New drugs, drug combinations and improved diagnostics for the control of helminthic infections

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Summary

Neglected Tropical Diseases (NTDs) represent one of the biggest health challenges in tropical and subtropical countries. They are most commonly caused by helminths, including the soil-transmitted helminths (STH) (*Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* and *Necator americanus*), *Schistosoma spp* (*S. haematobium, S. mansoni, S. japonicum* being the most common) and *Strongyloides stercoralis*.

Helminth infections are widely distributed but prevalence is highest in low-resource settings in subSaharan Africa, the Americas and Asia. Helminthic infections are more common in children, and, when chronic may be responsible for severe morbidities that interfere with normal growth and cognitive development. Preventive chemotherapy (PC) is the main strategy promoted by the World Health Organization to control morbidities linked to NTDs. This intervention is based on regular anthelminthic treatment of in-need populations in endemic areas.

Diagnostic methods for the detection of these infections suffer from low sensitivity and new approaches, such as molecular techniques, that may improve sensitivity, often are not applicable in field laboratories where expensive tools are not affordable.

The treatment of NTDs relies on few drugs: benzimidazoles (albendazole and mebendazole) for STH, praziquantel for schistosomiasis and ivermectin for onchocerciasis, lymphatic filariasis and strongyloidiasis. PC programs are highly effective but the regular use of the few available drugs in a vast population raises concern regarding development of resistance. Although confirmed resistance has not yet been proved, a lower sensitivity to praziquantel has been reported in populations who received several rounds of PC. Additionally, benzimidazoles are scarcely effective against *T. trichiura.* Therefore, there is a great need of new effective drugs to tackle these infections.

In the frame of my PhD program, I addressed the need of developing new drugs against helminthic infections by testing the efficacy of moxidectin, a macrocyclic lactone used in veterinary medicine, against STH, *S. haematobium, S. mansoni*, and *S. stercoralis* in exploratory randomized Phase 2 trials. We confirmed its good efficacy against *S. stercoralis* but not against *Schistosoma* spp. In our randomized non-inferiority Phase 2 clinical trial conducted with different drug combinations, moxidectin in co-administration with albendazole showed a good efficacy against STH infections. From a diagnostic point of view, we assessed the sensitivity of two DNA extraction methods for the detection of *S. stercoralis* using PCR; this technique showed a good potential but further studies are still necessary to improve its standardization. We also conducted an ultrasound assessment of the impact of different doses of praziquantel on urinary tract morbidity in *S. haematobium* infected children versus placebo. We found that light and moderate bladder morbidity has an early onset, and is present in pre-school-aged children. Six months after treatment we registered 90% of regression of morbidity in treated children and only 10% in the placebo group.

Our findings contribute to the development of moxidectin against helminthic infections. Our findings indicate that this drug is worth further evaluations before it can be promoted for public health campaigns. Furthermore, our data might foster the development of new potential diagnostics for NTDs such as *S. stercoralis.* Eventually, we confirmed the relevance of PC for the control of morbidity due to urogenital schistosomiasis that can be assessed in the field by relatively simple tools such as ultrasound.

Table of abbreviations

CCA	Circulating cathodic antigen
CI	Confidence interval
CR	Cure rate
DALYs	Disability-adjusted life years

EKBB	Ethical committee of Basel
ELISA	Enzyme-linked immunosorbent assay
EPG	Eggs per gram of stool
ERR	Egg reduction rate
ID	Identification number
IHA	Indirect hemagglutination
ITT	Intention-to-treat
LAMP	Loop-mediated isothermal amplification
MDG	Millennium development goal
N (or n)	Sample size
NVP	Negative predictive value
NTD	Neglected tropical disease
OR	Odds ration
PCR	Polymerase chain reaction
PHL-IdC	Public Health Laboratory-Ivo de Carneri
PPV	Positive predictive value
qPCR	Real-time quantitative PCR
SD	Standard deviation
STH	Soil-transmitted helminth
Swiss TPH	Swiss Tropical and Public Health Institute
WHO	World Health Organization

Chapter 1

General introduction

1. Epidemiology of soil-transmitted helminthiasis and schistosomiasis

Intestinal parasitic infections represent one of the biggest health challenges in tropical and subtropical countries [1]. The most common soil-transmitted helminths (STH) are the large roundworm (*Ascaris lumbricoides*), the whipworm (*Trichuris trichiura*), and the hookworms (*Ancylostoma duodenale* and *Necator americanus*). Another common intestinal parasite, but conventionally not named together with the other STH, is the dwarf threadworm *Strongyloides stercoralis* [1].

Soil transmitted helminthiasis and schistosomiasis (introduced below) are grouped among the neglected tropical diseases also because they thrive with poverty, and people who suffer from these diseases are mostly the poorest who live in mid-low resource settings [2–5].

According to WHO estimates from 2015, almost 2 billion people are affected by STH infections , of which 875 million children are in need of regular treatment [1,6,7]. Approximately 270 million preschool-aged children (PSAC) and more than 550 million school-aged children live in areas where these parasites are extensively transmitted [7]. Approximately 250 million girls and women of child bearing age (WCBA) are living in areas that are endemic for STH. Infections are widely distributed in all WHO regions, with the greatest numbers occurring in sub-Saharan Africa, the Americas and Asia. Overall, more than 100 countries are endemic for

STH infections [7]. This estimate excludes *S. stercoralis*, which alone infects up to 300 million people globally [4].

The number of years of healthy life lost attributable to a disease (or group of diseases) (known as Disability Adjusted Life Years: DALYs) is used as a measure of disease burden and provides a comparative indication of the importance of the disease in public health. For intestinal and urogenital parasitic infections this calculation is particularly cumbersome, as they often occur asymptomatic or with generic symptoms [3,8,9].

For STH it has been estimated that overall DALYs have decreased from 5 million in the 1990 to approximately

3 million in the past decades [8,10,11]. The same trend has been observed in case of schistosomiasis, with a previewed halving of DALYS (1.5 million to 750 000) in the upper-middle income countries. The slower adoption and implementation of recommended control strategies in the lowest income group may be related to multiple interrelated factors, including lack of human and financial resources, weak health systems, and epidemiological factors [10].

Schistosomiasis is a disease caused by the fluke of the genus *Schistosoma,* which is endemic in many tropical and subtropical countries. It is estimated that globally more than 770 million people are infected by at least one *Schistosoma* species (the most represented are: *S. mansoni, S. haematobium, S. intercalatum, S. japonicum*) [2,7]. As observed for STH, *Schistosoma spp* have similar high-risk population groups, being more prevalent among PSAC and SAC living in endemic countries and having limited access to clean water and sanitation [12].

The epidemiology of *S. stercoralis* is slightly different, being spread among different age ranges. Its life-cycle, as described below, differs slightly from the one of other STHs, being characterized by autoinfection, that leads to life-long carriage of the parasite, if untreated [13,14]. *S. stercoralis* is also called the neglected of the neglected tropical diseases [5], as few data are available on its prevalence and linked morbidity; actually, the true prevalence of this parasite is not well known and is often underestimated [5,13]. Similarly to the other intestinal parasites, *S. stercoralis* infection is mostly asymptomatic or characterized by unspecific symptoms

(diarrhoea, abdominal cramps, urticaria), and the diagnostic tools available for its detection are not accurate [5,15].

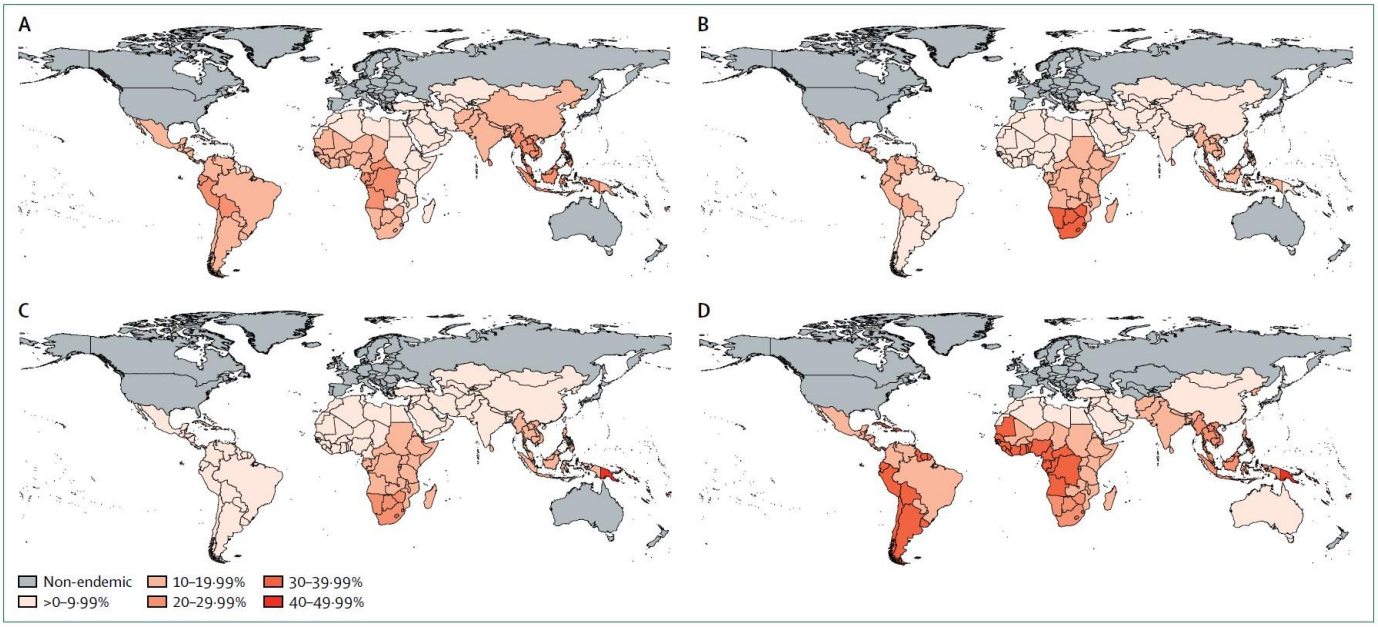

Fig 1. Prevalence by global regions of (A) *Ascaris lumbricoides* (for 2010), (B) *Trichuris trichiura* (for 2010), (C) hookworm *(Necator americanus and Ancylostoma duodenale; for 2010),* and (D) *Strongyloides stercoralis* (for 2011)

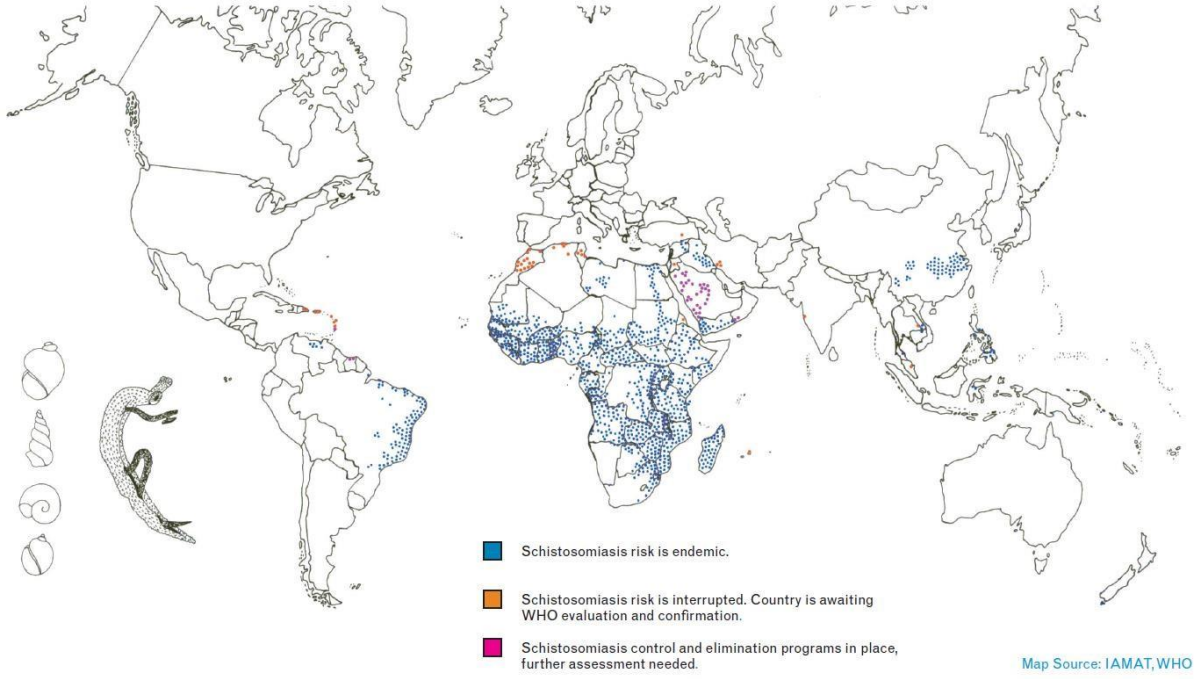

Fig 2. Prevalence of schistosomiasis, WHO 2015

2. Life cycle

2.1 Soil transmitted helminths

Eggs of STH are released through stools and contaminate the environment during open air defecation. In warm and moist soil, eggs can remain viable for years. Helminth eggs need a period of maturation in the soil (variable from weeks to months) before becoming infective. Infection of the host occurs through ingestion of infective eggs (either by eating raw and contaminated vegetables/ fruits or by ingestion of soil in the case of children). In the case of hookworm, free living larvae can infect people by penetrating unwounded skin, especially in areas were bare-foot walking is common practice. After ingestion, *A. lumbricoides* eggs disclose the larvae, which penetrate the intestinal mucosa and migrate to the lung circulation. Third stage larvae pass through tracheobronchial tree to the larynx, are swallowed and settle into the small intestine.

T. trichiura has a simpler life cycle. After infection, *T. trichiura* eggs hatch, worms develop and migrate first into the small intestine, and afterwards adult worms attach to the villi in the large intestine (caecum and ascending colon).

After penetration hookworm larvae are transported via the blood stream to the pulmonary circle, from where they pass into the larynx, are swallowed, and move into the small intestine. Adult worms can survive for months or even years in the host.

2.2 *Strongyloides stercoralis*

The life cycle of *S. stercoralis* is more complex compared to STH. This parasite has a free-living form which develops outside the human host. The larvae of *S. stercoralis* infect the host by penetrating unwounded skin or by being ingested. They are then transported to the pulmonary cycle, pass to the larynx and enter the small intestine. The adult worms lodge in the duodenal and jejunal, where they lay eggs that hatch within the human intestine and produce two kinds of larvae. The rhabditiform larvae migrate to the lumen and are released with faeces, whereas, the filariform larvae penetrate the intestinal wall or perianal skin, re-enter a new cycle and disseminate to different organs. Rhabditiform larvae can contaminate the environment and transform into infective filariform larvae or perpetuate a free-living cycle outside the host.

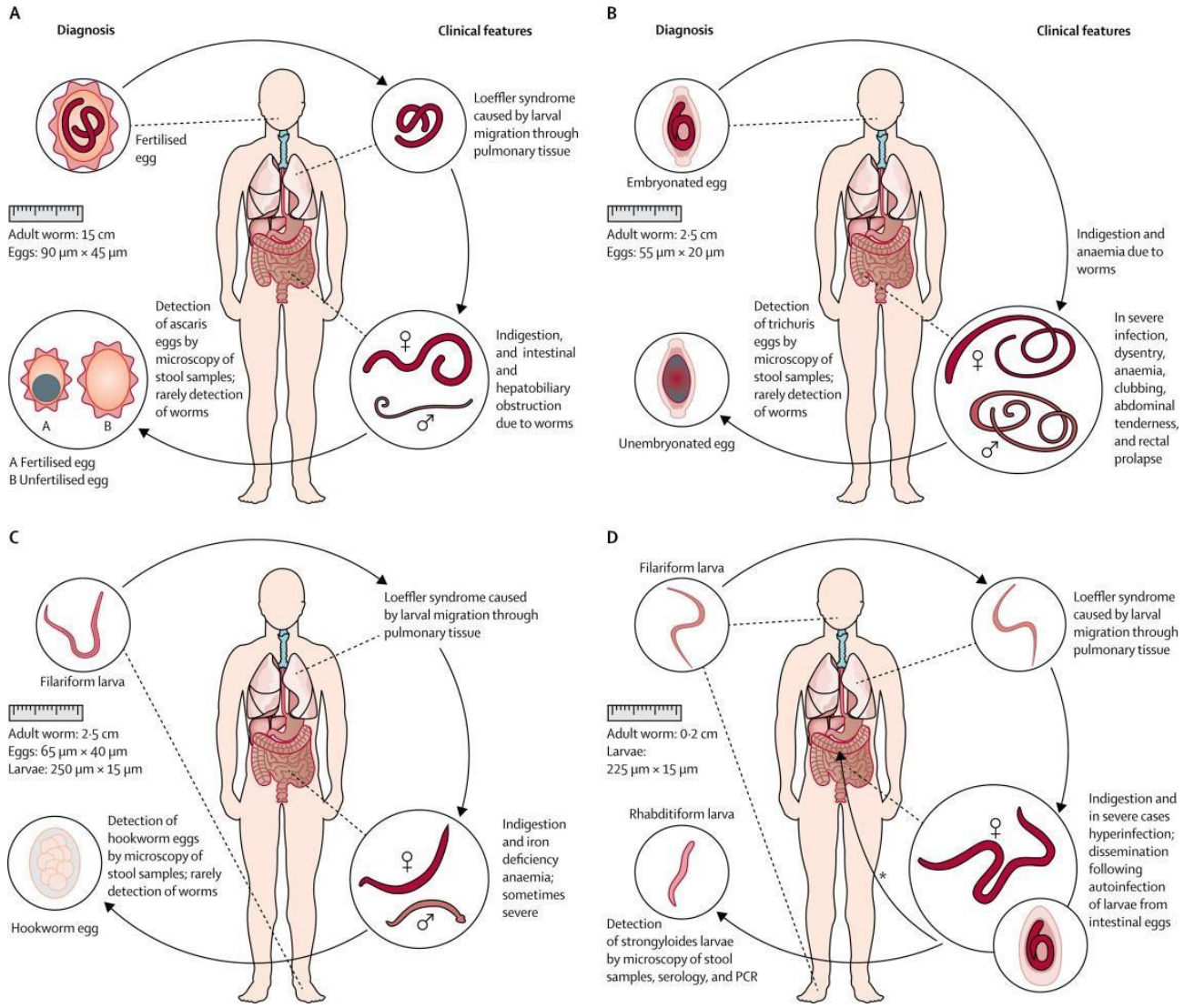

Fig 3*. Life cycle STH and *S. stercoralis

2.3 Schistosomes

Schistosome eggs are passed in stools or urine of infected individuals (depending on whether they are infected by intestinal or urogenital schistosomiasis) and need contact with fresh water for their development. In water, they transform into mobile miracidia that infect snail intermediate hosts (specific snails for each *Schistosoma* species). Miracidia multiply in the snails which release thousands of mobile infective larvae with forked tails, named cercariae. Cercariae infect human hosts by penetrating the skin into fresh water. After penetration, the parasite migrates through the lungs and develops into schistosomula (maturing larvae) that settle in the liver. After a few weeks, they migrate via the blood stream to their destinations which are either the perivesicular veins (*S. haematobium)* or the mesenteric

veins (*S. mansoni, japonicum, intercalatum* and others). Adult worms live *in copula* and the female only moves to release the eggs. Eggs penetrate the blood vessels, then the intestinal or bladder mucosa with the spike and reach fresh water through urine or stool.

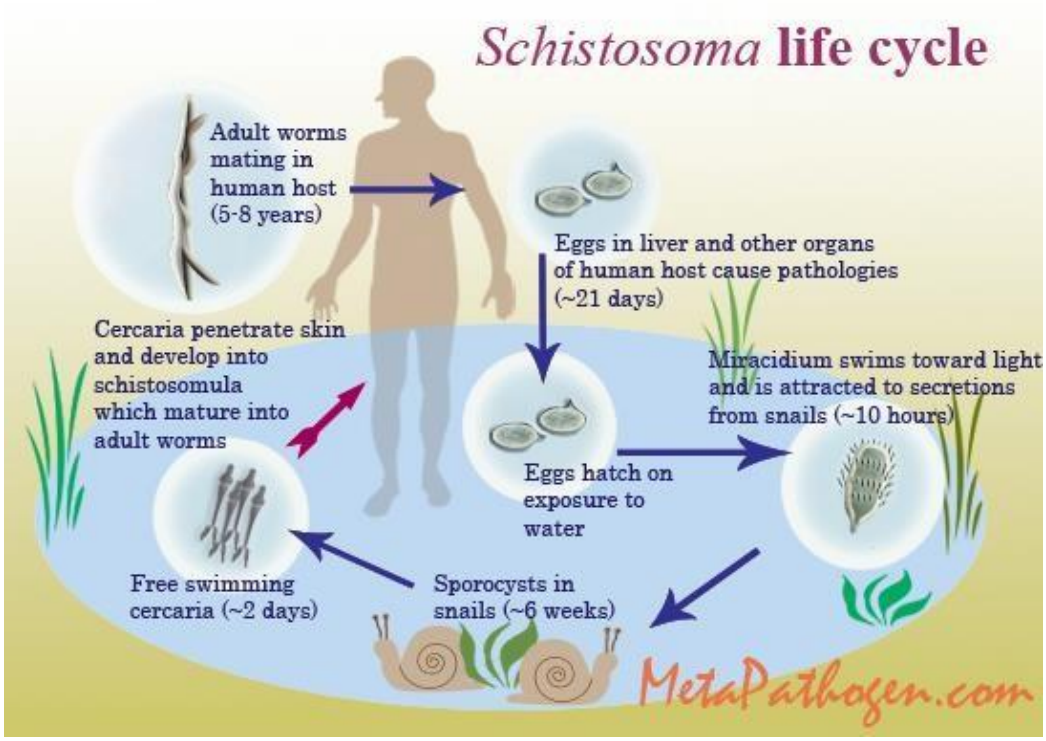

Fig 4. Life cycle of *Schistosoma* spp

3. Diagnosis

3.1 Direct methods

The diagnosis of intestinal parasites has always been cumbersome, with the most commonly used methods being burdened by low accuracy and sensitivity [16]. Moreover, intestinal parasites do not release eggs at a constant rate, therefore more than one sample on different collection days is needed in order to increase the accuracy of diagnosis. Up-to-date, there is no “gold standard” for the diagnosis of intestinal helminths infections; therefore, there is no clear indication on which is the best method to use.

In endemic countries, referral hospitals and laboratories at individual level diagnose these infections via a direct smear from fresh stool samples [17]. In settings were a centrifuge is available, samples are mixed with formalin and ether (to clean the sample from debris), centrifuged and the pellet is analysed on a slide

(formolether concentration method) [17,18]. These techniques allow the detection of all intestinal helminths and protozoa, but no indication on the intensity of infection (eggs per grams (EPG)) is given.

If public health trials or evaluations are conducted, direct, qualitative methods are inadequate and not reliable. The vast majority of trials focus on prevalence and intensity of infections, comparison of different diagnostic techniques and evaluation of drug efficacy. For such results to be obtained, more sophisticated and reliable techniques are needed. In drug efficacy trials it is crucial that quantitative methods are applied, especially when the egg reduction rate (ERR) is under evaluation. The ideal diagnostic method applied on the field must be fast, accurate, easy to use also in low resource settings and in trials where a large number of samples are screened.

Currently, the recommended quantitative method for the detection of STH and schistosomes is the duplicate Kato-Katz [19] ideally on two stool samples [18]. It is a quantitative method that allows counting eggs and classifying the infection intensity according to WHO classification (light, moderate or heavy). This method shows reasonable sensitivity at a high intensity of infection [18], however it loses sensitivity and reliability at low intensity infections [16,20]. An important advantage of this method is that it is cheap and the equipment required to perform it is easily available [21,22]. In order to enhance its sensitivity, multiple examination of the same sample and, ideally, multiple sampling on different days are performed [23,24].

Other diagnostic methods have been recently developed in order to increase sensitivity and overcome the Kato-Katz's limitations. The FLOTAC and Mini-FLOTAC are novel tools based on the principle of flotation techniques used in veterinary medicine and adapted to human samples [17]. Both are quantitative methods that detect intestinal helminths and protozoa infections [16,17,21,25]. The FLOTAC has proved to have a higher sensitivity, especially at low intensity infections, mainly due to the fact that the eggs are separated from the faecal material through centrifugation and the slide is easier to read than those prepared with the Kato-Katz method [26–28]. The biggest limitation of the application of the FLOTAC in field studies is represented by the required equipment, which is not available in peripheral laboratories and is rather expensive [29]. The Mini-FLOTAC is a simpler device developed from the FLOTAC philosophy but more

affordable at field level [30]. This technique is faster, as sensitive as the Kato Katz and cheaper than the FLOTAC technique [18]. In a field evaluation of Mini-FLOTAC and Kato-Katz, it was demonstrated that the former detected higher mean faecal egg counts [31].

For the diagnosis of *S. stercoralis,* WHO recommends both the Baermann methods and Koga-agar plates. Both methods have only moderate sensitivity, thus a combination of the two methods is often suggested. Since both methods take long to perform and require specific equipment, few epidemiological studies are conducted and, therefore, few data are available on *S. stercoralis* prevalence [4,5].

3.2 Non-direct methods

3.2.1. Serology

Serology based diagnostic is increasingly used in non-endemic areas for patient evaluation. It allows faster diagnosis, being the sample easy to collect and multi-diagnosis possible. Serology can be applied for diagnosis of *S. stercoralis*, when faecal-based methods fail to detect the infection [14]. In the past years different serological tests have been evaluated to test the performance in detecting the decline of antibody titre after treatment [32]. In areas where re-exposure is excluded, negativization of antibody titre is a better indication of cure than negative stool samples [32]. When drug efficacy is tested, the main limitation for serology is that the titre takes few months to decrease, which means a long follow-up of the patient is needed [32,33]. A difficult situation is when it occurs hyper-infestation in immunosuppressed patients (for instance, with HIV/AIDS, taking immunosuppressive therapy or with cancer), in which immune response is impaired and false negative results can occur and be misleading [14].

