies, such as the Risk Of Bias In Non-randomised Studies – of Interventions (ROBINS-I) and the Grading of Evidence for Public Health Interventions (GEPHI), which is based on the GRADE system, but systemic changes in research need to be made to overcome a "gold standard" only approach to evidence synthesis [86, 87].

6.4. References

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7. Conclusions

While strides have been made in the past decades to greatly reduce the burden of STHs, greater steps need to be taken if elimination is to be reached in the coming future. The aim of this PhD research was to inform decision and policymakers on best treatment and monitoring practices for the control of STHs in Côte d'Ivoire and globally. The goal of this PhD was to evaluate optimal treatment regimens and dosages against STH infections and to assess the potential markers of STH morbidity.

Given the complex landscape of high drug pressure, slow drug development and reduced efficacy of mainstay treatments, the conclusions of this thesis lead to recommending alternative treatment combinations for the control of STHs, rather than monotherapy alone, while continuing ongoing research and development of new drugs. The combination of albendazole and ivermectin is promising in many settings; however, this combination is not efficacious in Côte d'Ivoire. Further investigation into potential other combinations such as a higher dose (800 mg) of albendazole combined with a weight independent dose of either oxantel pamoate (500 mg) or moxidectin (8 mg) should be conducted in multiple low transmission settings. Of course, the cost-effectiveness of the aforementioned combinations would be higher than monotherapy, but, as proven by the elimination of lymphatic filariasis in many countries over the past two decades, is worthwhile on the road to elimination. This would require the cooperation and coordination of various stakeholders, including pharmaceutical companies, regulatory agencies and funders, to ensure oxantel pamoate and moxidectin can be approved, produced and made available at low costs for the use of STH control and elimination through mass drug administration.

As many countries switch to a more integrated approach to NTD management, it is important to consider the evaluation of the impact of various treatments together. For example, in Côte d'Ivoire, there were ongoing programs for helminthiasis, schistosomiasis, malaria and lymphatic filariasis control at the time this PhD research began. Evaluation of morbidity targets

outside of prevalence should be a planned and coordinated part of integrated NTD control programs since there is an opportunity to reach and collect ongoing information on children and adults with multiple infectious diseases. Morbidity data from national control programs should be made available on a global level, which requires improved technology and infrastructure, capacity building in data science, increased access to individual patient data in research and collaboration with global agencies to ensure data quality and assurance.

Lastly, to accomplish the changes in current treatment guidelines and evaluation of control programs, it is recommended that alternative trial designs should be used in STH research, especially in Phase II clinical research and in settings of low endemicity. For trials of drugs seeking regulatory approval, a more classic approach should be used; however, pre-planning and setting choice is key when deciding where to access the efficacy of an anthelminthic. Furthermore, observational studies should not be overlooked and small pilot studies could avoid costly and unethical trial designs.

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Profile

Public health professional with strong skill set in clinical project and program management in the field of epidemiology seeking employment in the Public Health sector. Over eight years of experience in coordinating and monitoring diverse health projects with expertise in infectious disease. Demonstrated clear communication with internal team and external stakeholders.

Certifications and Skills

- **Certifications:** Good Clinical Practice, Strategic Project Management, Research Data Management, Conflict Resolution.
- **Software proficiency:** Microsoft Office (Word, Excel, Powerpoint, Access), STATA, ODK, R.
- **Languages:** English (native), Spanish (intermediate), French (intermediate), Gujurati (native).

Select Professional Experience

Research Assistant in Epidemiology Mar 2018 – Feb 2021
Department of Medical parasitology and Infection Control, Swiss Tropical Institute, Basel, Switzerland

- Developed protocols for monitoring, evaluation, surveillance, quality control and safety in a clinical framework.
- Coordinated and managed international and multidisciplinary teams across research, financial and regulatory authorities.
- Created and implementing health project plans in clinical and laboratory settings.

Research Associate in Clinical Epidemiology Apr 2017 – Feb 2018
Basel Institute of Clinical Epidemiology and Biostatistics, University Hospital, Basel, Switzerland

- Consulted and advised on study design and data analysis plans.
- Performed meta-analyses and individual patient data analyses on relevant clinical topics.
- Conducted health technology assessments and systematic reviews for public and private health authorities.

Health Planner and Program Manager Jan 2015 – Sep 2016
Health Council of Southeast Florida, Palm Beach Gardens, FL, USA

- Evaluated and audited health programs led by various hospitals and health departments.
- Maintained and generated agency databases, including emergency room and inpatient hospital utilization.
- Leveraged data and research to devise community health assessments and improvement plans.
- Devised health education and information dissemination plans for various initiatives in Palm Beach County.

Breast Cancer Researcher Aug 2014 – Jan 2015
Department of Epidemiology, University of Florida, Gainesville, FL, USA

- Created and submitted protocol and study documents to ethical and regulatory committees.
- Collected, organized, and compiled data for literature reviews on breast cancer incidence and survival.
- Assisted in grant preparation for funding from the National Institute of Health and the National Cancer Institute.

Study Navigator Nov 2013 – July 2014
Department of Epidemiology, University of Florida, Gainesville, FL, USA

- Recruited, screened and tracked participants based on recruitment criteria for clinical studies.
- Monitored recruitment timelines and goals for industry-funded and investigator-initiated trials.
- Assisted in maintaining a participant recruitment database.

Education

M.S. in Public Health 2014
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

B.S. in Biology and B.A. in Art History 2011
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