Serology has also been applied for the detection of other intestinal parasitic infections. Indirect hemagglutination (IHA) has been used for the diagnosis of schistosomes with good results [34], reaching a sensitivity of 88% for *S. mansoni* and 80% for *S. haematobium* with an overall specificity of 95%. Another successful application of serologic methods is the enzyme-linked immunosorbent assay (ELISA), which has shown good performance (>90% sensitivity) for both species as well as high specificity (98%) [34]. For the

detection of *Schistosoma* a dipstick that employs a monoclonal antibody against *S. mansoni* tegumental antigen is also available; it has shown good sensitivity and specificity (86.7% and 90%, respectively) compared with ELISA sandwich [34].

3.2.2 CAA and CCA

A rapid diagnostic test for the *S. mansoni* diagnosis has recently been developed and tested for accuracy and sensitivity in the field against the recommended techniques. This test is based on the detection of Circulating

Cathodic Antigen (CCA) and Circulating Anodic Antigen (CAA) in urine samples described since late '70s [35]. Importantly, detection of these antigens in either blood or urine is evidence for an ongoing active infection as both antigens are subject to rapid renal clearance from the human circulation [35]. Both methods have shown a large ranges of both sensitivity (from 39% to 89% for CCA and from 47% to 94% for CAA) and specificity (from 8% to 100% for both methods) when compared with the microscopy considered as "gold standard", which underlines the need of further research [36–38]. One of the limitations of CCA is the risk of potential for cross-reaction with other parasite antigens and some human cancers [39,40]. Studies conducted on migrants, have shown that a combination of urine CCA and serology tests could find application in nonendemic settings, to determine both the previous state of infection and as marker of cure if treated [41]. The CAA test is applicable to all *Schistosome* species, not only to the most common (*S. mansoni* and *S. haematobium)* [42]. The concentration detected by CAA test is a good proxy on the number of worms present in the host. This method is based on luminescent up-converting phosphor reporter technology and includes different formats, depending on the matrix and sample volume used for testing [42]. In Morocco it was recently adopted on serum to test its accuracy in low-endemic settings [43]. In addition, a test conducted in Tanzania showed good results of CAA both on fresh blood samples and dried blood spots, being the latter more practical for field handling [44]. Knopp et al evaluated its performance on urine samples and results indicated high sensitivity and specificity (97% and 90% respectively) [45]

3.3 Molecular diagnosis

Because of the limited sensitivity of direct methods, the interest towards molecular diagnosis has increased lately and different DNA amplification based assays have been developed [34,46–48]. The main problem in evaluating molecular techniques is the lack of a "true gold standard" among direct methods [49]. The molecular diagnostic techniques are the loop-mediated isothermal amplification (LAMP) and polymerase chain reaction (PCR). The main challenge of applying these techniques at a wide scale, is overcoming the difficulties in DNA extraction, mainly hindered by the tough egg shell [50]. This fact decreases the sensitivity and accuracy of the methods. Furthermore, the presence of high amounts of suspended debris in most of the sample matrices impedes nucleic acid extraction and may inhibit PCR reactions [51]. The method of choice for DNA extraction varies between studies and depends on the material of the sample, as DNA can be extracted from biological samples but also from soil, water, plants, and compost [52]. LAMP is characterized by the use of a DNA polymerase that has low sensitivity to inhibitors and comprises a set of four primers specially designed to recognize six different sequences on the target gene [52]. The technique is based on an isothermal reaction; therefore, it eliminates the need for expensive thermocyclers used in conventional PCR. It has been observed that in low resource settings LAMP might be more effective, as it is cheaper and more practical to handle, alike a point-of-care diagnostic test with a sensitivity comparable to the conventional PCR [52,53]. As an example, in the detection of *Taenia spp* LAMP performed even better than conventional PCR, showing a sensitivity of 88% vs 37% [52].

PCR is the most used molecular technique in virology and microbiology, whereas its application in parasitology is still under development. However, conventional PCR is a non-quantitative technique, so it is not useful in settings aiming at evaluating drug efficacy or control of transmission and elimination. There are other important limitations of this technique that is worth to be taken into consideration: it is sensitive to inhibitors present in the sample, it relies largely on the setting and diet of the population, on sampling and collection, on the procedure of pipetting and it is largely operator-dependent [52]. Despite these limitations, conventional PCR is the method that changed the diagnostic perspective and improved the diagnostic tools for parasitic infections. One of the greatest advantages of PCR is its specificity for a species

or group of species, according to the target gene sequences chosen. Moreover, new multiplex PCR has developed a high sensitivity at detecting different parasites at the same time, rendering the diagnosis fast and efficacious, even if singleplex remains more accurate and sensitive [49]. The targets for amplification are multiple, though ribosomal sequences and mitochondrial targets are most studied because of the tandem repeats easily found in eukaryotic cells [54,55]. However, while effective, such diagnostic targets are often sub-optimal: highly repetitive DNA elements frequently make up a substantial portion of the genome, and are often present at thousands of copy-numbers per haploid genome. Due to such over-representation, non-coding repetitive sequence elements have become the targets of choice for many PCR-based diagnostic assays. Realtime quantitative PCR (qPCR) is a step forward from conventional PCR, as it allows an accurate count of DNA copies that correlates with egg counts of conventional direct methods. The performance of qPCR is still debated, but several studies have shown a good performance of this molecular method for intestinal parasites and more accurate results compared to Kato-Katz [47,50,56].

4. Morbidity linked to intestinal and urogenital infections

Morbidity arising from schistosome and STH infection is defined as complications linked to the primary infection of intestinal and urogenital parasites that lead to impairment of health and, therefore, of a healthy life. DALYs are usually considered a good indication of morbidities due to the infection [8,9,57]. Calculation of an accurate DALY value implies that it is possible to quantify an accurate measure of disease impact, as well as an accurate estimate of disease incidence and duration, or of current prevalence which is hard to determine for intestinal helminthic infections. STH infections are often asymptomatic or with light and unspecific symptoms, which misleads diagnosis and jeopardizes an accurate evaluation of morbidities. Acute syndromes are characterised by eosinophilia, cough, urticaria and gastric discomfort [1,15]. Chronic infections are also characterized by non-specific symptoms such as diarrhoea, abdominal discomfort, anaemia and occult or fresh faecal blood are common accompanied by mucosal haemorrhage, and abdominal distension [1,58,59]. *S. stercoralis* infection is commonly asymptomatic in otherwise healthy individuals, or presents mild and systemic symptoms. Hyperinfestation, on the other hand, can be severe

and eventually lethal [1,4,60]. Chronic STH infections induce a permanent inflammation which compromises growth at different levels: chronic loss of blood, impaired intake, digestion and absorption of nutrients

[59,61–63]. In detail, ascariasis (and trichuriasis in some cases) is associated with low serum vitamin A (retinol) by interfering with vitamin A absorption [62]. Moreover, studies on cognitive development have been carried out, and showed that STH infections negatively correlate with mental performances [64,65]. Children diagnosed with Trichuris Dysentery Syndrome, a severe form of trichuriasis, showed a severe decrease in mental and cognitive skills and development compared to the match controls [66–68]. Hookworm disease is characterised by moderate-severe iron-deficiency anaemia due to small yet multiple hemorrages in the small intestine caused by the worms, which leads to protein and chronic blood loss. *A. duodenale* causes 2-10 folds higher blood losses than *N. americanus* [69].

All evidence suggests that morbidity of schistosomiasis is due to the presence of the parasite eggs in tissues and not due to the adult worms [12,70]. Not all the eggs produced are excreted; therefore, they are lodged in the intestinal or hepatic tissue (*S. mansoni, japonicum, intercalatum, mekongi*) or in the urogenital system (*S. haematobium*). The eggs induce a granulomatous immune response, which contains egg enzymes that avoid tissue necrosis but produce a chronic tissue inflammation [70,71]. This form of the disease presents as non-specific intermittent abdominal pain, diarrhoea, and rectal bleeding, with the frequency of symptoms often being related to the intensity of infection. Such gastrointestinal features are often focal with isolated mucosal hyperplasia, pseudopolyposis, and polyposis interspersed with normal bowel [72]. The scarce immunoregulation often leads to extensive fibrosis and hepatosplenic disease with periportal fibrosis [71,73]. Clinical features include upper abdominal discomfort with palpable nodular and hard hepatomegaly, often with splenomegaly [74]. Ascites and haematemesis from oesophageal varices, a complication of portal hypertension, can rapidly lead to death [75]. The time from initial infection to advanced fibrosis is usually 5 –15 years [76]. Nonetheless, this clinical feature can be observed in children of 6 years of age [77], which underlines the importance of screening and periodic treatment. Periportal fibrosis is reversible after antischistosomal treatment.

The clinical presentation of urogenital schistosomiasis is characterized by persistent haematuria, urinary frequency and suprapubic discomfort [78]. As for intestinal schistosomiasis, severe urogenital schistosomiasis is a result of poor immunoregulation response to schistosomes-egg, leading to chronic fibrosis of urinary tract, presenting as obstructive uropathy up to hydronephrosis. This condition is a fertile field for bacterial superinfection and renal disfunction with potential lethal consequences [79,80]. Squamous cell carcinoma of the bladder is also strongly associated with *S. haematobium* infection [81]. Female urogenital schistosomiasis can severely affect fertility. Schistosome eggs lodged in the urogenital system induce inflammatory response, that damages the reproductive tract [82]. Unfortunately, treatment might not resolve these advanced forms of genital tract damage and there is growing evidence that such lesions can increase transmission of HIV [12,83]. For men urogenital schistosomiasis could present with haematospermia, orchitis and fertility impairment [84]. These conditions resolve more readily after antischistosomal treatment than those of female genital schistosomiasis.

Similar to STH infections, schistosomiasis has also systemic clinical morbidities such as anaemia (mainly due to minimal but chronic bleeding), malnutrition and impaired childhood development, as a result of the chronic inflammation status on iron metabolism, physical fitness and cognitive function [64,85,86]. Treatment can lessen these symptoms and preventive chemotherapy (PC) is crucial to decrease the onset of such severe clinical presentation.

An important epidemiologic feature common to most helminth infections, with the exception of *S. strercoralis*, is that parasites do not multiply in the host; therefore, morbidity is linked to the intensity of infection (worm load) which is proportional to the time and extent of exposure to transmission. Thus, the aim of regular treatment is to reduce worm load and control/eliminate morbidity in spite of re-infection, while for *S. stercoralis* the infection must be effectively cured in order to eliminate morbidity.

5. Treatment and strategies for control

5.1 Drugs available

The benzimidazoles (albendazole and mebendazole) are the mainstay of treatment for the reduction of STH prevalence and burden. These drugs have firstly been launched in the '80s and still represent the first-choice regimen for STH treatment. They are administered as single dose in public health interventions. Benzimidazoles have excellent safety records and can be administered in both young children and pregnant women except from the first trimester. Both drugs have high efficacy against *A. lumbricoides*, albendazole is efficacious against hookworm, but both are less efficacious against *T. trichiura* [87–89]. Currently, the recommended treatment for *S. stercoralis* infection is single dose ivermectin, which has high efficacy (CR >95%). Albendazole administered over three days could be an alternative although it is scarcely efficacious [90].

Praziquantel is the only drug of choice against *Schistosoma spp.,* It is active against adult worms, but has no activity against juvenile stages [91]. A standard dose of 40 mg/kg is thought to be effective for treatment of *S. haematobium* and *S. mansoni* and can safely be used in pregnancy after the first trimester. For other species (*S. japonicum* and *S. mekongi*), the recommended dose is 60 mg/kg. A dose pole is used in the field to determine the number of tablets to use by height [92].

A dose pole for pre-school aged children extended below 94 cm is also available since a few years. Cure rates are low perhaps because the extrapolation of adults' dose is not appropriate for children [93–95]. A paediatric formulation of praziquantel is under development; currently praziquantel is administered to children by crushing tablets in juice to counterbalance the bitter taste of the drug. The evidence collected so far from studies conducted on efficacy of praziquantel in pre-school aged children, show a flat dose-response against *S. mansoni* infection and the overall efficacy is lower in pre-school than in school-aged children [96]. This stress the need of more studies conducted in the paediatric population. Common side effects of praziquantel are abdominal pain and cramps, vomiting, headache and dizziness, especially in patients with moderate-heavy infections [96].

5.2 Preventive chemotherapy

PC is the regular anthelminthic treatment of in-need populations in endemic areas. This strategy is necessary due to the high rate of infection and severe morbidities caused by helminthiasis. In 2012, the London Declaration on Neglected Tropical Diseases announced a cross-sectoral commitment by several partners, including global partnerships and drug manufacturers, to help eliminate or control preventable neglected tropical diseases by 2020, inspired by WHO roadmap targets [2,6,89,97,98]**.** This commitment included a goal of treating 75% of children at risk of STH infections and schistosomiasis in all endemic countries. To this end, 600 million doses of albendazole and mebendazole are donated annually by pharmaceutical companies, which is enough to treat nearly 70% of the 876 million at-risk children worldwide. In 1984, WHO endorsed a strategy of control of morbidities caused by schistosomiasis through regular administration of praziquantel. Merck KGaA, (Darmstadt, Germany) is bound to donate approximately 250 million tablets of praziquantel per year.

The main focus of PC are school-aged children (5-14 years old). Current WHO guidelines suggest to widen the spectrum and include pre-school-aged children as well in order to tackle morbidities at cognitive and physical development stages. PC is distributed through school infrastructures, which has been demonstrated to be the most cost-effective strategy to reach such a vast population. Modelling done to measure the effects of the programme have erased the idea of extending the coverage to the whole population, in order to fight reservoirs (i.e. adults are considered to be a source of transmission for hookworm) [99,100]. In fact, the highest the coverage of in-need population, the greater the associated benefits in the non-targeted population in terms of reduced prevalence and transmission of infections. Although highly efficacious, PC has limitations that should be addressed and improved in order to maximise and sustain its benefits [89,101,102].

The first limitation is linked to the drugs currently used. PC is based on the administration of a single benzimidazole tablet in monotherapy yearly or twice a year in areas endemic for STH, but the cure rate of these drugs against STH is poor. Mebendazole has low efficacy against hookworm infections [103,104] and both benzimidazoles are ineffective against *T. trichiura* [87,103,104].

Reinfection shortly after treatment is another challenge. Several studies have shown that prevalence and intensity of infections, especially for *A. lumbricoides* and *T. trichiura*, rapidly (within 4-12 months from treatment) reverse back to the pre-treatment status [105–107], while the hookworm reinfection rate is lower [108]. In settings were PC is conducted systematically, helminths prevalence and intensity of infections decreased [109–111]. Outcomes of PC are different depending on which worm is targeted; for instance on Pemba Island, Tanzania, where one of my PhD studies has been conducted, the control programme against schistosomiasis started in 1986 [112]. The integrated program of PC and community involvement together with snail control [113–116], has been successful and *S. haematobium* prevalence has dropped from 50% to less than 10% [114,117]. On the same trend, it has been shown that there was a "collateral benefit" of the ivermectin community-based administration for the elimination of lymphatic filariasis on *S. stercoralis* prevalence, which has dramatically decreased after 6 years of systematic PC [118]. On the contrary, on this same island, data on *A. lumbricoides* and *T. trichiura* prevalence and intensity of infection are not encouraging: despite 20 years of regular PC, *T. trichiura* prevalence is still above 90% [87] and *A. lumbricoides* above 40% [108].

Another limitation of the ongoing STH control programmes is the complete lack of intervention against *S. stercoralis* infection, despites the results from recent studies reporting the high prevalence of this infection not only in tropical climates but at all latitudes [4,15]. Recent reports have demonstrated that its eradication is possible with pharmacological control with ivermectin [119]. A significant decrease of *S. stercoralis* prevalence has been reported, in areas endemic for lymphatic filariasis and onchocerciasis, where PC was distributed at community level [118,120].

Moreover, PC coverage is still lagging behind the 2020 goal: in 2015 it was reported to be 48.3% in PSAC and 63.2% in SAC for STH, for schistosomiasis the coverage was 41.8% in SAC and 10.3% in adults [7]. A survey recently conducted on praziquantel coverage in Pemba island, revealed that school- based treatment was high (more than 80% of coverage), whereas adult coverage (reflecting also WHO reports [7]) was low [101,114,121]. Many factors contribute to low PC coverage, Knopp et al., highlighted some such as hard-toreach areas, breastfeeding, pregnancy, absenteeism and community fatigue. The mentioned factors

should be taken into consideration when evaluating the state of the art of intestinal helminth control and when planning a specific strategy to implement the program, according to local needs and addressing constraints [97,114,122,123]. On the other hand, high coverage and frequent treatments , despite averting severe morbidity, decreasing rate of infection and transmission, and preventing severe and chronic consequences in young population, may have a downside which is represented by the increased danger of developing resistance due to high drug pressure [99,124].

In selected areas, the aim of PC programme is the elimination of the parasite. For example, elimination of schistosomiasis has been pursued and obtained in some endemic areas (such as Morocco, Tunisia, China) by joining forces between PC and other interventions for interruption of transmission such as vector control and water sanitation and hygiene (WASH) [102,116,125–129]. During the first 50 years of large-scale efforts to control schistosomiasis, snail control was the primary intervention used to prevent infection, as no drugs were suitable for mass distribution [111]. At present PC is the most widely used intervention, and molluscicides are applied in selected high-transmission water bodies. The limited application of pesticides is mainly due to environmental damages. Niclosamide is a good example, it is non-toxic to people, it kills snails at low concentrations, but it is harmful to some freshwater fish and amphibians. The use of this compound is licensed in the USA, and when used in suitable habitats, it has been an important contributor to schistosomiasis elimination campaigns [130–132]. In schistosomiasis endemic areas, snail control should be considered as an integral part of the intervention, especially in high transmission sites [99,123,126,127,129]

Lately, behavioural change is one of the most discussed topics with promising results [100,123,125,126,133].

The implementation of WASH, education and communication programmes should act harmoniously with PC. Studies are currently examining data collected from the implementation of PC together with WASH and educational approaches [125,126,133]. Results are still controversial, but such integrated interventions will have stronger and longer-term impact. Updated WHO guidelines tailored on specific needs would be highly

beneficial: for instance, high endemic settings that aim at transmission control, should focus on implementing

PC targeted on PSAC and maybe involving adult population when at high-risk of fresh water contact. In contrast, low endemicity settings, that aim at elimination of transmission, should focus on wide arrays of interventions, such as PC, WASH, behavioural changes and snail control [97,115,123,125,133–138].

5.3 Promising drug alternatives

The research for new drugs and compounds for intestinal parasite infections has languished in the past decades; currently, no candidates are in the pipelines of pharmaceutical companies, which could be soon put on the market to improve efficacy of existing products or in case resistance against available drugs develops [91,103].

Moxidectin is a macrocyclic lactone derived from the actinomycete *Streptomyces cyanogriseus* spp. *noncyanogenus* used in veterinary practice against filariasis since the ’90. Considering these promising results, it has recently been adopted by human medicine for the treatment of onchocerciasis with good results in terms of efficacy against microfilariae and safety profile. Its mechanism of action is still under debate, but studies on its pharmacokinetics have demonstrated that it influences the glutamate-gated chloride channels and the gamma-aminobutyric acid receptor complex, which, consequently, causes a paralysis and death of the parasite [139,140]. Studies conducted on moxidectin pharmacokinetic in humans have shown that both liquid and tablet formulation are quickly absorbed [82], are extensively distributed in body fluids and have a long half-life [140]. Korth-Bradley *et al*. have reported an increased bioavailability of the drug by the simultaneous consumption of food [141]. Given this scenario, Attah *et al.* have conducted a pilot study to assess the efficacy of moxidectin against the main intestinal parasites (STHs, *S. mansoni*), with encouraging results [142].. Moxidectin was for the above-mentioned reasons, one of the main focus of my PhD project.

Another promising group of drugs are the oxolanes. Oxolanes have been studied as potential alternatives, due to their *in vitro* and *in vivo* effects against *Schistosoma spp.* and *Clonorchis sinensis* [143,144]. In more

detail, OZ277 (arterolane maleate) has been shown to be effective against the adult and juvenile stages of *S. mansoni* and *S. japonicum* [143]. In 2011, arterolane maleate in combination with piperaquine was licensed in India (Synriam) for treatment of uncomplicated *P. falciparum* malaria. Synriam was a drug candidate evaluated during my PhD project.

As mentioned above, one of the main concerns of implementation of PC is the potential occurrence of drug resistance. In fact, extensive use of the few drugs available on the market as monotherapy might repeat in humans the dramatic scenario that we are witnessing in veterinary medicine [145–148]. Mathematical modelling revealed that the likelihood of resistance development is significantly delayed when drug combinations with different modes of action are administered [149–151]. Differently from other fields, such as bacteriology and virology, where combination of drugs that act on different pathogen's targets is a longtime established treatment strategy, only few clinical trials have been conducted to explore the efficacy of drug combination against helminthic infections [149,150,152,153]. One of the most striking evidence of efficacy of the combined treatment of albendazole/oxantel pamoate against *T. trichiura* infection was recently published [87]. This good outcome led the attention to the need of more research into drug combinations instead of single drug use. Lately, several trials have been conducted testing the efficacy and safety of anthelminthic drug combinations. For example, the co-administration of ivermectin and albendazole was revealed to enhance the efficacy of these drugs against *T. trichiura* and STH coinfections [154–156]. Moreover the co-administration of ivermectin and tribendimidine showed a non-inferiority efficacy to albendazole/oxantel pamoate against hookworm infections [157].

Chapter 2

Aim and objectives

2.1 Aim and objectives

The main goal of my PhD project was to evaluate the efficacy of new drugs and drug combinations against intestinal parasitic infections. Further goals stretched from the application of different diagnostic methods and their comparison (DNA extraction methods and PCR protocols) to clinical evaluation of urinary tract morbidity in children infected with *S. haematobium.* The specific objectives of my PhD are listed in more detail below:

1. **Efficacy of moxidectin and Synriam®** against *S. mansoni* and *S. haematobium* infections**:** to determine the efficacy and safety of (i) moxidectin alone and (ii) Synriam® (Arterolane (OZ277) and piperaquine) alone and (iii) Synriam®-praziquantel combination compared to (iv) praziquantel against *S. mansoni* and *S. haematobium* infections (Chapter 4).
2. **Efficacy of moxidectin** against *S. stercoralis* infection**:** to evaluate the efficacy and safety of (i) moxidectin compared to (ii) ivermectin against *S. stercoralis* infection (and co-infection with *O. viverrini*) (Chapter 5a).
3. **Compare**qPCR versus duplicate Baermann method in the diagnosis of *S. stercoralis*: to assess the sensitivity and specificity of two DNA extraction methods analysed with qPCR versus duplicate Baermann method in the diagnosis of *S. stercoralis* (Chapter 5b).
4. **Efficacy of different moxidectin drug combinations** against *T. trichiura* infection: to evaluate the efficacy and safety of (i) moxidectin, (ii) moxidectin plus tribendimidine, (iii) moxidectin plus albendazole versus (iv) albendazole plus oxantel pamoate against *T. trichiura* infection and concomitant STH infections in adolescents (Chapter 6).
5. **Clinical evaluation of** urinary tract morbidity in *S. haematobium* infected PSAC and SAC **:** to assess the evolution of urinary tract morbidity in *S. haematobium* infected PSAC and SAC six months after treatment with different doses of praziquantel and placebo (control group) (Chapter 7).

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Chapter 3

Efficacy and safety of Moxidectin, Synriam, Synriam-Praziquantel versus Praziquantel against *Schistosoma haematobium* and *S. mansoni* infections: a randomized, exploratory phase 2 trial

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Efficacy and Safety of Moxidectin, Synriam, Synriam-Praziquantel *versus* Praziquantel against *Schistosoma haematobium* and *S. mansoni* Infections: A Randomized, Exploratory Phase 2 Trial

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Abstract

Background

Schistosomiasis affects millions of people, yet treatment options are limited. The antimalarial Synriam (piperaquine 150 mg/arterolane 750 mg) and the anthelminthic moxidectin revealed promising antischistosomal properties in preclinical or clinical studies.

Methodology

We conducted two single-blind, randomized exploratory Phase 2 trials in *Schistosoma mansoni* and *S. haematobium*-infected adolescents in northern and central Côte d'Ivoire. Our primary endpoints were cure rates (CRs) and egg reduction rates (ERRs) based on geometric mean and safety. Each subject was asked to provide two stool samples (*S. mansoni* trial) for Kato-Katz analysis or three urine samples (*S. haematobium* trial) for urine filtration and one finger prick for malaria screening at baseline and follow-up. Participants were randomly assigned to either moxidectin, Synriam, Synriam plus praziquantel or praziquantel.

Principal Findings

128 adolescents (age: 12–17 years) were included in each study. Against *S. haematobium* moxidectin and Synriam revealed low efficacy. On the other hand, Synriam plus praziquantel and praziquantel yielded CRs of 60.0% and 38.5% and ERRs of 96.0% and 93.5%, respectively. CRs observed in the treatment of *S. mansoni* were 13.0%, 6.7%, 27.0%, and

27.6% for moxidectin, Synriam, Synriam plus praziquantel and praziquantel, respectively. ERRs ranged from 64.9% (Synriam) to 87.5% (praziquantel).

Conclusion/Significance

Synriam and moxidectin show low efficacy against *S. haematobium*, hence an ancillary benefit is not expected when these drugs are used for treating onchocerciasis and malaria in co-endemic settings. Further studies are needed to corroborate our findings that moxidectin and Synriam show moderate ERRs against *S. mansoni*.

Author Summary

Schistosomiasis is a parasitic infection that affects millions of people all over the world and it is due to schistosomes, helminths (worms) that infect the intestine and the urinary bladder. Treatment options are limited, with praziquantel being the only used drug. The antimalarial Synriam and the anthelminthic moxidectin revealed good action against this worm in previous studies. We conducted two studies in *Schistosoma mansoni* and *S. haematobium*-infected adolescents in Côte d'Ivoire. Subjects positive for the infection were allocated by chance to the four groups of treatment (moxidectin, Synriam, Synriam plus praziquantel or praziquantel); participants did not know which drug they took. Our aim was to calculate how many participants were negative after the treatment and how did the intensity of infection change before and after treatment. Each subject provided stools and urines for examination. 128 adolescents were included in each study. Moxidectin and Synriam did not work well against *S. haematobium*. Against *S. mansoni*, only a small part of the participants were negative after treatment in all treatment groups, but the intensity of infections were reduced. Further studies are needed to better understand this result.

Introduction

Schistosomiasis caused by the three main species *Schistosoma haematobium*, *S. japonicum* and *S. mansoni* is a disease known since ancient times. An estimated 230 million people are infected and the disease causes a burden of 3.0 million disability-adjusted life years lost [1, 2]. Despite the enormous public health problem with regard to symptomatology and morbidity, it is listed among the so-called neglected tropical diseases (NTDs) [3]. The treatment of schistosomiasis relies on one drug only, praziquantel, which is active against adult schistosomes, but has little activity against juvenile worms [3]. The high drug pressure resulting from the widespread administration of praziquantel in the framework of preventive chemotherapy programs could lead to drug resistance [4]. However, at the moment there are no viable alternatives to praziquantel [5]. Even so, drug discovery has languished, and no drug is currently undergoing clinical testing [6]. Therefore, the discovery and development of new drugs for the treatment of schistosomiasis is a high priority.

The antischistosomal activity of the artemisinins was described more than 30 years ago [7]. Subsequently, many studies have been conducted to elucidate the antischistosomal effect of the artemisinins and synthetic peroxides [8, 9]. The 1,2,4-trioxolanes in particular, characterized by improved pharmacokinetic parameters compared to the artemisinins [10], were the focus of different *in vitro* and *in vivo* studies, which demonstrated efficacy not only against *Schistosoma* spp. but also against *Echinostoma caproni*, *Fasciola hepatica* and *Clonorchis sinensis* [9, 11]. In

more detail, OZ78, OZ277 and OZ209 were first studied in *S. mansoni* and *S. japonicum* rodent models more than a decade ago. A particularly high activity of the 1,2,4-trioxolanes was observed against juvenile *S. mansoni* and *S. japonicum* infections in the mouse model and against both juvenile and adult stages of the worms in the hamster model [11]. After licensing OZ277 (arterolane maleate) in combination with piperaquine in 2011 (Synriam) [12–14] Mossallam and colleagues studied the efficacy of OZ277 in combination with piperaquine in rodents infected with *S. mansoni*, which confirmed the excellent activity of this trioxolane derivative particularly against the worms' juvenile stages [15].

Moxidectin is widely used in veterinary medicine for heartworms [16]. Studies are ongoing to develop moxidectin as potential alternative to ivermectin for the treatment of onchocerciasis [17]. In the framework of this drug development program effects of moxidectin on concomitant helminths were studied [18], revealing cure rates (CR) and egg reduction rates (ERR) of 64% and 66%, respectively in *S. mansoni*-infected patients [19].

We conducted two single-blinded, randomized exploratory Phase 2 trials in *S. haematobium* and *S. mansoni*-infected adolescents to assess the efficacy of moxidectin and Synriam. One group of children was treated with a combination of Synriam and praziquantel since this combination might offer effects against pre-patent and patent infections. Finally, praziquantel treated participants served as active control.

Methods

Ethics statement

Ethical clearance was obtained from the ethics committee of Northern and Central Switzerland (EKNZ; reference no. 15/01) and from the Comité National d'Éthique et de la Recherche du Ministère de la Santé et de l'Hygiène Publique (reference no. 026) in Côte d'Ivoire. The trial is registered with Current Controlled Trials (ISRCTN 63657086). Participants aged 12–18 years old were eligible for inclusion in the trial. Written informed consent was obtained before enrolment by the children aged 18 years and by parents or legal guardians of the children below 18 years old. The latter assented orally.

Study setting and population

The single-blind, randomized, exploratory, four arm Phase 2 trials were conducted in May and June 2015, in the health districts of Toumodi (Moronou village (geographical co-ordinates 06° 19'0" N latitude, 04°58'0" W longitude), endemic for *S. haematobium*) and Man (villages of Bigouin (7°24'01" N, 7°33'11" W) and Biakalé (7°27'07" N, 7°41'32"), endemic for *S. mansoni*) of Côte d'Ivoire. In all locations, village based recruitment was implemented.

Randomization and drugs

We used a computer-generated block randomization code stratified by baseline infection intensities (block size of 8) provided by an independent statistician. Enrolled subjects were randomly allocated to the four treatment arms i) single dose of moxidectin liquid formulation 8 ml (8 mg), ii) Synriam (150 mg arterolane plus 750 piperaquine): three doses administered for three consecutive days, iii) Praziquantel 40 mg/kg single dose plus Synriam (150 mg arterolane plus 750 piperaquine) three doses administered for three consecutive days, iv) Praziquantel 40 mg/kg single dose. Since drug interactions were not yet studied, the combination treatment group received praziquantel in the morning followed by Synriam in the late afternoon (and two additional Synriam courses over the next two days). Only the study investigator was aware of the treatment assignments, while children and laboratory technicians were blinded. Moxidectin

was administered as oral suspension (Cydectin 0.1% Zoetis, Switzerland) mixed with equal amounts of mint syrup (sweetener RK50 (E952 (Sodium Cyclamate), E954 (Sodium Saccharin); Peppermint Plus Aroma and Citrus Plus Aroma [Rohner Konzept, Switzerland]) to mask the bitter taste of the drug. Praziquantel was administered based on subjects' weight using 600 mg tablets (Cesol, Merck).

Study procedures

Community meetings were conducted to explain the purpose, procedures, potential risks and benefits of the study. At baseline three urine samples (*S. haematobium* cohort) and two stool samples (*S. mansoni* cohort) were collected from participants. Schistosome-positive adolescents, who had provided 2 stool and 3 urine samples were eligible to participate in the trials. One stool sample was collected from each participant in the *S. haematobium* trial and one urine sample from each patient participating in the *S. mansoni* trial to evaluate co-infections.

The standard urine filtration method (10 ml of urine) was used for appraisal of *S. haematobium* infections [3, 20]. Microhematuria was assessed on one urine sample using Hemastix (Siemens, Munich, Germany) dipsticks. The Kato-Katz technique was used for the quantitative assessment of *S. mansoni* infections. Duplicate Kato-Katz was performed on each stool sample using the 41.7 mg template according to the standardized method [21]. All slides were double-checked by a second laboratory technician; slides were considered negative only if no parasites were detected by the two independent microscopists. Concomitant infections with soil-transmitted helminth (STH) infections were recorded. In addition, we used the circulating cationic antigen (CCA) tests (ICT diagnostics, Cape Town, South Africa) on one urine sample for *S. mansoni* diagnosis. The POC-CCA cassette (batch 33112) was performed according to the manufacturer's instructions. The test results were scored as negative or positive, the latter stratified into trace (very light color band), 1+, 2+ (light infection), and 3+ (heavy infection) according to the visibility of the color reaction. On the day of treatment and physical examination, participants provided one finger prick sample for malaria and hemoglobin testing. The hemoglobin concentration was determined using a portable Hemocue 301 (HemoCue AB; Ängelholm, Sweden). Additionally, a rapid malaria diagnostic test (RDT) (ICT diagnostics) was employed. Thick and thin blood smears were prepared on a microscope slide for subsequent appraisal of malaria parasitemia. The medical history of participants was assessed with a standardized questionnaire, in addition to a clinical examination carried out by the study clinician. Height was measured with a standard meter (to the nearest 1 cm) and weight with an accurate electronic balance (to the nearest 0.1 kg). After treatment adverse events were monitored at 3, 24, 48 and 72 hours after each dose of treatment was administered. Participants were excluded if they suffered from any systematic illness (e.g. clinical malaria).

At day 21 after the last treatment dose was provided we sampled again 3 urine and 2 stool specimens for analysis of *S. haematobium*, *S. mansoni* and STH infections, together with a finger prick for the diagnosis of malaria infection. At the end of the study all participants positive for *S. mansoni*, *S. haematobium*, and STH infections were treated with albendazole (400 mg) and/or praziquantel (40 mg/kg) and artesunate and lumefantrine following national guidelines for malaria treatment.

Sample size and statistical analysis

We aimed for 25 participants per treatment arm, a common sample size for exploratory Phase 2 trials [22]. Allowing for an attrition rate of up to 20%, it was planned to include 30 participants per treatment arm. For the estimation of the prevalence of *S. mansoni* and *S. haematobium* infection in these settings we based our calculation on a previously reported prevalence

of 60–70% at nearby sites [23, 24]. However, we used a conservative estimate of 60% for our sample size determination and hence we planned to screen 200 subjects in each study cohort to detect at least 120 eligible participants infected with *S. mansoni*/*S. haematobium*.

Results were double entered in a database (Excel 2010), cross-checked and analyzed with Stata 12.0 (Lakeway Drive College station, TX, Unites States of America). An available case analysis was performed, which included all children with primary outcome data. The intensity of *S. mansoni* infection (number of eggs per gram (epg) of feces) was assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears (from baseline and follow-up separately) and multiplying this number by a factor of six [25]. The intensity of infection for *S. haematobium* was assessed by calculating the average of the egg counts from the triplicate urine filtration. Infection intensity was classified following WHO cutoffs [26]. Geometric and arithmetic-mean egg counts were calculated for each group before and after treatment. Egg reduction rates (ERRs) were calculated by the following formula (ERR = (1-(geometric mean at follow-up/geometric mean at baseline))*100). Bootstrap resampling method with 2,000 replicates were used to calculate 95% confidence intervals (CIs) for ERRs. Differences in ERRs were determined under the assumption that non-overlapping CIs indicate statistical significance. Cure rates (CRs) were calculated as the percentage of children who became egg-negative after treatment, being egg-positive at baseline.

Results

Study flow

The study flowcharts are presented in Figs 1 and 2. In the *S. haematobium* study we screened 268 children/adolescents of which 52 were negative for infection, 23 did not provide any urine sample, 60 delivered less than 3 urine samples, and 5 were excluded at physical examination because they did not meet the inclusion criteria. In total 128 participants were enrolled and randomly assigned to one of the four treatments as follows: 31 subjects received moxidectin, 32 were treated with Synriam plus praziquantel, 33 were administered Synriam and 32 were treated with praziquantel. Of all participants, 110 were present at the follow up examination and 18 were lost to follow up (Fig 1).

In the *S. mansoni* study we screened 388 subjects among whom 25 were negative for a *S. mansoni* infection, 158 did not provide 2 stool samples, and 72 did not appear at the treatment venue. Five subjects were excluded because they did not meet the inclusion criteria.

Participants were randomly assigned to either moxidectin (n = 34), Synriam plus praziquantel (n = 30), Synriam (n = 32) and praziquantel (n = 32). In total we treated 128 subjects and 116 were present at the follow up examination (12 lost to follow up) (Fig 2).

Baseline characteristics

Demographic and clinical baseline characteristics are summarized in Tables 1 and 2. Treatment groups in the *S. haematobium* trial were well balanced in terms of age (mean age: 13.5 years, range: 12–17 years), sex (58.6% male participants), weight (mean weight: 39.4 kg), height (mean height: 149.2 cm) and baseline infection intensity. The arithmetic and geometric mean number of *S. haematobium* eggs per 10 ml was 53.4 and 17.1, respectively. 70% of participants had light and 30% moderate to heavy infections. Twenty-eight (21.9%) of the 128 treated subjects had a coinfection with STH. Microhematuria was detected in 61 (49%) subjects. Eighty-two (65.6%) of the 128 treated subjects were malaria positive based on thick and thin smears and 72.6% based on RDT.

Among the participants in the *S. mansoni* trial, 55.5% were male and the mean age was 12.8 (12–17) years (Table 2). No differences among treatment arms were observed in terms of

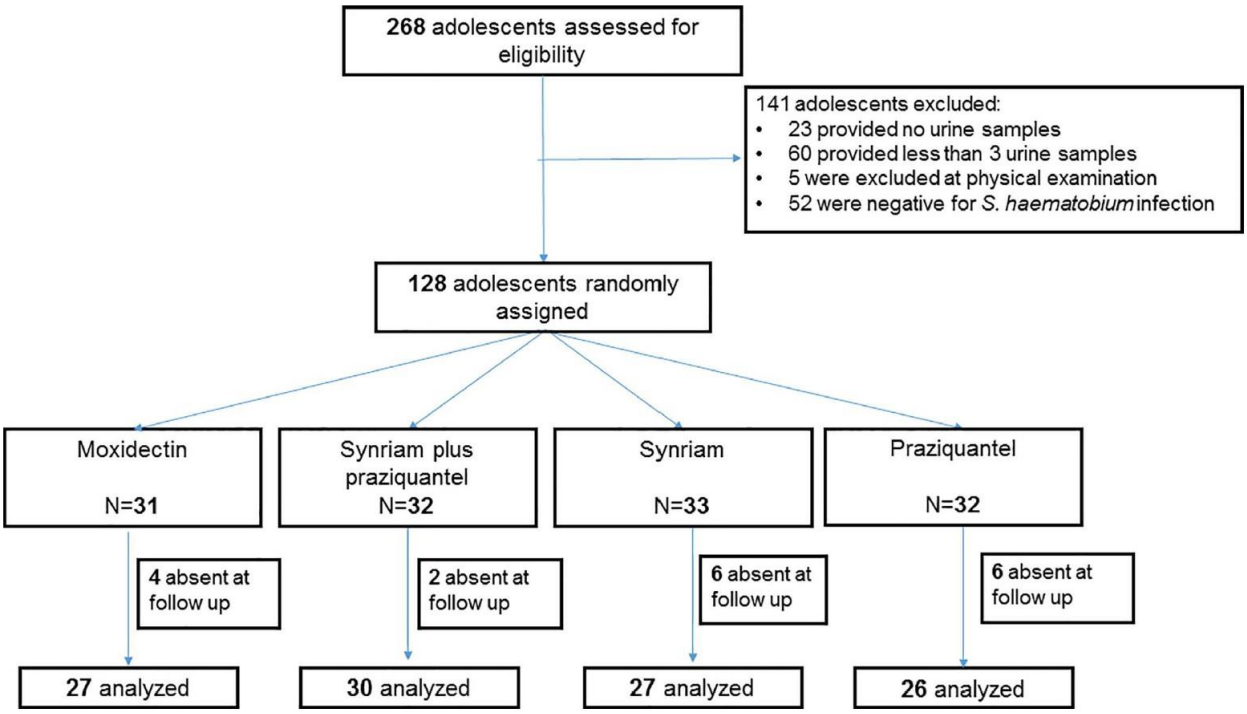

Fig 1. Study flow in the *S. haematobium* study.

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weight (mean weight 35.2 kg), height (139 cm), gender (71 males) except from the Synriam plus praziquantel treatment arm, in which 73% of participants were males. The arithmetic and geometric mean *S. mansoni* infection load was 216.6 and 109.1 epg, respectively. 59 (46.1%) of adolescents had light infections and 69 (53.9%) moderate to heavy infections. 49 (38.3%) of participants had a coinfection with STH. 101 (78.1%) of the *S. mansoni*-infected adolescents were malaria positive according to thick and thin smear and 99 (77.3%) according to the RDT.

Efficacy against *S. haematobium* and *S. mansoni*

We observed a low efficacy for the two novel treatments in the *S. haematobium* cohort (Table 3). In more detail, moxidectin and Synriam achieved CRs of 14.8% (95% CI 0.04–0.3) and 11.1% (95% CI 0.02–0.3), and ERRs of 8.7% (95% CI -0.4–0.6) and 0% (95% CI -0.8–0.6), respectively. The two treatment arms containing praziquantel (Synriam plus praziquantel and praziquantel) yielded CRs of 60% (95% CI 0.4–0.8) and 38.5% (95% CI 0.2–0.6) and ERRs of 96% (95% CI 0.8–1.0) and 93.5% (95% CI 0.8–1.0), respectively.

Results of the *S. mansoni* cohort are reported in Table 4. ERRs were as follows: moxidectin 70.9% (95% CI 0.4–0.9), Synriam 64.9% (95% CI 0.4–0.8), Synriam plus praziquantel 77.6% (95% CI 0.5–1.1), and praziquantel 87.5% (95% CI 0.8–1.0). The CRs based on Kato-Katz were 12.9% (95% CI 0.03–0.3) for moxidectin, 6.7% (95% CI 0.01–0.2) for Synriam, 27.0% (95% CI 0.1–0.5) for Synriam plus praziquantel, and 27.6% (95% CI 0.1–0.5) for praziquantel. Praziquantel showed a moderate CR of 50.0% in adolescents harboring a low intensity infection. CRs according to CCA (trace results considered as positive) were 17.9%, 30.4%, 20%, 14.3% for moxidectin, Synriam plus praziquantel, Synriam, and praziquantel, respectively.

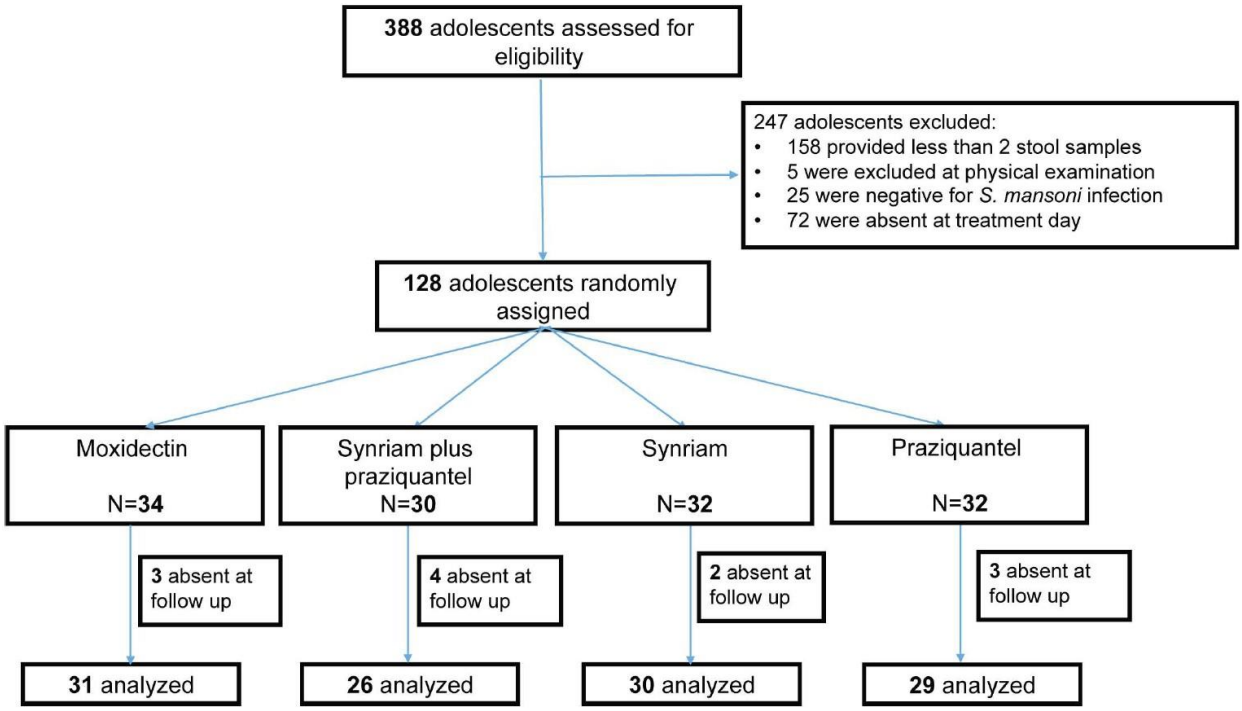

Fig 2. Study flow in the *S. mansoni* study.

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Table 1. Baseline characteristics of adolescents infected with *S. haematobium* stratified by treatment group. The study was carried out in the villages of Moronou in central Côte d'Ivoire, between May and June 2015.

	Moxidectin (N = 31)	Synriam plus praziquantel (N = 32)	Synriam (N = 33)	Praziquantel (N = 32)	Total (N = 128)
Age (years) [mean (SD)]	13.8 (0.25)	13.6 (0.2)	13.5 (0.2)	13.3 (0.2)	13.5 (1.1)
Males N (%)	20 (64.5)	19 (59.4)	20 (60.6)	16 (50)	75 (58.6)
Hemoglobin (g/dl) [mean (SD)]	11.9 (0.1)	11.9 (0.2)	11.7 (0.1)	11.8 (0.2)	11.9 (0.1)
Weight (kg) [mean (SD)]	41.0 (1.6)	38.8 (1.3)	38.0 (1.8)	39.9 (1.9)	39.4 (0.8)
Height (cm) [mean (SD)]	150.0 (2.0)	148.8 (1.5)	148.9 (1.9)	148.9 (1.9)	149.2 (0.9)
EPG (AM)	55.0	52.4	53.9	52.1	53.4
EPG (GM)	16.8	16.6	16.9	18.2	17.1
Infection intensity N (%)					
Light	22 (71.0)	23 (72.0)	23 (70.0)	22 (69.0)	90 (70.3)
High	9 (29.0)	9 (28.0)	10 (30.0)	10 (31.0)	38 (29.7)
Microhematuria positive N (%)	17 (57.0)	14 (46.0)	16 (59.0)	14 (44.0)	61 (49.0)
STH coinfection N (%)	5 (16.1)	7 (21.9)	9 (27.3)	7 (21.9)	28 (21.9)
Malaria RDT positive N (%)	20 (64.5)	22 (68.7)	26 (78.7)	25 (78.1)	93 (72.6)
Malaria direct smear positive N (%)	20 (64.5)	22 (68.7)	18 (58.0)	22 (71.0)	82 (65.6)

SD, standard deviation; AM, arithmetic mean; GM, geometric mean, EPG, eggs per gram, RDT, rapid diagnostic test

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Table 2. Baseline characteristics of adolescents infected with *S. mansoni* stratified by treatment group. The study was carried out in the villages of Bigouin and Biakalé in western Côte d'Ivoire, between May and June 2015.

	Moxidectin (N = 34)	Synriam plus praziquantel (N = 30)	Synriam (N = 32)	Praziquantel (N = 32)	Total (N = 128)
Age (years)	12.9 (0.2)	12.7 (0.2)	12.8 (0.3)	12.8 (0.3)	12.8 (0.1)
Males N (%)	17 (50.0)	22 (73.3)	15 (46.8)	17 (53.1)	71 (55.5)
Hemoglobin (g/dl) [mean (SD)]	12.2 (0.1)	11.7 (0.2)	11.7 (0.2)	11.8 (0.2)	11.8 (0.1)
Weight (kg) [mean (SD)]	34.4 (1.5)	32.8 (1.5)	39.8 (2.2)	33.97 (1.2)	35.2 (0.8)
Height (cm) [mean (SD)]	138.5 (2.0)	136.2 (2.5)	144.7 (2.1)	137.8 (1.8)	139.3 (1.1)
EPG AM	207.5	249.8	191.7	219.8	216.6
EPG GM	100.9	103.2	99.2	139.9	109.1
Light intensity N (%)	17 (50)	13 (43.3)	15 (46.8)	14 (43.8)	59 (46.1)
Moderate intensity N (%)	13 (38.2)	11 (36.7)	13 (40.6)	12 (37.5)	49 (38.3)
High intensity N (%)	4 (11.8)	6 (20.0)	4 (12.5)	6 (18.7)	20 (15.6)
CCA N (%)	29 (90.6)	27 (90.0)	26 (86.7)	29 (90.0)	111 (89.5)
Trace N (%)	2 (6.3)	4 (13.3)	4 (6.7)	0 (0)	8 (6.5)
Low intensity N (%)	5 (15.6)	2 (6.7)	4 (13.3)	3 (3.4)	14 (11.3)
High intensity N (%)	22 (68.8)	21 (70)	20 (66.7)	26 (81.3)	89 (71.8)
STH coinfection N (%)	12 (35.3)	16 (53.3)	13 (40.6)	8 (25.0)	49 (38.3)
Malaria RDT positive N (%)	24 (70.6)	24 (80)	26 (81.2)	25 (78.1)	99 (77.3)
Malaria direct smear positive N (%)	28 (82.3)	22 (73.3)	24 (75)	27 (84.4)	101 (78.1)

SD, standard deviation; AM, arithmetic mean; GM, geometric mean, EPG, eggs per gram, RDT, rapid diagnostic test

doi:10.1371/journal.pntd.0005008.t002

Table 3. Effect of moxidectin, Synriam, Synriam plus praziquantel and praziquantel against *S. haematobium* and malaria co-infections. The study was carried out in the village of Moronou in central Côte d'Ivoire, between May and June 2015.

	Moxidectin (N = 27)	Synriam plus Praziquantel (N = 30)	Synriam (N = 27)	Praziquantel (N = 26)
***S. haematobium* infection**				
Children cured (%) (CI)	4 (14.8) (0.04–0.3)	18 (60) (0.4–0.8)	3 (11.1) (0.02–0.3)	10 (38.5) (0.2–0.6)
Children cured with high infection intensity (%)	0/8 (0)	3/9 (33.3)	0/8 (0)	2/7 (28.6)
Children cured with low infection intensity (%)	4/19 (21.1)	15/21 (71.4)	3/19 (15.8)	8/19 (42.1)
EPG before treatment AM	56.3	54	52.7	47
EPG after treatment AM	117.6	2.8	83.8	2.07
EPG before treatment GM	17.2	16	16.1	15.2
EPG after treatment GM	15.7	0.6	17.7	0.98
Egg reduction rate (%) (95% CI)	8.7 (-0.4–0.6)	96 (0.8–1.0)	0 (-0.8–0.6)	93.5 (0.8–1.0)
Microhematuria positive before treatment N (%)	16 (61.5)	13 (44.8)	14 (53.8)	12 (46.2)
Microhematuria negative after treatment N (%)	8 (29.6)	19 (63.3)	9 (33.3)	15 (57.7)
***Plasmodium falciparum* infection**				
Number RDT positive before treatment (%)	18 (66.7)	20 (66.7)	21 (77.8)	21 (80.7)
Number children negative based on RDT (%)	10/18 (55.5)	20/20 (100.0)	20/21 (95.0)	14/21 (66.6)
No. malaria direct smear positive before treatment (%)	18 (66.7)	20 (66.7)	17 (63.0)	19 (73.1)
No. children negative based on malaria smear (%)	10/18 (55.5)	20/20 (100.0)	17/17 (100.0)	12/19 (63.2)

CI, confidence interval; AM, arithmetic mean; GM, geometric mean, EPG, eggs per gram, RDT, rapid diagnostic test

doi:10.1371/journal.pntd.0005008.t003

Table 4. Effect of moxidectin, Synriam, Synriam plus praziquantel and praziquantel against *S. mansoni* and malaria co-infections. The study was carried out in the villages of Bigouin and Biakalé in western Côte d'Ivoire, between May and June 2015.

	Moxidectin (N = 31)	Synriam plus Praziquantel (N = 26)	Synriam (N = 30)	Praziquantel (N = 29)
***S. mansoni* infection**				
Children cured (%) (95% CI)	4 (12.9) (0.03–0.3)	7 (27.0) (0.1–0.5)	2 (6.7) (0.01–0.2)	8 (27.6) (0.1–0.5)
Children cured with high infection intensity (%)	0/3 (0)	2/6 (33.3)	0/4 (0)	1/5 (20.0)
Children cured with moderate infection intensity (%)	2/13 (15.4)	2/9 (22.2)	0/13 (0)	1/12 (8.3)
Children cured with low infection intensity (%)	2/15 (13.3)	3/11 (27.3)	2/13 (15.4)	6/12 (50.0)
EPG before treatment AM	216.9	272.7	201.6	221.6
EPG after treatment AM	159.3	176.3	101	121.6
EPG before treatment GM	106.7	108.4	107	143.6
EPG after treatment GM	33.5	24.3	37.4	17.9
Egg reduction rate (%) (95% CI)	70.9 (0.4–0.9)	77.6 (0.5–1.1)	64.9 (0.4–0.8)	87.5 (0.8–1)
CCA positive (tr+) before treatment N (%)	28 (93.3)	23 (88.5)	25 (89.3)	28 (96.5)
CCA positive (tr-) before treatment N (%)	26 (86.6)	20 (76.9)	23 (82.0)	28 (96.5)
CCA negative (tr+) after treatment N (%)	5 (17.9)	7 (30.4)	5 (20.0)	4 (14.3)
CCA negative (tr-) after treatment N (%)	7 (27.0)	8 (40.0)	5 (21.7)	7 (25.0)
***Plasmodium falciparum* infection**				
No. RDT positive before treatment (%)	22 (71.0)	20 (77.0)	24 (80.0)	23 (79.0)
No. children negative based on RDT (%)	1 (4.5)	16 (80.0)	23 (95.8)	5 (21.7)

CI, confidence interval; AM, arithmetic mean; GM, geometric mean, EPG, eggs per gram; tr+, trace considered as positive; tr-, trace considered as negative; RDT, rapid diagnostic test

doi:10.1371/journal.pntd.0005008.t004

Efficacy against co-infections

In the *S. haematobium* cohort all subjects in both Synriam treatment groups were cured from malaria infection according to direct smear technique, 5% (1/21) remained positive according to RDT. At follow up 55.5% of subjects in the moxidectin group were *Plasmodium* spp. negative based on both techniques. In the praziquantel treatment group 66.6% and 63.2% of participants were negative according to RDT and direct smear, respectively.

In the *S. mansoni* cohort overall 50% of subjects were *Plasmodium* spp. negative at follow up. CR in the groups treated with Synriam (praziquantel plus Synriam, Synriam respectively) were 80% and 95.8%.

In both cohorts none of the treatments showed any efficacy against STH infections (data not shown).

Tolerability

At clinical examination all 128 subjects in the *S. haematobium* cohort reported symptoms. The number of adverse events stratified by treatment arm and evaluation time point are summarized in Table 5. Overall, recorded clinical symptoms decreased from 100% prior to treatment to 48% (3 hours post-treatment) and 21% (72 hours post-treatment). Three days after the last treatment administered, none of the participants reported any adverse events. The highest number of mild adverse events 3 hours post-treatment were recorded in the praziquantel treatment group (58%) and the lowest number in the Synriam plus praziquantel treatment group (41%). The most commonly observed adverse events were stomach ache (30%) and headache (19%) (S1 Table).

Table 5. Number of adolescents with clinical symptoms observed prior to treatment and adverse events in the *S. haematobium* study among the four different treatment arms, assessed at different time points. The study was carried out in the villages of Moronou, Bigouin and Biakalé in central Côte d'Ivoire, between May and June 2015.

Evaluation time points	Moxidectin (N = 31)	Synriam plus praziquantel (N = 32)	Synriam (N = 33)	Praziquantel (N = 32)	Overall (n = 128)
Clinical symptoms before treatment	31 (100.0)	32 (100.0)	33 (100.0)	32 (100.0)	128 (100.0)
3 hours after first treatment	14 (45.0)	13 (41.0)	17 (52.0)	18 (58.0)	62 (48.4)
24 hours after first treatment	9 (29.0)	9 (28.0)	4 (12.0)	6 (19.0)	28 (21.8)
72 hours after first treatment	7 (23.0)	9 (28.0)	6 (18.0)	5 (16.0)	27 (21.1)
3 hours after second treatment	NA	3 (9.0)	1 (3.0)	NA	4/65 (6.2)
24 hours after second treatment	NA	1 (3.0)	0 (0)	NA	1/65 (1.5)
72 hours after second treatment	NA	0 (0)	0 (0)	NA	0/65 (0)
3 hours after third treatment	NA	1 (3.0)	0 (0)	NA	1/65 (1.5)
24 hours after third treatment	NA	1 (3.0)	0 (0)	NA	1/65 (1.5)
72 hours after third treatment	NA	0 (0)	0 (0)	NA	0/65 (0)

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In the *S. mansoni* cohort 95 subjects reported symptoms (74%) at clinical examination (Table 6). Overall, 51% of participants had mild symptoms at the first evaluation time point, ranging from 44% in the moxidectin group to 57% in the praziquantel treatment group. 15% of participants had symptoms 72 hours post-treatment and 3 days after the last treatment dose none of the participants reported symptoms.

All events reported were mild, the most common symptoms were stomach ache (29%) and headache (10%). Only one subject had a moderate adverse event, namely eye swelling, which was resolved following antihistamine treatment. S1 Table and S2 Table summarize clinical symptoms stratified by treatment arm and assessment time for the *S. haematobium* and *S. mansoni* studies.

Discussion

To our knowledge we have for the first time assessed the efficacy of Synriam and moxidectin against *S. mansoni* and *S. haematobium* infections. It is crucial to develop alternatives to praziquantel for the treatment of schistosomiasis, but lately no new drug candidates entered the

Table 6. Number of adolescents with clinical symptoms observed prior to treatment and adverse events in the *S. mansoni* study among the four different treatment arms, assessed at different time points. The study was carried out in the villages of Moronou, Bigouin and Biakalé in central Côte d'Ivoire, between May and June 2015.

Evaluation time points	Moxidectin (N = 34)	Synriam plus praziquantel (N = 30)	Synriam (N = 32)	Praziquantel (N = 32)	Overall (N = 128)
Clinical symptoms before treatment	26 (76.0)	22 (73.0)	26 (81.0)	21 (65.0)	95 (74.2)
3 hours after first treatment	15 (44.0)	17 (57.0)	16 (50.0)	17 (53.0)	65 (50.7)
24 hours after first treatment	10 (29.0)	9 (30.0)	2 (6.0)	6 (19.0)	27 (21.1)
72 hours after first treatment	7 (21.0)	6 (20.0)	3 (9.0)	3 (9.0)	19 (14.8)
3 hours after second treatment	NA	1 (3.0)	0 (0)	NA	1/62 (1.6)
24 hours after second treatment	NA	1 (3.0)	0 (0)	NA	1/62 (1.6)
72 hours after second treatment	NA	1 (3.0)	0 (0)	NA	1/62 (1.6)
3 hours after third treatment	NA	1 (3.0)	0 (0)	NA	1/62 (1.6)
24 hours after third treatment	NA	1 (3.0)	1 (3.0)	NA	2/62 (3.2)
72 hours after third treatment	NA	0 (0)	0 (0)	NA	0/62 (0)

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drug discovery and development pipeline for this NTD [6]. Repurposing of existing drugs with different treatment indications as in the present exploratory trial, might be the way forward in order to find alternatives to praziquantel in a fast and cost-effective manner [27].

Based on promising findings in preclinical or clinical studies, two drugs were selected for our study, Synriam a new generation antimalarial drug and moxidectin, which will soon be marketed for the treatment of onchocerciasis [19, 28]. Disappointingly, we observed low efficacy of moxidectin and Synriam in terms of ERR and CR against *S. haematobium* though infection intensities in study participants were low. The highest CR and ERR (60% and 96%) were observed for Synriam plus praziquantel treated participants. Though the drugs were not administered simultaneously since possible drug interactions have not been studied to date, both treatments might have positively influenced each other. It could be hypothesized that the damage caused by praziquantel on *S. haematobium* was exacerbated by treatment with Synriam, which resulted in a slightly better efficacy when the two drugs were administered together. However, our study was not powered to detect statistical differences among treatment arms, hence studies with larger samples sizes would be necessary to confirm this finding.

Interestingly, against *S. mansoni* both moxidectin and Synriam alone performed better than against *S. haematobium* with higher ERRs observed but not CRs. The better efficacy of moxidectin and Synriam against *S. mansoni* in terms of ERRs cannot be explained at the moment. Fluctuations in egg counts might also play a role. However, the ERR observed for moxidectin in the present study is in line with previous results on *S. mansoni* [18]. In contrast with our findings in *S. haematobium* treated adolescents, in the *S. mansoni* study no increased efficacy was observed with Synriam plus praziquantel over praziquantel alone.

The findings on praziquantel observed in the two trials warrant further discussion. First, observed ERRs of praziquantel were similar in both cohorts (87.5–93.5%) and in accordance with previous findings [5, 29]. However, strikingly, praziquantel showed low CRs in participants infected with *S. haematobium* (38.4%) and *S. mansoni* (27.6%). These CRs are notably lower (except for one of our own studies in a nearby setting [5]) than in previous reports, which have documented CRs (for a single dose of praziquantel (40 mg/kg)) ranging from 51 to 100% against *S. mansoni* and *S. haematobium*. Our findings underline the great variability of CRs linked to praziquantel treatment [30]. As discussed elsewhere [31,32] the low CRs of praziquantel in the *S. mansoni* cohort might be due to the fact that half of participants suffered from moderate/heavy infection intensity. Praziquantel revealed a higher CR of 50% in adolescents with low intensity *S. mansoni* infections. Similarly, a higher CR (42%) was observed in participants with a low *S. haematobium* infection intensity.

Many clinical trials have been conducted using artemether and artesunate alone and in combination with praziquantel [33]. The moderate efficacies against *S. mansoni* and *S. haematobium* observed with the antimalarial Synriam in our trial are in line with previous studies using peroxidic drugs as monotherapy against chronic schistosome infections [34, 35]. For example, a recent meta-analysis determined that artesunate has a low CR against chronic *S. haematobium* infection (25%) [34, 36]. This finding is not surprising since artemisinin derivatives mainly act on juvenile schistomes [8]. We had therefore initially planned a second follow up at 50 (*S. mansoni*) and 80 days (*S. haematobium*) after treatment, to assess the efficacy of Synriam against prepatent infections. However, this additional survey was not conducted, due to the low efficacy observed at the first follow up, which made it impossible to assess activity against juvenile schistosome infections. Follow up studies could be planned to assess the prophylactic effect of Synriam and Synriam plus praziquantel against infections with *S. mansoni* and *S. haematobium*.

In the *S. haematobium* cohort we used both filtration and assessment of microhaematuria. Our data confirm [37] that the evaluation of microhematuria for the diagnosis of *S.*

haematobium has a lower sensitivity compared to the filtration method. However, urine filtration was performed on three samples while microhaematuria was investigated on only one. Furthermore, the majority of participants in our study had low *S. haematobium* intensity infections.

In both settings we examined *Plasmodium* infections: based on RDT in both cohorts infected adolescents treated with Synriam were all cured with the exception of four patients (CRs 80–100%). CRs with the thin and thick smears were slightly lower. To date few studies have been conducted with Synriam against *Plasmodium* infections: two on the African and Asian continents reported CRs of 90–99% 28 days after treatment, a second follow up was performed 42 days after the last dose of drug and 100% of participants had no gametocytes [38]. The rate of reinfection was lower than 0.5% at 28 days [38]. Similar results were found in another study comparing artemether plus lumefantrine and Synriam, with all patients being cured 28 days post-treatment [39]. The moderate CRs observed in the praziquantel and moxidectin treated groups in the *S. haematobium* setting cannot fully be elucidated. Spontaneous clearance might have occurred, however we also cannot rule out self-treatments.

Interestingly, in both trials a higher number of clinical symptoms was observed before treatment compared to post-treatment. For example, before treatment 41% (*S. haematobium* cohort) and 54% (*S. mansoni* cohort) participants reported headache, whereas 3 hours after treatment only 19% and 10% reported this symptom in the two cohorts, respectively. A similar finding was observed for stomach ache, which rapidly improved after treatment. These results are contradictory to most studies, which observe a worsening of gastrointestinal symptoms following treatment with praziquantel most likely due to dying worms [29]. We cannot fully explain our findings, however it might be worth highlighting that the number of participants complaining about symptoms prior to treatment was very high, higher than in previous studies, which makes it difficult to present a clear picture on the occurrence of adverse events. In addition, it is not possible to distinguish between an actual improvement in their conditions (which however seems to have occurred too fast) or a perceived improvement, driven by participants' expectations or other factors.

In conclusion, the two drugs tested (moxidectin and Synriam) showed low efficacy against *S. haematobium* infections. We therefore do not expect a significant ancillary benefit when these two drugs are used for the treatment of onchocerciasis and malaria in settings co-endemic for *S. haematobium*. We observed a better performance of both drugs in terms of ERR against *S. mansoni* infection. Studies with larger sample size are needed to confirm our finding and to rule out that these results have occurred by chance.

Supporting Information

S1 Checklist. Consort checklist.
(PDF)

S1 Protocol. Trial protocol.
(PDF)

S1 Table. Number of adolescents with clinical symptoms prior to treatment and adverse events among the four different treatment arms assessed at different time points in the *S. haematobium* cohort.
(DOCX)

S2 Table. Number of adolescents with clinical symptoms prior to treatment and with adverse events among the four different treatment arms assessed at different time points in

the *S. mansoni* cohort.
(DOCX)

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Author Contributions

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Investigation: BB JTC JK.

Methodology: JHu MP.

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Writing – review & editing: JTC MP JHa JHu.

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Chapter 4

Efficacy of moxidectin versus ivermectin against *Strongyloides stercoralis* infections: a randomized, controlled non-inferiority trial

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MAJOR ARTICLE

Efficacy of Moxidectin Versus Ivermectin Against *Strongyloides stercoralis* Infections: A Randomized, Controlled Noninferiority Trial

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Background. Infections with *Strongyloides stercoralis* are of considerable public health relevance. Moxidectin, a well-established drug in veterinary medicine under consideration for regulatory submission for the treatment of onchocerciasis, might serve as an alternative to the widely used ivermectin.

Methods. We conducted an exploratory, randomized, single-blind trial to evaluate the efficacy and safety of moxidectin (8 mg) vs ivermectin (200 μg/kg) against *S. stercoralis* infections. Cure rate (CR) against *S. stercoralis* was the primary outcome. Safety and efficacy against coinfections with soil-transmitted helminths and *Opisthorchis viverrini* were secondary outcomes. Noninferiority required the lower limit of the 95% confidence interval (CI) of the differences in CRs not exceed 7 percentage points.

Results. A total of 127 participants were enrolled and randomly assigned to the 2 treatments whereby 1 participant per arm was lost to follow-up. We observed a CR of 93.7% (59/63) for moxidectin compared to 95.2% (59/62) for ivermectin. Differences between CRs were estimated as –1.5% percentage points (95% CI, –9.6 to 6.5), thus the lower limit of the CI exceeds the noninferiority margin of 7 percentage points. No side effects were observed. CRs against hookworm infection were 57% (moxidectin) and 56% (ivermectin). Low efficacy for both drugs against *O. viverrini* was observed.

Conclusions. Moxidectin might be a safe and efficacious alternative to ivermectin for the treatment of *S. stercoralis* infection, given that only slight differences in CRs were observed. However, noninferiority could not be demonstrated. Larger clinical trials should be conducted once the drug is marketed.

Clinical Trials Registration. Current Controlled Trials: ISRCTN11983645

Keywords. *Strongyloides stercoralis*; moxidectin; ivermectin; *Opisthorchis viverrini*.

Strongyloides stercoralis is a soil-transmitted nematode and one of the most overlooked helminths among the neglected tropical diseases. It exists throughout the world, excluding only the far North and South, yet estimates of its prevalence (about 100 million people) are often only little more than educated guesses and probably largely underestimated [1–3]. Compared to other major soil-transmitted helminths (STHs), information on *S. stercoralis* is scarce [4]. *Strongyloides stercoralis* is an exception among helminthic parasites as it can reproduce within a human host (endogenous autoinfection), which may result in long-lasting infections. Some studies have reported on individuals who had *S. stercoralis* infections sustained for more than 75 years. *Strongyloides stercoralis*' ability to cause systemic infection is another exceptional feature of this threadworm [3]. However, most infections, chronic, low-intensity infections in particular, remain asymptomatic. It has been found that *S. stercoralis* infection occurs often in adults [4, 5].

The current recommended treatments are a single dose of ivermectin or albendazole for 3 consecutive days, which has a lower efficacy [1, 5, 6]. Ivermectin is highly effective against *S. stercoralis* infection, characterized by a high cure rate (CR). Several trials conducted in Southeast Asia on *S. stercoralis* reported a CR for ivermectin of 97%–99% [6–10]. Despite this, new drugs are needed. Among new candidates in the human anthelminthic drug development pipeline is moxidectin, a macrocyclic lactone that is well established in veterinary practice [11]. In vivo studies on *Strongyloides fuelleborni* conducted on rhesus macaques infections reported the efficacy of moxidectin to be similar to that of ivermectin [12]. Moxidectin is currently under consideration by the US Food and Drug Administration (FDA) for use against onchocerciasis in humans. The drug

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might offer some advantages over ivermectin. First, moxidectin use is weight independent at an 8-mg fixed dose, simplifying administration, especially when treating large communities. Second, moxidectin has been shown to have a lower neurotoxic potential than ivermectin [13]. Finally, and most importantly, moxidectin has been used successfully in veterinary medicine against ivermectin-resistant strains of *Haemonchus contortus* [14].

Our aim in this study was to assess, for the first time, the efficacy of moxidectin against *S. stercoralis* infections. Ivermectin served as the comparator. The safety of moxidectin and its efficacy against coinfections with *Opisthorchis viverrini* and STHs were evaluated as secondary outcomes.

METHODS

Ethical Considerations

Ethical clearance was obtained from the ethics committee of Northwestern and Central Switzerland and from the Lao National Ethics Committee on Health Research Ministry of Health. The trial is registered with Current Controlled Trials (ISRCTN11983645). Participants aged 12–60 years were eligible for inclusion. Written informed consent was collected from all participants or legal guardians for children before enrollment.

Randomization and Drugs

We used a computer-generated block randomization code (block size of 4) provided by an independent statistician. Enrolled participants were randomly allocated to the following 2 treatment arms: ivermectin 200-μg/kg single dose or moxidectin 8-mg single dose. Moxidectin was administered as an oral suspension (Cydectin 0.1%; Zoetis, Switzerland) mixed with equal amounts of mint syrup (sweetener Premix CY/SA S741 from Sanaro SA, Switzerland) containing E952 (sodium cyclamate), E954 (sodium saccharin), and peppermint aroma (Permaseal from Givaudan AG, Switzerland) to mask the drug's bitter taste. Ivermectin (Iver P; 3-mg tablets), obtained from Elea, Argentina, was administered based on patient weight (200 μg/kg). Only the principal investigator was aware of the treatment assignments, while laboratory technicians were blinded. Patients were not informed whether they would receive ivermectin or moxidectin, though we cannot exclude the possibility that the patients recognized ivermectin tablets if they had been treated with it in earlier treatment campaigns.

Study Procedures and Diagnosis

This exploratory, phase 2, randomized, single-blind study was conducted between April and June 2016 in the district of Pathoumphone, Lao People's Democratic Republic, which is endemic for *S. stercoralis* infection. CR against *S. stercoralis*, determined 21 days after treatment, was the primary outcome. Safety, CR, and egg reduction rate (ERR) against coinfections with STHs and *O. viverrini* were the secondary outcomes. In both locations, village-based recruitment was implemented.

At baseline 2 fecal samples on 2 consecutive days were collected from participants. Samples were examined with the Baermann method for the detection of *S. stercoralis* larvae. The Baermann method was performed following the World Health Organization standard procedure [15]. Only participants positive for the infection were included in the study. Concomitant infections with STHs (*Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm) and *O. viverrini* were assessed using the Kato-Katz method [16]. Height was measured with a standard meter (to the nearest 1 cm) and weight with an electronic balance (to the nearest 0.1 kg). The medical history of participants was assessed with a standardized questionnaire in addition to a clinical examination carried out by the study clinician. Participants who had chronic diseases, were aged <12 years, were pregnant women, or were considered not healthy at physical examination were excluded from the trial but still given the recommended treatment. Side effects were monitored at 3, 24, and 48 hours after treatment.

Between day 21 and day 25 after treatment, we resampled 2 stool specimens for analysis of *S. stercoralis*, STHs, and *O. viverrini.* At the end of the study, all participants who were still positive for *S. stercoralis*, STHs, and/or *O. viverrini* infections were treated with ivermectin (200 μg/kg), albendazole (400 mg) and/or praziquantel (40 mg/kg) according to local guidelines.

Sample Size and Statistical Analyses

This study was designed as a binary outcome noninferiority trial. The sample size determination was based on the assumptions that the efficacy of moxidectin against *S. stercoralis* has not yet been studied and that it is well known that the efficacy of ivermectin is high (97%–99%) [7–10, 17]. Since the mode of action of both drugs is similar, we assumed a CR of 98% for both drugs. The noninferiority limit was set to 7 percentage points. With no difference between both drugs, 100 patients (50 per arm) would yield an upper limit of the 95% confidence interval (CI) that excludes a difference of more than 7% with a power of 80%. The sample size was increased to 60 per arm to account for a potential loss to follow-up of 15%.

We based the screening on reported prevalence data of 40% on Mekong islands [18]. Hence, we anticipated screening 350 participants for the detection of at least 120 infected with *S. stercoralis*, including a safety margin. However, this number had to be increased since the proportion of participants who provided 2 stool samples was lower than expected.

Data were digitally collected on tablets using CommCare ODK, version 2.8. The questionnaires and forms were developed in the CommCare server (www.commcarehq.org) and tested previous to the field activity. A mobile user was created for each field data collector allowing access to a specific form. A completed form was immediately synchronized to the server

for real time data monitoring. After fieldwork, data was downloaded from the server into Excel (version 2011). All data was crossed-checked for completeness and consistency. A hard copy of the forms was also completed during data collection and used to cross-check 10% of the electronically collected data. Validated data were cross-checked and analyzed with Stata 12.0 (College Station, Texas). A barcode generating system was applied using a free-barcode generator software available at www.free-barcode.com. A generated barcode containing the UID of each patient was placed on the stool containers before handing them to patients. Once a filled stool container arrived at the research station, a research team member scanned the attached barcode for sample registration and subsequently the system automatically generated a specific form for further data entry. An available case analysis, which included all participants with primary outcome data, and a per-protocol analysis were planned. CRs were calculated as the percentage of participants who became larvae-negative after treatment, being larvae positive at baseline. Bootstrap resampling methods with 2000 replicates were used to calculate 95% CIs for ERRs. CIs indicate statistical significance. For the secondary outcome parameters, the intensity of infection of *O. viverrini* and STHs in terms of eggs per gram (EPG) was assessed by adding up the egg counts from the quadruplicate Kato-Katz thick smears (from baseline and follow-up separately) and multiplying this number by a factor of 6. Geometric and arithmetic mean egg counts were calculated for each group before and after treatment for *O. viverrini* and STHs infections. Intensity of infection for *O. viverrini* was categorized considering 600, 1500, and 6000 EPG as cutoffs [19]. Intensity of infection for hookworm was categorized considering 2000 and 4000 EPG as cutoffs [20]. ERRs were calculated using the following formula: ERR = [1 – (geometric mean at follow-up/geometric mean at baseline)] × 100. CRs for STHs and *O. viverrini* were calculated as the percentage of participants who became egg-negative after treatment, being egg-positive at baseline. Analyses were performed with Stata (version 12.1).

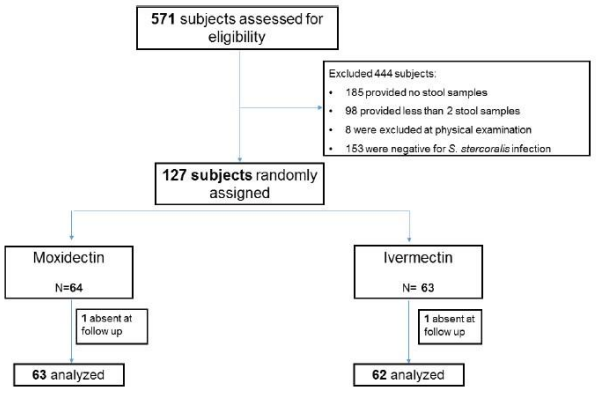

Figure 1. Flow chart of the study conducted in the villages of Morphu and Phakpheo in Champasack Province, Lao People's Democratic Republic, between April 2016 May 2016.

RESULTS

Baseline Characteristics and Study Flow

The study flow chart is presented in Figure 1. We screened 571 participants, of which 153 were negative for infection, 283 did not provide any/enough stool sample, and 8 were excluded at the physical examination because they did not meet the inclusion criteria. In total, 127 participants were enrolled and randomly assigned to 1 of the 2 treatments as follows: 64 received moxidectin (8 mg) and 63 were treated with ivermectin (200 µg/kg). In each treatment arm 1 patient was not present at the follow-up examination (Figure 1). No deviations from the treatment protocol were observed; therefore, the available case analysis is identical with the per-protocol analysis.

Demographic and clinical baseline characteristics are summarized in Table 1. Treatment groups were well balanced in terms of age (mean age, 40 years), sex (51% male participants), weight (mean weight, 54 kg), and height (mean height, 158 cm).

Coinfections were more often observed in the moxidectin arm, in which the proportions of *O. viverrini* and hookworm infections were 89% and 58%, respectively, compared to 75% and 56% of patients, respectively, in the ivermectin arm. Most infections with *O. viverrini* and hookworm were of light infection intensity. No coinfections with other helminths were detected among participants in both treatment arms.

Efficacy Against *S. stercoralis*

We observed a high efficacy of both drugs against *S. stercoralis* infection. Moxidectin achieved a CR of 93.6% (59/63; 95% CI, 84.5 to –98.2) compared to a CR of 95.1% (59/62; 95% CI, 86.5 to 99.0) calculated for ivermectin (Table 2). Differences between CRs were estimated as –1.5 percentage points (95% CI, –9.6 to –6.5). Therefore, the lower limit of the 95% CI exceeds the preset noninferiority margin of 7 percentage points.

Efficacy Against Coinfections

A moderate efficacy was observed against hookworm infection in both treatment arms. The CRs and ERRs for moxidectin and ivermectin were 56.7% (21/37; 95% CI, 55.9% to 79.7%) and 55.9% (19/34; 95% CI, 52.1% to 84.7%) and 74.6% (95% CI, 61% to 90%) and 79.4% (95% CI, 61% to 88%), respectively. None of the drugs showed activity against *O. viverrini*. CRs were 17.8% (10/56; 95% CI, 11.2% to 32.2%; moxidectin) and 6.5% (3/46; 95% CI, 6.4% to 25.4%; ivermectin) with corresponding ERRs of 12.5% (95% CI, –2% to 30%) and 0% (95% CI, –40% to 2%; Table 2).

Safety

At clinical examination, 37 (29.1%) participants reported symptoms before treatment. Most had vertigo (13.4%) and headache (8.6%). In addition, a few participants reported

Table 1. Baseline Characteristics of *Strongyloides stercoralis*–Infected Participants Stratified by Treatment Group

Characteristic	Moxidectin (N = 64)	Ivermectin (N = 63)
Age [y], mean (SD)	39.4 (12.9)	40.7 (10.9)
Males, N (%)	31 (48.4)	34 (54.0)
Weight [kg], mean (SD)	54.4 (10.2)	52.5 (9.3)
Height [cm], mean (SD)	157.5 (7.5)	158.2 (7.5)
Temperature [°C], mean (SD)	36.4 (0.5)	36.4 (0.4)
Coinfection with *Opisthorchis viverrini*, N (%)	57 (89.1)	47 (74.6)
Coinfection with hookworm, N (%)	37 (57.8)	35 (55.6)

Abbreviation: SD, standard deviation.

Table 2. Efficacy of Moxidectin and Ivermectin Against *Strongyloides stercoralis* and Coinfections

Study Parameter	Moxidectin (N = 63)	Ivermectin (N = 62)
Strongyloides stercoralis		
Participants cured, N (%) (CI)	59/63 (93.6) (84.5 to 98.2)	59/62 (95.1) (86.5 to 99.0)
Opisthorchis viverrini		
EPG before treatment AM (CI)	276.7 (51.3 to 502.1)	248.1 (65.5 to 430.8)
EPG after treatment AM (CI)	169.1 (51 to 287.2)	191.2 (58.6 to 323.7)
EPG before treatment GM (CI)	44.6 (27.1 to 73.2)	32.1 (17.1 to 59.3)
EPG after treatment GM (CI)	27.6 (15.7 to 47.9)	49.7 (23.8 to 65.8)
Egg reduction rate (%) (CI)	12.5 (–2 to 30)	0 (–40 to 2)
Participants cured, N (%) (CI)	10/56 (17.8) (11.2 to 32.2)	3/46 (6.5) (6.4 to 25.4)
Hookworm		
EPG before treatment AM (CI)	149.1 (47.9 to 250.3)	432.9 (0 to 1192.2)
EPG after treatment AM (CI)	34.8 (11.9 to 57.7)	23.7 (8.1 to 39.3)
EPG before treatment GM (CI)	11.4 (5.7 to 22.1)	7.3 (3.6 to 13.9)
EPG after treatment GM (CI)	2.9 (1.0 to 4.4)	1.5 (0.6 to 3.0)
Egg reduction rate (%) (CI)	74.6 (61 to 90)	79.4 (61 to 88)
Participants cured, N (%) (CI)	21/37 (56.7) (55.9 to 79.7)	19/34 (55.9) (52.1 to 84.7)

Abbreviations: AM, arithmetic mean; CI, 95% confidence interval; EPG, eggs per gram; GM, geometric mean.

nausea, diarrhea, abdominal discomfort, and skin lesions. One adult reported blood in stool. Participants were checked at 3, 24, and 48 hours after drug administration for side effects. None of the participants reported any side effect from treatment at any time point.

DISCUSSION

This is the first randomized trial to assess the efficacy of moxidectin against *S. stercoralis* infection, which is a neglected yet considerable public health problem. Despite the high efficacy and safety of ivermectin, which is the current drug of choice, it is crucial to develop and find alternative treatments in case ivermectin resistance arises. Other available drugs, that is, the benzimidazoles (albendazole), need longer treatment courses and are less efficacious [6]. No new drugs are under development for *S. stercoralis* infection [21]. Repurposing of drugs currently used or under registration for different indications might be a fast and cost-effective way to discover novel molecules effective against this infection [22].

Moxidectin, which is widely used in veterinary medicine [11], is a "low hanging fruit" to be repositioned for treatment of *S. stercoralis* infection, given the good results observed in vivo against *S. fuelleborni* infection in macaques [12] and the nearly completed FDA registration for treatment of onchocerciasis in humans.

Moxidectin showed promising efficacy against *S. stercoralis* infection in our trial, comparable to that of ivermectin (94% vs 95%, respectively). Of note, although CRs for ivermectin of between 97% and 99% have been repeatedly reported in similar settings [9, 10], the sample size relied on the optimistic assumption of 98% CR. Since the observed CRs were lower, the study was underpowered and noninferiority could not be demonstrated at the prespecified margin.

Both drugs were very well tolerated in our study; none of the participants reported side effects after treatment. As reported in the literature, ivermectin was well tolerated, with a similar number of side effects observed in the ivermectin and placebo groups [23–25]. A recent study that used the same formulation of moxidectin in children infected with *Schistosoma mansoni* and *Schistosoma haematobium* reported mild side effects including nausea, headache, and abdominal discomfort [26]. One possible explanation might be related to the age of participants (adults vs school-age children) who perceive symptoms and physical discomfort in different ways. Moreover, adults better understand physical symptoms and are more critical and reliable in reporting them. Another possible reason might be linked to the fact that in the cited study, all participants reported similar symptoms before and after treatment, hence it cannot be determined whether symptoms are treatment related or not.

As highlighted above, one key advantage of moxidectin over ivermectin is that it might be effective against ivermectin-resistant *S. stercoralis*. Fortunately, anthelminthic resistance has not yet been observed in humans. Nevertheless, it is worth highlighting that resistance has been demonstrated in sheep infected with *Strongyloides* spp. (40%) following treatment with ivermectin, even at low frequency [27]. Different studies in veterinary medicine have demonstrated that moxidectin is effective against ivermectin-resistant strains of parasites [14]. One trial conducted on lambs showed an efficacy of >99% against resistant *Haemonchus contortus*, whereas the CRs of

ivermectin were only 38%–53% [28]. Resistance against ivermectin was found to be a dominant trait, while it might be rendered incompletely dominant or recessive for moxidectin [29]. Cross-resistance among macrocyclic lactones has been reported in livestock; this impairs efficacy of multiple compounds [14, 27, 30]. Despite this fact, it has been shown that drugs develop resistance at different speed, and resistance toward moxidectin occurs more slowly than for ivermectin. Moreover, moxidectin at recommended dosages was shown to be effective against ivermectin-resistant parasites as well as several macrocyclic lactone–resistant parasites [27].

We evaluated the efficacy of moxidectin against coinfections with hookworm and *O. viverrini*. A low efficacy was observed against *O. viverrini* for both drugs (CRs of 18% for moxidectin and 6.5% for ivermectin). Moderate CRs of 57% and 56% against hookworm infection were recorded for moxidectin and ivermectin, respectively. Of note, we did not distinguish the activity against different hookworm species, including *Ancylostoma ceylanicum*, which is common in the study area [31] and should be evaluated in follow-up studies. Studies on the efficacy of ivermectin against hookworm infections in humans are scarce. Three studies revealed low CRs of 11.8%–33.1% for ivermectin against hookworm infections [32–34]. In veterinary medicine, on the other hand, both drugs are successfully used for the treatment of *Ancylostoma* spp. and other gastrointestinal parasites [35, 36]. In dogs, for example, ivermectin was administered against *Ancylostoma caninum* infections, yielding CRs of 100% [35]. Similarly, moxidectin as pour-on or oral formulation demonstrated a high efficacy against gastrointestinal nematodes in beef cattle [36]. The most widely used strategy for protecting against drug resistance is to use drug combinations [37]. Hence, it might be worth exploring the use of moxidectin in combination with albendazole. Considering the moderate effect of moxidectin against hookworm and the moderate efficacy of albendazole against *S. stercoralis* together with the similar distribution of these parasites, the combination might be effective in tackling the mentioned infections, while potentially delaying drug resistance.

We conclude that moxidectin might be a safe and efficacious alternative to ivermectin for the cure of *S. stercoralis* infection. We did not observe any ancillary benefit against coinfection with *O. viverrini* and moderate efficacy against hookworm. Larger trials are needed to confirm our findings once the drug has successfully passed FDA registration and is marketed for human use.

Notes

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Chapter 5

Efficacy and tolerability of moxidectin alone and in coadministration with albendazole and tribendimidine versus albendazole plus oxantel pamoate against Trichuris trichiura infections: a randomised, non-inferiority, single-blind trial.

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Efficacy and tolerability of moxidectin alone and in co-administration with albendazole and tribendimidine versus albendazole plus oxantel pamoate against *Trichuris trichiura* infections: a randomised, non-inferiority, single-blind trial

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Summary

Background The recommended anthelmintics show low efficacy in a single-dose regimen against *Trichuris trichiura*. Moxidectin, a new treatment for river blindness, might complement the drug armamentarium for the treatment and control of soil-transmitted helminthiasis. However, its efficacy against *T trichiura* has not yet been studied. The aim of the study was to assess the efficacy of moxidectin alone and in co-administrations against *T trichiura* infection.

Methods A randomised, single-blind, non-inferiority trial was done in two primary schools and one secondary school in Pemba, Tanzania. Adolescents aged 12–18 years who tested positive for *T trichiura* were randomly assigned (5:5:3:3) with a computer-generated sequence to receive moxidectin (8 mg) plus albendazole (400 mg), albendazole (400 mg) plus oxantel pamoate (25 mg/kg; reference treatment), moxidectin (8 mg) plus tribendimidine (200 mg or 400 mg), or moxidectin (8 mg) alone. Study group assignments were masked from participants and laboratory technicians. The primary outcome was non-inferiority with a 2 percentage point margin for egg reduction rate (ERR) against *T trichiura* assessed as the relative change in the geometric mean egg counts from baseline to 14–21 days after treatment with the Kato-Katz method, based on the available case population. Cure rates (CR) and tolerability (assessed 3, 24, and 48 h post treatment) were secondary outcomes. The study is registered at ISRCTN (number 20398469) and is closed to accrual.

Findings 701 students were enrolled between April 1, and Aug 7, 2017. Primary outcome data were available for 634 students. We observed ERRs of 98·5% for moxidectin plus albendazole and 99·8% for albendazole plus oxantel pamoate, resulting in an absolute difference of −1·2 percentage points (95% CI −1·8 to −0·8), meeting the non-inferiority margin. 100 (51%) of 197 students receiving moxidectin plus albendazole and 166 (83%) of 200 receiving albendazole plus oxantel pamoate were cured, indicating a difference of 32 percentage points (odds ratio 5·3, 95% CI 3·3 to 8·7). ERRs were 91·6% for moxidectin–tribendimidine and 83·2% for moxidectin. Only mild adverse events (mainly headache and stomach pain) were reported. The largest number of adverse events (126 [20%] of 632 students) was observed 24 h post treatment, with no difference among the individual treatment arms (ranging from 23 [19%] of 118 students treated with moxidectin to 38 [19%] of 199 with moxidectin plus albendazole).

Interpretation Moxidectin plus albendazole showed non-inferiority to albendazole plus oxantel pamoate in terms of ERR; however, albendazole plus oxantel pamoate showed a considerably higher cure rate. Dose-optimisation studies with moxidectin and moxidectin plus albendazole should be considered since the efficacy of the dose used for the treatment of onchocerciasis (8 mg) in this study might not be optimal for the treatment of *T trichiura* infections.

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Introduction

Soil-transmitted helminthiases are infections caused by intestinal nematodes: *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm (*Ancylostoma duodenale* and *Necator americanus*). Estimates suggest that up to 1·5 billion people are infected with one or several of the common soil-transmitted helminths (STHs).[1,2] Data from the Global Burden of Diseases Study (2016) indicate that STH infections resulted in 3·3 million disability-adjusted life-years (DALYs).[3] *T trichiura* infections were responsible for 337 000 DALYs in 2016.[3] Infections are typically most intense and debilitating in school-aged children and adolescents, and children with chronic infection might suffer from malnutrition, physical and cognitive retardation, and reduced work performance in adulthood.[4]

Benzimidazoles (ie, albendazole and mebendazole) are the drugs of choice against STH infections[1,5] and are used in preventive chemotherapy[7] programmes, which is the regular administration of anthelmintic drugs to

Research in context

Evidence before this study

We searched PubMed for all articles published before Sept 1, 2016, using different combinations of the search terms "*T trichiura*", "soil-transmitted helminthiasis", "moxidectin", and "efficacy" without language restriction. Our search found 23 studies on moxidectin against *Trichuris* spp in veterinary medicine: none of the papers were on human medicine. However, secondary outcomes from a randomised, controlled, double-blind, phase 3 trial on moxidectin versus ivermectin for *Onchocerca volvulus* published in January 2018, showed a promising activity of moxidectin against soil-transmitted helminthiasis, primarily against *Trichuris trichiura*, with an egg reduction rate of 99% and a cure rate of 91%.

Added value of this study

This study provides the first clinical data on the efficacy of moxidectin alone and in co-administration with albendazole and tribendimidine against soil-transmitted helminth infections. The results from this randomised, controlled, single-blind, non-inferiority trial confirmed the good safety profile of moxidectin, with only a few and mainly mild adverse events reported. The efficacy profile in terms of egg reduction rate (geometric mean) of moxidectin in co-administration with albendazole resembles albendazole plus oxantel pamoate, which is currently the best treatment option against *T trichiura* infection. The efficacy of the co-administration is superior to monotherapy with moxidectin against *T trichiura* infection, hence it suggests a synergism.

Implications of all the available evidence

The strategy to control soil-transmitted helminth infections is mainly based on preventive chemotherapy (annual or biannual treatment of at-risk populations). The benzimidazoles (albendazole and mebendazole) have been used for more than three decades, which leads to a high drug pressure and risk of emerging benzimidazole resistance. Moxidectin, which is undergoing US Food and Drug Administration registration against onchocerciasis, is a promising treatment alternative. Our data suggests that moxidectin will be a useful addition to preventive chemotherapy programmes, although further studies are required.

at-risk populations. The goal of preventive chemotherapy is to control morbidity due to chronic infections by decreasing the prevalence of moderate and high infection intensities below 1% among target groups.[8,9]

Although albendazole and mebendazole have a considerable effect on *A lumbricoides* and albendazole against hookworm infections, these drugs are characterised by only low efficacy against *T trichiura*.[9,10] Alternative treatments or co-administration of drugs that could increase the efficacy against *T trichiura* are therefore required. Additionally, the increased drug pressure exerted by regular large-scale administration of the same drug might select for drug resistance.[11,12]

Moxidectin is a macrocyclic lactone, a member of the milbemycin family[13] developed in the 1980s, and is widely used in veterinary medicine for heartworm, whipworms, and lungworms. The drug is undergoing registration at the US Food and Drug Administration for the treatment of onchocerciasis.[14] The success of moxidectin relies on a broad spectrum of activity, including efficacy against benzimidazole-resistant strains of helminths.[15,16] Moxidectin has a long half-life, mainly because of its lipophilicity, is minimally metabolised, and has low susceptibility to transport by ATP-binding cassette transporters. Moxidectin interacts with chloride ion channels through high affinity binding.[17] Preliminary results from a randomised, controlled, double-blind, phase 3 trial of moxidectin versus ivermectin for *Onchocerca volvulus* showed a promising activity of moxidectin against STHs, primarily against *T trichiura*, with an egg reduction rate (ERR) of 99% and a cure rate (CR) of 91%.[14] The drug was shown to be safe and well tolerated.[13,14,18–20]

The primary objective of the study was to assess the efficacy of moxidectin alone and in co-administrations against *T trichiura* infection. Because of the reported low efficacy of the drug against hookworm (ERR 81%, CR 47%),[21] we chose tribendimidine and albendazole as partner drugs in co-administrations to broaden the efficacy spectrum.[22] The efficacy of these co-administrations was also assessed against concomitant STH infections and their safety investigated. The co-administration of albendazole plus oxantel pamoate, the most effective single-dose treatment against *T trichiura*[23] and concomitant STH infections, served as comparator.

Methods

Study design and participants

We did a randomised, single-blind, non-inferiority trial from April 1, to Aug 7, 2017, on Pemba Island, Tanzania. Adolescents aged 12–18 years were invited to participate in the study. Students were recruited from two primary schools and one secondary school in Mkanyagent and Mchokocho. Participants were asked to provide two stool samples, and those who tested positive for *T trichiura* infection according to the Kato-Katz method were considered eligible for inclusion in the trial. The students were asked about their medical history, and each participant had a physical examination. Participants who had any systemic illness (eg, clinical malaria or any chronic disease), as assessed by a medical doctor at the initial clinical assessment, were excluded from the trial.

Ethics approval was obtained from the Ministry of Health and Social Welfare of Zanzibar, Tanzania, and from the Ethics Commitee Nordwest and Central Switzerland, Switzerland (ref 2016–00839).

Written informed consent was obtained from all the parents or guardians, and all the adolescents provided verbal assent.

Randomisation and masking

An independent statistician provided a computer-generated list, stratified according to baseline infection (light or moderate plus heavy infections), using a block size of 16, without allocation concealment. Students were randomly assigned (5:5:3:3) to receive one of four treatments: moxidectin (8 mg) plus albendazole (400 mg), albendazole (400 mg) plus oxantel pamoate (25 mg/kg), moxidectin (8 mg) plus tribendimidine (200 mg for students younger than 15 years or 400 mg for those older than 15 years), and moxidectin (8 mg) alone. Study-group assignments were masked from participants and laboratory technicians; however, participants might have noticed a difference between treatments because of the differing colours, shape, and number of tablets.

Procedures

Oxantel pamoate (400 mg)[10,12] and moxidectin tablets were manufactured by the Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland. Moxidectin (Livzon New North River Pharmaceutical Co, Qingyuan, China; 8 mg) was admixed with Ormyapharm functionalised calcium carbonate based Ready Mix formulation[13] (Basel, Switzerland) and compacted to oval tablets. Tribendimidine (200 mg) and albendazole (400 mg) tablets were obtained from Shandong Xinhua Pharmaceutical Co (Shandong, China) and GlaxoSmithKline (London, UK), respectively.

At baseline, information meetings with all parents or guardians of eligible adolescents were organised. We explained the purpose and procedures of the study, including potential benefits and risks to the parents or guardians. Eligible students were asked to provide two stool samples obtained over consecutive days. Stool samples were transferred to the Public Health Laboratory–Ivo de Carneri. From each sample, duplicate Kato–Katz thick smears were prepared and examined for STH eggs by experienced laboratory technicians (all of whom were unaware of the treatment assignments). For quality control, 10% of the slides were randomly chosen and re-examined by the technicians,[14] revealing an agreement of more than 95%. A sub-sample of 1 day of collection was preserved with ethanol and shipped to the Swiss Tropical and Public Health Institute for PCR analysis (data not reported here). Before treatment, children were asked about clinical signs and symptoms, and their weight and height were measured. Adverse events were assessed and graded by active questioning at 3, 24, and 48 h after treatment. At each timepoint, the answers in the questionnaire were recorded and calculated as percentages with STATA version 12.0. Treatment efficacy was assessed 14–21 days after treatment, when participants submitted another two stool samples. At the end of the study, all participants who tested positive for STHs were offered albendazole (400 mg) in accordance with national guidelines.

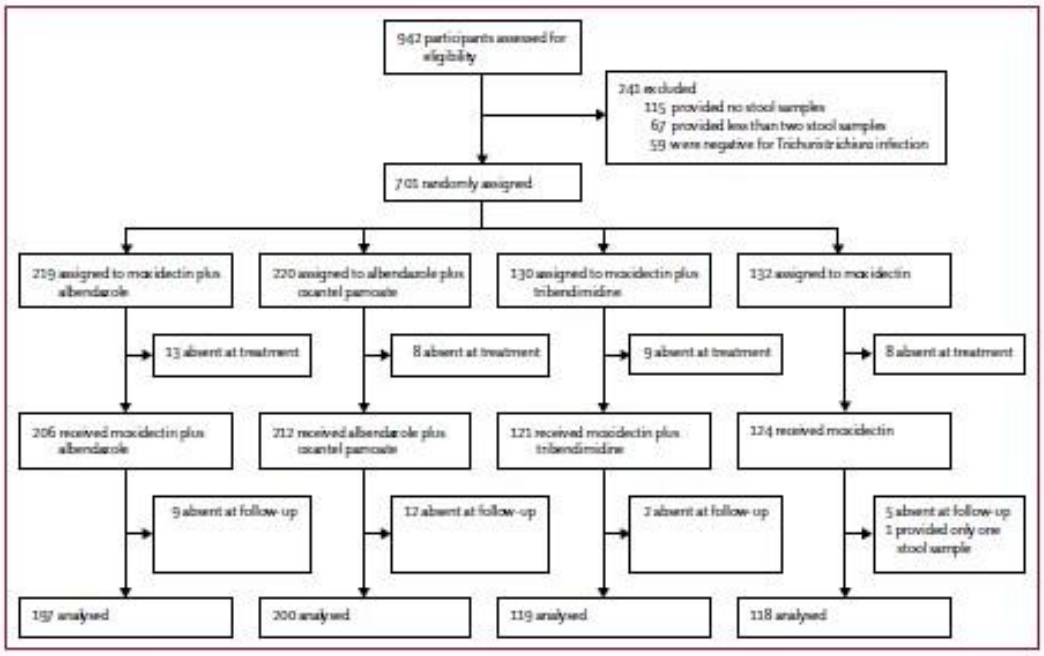

Figure 1: Trial profile

	Moxidectin plus albendazole (n=219)	Albendazole plus oxantel pamoate (n=220)	Moxidectin plus tribendimidine (n=130)	Moxidectin (n=132)	Total (n=701)
Age, years	14·0 (2·0)	13·9 (1·8)	14·2 (2·0)	13·9 (1·9)	14·0 (1·9)
Sex*					
Girls	130 (59%)	124 (56%)	71 (55%)	76 (58%)	400 (57%)
Boys	88 (40%)	96 (44%)	59 (45%)	56 (42%)	298 (43%)
Weight, kg	39·5 (9·6)	39·7 (10·4)	39·6 (9·2)	40·7 (9·5)	40·2 (10·4)
Height, cm	147·4 (10·5)	148·0 (11·4)	148·4 (11·2)	148·5 (11·2)	148·2 (11·0)
Trichuris trichiura infection					
Median EPG	522 (119–702)	600 (184·5–1237·5)	588 (205·5–1204·5)	465 (144–1218)	534 (183–1212)
EPG geometric mean	501·4	478·6	493·0	451·5	482·9
EPG arithmetic mean	1191·7	1078·3	1271·4	1263·4	1184·6
Infection intensity					
Light, 1–999 EPG	150 (68%)	150 (68%)	90 (69%)	91 (69%)	481 (69%)
Moderate, 1000–9999 EPG	67 (31%)	70 (32%)	39 (30%)	37 (28%)	213 (30%)
Heavy, ≥10 000 EPG	2 (1%)	0	1 (1%)	4 (3%)	7 (1%)
Hookworm infection					
Children infected	95 (43%)	94 (43%)	56 (43%)	51 (39%)	296 (42%)
Median EPG	126 (48–282)	120 (54–216)	105 (28·5–369)	144 (42–507)	126 (42–288)
EPG geometric mean	117·7	113·0	105·9	150·2	119·6
EPG arithmetic mean	275·2	219·2	316·3	474·3	233·5
Infection intensity					
Light, 1–1999 EPG	93 (98%)	93 (99%)	54 (96%)	48 (94%)	288 (97%)
Moderate, 2000–3999 EPG	2 (2%)	1 (1%)	2 (4%)	2 (4%)	7 (2%)
Heavy, ≥4000 EPG	0	0	0	1 (2%)	1 (<1%)
Ascaris lumbricoides infection					
Children infected	133 (61%)	128 (58%)	77 (59%)	71 (54%)	409 (58%)
Median EPG	3792 (738–9492)	4179 (945–10 886)	4110 (258–10 788)	3246 (927–7563)	3900 (657–10 014)
EPG geometric mean	1970·4	2712·6	2056·1	2079·9	2067·0
EPG arithmetic mean	9046·4	9605·3	10 061·7	9250·8	8957·2
Infection intensity					
Light, 1–4999 EPG	79 (59%)	78 (61%)	42 (55%)	44 (62%)	243 (59%)
Moderate, 5000–49 999 EPG	49 (37%)	46 (36%)	30 (39%)	24 (34%)	149 (36%)
Heavy, ≥50 000 EPG	5 (4%)	5 (4%)	5 (6%)	3 (4%)	18 (4%)

Data are mean (SD), n (%), or median (IQR). EPG=eggs per g of stool. *Sex was not registered for all participants.

Table 1: Baseline characteristics

Outcomes

The primary outcome was ERR against *T trichiura*, defined as the relative change in the geometric mean egg counts from baseline to 14–21 days after treatment by use of the Kato-Katz method of the following four oral treatment regimens: moxidectin plus albendazole, albendazole plus oxantel pamoate, moxidectin plus tribendimidine, and moxidectin alone.

The secondary outcomes were to assess the safety of all the treatment arms, their CRs against *T trichiura*, and their efficacy in terms of CR and ERR against *A lumbricoides* and hookworm co-infections. CR was calculated as the percentage of cured participants (egg negative) at follow-up who were infected (egg positive) at baseline. The comparison between Kato-Katz and PCR techniques was another secondary outcome with analyses ongoing.

Statistical analysis

The required sample size was determined via computer simulations with data from previous trials. The primary hypothesis was that the co-administration of moxidectin plus albendazole was not inferior in ERR against *T trichiura* compared with the co-administration of albendazole plus oxantel pamoate. We postulated, on the basis of expert opinion, that a difference in ERRs of 2 percentage points could be judged as clinically equivalent and set the non-inferiority margin to 2 percentage points. The secondary hypothesis was that moxidectin plus albendazole would be superior against *T trichiura* and hookworm compared with moxidectin monotherapy. The third hypothesis aimed to assess the difference between the efficacy of moxidectin plus albendazole and moxidectin plus tribendimidine against *T trichiura* infection.

	Moxidectin plus albendazole (n=197)	Albendazole plus oxantel pamoate (n=200)	Moxidectin plus tribendimidine (n=119)	Moxidectin (n=118)
Trichuris trichiura infection				
Children positive for infection				
Before treatment	197	200	119	118
After treatment	97	34	92	101
Median EPG	0 (0–66)	0	78 (6–258)	114 (24–329)
EPG geometric mean				
Before treatment	504·8	463·6	494·2	431·0
After treatment	7·4	1·1	41·7	72·2
EPG arithmetic mean				
Before treatment	1220·1	1055·1	1290·8	1273·8
After treatment	116·6	31·1	308·3	405·2
ERR (95% CI) based on geometric mean	98·5% (98·0–98·9)	99·8% (99·6–99·9)	91·6% (88·2–93·9)	83·2% (77·9–87·6)
ERR based on arithmetic mean	90·4%	97·1%	76·1%	68·2%
Cure rate (95% CI)	50·8% (43·6–57·9)	83·0% (77·1–87·9)	22·7% (15·5–31·3)	14·4% (8·6–22·1)
Children cured/total with infection (%)				
Light infection, 1–999 EPG	86/134 (64%)	121/137 (88%)	24/83 (29%)	16/82 (20%)
Moderate infection, 1000–9999 EPG	14/48 (23%)	45/63 (71%)	3/35 (9%)	1/32 (3%)
Heavy infection, ≥ 10000 EPG	0/2 (0%)	–	0/1 (0%)	0/4 (0%)
Hookworm infection				
Children positive for infection				
Before treatment	82	83	51	47
After treatment	19	20	6	31
Median EPG	0	0	0	36 (0–135)
EPG geometric mean				
Before treatment	114·1	118·6	98·0	146·3
After treatment	1·2	1·7	0·6	19·2
EPG arithmetic mean				
Before treatment	270·3	229·9	279·8	471·5
After treatment	10·7	34·2	5·0	194·2
ERR (95% CI) based on geometric mean	98·9% (98·0–99·5)	98·6% (97·4–99·3)	99·4% (98·7–99·8)	86·8% (72·7–93·9)
ERR based on arithmetic mean	95·8%	81·9%	97·2%	58·4%
Cure rate (95% CI)	76·8% (66·2–85·4)	75·9% (65·3–84·6)	88·2% (76·1–95·6)	34·0% (20·8–49·3)
Children cured/total with infection (%)				
Light infection, 1–1999 EPG	61/93 (66%)	62/93 (67%)	44/54 (82%)	14/48 (29%)
Moderate infection, 2000–3999 EPG	2/2 (100%)	1/1 (100%)	1/1 (100%)	1/2 (50%)
Heavy infection, ≥ 4000 EPG	–	–	–	1/1 (100%)

(Table 2 continues on the next page)

For the primary hypothesis, assuming a true ERR of 97%,[19] the results suggested that if no true difference exists between the treatment arms, then 160 participants are required to be 80% sure that the lower limit of the two-sided 95% CI will exclude a difference of more than 2 percentage points. For the secondary hypothesis, since we assumed a significant effect against hookworms (ERRs 88% *vs* 95%), our simulation results showed that we would require 70 adolescents in the moxidectin monotherapy arm to detect a significant difference in ERR with a power of 80%. Because not all students were co-infected with hookworms, we increased the sample size to 96. No formal sample size calculation was done for the third hypothesis because the expected difference was assumed to be of moderate dimension, which would have required an unrealistic sample size; thus, the sample size was fixed at 96.

To account for a potential loss to follow-up of 20%, the sample size was adjusted to 640 (200 in the moxidectin plus albendazole and albendazole plus oxantel pamoate arms, and 120 in the moxidectin plus tribendimidine and moxidectin monotherapy arms). In adolescents, the expected prevalence was estimated to be 80% for *T trichiura*, 65% for hookworm, and 45% for *A lumbricoides*. For the estimation of the prevalence of *T trichiura* infection in adolescents in Pemba, two previous studies done at nearby sites were considered and reported a prevalence of infection of 97%[20] and 95%.[21] However, we used a conservative estimate of 80% and planned to screen 800 adolescents to detect at least

	Moxidectin plus albendazole (n=197)	Albendazole plus oxantel pamoate (n=200)	Moxidectin plus tribendimidine (n=119)	Moxidectin (n=118)
(Continued from previous page)				
Ascaris lumbricoides infection				
Children positive for infection				
Before treatment	118	116	71	63
After treatment	4	4	2	1
Median EPG	0	0	0	0
EPG geometric mean				
Before treatment	1914·1	2485·3	2149·5	2147·9
After treatment	0·1	0·3	0·2	0·1
EPG arithmetic mean				
Before treatment	9544·7	8832·5	9580·6	8808·6
After treatment	11·1	73·0	12·9	19·2
ERR (95% CI) based on geometric mean	>99·9% (99·98–99·99)	>99·9% (99·97–99·99)	>99·9% (99·9–100·0)	>99·9% (99·9–100·0)
ERR based on arithmetic mean	99·7%	>99·9%	>99·9%	>99·9%
Cure rate (95% CI)	96·6% (91·5–99·1)	96·6% (91·4–99·1)	97·1% (90·2–99·6)	98·4% (91·4–99·9)
Children cured/total with infection (%)				
Light infection, 1–4999 EPG	67/79 (85%)	67/78 (86%)	39/42 (93%)	37/44 (84%)
Moderate infection, 5000–49 999 EPG	42/49 (86%)	42/46 (91%)	26/30 (87%)	23/24 (96%)
Heavy infection, ≥50 000 EPG	5/5 (100%)	3/5 (60%)	5/5 (100%)	2/3 (67%)

Data are n/N (%) or median (IQR) unless otherwise stated. EPG=eggs per g of stool. ERR=egg reduction rate.

Table 2: Cure rates and egg reduction rates

640 individuals infected with *T trichiura* to reach the anticipated sample size.

Data were double entered into a spreadsheet (Excel 2010, Microsoft), cross-checked, and analysed with R version 3.2.0 and STATA version 12.0. An available case analysis was done, which included all children with primary outcome data. ERR, which was the primary outcome, was calculated with the geometric mean for the treatment groups (before and after treatment) and expressed as a percentage. We used the following formula (Equation 1):

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

For hookworm and *A lumbricoides*, we calculated ERR with a geometric and arithmetic mean of positives at baseline. A bootstrap resampling method with 5000 replicates was used to estimate 95% CIs of the geometric means for the ERR. Intention-to-treat analysis was calculated for the primary hypothesis, by considering those lost at follow-up as participants with no change in egg counts after treatment. Odds ratios (OR) and corresponding 95% CIs were estimated by logistic regression models.

See Online for appendix

The study is registered at ISRCTN, number 20398469.

Role of the funding source

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

Results

Of 942 adolescents who were invited to participate (figure 1), 760 (81%) had complete baseline data. Of these, 701 (92%) were positive for *T trichiura* infection. 409 (58%) of 701 participants were infected with *A lumbricoides* and 296 (42%) with hookworm, and 198 (28%) had a triple STH infection. Most students had low intensity *T trichiura* (69%) and hookworm (97%) infections, whereas *A lumbricoides* infections were moderate to high intensity. Classifications of infection intensities according to WHO cutoffs are presented in table 1. Of 67 children who were lost to follow-up after random allocation, 38 (57%) did not show up at the treatment venue, 28 (42%) were absent and did not provide any stool samples, and one (1%) provided only one of two required samples after treatment and hence was not considered for analysis. Demographic and baseline laboratory characteristics of the 701 children randomly assigned are summarised in table 1, and baseline characteristics (age, sex, weight, and height) are presented in the appendix. Treatment groups were well balanced with respect to age, sex, weight, and height.

ERRs and CRs are shown in table 2. The co-administrations of moxidectin plus albendazole and moxidectin plus tribendimidine revealed ERRs of 98·5% and 91·6% respectively; the comparator

co-administration of albendazole plus oxantel pamoate resulted in the highest ERR of 99·8%, and moxidectin monotherapy had the lowest ERR of 83·2%.

Our results showed that the co-administration of moxidectin plus albendazole was not inferior to the most efficacious co-administration, albendazole plus oxantel pamoate (difference −1·2 percentage points, 95% CI −1·8 to −0·79; figure 2). Additionally, moxidectin plus albendazole was superior to moxidectin monotherapy (difference −15·3 percentage points, 95% CI −21·1 to −11·0; figure 2). Different from the available case analysis, according to the intention-to-treat analysis, non-inferiority was not confirmed since the lower bound of the CI exceeds 2 percentage points (difference −1·8 percentage points, 95% CI −2·7 to −0·1).

The moxidectin plus albendazole group had a significantly lower CR than albendazole plus oxantel pamoate (CR 50·8% *vs* 83·0%, OR 4·7, 95% CI 3·0–7·6, p<0·0001), but significantly higher than moxidectin monotherapy (CR 14·4%, OR 6·1, 95% CI 3·5–11·3, p<0·0001). None of the point and interval estimates of the adjusted logistic regression models differed considerably from the unadjusted estimates. CRs from light and moderate infection were 64% and 23%, respectively, for moxidectin plus albendazole, 29% and 9%, respectively, for moxidectin plus tribendimidine, and 20% and 3%, respectively, for moxidectin alone. None of the high-intensity infections of *T trichiura* were cured by any treatment.

263 (41%) of 634 adolescents were infected with hookworm. The co-administrations of moxidectin plus tribendimidine and moxidectin plus albendazole had the highest ERR against hookworm (99·4% and 98·9%, respectively), followed by albendazole plus oxantel pamoate (98·6%) and moxidectin alone (86·8%). This finding confirmed the superiority of moxidectin plus albendazole over moxidectin monotherapy in terms of ERR (difference −12·1 percentage points, 95% CI −26·7 to −4·7; figure 2) and CR (76·8% *vs* 34·0%; OR 6·42, 95% CI 2·96–14·5, p<0·0001). The highest CR against hookworm was 88·2% (moxidectin plus tribendimidine). Almost all infections of high to moderate intensity were cured in the co-administration arms (table 2) and only 50% were cured in the monotherapy arm. 368 (58%) of 634 participants were co-infected with *A lumbricoides*. All treatment arms showed a high efficacy against *A lumbricoides* in terms of ERRs (>99·9%) and CRs above 96%.

No serious adverse events were reported during the study. All reported adverse events were mild and did not require any intervention. Before treatment, 66 (10%) of 634 participants reported symptoms, mainly stomach pain and headache. 3 h after treatment, seven (5%) of 118 students reported mild symptoms in the moxidectin group, eight (7%) of 119 in the moxidectin plus tribendimidine group, 24 (12%) of 196 in the moxidectin plus albendazole group, and 16 (8%) of 199 in the albendazole plus oxantel pamoate group. The highest percentage of adverse events (18–24%) was reported 24 h after the administration of all treatments (table 3). 48 h after treatment, mild symptoms were reported by the participants, which accounted for less than 10% in all arms. Stomach pain, constipation, and headache were the most frequently reported symptoms 3 h and 24 h after treatment (figure 3).

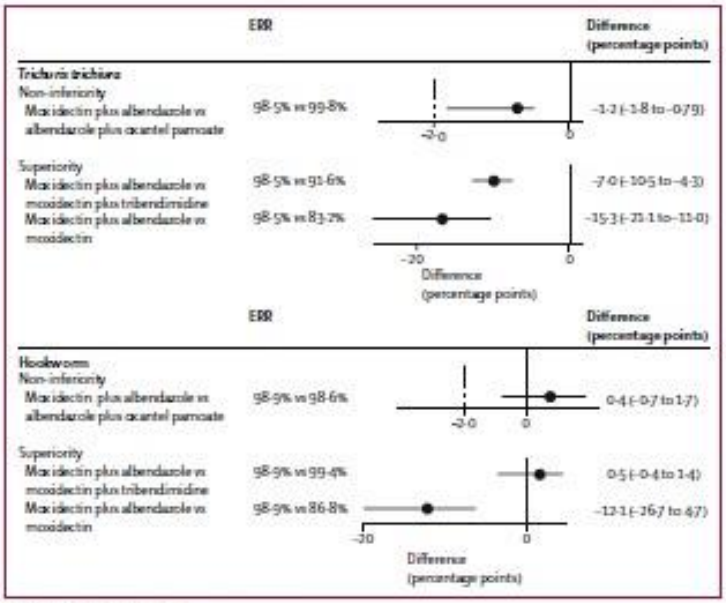

Figure 2: **ERR of treatments**
Data are ERR, differences in ERR (95% CIs), and a graphical representation of differences in ERRs (95% CIs) against *Trichuris trichiura* and hookworm with moxidectin plus tribendimidine, albendazole plus oxantel pamoate, and moxidectin alone, versus moxidectin plus albendazole. ERR=egg reduction rate.

	Moxidectin plus albendazole (n=197)	Moxidectin plus oxantel pamoate (n=200)	Moxidectin plus tribendimidine (n=119)	Moxidectin (n=118)	Total (n=634)
Before treatment	20/197 (10%)	22/200 (11%)	11/119 (9%)	13/118 (11%)	66/634 (10%)
After treatment					
3 h	24/196 (12%)	16/199 (8%)	8/119 (7%)	7/118 (5%)	55/632 (9%)
24 h	36/196 (18%)	38/199 (19%)	29/119 (24%)	23/118 (19%)	126/632 (20%)
48 h	12/196 (6%)	4/199 (2%)	5/119 (4%)	4/118 (3%)	25/632 (4%)

Data are n/N (%).

Table 3: **Number of adverse events and related symptoms assessed at three timepoints**

Discussion

New anthelmintic drugs need to be developed because the few available treatments have suboptimal efficacy, particularly against *T trichiura*, and resistance is a concern. Moxidectin is one of the few drug candidates in the research and discovery pipeline. This drug has shown promising efficacy in a single dose against *T trichiura* infections and is being registered for the

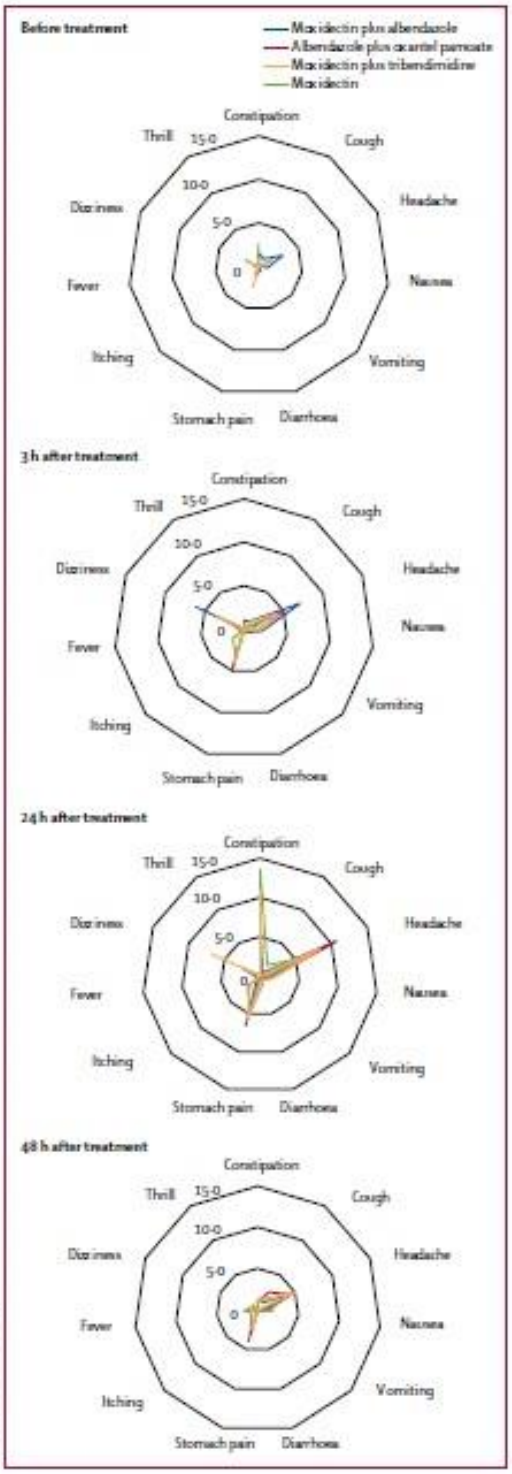

Figure 3: Clinical symptoms and adverse events
Spider plots indicate the percentage of symptoms before treatment and adverse events after treatment (3, 24, 48 h) in the four treatment arms.

treatment of onchocerciasis.[14] Moreover, moxidectin has shown high efficacy against *Strongyloides stercoralis* infection.[15] We therefore designed a randomised clinical trial combining moxidectin with albendazole and tribendimidine, which are the two anthelmintic drugs characterised by the highest efficacy against hookworm.[25] 8 mg of moxidectin were used, which is the dose used for the treatment of onchocerciasis.[14]

The co-administration of moxidectin and albendazole revealed non-inferiority in terms of ERR by use of a 2 percentage points non-inferiority margin against *T trichiura* compared with albendazole plus oxantel pamoate, known for its excellent and broad-spectrum activity against STHs.[10] However, the margin of 2 percentage points showed that non-inferiority should be carefully interpreted because the ERR for albendazole plus oxantel pamoate against *T trichiura* was higher than expected and previously experienced,[26] probably because of the higher dose of oxantel pamoate used (25 mg/kg *vs* 20 mg/kg) in this study. Hence, this margin is not the anticipated interpretation of clinical equivalence—ie, a difference of 2 percentage points would be considered as equivalent if the ERR was about 97%. However, this assumption does not hold true for an ERR of about 100%. Therefore, even though non-inferiority in terms of ERR was confirmed, clinical equivalence between the two combinations cannot be ascertained since the CR of moxidectin plus albendazole against *T trichiura* was considerably lower than the CR observed for albendazole plus oxantel pamoate (51% *vs* 83%).

Although all groups responded well to treatment against *A lumbricoides*, the co-administrations had higher efficacy than moxidectin monotherapy against hookworm and *T trichiura* infections, which suggests a synergistic effect. The synergism might be explained by the pathways of efflux of these drugs.[11,12,27] The transporter pumps are ATP-binding cassettes located in the apical side of the cells of barrier epithelia and consist of transmembrane proteins that use ATP hydrolysis as an energy source for the active extrusion of a variety of drugs, carcinogens, and toxins across the cellular plasma membrane. P-glycoprotein, multidrug resistance associated proteins (of the ATP-binding cassette family), and breast cancer resistance protein are among the best characterised drug transporters.[28] Ivermectin is transported by p-glycoprotein[29] and albendazole sulphoxide and moxidectin by the breast cancer resistance protein.[27] Inhibition of the ATP-binding cassette transporters increases moxidectin bioavailability.[27] Therefore, albendazole sulphoxide and moxidectin are likely to interact with the breast cancer resistance protein, which would explain the synergistic action of albendazole plus moxidectin when administered together and therefore the higher efficacy of the co-administration than that of the monotherapy.

As expected and shown in earlier anthelmintic trials,[30] CRs and ERRs for the four treatments against *T trichiura* were higher for low intensities of infections. Whereas,

for *A lumbricoides*, we noticed a good efficacy of all four treatment arms against infections of moderate and high intensity. A note of concern is the persistence of *A lumbricoides* infections of moderate and high intensity in the study setting, despite several rounds of anthelmintic treatment during the past 25 years. This finding might suggest either an extremely high transmission or a potential resistance of this parasite, even if the latter hypothesis is not probable since *A lumbricoides* has always shown to be quite sensitive to the commonly used anthelmintic treatment regimens.

This study has some limitations. A double-blind trial would have been a better design, but in our case, this would not have been feasible since our study included one weight-dependent (oxantel pamoate) and one age-dependent (tribendimidine) treatment arm. Another limitation is that hookworm infections on Pemba Island are mainly of light intensity, and, in our trial, we registered only a few moderate or heavy infections making it difficult to thoroughly assess the efficacy of the tested treatments against moderate and high intensities of hookworm infection.

Adverse events were thoroughly monitored and adolescents were followed up at several timepoints after drug intake. Our results show that moxidectin is a safe treatment, which confirms findings from previous studies.[9,10] All events reported were of mild intensity and did not require any mitigation measure. However, since moxidectin has a long half-life, further studies might be necessary to confirm its safety at longer follow-up points. This assessment was not feasible during our study for logistical reasons (ie, flooding of some paths that made access to the school difficult) and upcoming school holidays. Additionally, thorough safety studies will be required in the paediatric population because preschool and school-aged children are the main target group for preventive chemotherapy programmes.

Co-administration of moxidectin with albendazole was non-inferior to albendazole plus oxantel pamoate. However, given the observed difference in CRs, further optimisation of this co-administration is necessary. Further studies could assess escalating doses of moxidectin and moxidectin plus albendazole in the framework of a dose-finding study, as was successfully done for oxantel pamoate,[22] as well as provide more insight on the drug disposition of this combination.

Contributors

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

Declaration of interests

We declare no competing interests.

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Chapter 6

Evaluation of two PCR extraction methods on the detection of *Strongyloides stercoralis* infection

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PARASITOLOGY

Evaluation of Two DNA Extraction Methods for Detection of *Strongyloides stercoralis* Infection

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ABSTRACT *Strongyloides stercoralis* is present worldwide, but its prevalence is still uncertain, mainly due to the lack of sensitivity of diagnostic methods. Molecular techniques are under development, but a standardized protocol is still unavailable. We compared the sensitivity of real-time PCR, using two extraction protocols, with that of the Baermann technique. Samples were collected in the framework of the baseline screening of a randomized clinical trial evaluating moxidectin against *S. stercoralis* in Lao People's Democratic Republic. Two stool samples from each participant were processed by the Baermann method, and one subsample was processed by PCR. DNA was extracted using the QIAamp DNA stool minikit based on the standard protocol for the QIAamp DNA minikit (QIA) and using a modification of the QIA procedure (POL). Subsequently, all extracted samples were analyzed by real-time PCR. Overall, 95 samples were analyzed by the three diagnostic methods. Sixty-nine (72.6%) samples were positive according to the Baermann method, 25 (26.3%) by the QIA method, and 62 (65.3%) by the POL method. The sensitivities were 86% (95% confidence interval [CI], 76.7 to 92.9), 31.0% (95% CI, 21.3 to 42.6), and 78.0% (95% CI, 66.8 to 86.1) for the Baermann, QIA, and POL methods, respectively. The sensitivities calculated for each day of the Baermann method separately were 60% (48.4 to 70.8%) and 64% (52.2 to 74.2%) for days 1 and 2, respectively. In conclusion, the POL method revealed a good performance and was comparable to the Baermann test performed on two stool samples and superior to the Baermann method performed on one stool sample. Additional studies are needed to standardize a PCR protocol for *S. stercoralis* diagnosis.

KEYWORDS PCR, *Strongyloides stercoralis*, diagnosis

The threadworm *Strongyloides stercoralis* is known to be present worldwide except for Antarctica (1). However, its real prevalence is still an estimated guess, and epidemiology data vary from 100 million to 300 million infected individuals (2, 3). In the last decade, migration flows and travels to countries where this worm is endemic have changed the geography of infection, contributing to a further increase in spreading of *S. stercoralis* (2, 4). Initial studies in the 1980s on this parasitic infection were deemed by Genta et al. (5) inspired guesses: data were considered not reliable because knowledge on the presence of *S. stercoralis* at the country level was poor. This was due mainly to the fact that the diagnostic methods used were unsuitable for accurate detection of *S. stercoralis* (4, 5). The situation has not changed much since then (1, 6), with *S. stercoralis* being one of the most underdiagnosed and neglected infections of humans. One of the peculiar aspects of this helminth is that it replicates within the human host, with eggs developing into rhabditiform larvae. Those either can be passed in the stool or can cause autoinfection by developing into infective filariform larvae.

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Infective larvae can reinfect the host by penetrating either the intestinal mucosa or the perirectal skin.

Weakening of the immune system (due to infection with HIV/AIDS or human T-cell lymphotropic virus [HTLV]) plays an important role in the proliferation of the parasite and maintenance of the infection, which can become disseminated and occasionally fatal (3, 4, 7). Furthermore, the increased iatrogenic immunosuppression secondary to the broader use of corticosteroids and chemotherapy, and organ transplantation also in countries where the worm is endemic, contributes to increase the burden of *S. stercoralis* infection. This highlights the importance of detecting *S. stercoralis* infection, which acts synergistically with immunosuppression and considerably increases morbidity and mortality also in countries where this infection is not endemic.

S. stercoralis infections have a different range of clinical presentations, from asymptomatic infection or mild nonspecific symptoms (8) to a life-threatening dissemination of larvae to internal organs (1, 6). In terms of treatment and diagnosis, *S. stercoralis* infection is considered one of the most neglected diseases among the neglected tropical diseases. Direct methods, such as the Baermann method and Koga agar plate culture, the two WHO-recommended methods, are still the diagnostic methods used most in countries where this infection is endemic, yet they are time-consuming and cumbersome and show only moderate sensitivity (2, 9). In addition, for both methods specific equipment and conditions are crucial for good performance: an incubator, stable electricity, sufficient space for sample incubation with funnels and tubes, and mesh and gauze for incubation (10–12).

Serological methods have recently demonstrated good sensitivity in countries where this infection is not endemic, and they can be easily used in advanced laboratories for diagnosis and screening (13, 14). The main limitation of serology is that it cannot be used to assess drug efficacy. While larva excretion stops a few days after successful treatment (15), serology titers decrease only 6 to 12 months after treatment (16). Therefore, assessment of drug efficacy by serology in areas where the infection is endemic, where a high rate of reinfection is common, is not feasible.

Molecular techniques for the diagnosis of *S. stercoralis* are still under development (17, 18). They offer many advantages compared to the Baermann method: only one sample is needed, the amount of stool required is smaller, multiple infections can be detected (19, 20), fixation of the sample avoids contamination, and, finally, the technique, once standardized, is objective and is quicker to perform. Another advantage of PCR is the fact that DNA from dead or live larvae will be detected, whereas the Baermann method, although analyzing a bigger amount of stool and therefore having a greater chance to detect infection, relies on the fact that the larvae have to be alive in order to migrate into the collection tube.

However, initial studies conducted with PCR on *S. stercoralis* obtained discordant results (17, 21). In addition, protocols for DNA extraction and PCR are still not well defined, being mainly based on in-house procedures rather than on standardized kits (21). The sensitivity and specificity of PCR methods have been compared with those of traditional direct diagnostic methods (9, 18). The specificity turned out to be very high (2, 9, 17, 22), but the sensitivity was shown to vary. While in some studies PCR, especially real-time PCR (23), showed a better performance than direct methods (23–25), other studies combining direct methods revealed a higher sensitivity (2, 9, 22). In addition, recently PCR has been compared with serology and was shown to be less sensitive (4).

The aim of this study was to compare the sensitivity of real-time PCR with that of the Baermann technique. Moreover, two well-established DNA extraction protocols were evaluated (9, 17). Finally, we evaluated the advantage of a receiver operating characteristic (ROC) analysis for an individual evaluation of cycle threshold (C_T) in real-time PCR and its impact on sensitivity and specificity.

MATERIALS AND METHODS

Ethical consideration. Stool samples were collected in the framework of the baseline screening of an exploratory phase II, randomized, single-blind clinical trial evaluating the safety and efficacy of moxidectin versus ivermectin against *S. stercoralis* infection (26). The trial was performed between April and June 2016 in the Lao People's Democratic Republic in the district of Pathoumphone, where *S. stercoralis* infection is endemic.

Ethical clearance was obtained from the Ethics Committee of Northwestern and Central Switzerland (EKNZ; reference no. 15/103) and the Lao Ministry of Health (reference no. 075/2016). The trial is registered with Current Controlled Trials (ISRCTN11983645). Participants 12 to 60 years old were eligible for inclusion in the trial. Written informed consent was collected before enrollment from all participants. At the end of the study, all participants positive for infection were treated according to local guidelines.

Laboratory procedures. Two stool samples obtained from 95 participants were examined with the Baermann method for the detection of *S. stercoralis* larvae. The Baermann method was carried out following the WHO standard procedure (27).

A subsample of the first sample of stool (~200 mg) was preserved in ethanol and shipped to the Swiss Tropical and Public Health Institute (TPH) in Basel, Switzerland, for PCR analyses. Preserved samples were processed with two different protocols for DNA extraction. One DNA extraction (QIA method) was performed using the QIAamp DNA stool minikit (Qiagen; Hilden, Germany) by following the manufacturer's protocol, with minor modifications (9). The second DNA extraction (POL method) was done using the QIAamp DNA minikit with modifications according to Polley et al. (28). In brief, samples were washed once with phosphate-buffered saline (PBS); 400 μl of animal tissue lysis (ATL) buffer with 40 μl of proteinase K was added, followed by 2 h of incubation at 56°C. During this period, the samples were briefly vortexed every 30 min. After incubation, the samples were pelleted and 200 μl of supernatant was processed according to the kit protocol. All samples were analyzed with real-time PCR for detection of *S. stercoralis*. The 18S rRNA *S. stercoralis*-specific real-time PCR protocol was conducted using TaqMan GeneExpression MasterMix (Thermo Fisher, Switzerland), sense and antisense primers (5′-to-3′ forward primer, GGA ATT CCA AGT AAA CGT AAG TCA TTA [modified from reference 17], and 5′-to-3′ reverse primer, GTT ACG ACT TTT GCC CGG TTC) and the respective probe (6-carboxyfluorescein [FAM]-TAT ATT AAA TCC TTC CAA TCG CTG TTG-BHQ1) (Eurofin Genomics, Ebersberg, Germany) to amplify a specific 184-bp fragment of *S. stercoralis*. The thermoprofile on the 7500 ABI real-time machine (Thermo Fisher) was 2 min at 50°C and 10 min at 95°C followed by 45 cycles of 15 s at 95°C and 1 min at 58°C. The specificity of these primers was previously tested on a variety of DNAs from stool samples confirmed by light microscopy at the diagnostic center of the Swiss TPH to be infected with *Ascaris lumbricoides*, *Blastocystis hominis*, *Cryptosporidium* spp., *Cyclospora* spp., *Entamoeba coli*, *Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba histolytica*, *Entamoeba moshkovskii*, *Endolimax nana*, *Giardia lamblia*, *Iodamoeba bütschlii*, and *Schistosoma mansoni* and was found to be 100%. On each real-time PCR plate, we included negative and positive controls with different plasmid concentrations (10^2, 10^4, and 10^6 plasmids/μl) containing an insert with the sequence of the *S. stercoralis* real-time PCR product. Each DNA sample was further tested for inhibition by addition of 2 μl of a known plasmid concentration (10^2 plasmids/μl). In case of inhibition, the sample was diluted 1:2 and 1:5 and retested.

Statistical analysis. Data for the amplification curves were entered in an Excel file, and statistical analyses were conducted using Stata 12.0 (Lake Drive College Station, Texas).

The cycle threshold (C_T) cutoff value was defined as the number of PCR cycles required for the detection of fluorescence signal of the amplified products to exceed the set threshold value. As a consequence, higher quantities of DNA resulted in lower C_T values and vice versa (29).

Because of possible unspecific amplification and to exclude any cross-contamination from highly positive samples, PCR results were considered negative if C_T values were more than 40 or if no amplification was detected (18). Because no quantification in the Baermann method was conducted and thus no quantitative correlation with real-time copy numbers was feasible, mean and median copy numbers by DNA extraction method are reported in the supplemental material.

In the absence of a true "gold standard," we calculated the sensitivity of the methods on the basis of positive results obtained by the Baermann method, performed on 2 days. Assuming that PCR has a specificity of >90% (9, 18, 20, 30), we also calculated sensitivity on the basis of any positive finding with any of the three diagnostic methods. We compared the positivity rates of the tests with the McNemar test. The test was considered significant if its P value was <0.05. Both PCRs were compared separately with the Baermann method, both as the mean of 2 samples collected on 2 different days and for only 1 out of the 2 days of collection. A Wilcoxon rank sum test was used to compare the C_T values between microscopy-positive and -negative tests, with a C_T of >0.

After establishing that the POL DNA extraction showed a better performance than the QIA extraction when using a cutoff value of 40 cycles, we determined the optimal cutoff for the new test based on its ROC curve with the results obtained by the Baermann method over 2 days by maximizing the index of Youden (i.e., sensitivity + specificity − 1).

RESULTS

Complete data from the three diagnostic methods/protocols were available for 95 participants.

Fifteen (15.8%) participants were classified as negative by all methods and 80 participants were positive by at least one method. Sixty-nine (72.6%) were positive

TABLE 1 Results for both extraction methods (QIA and POL) compared to the Baermann method results

Test and result	No. of samples with indicated Baermann method result					
	Days 1 and 2		Day 1		Day 2	
	Negative	Positive	Negative	Positive	Negative	Positive
QIA						
Negative	20	50	37	33	33	37
Positive	6	19	10	15	11	14
POL						
Negative	17	16	23	10	21	12
Positive	9	53	24	38	23	39

according to the Baermann method based on 2 days of collection, 25 (26.3%) by the QIA method, and 62 (65.3%) by the POL method (Table 1).

The sensitivities estimated on the basis of a positive Baermann result (2 days; 69/95) were 70% (95% CI, 57 to 80%) and 74% (95% CI, 62 to 84%) for a single Baermann test, 27.5% (95% CI, 16 to 40%) for the QIA method, and 77.0% (95% CI, 65 to 86%) for the POL method (Table 2). The sensitivities estimated on the basis of any positive result obtained by any method (80/95) were 86.3% (95% CI, 76.7 to 92.9%) for 2 days of Baermann method testing, 60% (95% CI, 48.4 to 70.8%) for the Baermann first-day sample, 63.8% (95% CI, 52.2 to 74.2%) for the second-day sample, 31.3% (95% CI, 21.3 to 42.6%) for the QIA method, and 77.5% (95% CI, 66.8 to 86.1%) for the POL method. The McNemar test confirmed that the Baermann method performed on a single day had significantly lower positivity rates than analyses of 2 stool samples by the Baermann and the POL methods ($P = 0.02$ and 0.03, respectively) (Table 2). The Baermann (2 days) and POL methods were exactly the same in terms of sensitivity, as the POL method could confirm 77% of the Baermann method (2 days)-positive samples and vice versa (Table 2). The two extraction methods were significantly different, and the POL method was significantly more sensitive than the QIA method ($P < 0.005$). The combination of PCR and direct method showed the best sensitivity (Table 3), reaching 97.5% when the POL method and the Baermann method (2 days) are considered. The POL method combined with a single test by the Baermann method has a sensitivity up to 92.5% (Table 3), which is not significantly different from that for testing 2 samples by the Baermann method plus the POL method ($P > 0.005$).

The median C_T values were 36.2 (range, 31.1 to 39.8) and 28.6 (range, 17.3 to 39.5) for the QIA and POL methods, respectively. C_T cutoff calculated based on the ROC curve estimated that the optimal threshold was at 30.5 for the POL method. With this definition, the POL method has a sensitivity of 58.0% in confirming Baermann method positivity and a specificity of 88.5% in confirming Baermann method negativity. Similar results were found by analyzing the copy numbers obtained by the extraction method in comparison to the Baermann method (supplemental material).

Figure 1 shows that even if the difference was not statistically significant, PCR-

TABLE 2 Sensitivities of the three diagnostic methods[a]

Method	% of positive samples (no./total)	% sensitivity in comparison to Baermann (day 1 and 2) positive result (95% CI) ($n = 69$)	% sensitivity in comparison to any positive test (95% CI) ($n = 80$)
Baermann, days 1 and 2	72.6 (69/95)		86.3 (76.7–92.9)**
Baermann, day 1	50.5 (48/95)	69.6 (57.3–80.0)	60.0 (48.4–70.8)*
Baermann, day 2	53.7 (51/95)	74.0 (62.0–84.0)	63.8 (52.2–74.2)*
QIA	26.3 (25/95)	27.5 (17.5–39.6)	31.3 (21.3–42.6)*
POL	65.3 (62/95)	77.0 (65.1–86.1)	77.5 (77.0–86.0)**

[a]Sensitivity was estimated on the basis of Baermann (collected on 2 days) method positivity and positivity detected by both the Baermann method (days 1 and 2) and PCR. *, statistically significant difference between the Baermann method (days 1 and 2 together and days 1 and 2 separately and QIA and between POL and the Baermann method (days 1 and 2) and the QIA method ($P < 0.05$); **, no statistically significant difference between the Baermann method (days 1 and 2) and the POL method ($P > 0.05$).

TABLE 3 Sensitivity of the combination of Baermann collected on one and two samples and QIA and POL methods[a]

Method	% of positive samples (no./total)	% sensitivity in comparison to any positive test (95% CI)
Baermann, days 1 and 2, + QIA	77.9 (74/95)	93.7 (86.0–97.9)
Baermann, days 1 and 2, + POL	82.0 (78/95)	97.5 (91.3–99.7)
Baermann, day 1, + QIA	61.0 (58/95)	72.5 (61.4–81.9)
Baermann, day 2, + QIA	65.3 (62/95)	77.5 (66.8–86.1)
Baermann, day 1, + POL	75.8 (72/95)	90.0 (81.2–95.6)
Baermann, day 2, + POL	65.2 (62/95)	92.5 (84.4–97.2)

[a]Significant difference between one group of combinations (1-2-5) and the other group of combinations (3-4-6) ($P < 0.05$).

positive but microscopy-negative samples had lower DNA loads (i.e., higher C_T values) than PCR-positive samples that were also microscopy positive ($P = 0.08$, Wilcoxon rank sum test) for both the QIA and POL methods.

DISCUSSION

S. stercoralis infection is considered the most neglected of the neglected tropical diseases (1). Its worldwide prevalence is underestimated (2, 3), mainly due to an asymptomatic course of infection and low sensitivity of diagnostic methods (31, 32). Currently the examination of multiple samples with combined techniques is used to improve diagnostic power (32, 33).

Hence, there is a need for alternative, standardized methods. We evaluated one of the standard coprological methods (the Baermann method) with a molecular approach, using two different DNA extraction methods. Lately, several studies have been performed with PCR in order to find a suitable alternative for *S. stercoralis* detection, but to our knowledge there have been only a few studies comparing different DNA isolation techniques (21). Among these, Repetto et al. describe a modification to the standard QIAamp stool minikit method that performs better than the original protocol (25). The same group reported an in-house extraction method which was more sensitive than the commercial Qiagen kit (21).

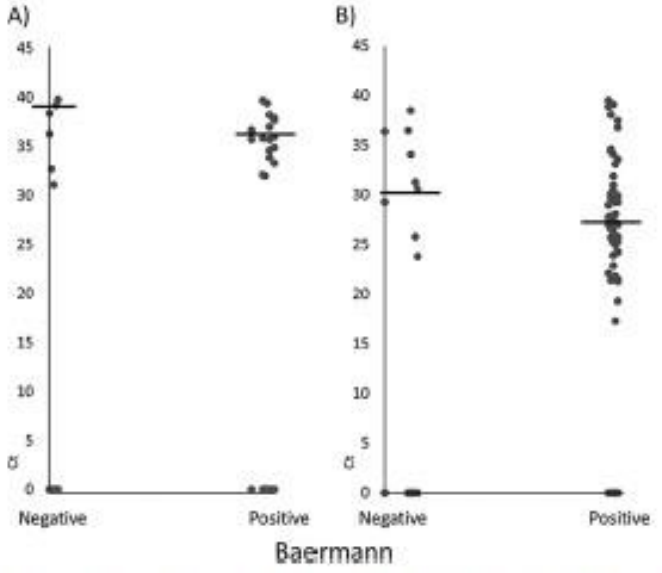

FIG 1 Distribution of C_T values among Baermann method positive and negative tests. Microscopy tests were based on two stool samples from consecutive days, and two extraction methods, the QIA (A) and POL (B) methods, were applied to the stool sample for the first day. Horizontal lines represent the means of C_T.

If only 1 day of Baermann method testing is taken into consideration, the POL method is more sensitive (78% versus 60%), while the QIA method (31% versus 60 to 64%) shows lower sensitivity than the Baermann method. Moreover, we have seen that the combination of the POL method and 2 days of Baermann method testing reaches a high sensitivity, but also the POL method with only 1 day of Baermann method testing has a sensitivity of 90%. Hence, it would be feasible to increase sensitivity and shorten the sample collection with a combination of PCR (POL extraction method) and the Baermann method on a single stool sample. The stool amount analyzed by the Baermann method being around 50 to 100 times larger than for molecular methods further explains the observed discrepancies between the methods, as the probability of the presence of a larva is higher when more stool is analyzed.

Our results are in accordance with reports from elsewhere that PCR performs well using fecal samples and that no consecutive samples are needed to improve the power of the diagnostic technique (9, 25, 34). We found that the POL method revealed a significantly higher sensitivity (78% versus 31%) than the widely and traditionally used QIA method. The POL method is similar to the technique of helminth DNA extraction described by Verweij (35) except the missing bead mill procedure, which was not available in our laboratories at the time of the study. The POL method was the only alternative method (with good results for protozoans) known, which could be used without a cell lysing instrument (9, 18, 22, 36).

In the literature, results on the performance of the QIA extraction method are controversial, with some authors reporting a sensitivity of PCR similar to that of microscopy and others reporting lower or higher sensitivity for the PCR than for microscopy (9, 18, 37, 38). Surprisingly, we observed a low performance of the QIA method. As reported previously (18), one possible explanation for lower sensitivity of the QIA method might be the short lysis period (5 min at 95°C) not being sufficient to lyse helminth eggs and worms. In our samples, an external inhibition control detected *S. stercoralis* plasmid DNA well; therefore, we suppose that there are no stool-related substances that inhibit the reaction (18).

The observed difference in sensitivities of the combined methods is interesting. Both extraction methods in combination with day 1 and day 2 Baermann testing show a range of sensitivities, suggesting a variety in day-to-day larva output. This is a limitation of the microscopic technique, which loses power of detection if only one sample is taken into consideration, whereas molecular methods have relatively good sensitivity even for single-day detection. In this regard, it would have been relevant to compare 2 days of Baermann testing with 2 days of PCR analyses to confirm that PCR is not affected by larva output. Although this would be interesting, the performance of 2 PCRs is too expensive to be considered for field application. Another advantage of molecular diagnosis over the Baermann method is that a differentiation between hookworm and *S. stercoralis* larvae is sometime difficult. Since our study was embedded in a larger clinical trial, data obtained by the Kato-Katz technique were available and coinfections were thoroughly checked. Moreover, samples were analyzed shortly after collection in order to minimize the risk of hatching of hookworm eggs.

One limitation of our study is that larva counting and larva staging were not performed; therefore, a correlation between intensity of infection and C_T values was not feasible. In previous studies, intensity of infection was, as expected, inversely proportional to C_T values (12).

A long-debated aspect of molecular diagnostics is the C_T cutoff: in the literature it is frequently set at 40 (17, 18, 37), but some authors set it at 35 (19) or do not mention it (9, 30, 34). A high C_T cutoff often results in unspecific amplification, which is more likely at later real-time PCR cycles, and possible cross-contamination of highly positive samples. We calculated sensitivity using 40 as the C_T cutoff, but considering the ROC curve between the Baermann and POL methods, we observed that a different threshold (30.5) might be more suitable for our study. On the other hand, if we adjusted the C_T cutoff to 30.5, the sensitivity of both PCR methods would further decrease. This

consideration demonstrates that more research is needed in order to reach standardization.

In conclusion, we found that the POL method outperforms the QIA method for *S. stercoralis* DNA detection in stool samples. We demonstrated that the combination of two diagnostic methods, with one sample tested by both the Baermann and POL methods, reaches a sensitivity of 90%, which is significantly higher than that of PCR or the Baermann method only. Our study confirmed that PCR has advantages over the Baermann method in terms of time to perform the method, possibility to standardize the protocol and to conduct multiple analyses with the same sample, and the small quantity of fecal material needed to perform the method.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01941-17.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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J.K., B.B., and R.W. designed the study; B.B., R.W., S.S., K.P., S.X., and K.K. performed field work and sample analyses; C.S. performed statistical analysis; and B.B., J.K., and R.W. wrote the manuscript.

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Chapter 7

Ultrasonographic evaluation of urinary tract morbidity in school aged and preschool-aged children infected with *Schistosoma haematobium* and its evolution after praziquantel treatment: A randomized controlled trial

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RESEARCH ARTICLE

Ultrasonographic evaluation of urinary tract morbidity in school-aged and preschool-aged children infected with *Schistosoma haematobium* and its evolution after praziquantel treatment: A randomized controlled trial

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Abstract

Background

Schistosoma haematobium infections are responsible for significant urinary tract (UT) complications. Schistosomiasis control programs aim to reduce morbidity, yet the extent of morbidity in preschool-aged children and the impact of treatment on morbidity reduction are not well studied.

Methodology

Our study was embedded in a randomized, placebo-controlled, single-blind trial in Côte d'Ivoire, which evaluated the efficacy and safety of three doses (20, 40 and 60 mg/kg) of praziquantel in school-aged (SAC) and preschool-aged (PSAC) children infected with *S. haematobium*. Enrolled children were invited to participate in an ultrasound examination prior and six months after treatment. At these time points 3 urine samples were collected for parasitological and clinical examinations.

Principal findings

162 PSAC and 141 SAC participated in the ultrasound examination at baseline, of which 128 PSAC and 122 SAC were present at follow-up. At baseline 43% (70/162) of PSAC had UT morbidity, mostly at bladder level and 7% had hydronephrosis. 67% (94/141) of SAC revealed mainly moderate UT pathology, 4% presented pseudopolyps on the bladder wall, and 6% had pyelectasis. At follow up, 45% of PSAC and 58% of SAC were *S. haematobium* positive, mostly harboring light infection intensities (41% and 51%, respectively).

Microhematuria was present in 33% of PSAC and 42% of SAC and leukocyturia in 53% and 40% of PSAC and SAC, respectively. 50% (64/128) of PSAC and 58% (71/122) of SAC presented urinary tract morbidity, which was mainly mild. A significant correlation ($p<0.05$) was observed between praziquantel treatment and reversal of *S. haematobium* induced morbidity. Progression of UT pathology decreased with increasing praziquantel dosages. A worsening of morbidity was observed among children in the placebo group.

Conclusion/Significance

Bladder morbidity is widespread among PSAC. Praziquantel treatment is significantly associated with the reversal of *S. haematobium* induced morbidity, which underscores the importance of preventive chemotherapy programs. These programs should be expanded to PSAC to prevent or decrease the prevalence of morbidity in young children. This trial is registered as an International Standard Randomized Controlled Trial, number ISRCTN15280205.

Author summary

Schistosoma haematobium is a parasite that infects the human genito-urinary tract. People get infected with the parasite through contact with fresh water and children are at major risk. The complications linked to this infection are due to an inflammation caused by accumulation of the eggs in peri-bladder veins. If not treated, infections can last years and different degrees of severity are observed. These range from thickening of the bladder wall and blurriness of the mucosa to more serious lesions such as pseudo polyps and masses in the bladder that can, with time, evolve in cancer of the bladder. We analyzed preschool-aged children (PSAC) and school-aged children (SAC) with ultrasound before and after praziquantel treatment. Children were randomly assigned to different doses of praziquantel (20, 40 or 60 mg/kg) or to placebo at baseline. Six months after treatment all children underwent another ultrasound of the urinary tract. We included 162 PSAC and 141 SAC at baseline, of which 128 PSAC and 122 SAC had a second ultrasound evaluation six months afterwards. In addition, urine was sampled at both time points for presence of blood, proteins and signs of infection (leukocytes and nitrates). Six months post-treatment 45% of PSAC and 58% of SAC were *S. haematobium* positive. Already at the first screening 43% of PSAC and 67% of SAC had bladder lesions. After treatment 50% of PSAC and 58% of SAC still had pathology linked to the infection. We found a correlation between the treatment dose and healing of bladder lesions. On the other hand, we experienced an aggravation of lesions in the placebo group. Praziquantel is given to SAC as preventive chemotherapy every year at national level, where this parasite is endemic. This program should be expanded and include PSAC as well in order to reduce the consequences of infection.

Introduction

Schistosomiasis primarily caused by *Schistosoma haematobium*, *S. japonicum and S. mansoni* is a significant public health problem in low-income tropical and subtropical countries. It is an ancient disease with first reports on schistosomiasis dating back 4000 years ago [1]. Yet, still today an estimated 230 million people are infected [2]. Adult *S. haematobium* settle in the

venous plexus of the genito-urinary tract of the infected host and produce fertilized eggs. Evidence suggests that morbidity is caused by the trapping of eggs within the urinary and genital tract, which induce a granulomatous host immune response. The granuloma formation induces a chronic inflammation resulting in disease manifestations. In more detail, morbidity includes a wide range of pathological presentations, from thickening of the bladder wall mucosa, ureteral dilatation and hydronephrosis, to presence of polyps and masses in the lumen, which could lead to bladder carcinoma in more severe cases [3,4].

S. haematobium infection is commonly detected by microscopic examination for eggs via urine filtration. Macro and microhematuria and proteinuria are indirect signs of infection, especially in school -aged (SAC) and preschool -aged (PSAC) children [5,6]. In addition, ultrasound examination of the urinary tract (UT) of infected subjects is an important tool to provide information on bladder and kidney lesions [7]. While intensity of infection as well as hematuria are important indirect indicators of morbidity [8,9], UT lesions could be quite different even at similar intensity of infection. Moreover this technique is useful not only at individual level, but also at community level, since it is well accepted, non-invasive and simple to perform [10,11]. Therefore, ultrasonography has been widely used to evaluate morbidity of UT due to *S. haematobium* infection [12–15] as well as its resolution after treatment [7,16–18]. It has been shown that UT lesions improve 12 months after treatment and, if not re-treated in case of reinfection, reappear 18 months after treatment [16]. It might be worth highlighting that studies in PSAC have been rare. To date, only few studies have included young children [4,15], which in general show a higher prevalence of morbidities in older children and adolescents. However, given that efforts are ongoing to include PSAC in preventive chemotherapy programs, it is crucial to have more data on the morbidity of PSAC and the impact of praziquantel in the prevention and reversal of morbidity at different follow up times, with the ultimate goal to define suitable control strategies. In addition, the optimal praziquantel dose in PSAC remains to be elucidated and findings on the reversal of morbidity might aid in the selection of optimal treatment dosages.

The aim of our study was therefore to evaluate morbidity in PSAC and SAC infected with *S. haematobium* and its resolution 6 months after treatment with different doses of praziquantel compared to placebo.

Methods

Ethics statement

Ethical approval for the study was obtained by the National Ethics Committee of the Ministry of Health in Côte d'Ivoire (CNER, reference no. 037/MSLS/CNER-dkn) and the Ethical Committee of Northwestern and Central Switzerland (EKNZ; reference no. 162/2014). Parents/ guardians of enrolled children were informed about the trial, and written informed consent as well as signed assent was obtained before the first child was enrolled. This trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN15280205. All children were treated with praziquantel at the end of the trial according to local guidelines (40 mg/kg).

Study design and population

Our study was embedded in a randomized, parallel-group, single-blind, placebo-controlled, dose ranging trial in PSAC (aged 2–5 years) and SAC (6–15 years) infected with *S. haematobium*. In both cohorts, 40 children per arm were randomized, using block randomization to 20, 40, 60 mg/kg praziquantel or placebo. The ultrasound evaluation was carried out in November 2015 and May 2016, in four different villages (Mopé, Diasson, Nyan, Massandji and Djiougbosso) in the Adzopè region of Côte d'Ivoire.

Study procedures

Details on the study procedures will be presented elsewhere. Briefly, all children provided three samples of urine on three different days at baseline, 21 days after treatment (follow up; not reported here) and six months after treatment. Urines were examined with the filtration method for detection of *S. haematobium* eggs according to standard procedures [19]. In addition, chemical examination of urines was performed using Multistix 10 SG Reagent Strips (Siemens Healthcare, Zurich Switzerland). From each child one stool sample was collected at baseline and 21 days post-treatment for the evaluation of co-infections with *S. mansoni* and soil-transmitted helminths. On the day of treatment all children provided one drop of blood for *Plasmodium spp* detection with rapid test (RDT) and hemoglobin measurement.

Before treatment all children underwent a physical examination performed by a physician and body temperature, blood pressure and pulse height and weight were recorded. Signs and symptoms of malaise were assessed with a questionnaire. *S. haematobium* egg-positive children fulfilling all inclusion criteria were assigned to one of the four following treatment arms: praziquantel 20 mg/kg (group 1), 40 mg/kg (group 2), 60 mg/kg (group 3) or placebo (group 4). Ultrasound was performed by a trained physician with Sonosite 180 Plus, probe Convex 3.5 mHz ultrasonography machine on the day of treatment. Children were asked to drink at least two full glasses of water before undergoing UT ultrasound. Ultrasound was performed if the bladder was at least 100 cc full and the ureter was considered dilated if its diameter measured >7 mm.

21 days and 6 months post-treatment all treated children were asked to provide three urine samples for detection of *S. haematobium* eggs and chemical examination. At the second follow up another sonography of urinary tract was performed from the same operator as at baseline.

Statistical analysis

Results were double entered in a database (Excel 2010), cross-checked and analyzed with Stata 12.0 (Lakeway Drive College station, TX, Unites States of America). The intensity of infection for *S. haematobium* was assessed by calculating the average of the egg counts from the triplicate urine filtration. Infection intensity was classified following WHO cutoffs [20].

Chi-squared analyses were performed to determine the associations between different markers of morbidity by sex, age, intensity of infection or markers of UT infections.

Results

Study flow

In November 2015 303 of the 348 children enrolled in the randomized controlled trial underwent an evaluation of the UT with ultrasound (Fig 1). Demographic, clinical and parasitological baseline data are presented in Table 1. Briefly, 162 of the 303 children were PSAC with a mean age of 3.8 (2–5) years. 46% of the preschoolers were male. 141 participants were SAC. Their average age was 8.9 (6–15) years and 44% were male. Six months after treatment (May 2016) 250 children (128 PSAC and 122 SAC) had an ultrasonography done for evaluation of UT lesions.

Clinical symptoms and parasitology

Baseline characteristics. 88% (142/162) of PSAC had a light *S. haematobium* infection, with a geometric mean of 7.8 eggs per 10 ml urine. 40% had a co-infection with *P. falciparum* and 18.5% with *S. mansoni*, no co-infection with soil-transmitted helminths was found.

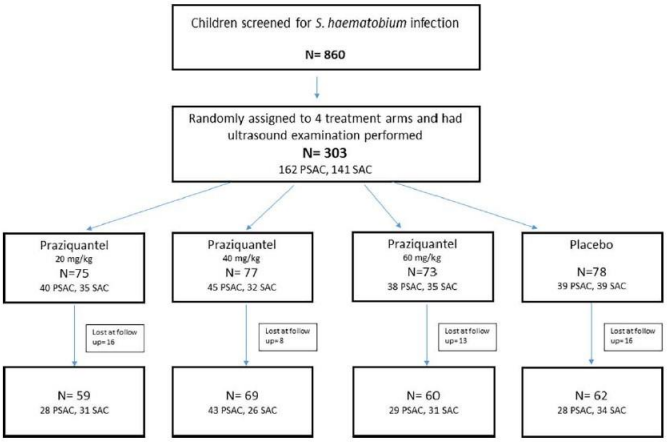

Fig 1. Flowchart of the study conducted in the Adzopè region of Côte d'Ivoire between November 2015 and May 2016.

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Urine analysis revealed that 62% of PSAC had microhematuria, 48% had leukocyturia, 37% had proteinuria and 15% had nitrites in urine (Table 1). The most common clinical symptoms among the PSAC at baseline were cough (32%) and headache (22%). Physical examination revealed that 36% had palpable splenomegaly and 33% hepatomegaly.

70% (98/141) of SAC had a light *S. haematobium* infection, with a geometric mean of 25.8 eggs per 10 ml urine. 42% had a co-infection with *P. falciparum* and 3% were co-infected with *S. mansoni*, no co-infection with soil-transmitted helminths was found. Urine examination revealed that 76% of SAC were positive for microhematuria, 72% revealed leukocyturia, 75% showed proteinuria and 3% had nitrites in their urines. When asked about presence of symptoms, 23% of SAC reported cough, 18% had fever and 16% documented headache. 28% had palpable splenomegaly and 34% hepatomegaly (Table 1).

Follow up at 6 months after treatment. Six months after treatment 45% of PSAC (ranging from 35% in the 40 mg/kg treatment arm to 64% in the placebo group) were positive for *S. haematobium* based on urine filtration with a geometric mean of 1.5 eggs/10 ml urine. 14.3% and 7% of PSAC who received placebo and 20 mg/kg praziquantel, respectively were characterized by high intensity of infection in contrast to children treated with 40 and 60 mg/kg praziquantel. Analysis of the urine revealed that 33% had microhematuria, 53% had leukocyturia, 6% had proteinuria and 48% had nitrites in their urines. In the placebo group hematuria was more prevalent (41%) than in the 60 mg/kg praziquantel treatment arm (29%). For the other chemical parameters the difference among the treatment arms was less pronounced (Table 2).

During physical examination the most frequently observed symptoms were cough (38%) and fever (19%). 13% of PSAC had palpable splenomegaly and 3% had hepatomegaly.

58% (71/122) of SAC were positive for *S. haematobium* based on urine filtration (geometric mean of 2.5 eggs/10 ml urine), ranging from 42% in the 40 mg/kg treatment arm to 82% in the placebo group. Infections were mostly light, with high intensity of infection mainly observed in the placebo group (24%). Analysis of the urine revealed that 42% of children had microhematuria, of which 65% were treated with placebo compared to 28% of children treated with 20 mg/kg praziquantel. 40% of children had leukocyturia, 8% had proteinuria and 24% had nitrites in their urines (Table 2).

Table 1. Baseline characteristics of children infected with *S. haematobium* stratified by treatment group. The study was conducted in the Adzopè region of Côte d'Ivoire in November, 2015.

		Preschool-aged children					School-aged children					Total
Baseline		**Placebo**	**20 mg/kg**	**40 mg/kg**	**60 mg/kg**	**Total**	**Placebo**	**20 mg/kg**	**40 mg/kg**	**60 mg/kg**	**Total**	
		N = 39	**N = 40**	**N = 45**	**N = 38**	**N = 162**	**N = 39**	**N = 35**	**N = 32**	**N = 35**	**N = 141**	**N = 303**
Demography	N Male (%)	16 (41.0)	18 (45.0)	25 (55.6)	15 (39.5)	74 (45.7)	16 (41.0)	18 (51.4)	14 (43.8)	13 (37.1)	61 (43.3)	135 (44.6)
	Age (SE)	3.8 (0.2)	3.7 (0.2)	4 (0.2)	4.0 (0.2)	3.8 (0.1)	8.4 (0.4)	8.9 (0.4)	9.3 (0.4)	9.2 (0.5)	8.9 (0.2)	6.2 (0.2)*
	Weight (kg) (SE)	14.9 (0.6)	15.1 (0.4)	15.3 (0.4)	15.4 (0.3)	15.2 (0.2)	23.2 (0.8)	24.1 (0.8)	25.1 (1.2)	25.7 (1.5)	24.4 (0.5)	19.5 (0.4)
	Height (cm) (SE)	97.9 (2.2)	99.0 (1.6)	101.2 (1.7)	99.6 (1.5)	99.5 (0.9)	125.2 (1.7)	127.3 (1.4)	126.9 (2.1)	127.3 (2.3)	126.6 (0.9)	112.2 (1.0)
S. haematobium infection	Light infection intensity N (%)	34 (87.2)	36 (90.0)	38 (84.4)	34 (87.2)	142 (87.7)	26 (66.7)	23 (65.7)	23 (71.9)	26 (74.3)	98 (69.5)	239 (78.8)
	High infection intensity N (%)	5 (12.8)	2 (5.0)	6 (13.3)	4 (10.5)	17 (10.5)	13 (33.3)	12 (34.3)	9 (28.1)	9 (25.7)	43 (30.3)	60 (19.8)
	EPG AM (CI 95%)	21.8 (8–35.6)	11.9 (6.9–16.9)	20.8 (10.6–30.9)	19.4 (12.5–26.2)	18.5 (13.8–23.2)	91.9 (21.2–162.6)	114.8 (0–252.4)	10.4 (22–58.8)	37.7 (20.7–54.8)	71.6 (33.7–109.5)	43.1 (25.2–61)
	EPG GM (CI 95%)	7.6 (4.7–11.9)	5.9 (3.7–8.9)	8.1 (5.2–12.5)	10.1 (6.4–15.5)	7.8 (6.2–9.6)	32.7 (21.4–49.7)	30.1 (18.7–48.1)	22.9 (15.5–33.5)	19.5 (12.8–29.3)	25.8 (21–31.7)	13.7 (11.7–16.1)
Co-infections	Soil-transmitted helminths	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	S. mansoni	2 (6.5)	7 (20.0)	9 (23.1)	6 (18.2)	24 (18.5)	0 (0.0)	3 (9.4)	0 (0.0)	1 (3.1)	4 (2.8)	28 (9.2)
	P. falciparum	15 (38.5)	16 (40.0)	20 (44.4)	13 (34.2)	64 (39.5)	16 (41.0)	14 (41.2)	17 (53.1)	12 (35.3)	59 (41.8)	123 (40.6)
Clinical findings	Hemoglobin (g/dl) (SE)	10.7 (0.2)	10.8 (0.2)	10.5 (0.2)	10.9 (0.2)	10.7 (0.1)	11.3 (0.2)	11.1 (0.2)	11.4 (0.2)	11.7 (0.2)	11.4 (0.1)	11 (0.1)
	Hematuria N (%)	23 (65.7)	21 (56.7)	24 (53.3)	25 (73.5)	93 (61.6)	34 (87.2)	26 (78.8)	22 (73.3)	25 (75.8)	107 (75.9)	200 (66.0)
	Proteinuria N (%)	11 (31.4)	11 (29.7)	18 (40)	16 (47.1)	56 (37.1)	29 (74.4)	25 (75.8)	26 (86.7)	26 (78.8)	106 (75.2)	162 (53.5)
	Leukocyturia N (%)	13 (37.1)	19 (51.4)	18 (40)	23 (67.7)	73 (48.3)	29 (74.4)	25 (75.8)	20 (66.7)	27 (81.8)	101 (71.6)	174 (57.4)
	Nitrituria N (%)	10 (28.6)	3 (8.1)	5 (11.1)	5 (14.7)	23 (15.2)	1 (2.6)	1 (3.0)	0 (0.0)	2 (6.1)	4 (2.8)	27 (8.9)
	Urinary tract morbidity N (%)	19 (48.7)	12 (30.0)	20 (44.4)	19 (50.0)	70 (43.2)	28 (71.8)	23 (65.7)	19 (59.4)	23 (65.7)	94 (66.7)	164 (54.1)*
	Urinary tract morbidity light N (%)	18 (46.2)	13 (30.0)	18 (40)	17 (44.7)	65 (40.1)	21 (53.9)	16 (45.7)	14 (43.8)	15 (42.9)	66 (46.8)	131 (43.2)
	Urinary tract morbidity severe N (%)	1 (2.6)	0 (0.0)	2 (4.4)	2 (5.3)	5 (3.1)	7 (18.0)	7 (20.0)	5 (15.6)	8 (22.9)	27 (19.1)	32 (10.6)
	Polyps/mass morbidity N (%)	1 (2.6)	1 (2.5)	1 (2.3)	0 (0.0)	3 (1.8)	1 (2.6)	1 (2.9)	2 (6.3)	2 (5.7)	6 (4.2)	9 (3.0)
	Kidney morbidity N (%)	4 (10.3)	2 (5.0)	4 (8.9)	1 (2.6)	11 (6.8)	2 (5.1)	4 (11.4)	1 (3.1)	2 (5.7)	9 (6.4)	20 (6.6)

* Significant correlation ($p<0.05$) between urinary tract morbidity at baseline and age. SE: standard error

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At physical examination, the most common symptoms reported were cough (38%) and headache (22%). 9% of SAC had palpable splenomegaly, while none had hepatomegaly.

Table 2. Follow up characteristics of children infected with *S. haematobium* assessed 6 months after treatment and stratified by treatment group.

		Pre-school aged children					School-aged children					Total
Follow up		Placebo	20 mg/kg	40 mg/kg	60 mg/kg	Total	Placebo	20 mg/kg	40 mg/kg	60 mg/kg	Total	
		N = 28	N = 28	N = 43	N = 29	N = 128	N = 34	N = 31	N = 26	N = 31	N = 122	N = 250
S. haematobium infection	Positive N (%)	18 (64.3)	13 (46.4)	15 (34.9)	12 (41.4)	58 (45.3)	28 (82.4)	15 (48.4)	11 (42.3)	17 (54.8)	71 (58.2)	129 (51.6) [3]
	Light infection intensity N (%)	14 (50)	11 (39.3)	15 (34.9)	12 (41.4)	52 (40.6)	20 (58.8)	15 (48.4)	11 (42.3)	16 (51.6)	62 (50.8)	114 (45.6) [1,3]
	High infection intensity N (%)	4 (14.3)	2 (7.1)	0 (0.0)	0 (0.0)	6 (4.7)	8 (23.5)	0 (0.0)	0 (0.0)	1 (3.2)	9 (7.4)	15 (6) [1, 3]
	EPG AM (CI 95%)	17.3 (6.5–28.1)	5.7 (1.3–10.2)	2.7 (0.5–4.8)	2.6 (0.5–4.7)	6.5 (3.7–9.2)	42.2 (21.1–63.2)	3.3 (0.8–5.7)	2.8 (0.2–5.4)	5.2 (0.1–10.4)	14.4 (7.8–20.9)	10.4 (6.8–13.9)
	EPG GM (CI 95%)	5.0 (2.2–10.5)	1.6 (0.5–3.4)	0.7 (0.2–1.3)	0.9 (0.3–1.7)	1.5 (1–2.2)	12.2 (6.1–23.3)	1.2 (0.5–2.1)	0.9 (80.3–1.9)	1.3 (0.5–2.4)	2.5 (1.7–3.6)	2 (1.4–2.5)
Clinical findings	Hematuria N (%)	9 (40.9)	8 (36.4)	11 (30.6)	7 (29.2)	35 (33.4)	20 (64.5)	7 (28.0)	9 (39.1)	10 (34.5)	46 (42.2)	81 (32.4)[2,3,4]
	Proteinuria N (%)	1 (4.6)	2 (9.1)	2 (5.6)	1 (4.2)	6 (5.8)	2 (6.5)	3 (12.0)	3 (12.0)	2 (6.9)	10 (8.1)	16 (6.4)
	Leukocyturia N (%)	13 (59.1)	14 (63.6)	17 (47.2)	11 (47.8)	55 (53.4)	17 (54.8)	10 (40.0)	8 (32.0)	9 (32.1)	44 (40.0)	99 (39.6)[4]
	Nitrituria N (%)	10 (45.5)	10 (45.5)	17 (47.2)	13 (54.2)	50 (48.1)	10 (32.3)	5 (20.0)	6 (24.0)	5 (17.9)	26 (23.6)	76 (30.5)
	Urinary tract morbidity N (%)	16 (57.1)	12 (42.9)	23 (53.5)	13 (44.8)	64 (50)	26 (76.5)	16 (51.6)	15 (57.7)	14 (45.2)	71 (58.2)	135 (54.0)[4] §
	Urinary tract morbidity light N (%)	12 (42.9)	11 (39.3)	21 (48.8)	13 (44.8)	57 (44.5)	19 (55.9)	15 (48.4)	12 (46.2)	12 (38.7)	58 (47.5)	115 (46.0)
	Urinary tract morbidity severe N (%)	4 (14.3)	1 (3.6)	2 (4.7)	0 (0.0)	7 (5.5)	7 (20.6)	1 (3.2)	3 (11.5)	2 (6.5)	13 (10.6)	20 (8.0)
	Polyps/mass morbidity N (%)	1 (3.6)	2 (7.1)	4 (9.3)	0 (0.0)	7 (5.5)	6 (17.6)	2 (6.4)	0 (0.0)	0 (0.0)	8 (6.5)	15 (6.0)
	Kidney morbidity N (%)	1 (3.6)	2 (7.1)	1 (4.7)	1 (3.5)	6 (4.7)	4 (11.8)	1 (3.2)	1 (3.9)	1 (3.2)	7 (5.7)	13 (5.2)

[1] Significant correlation ($p<0.05$) between *S. haematobium* intensity of infection, hematuria, leucocyturia at baseline and *S. haematobium* intensity of infection at follow up

[2]Statistical significant correlation ($p<0.05$) between *S. haematobium* intensity of infection at baseline and hematuria at follow up

[3] Statistical significant correlation ($p<0.05$) between *S. haematobium* intensity of infection and hematuria at follow up

[4]Statistical significant correlation ($p<0.05$) between UT morbidity at follow up and *S. haematobium* intensity of infection, hematuria and leucocyturia at follow up

§ Statistical significant correlation ($p<0.005$) between UT morbidity and different treatment dosage

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Ultrasound analysis. At baseline 43% (70/162) of PSAC had UT morbidity (Fig 2). The vast majority had light/ moderate bladder morbidity (40%), such as focal wall thickening or bladder heterogeneously echoic (Fig 2C and 2F), 2% (3/162) had polyps or masses on their bladder (Fig 2A, 2B, 2D and 2E) and 7% (11/162) had hydronephrosis (Table 1).

67% (94/14) of SAC presented UT pathology (Fig 3). The majority had light/moderate pathology (47%), such as heterogenous wall or focal thickening of the bladder (Fig 3A, 3B and 3F), 4% (6/141) presented polyps or masses on the bladder wall (Fig 3C, 3D, 3E and 3F) or dilated ureter (Fig 3C) and 6% (9/141) had pyelectasis.

30% of PSAC (48/162) and 1.4% of SAC (2/141) did not reach an adequate fill of the bladder to be analyzed with sonography.

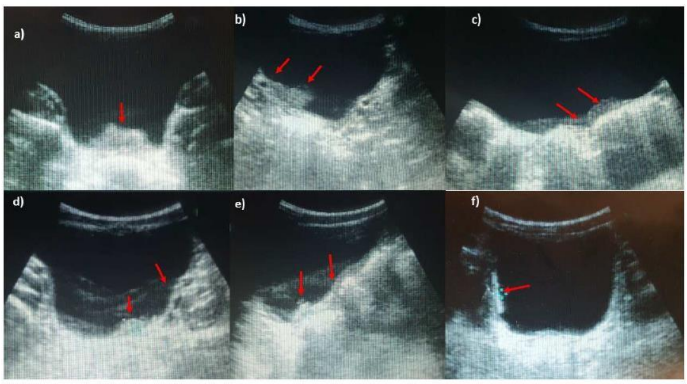

Fig 2. Ultrasonography images of urinary bladder of PSAC infected with *S. haematobium*. a) focal thickening of the bladder wall, longitudinal plane shows mass-like lesion (arrows); b) transverse plane image shows diffuse thickening of the bladder wall more evident in the right posterior wall; c) transverse plane image shows a focal heterogeneous echo of the bladder wall in absence of true thickening or mass-like lesions in the lumen; d) longitudinal plane shows a marked and diffuse thickening of the bladder left wall with a mass like lesion (arrow); e) image of diffuse and marked thickening of the bladder wall with pseudo-polyp lesion; f) focal thickening of the wall evident on the right wall and diffuse heterogeneity of bladder echo.

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At the six month follow up, 50% (64/128) of PSAC presented morbidity at bladder level, most of it (45%) was mild (thick/ semi-sonolucent mucosa), 6% (7/128) had polyps on the bladder wall and 5% (6/128) had pyelectasis. 58% (71/122) of SAC presented morbidity at bladder level, most of it (48%) was mild (thick/ semi-sonolucent mucosa), 7% (8/122) had polyps on the bladder wall and 6% (7/122) had kidneys dilatation.

Statistical analyses revealed significant correlations between UT morbidity and urine examination (leucocyturia and hematuria) and with intensity of infection both at baseline and at follow up (Table 2).

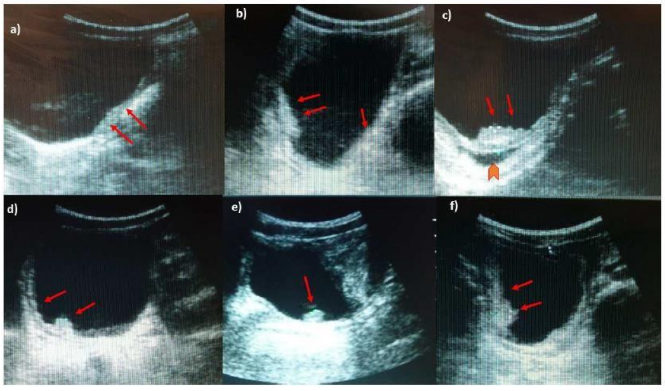

Fig 3. Ultrasonography images of urinary bladder of SAC infected with *S. haematobium*. a) Thickening of the bladder wall, transverse plane shows thickening of the left lateral wall (arrows); b) Diffuse thickening of the bladder wall more evident in the right posterior wall, echogenic snow in the lumen; c) In oblique longitudinal plane ultrasound image shows a mass-like lesion in the mucosa layer of the bladder. Block arrow indicates the dilation of the ureter; d) longitudinal plane shows a marked and diffuse thickening of the bladder wall with a mass-like lesion (arrow); e) mass-like lesion in the absence of a marked and diffuse thickening of the bladder wall; f) multifocal thickening of the wall, particularly evident on the right and posterior wall.

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Impact of praziquantel dose on morbidity. 19% (24/128) of PSAC and 22% (27/122) of SAC did not have any pathology at sonographic UT examination at baseline. A significant correlation ($p<0.05$) was observed between praziquantel treatment and reversal of *S. haematobium* induced morbidity in all children. In more detail and stratified according to treatment arm, among PSAC who did not experience any resolution of lesion at follow up 10% (2/21) had received 20 mg/kg, 43% (9/21) received 40 mg/kg, 23.8% (5/21) received 60 mg/kg praziquantel and 24% (5/21) placebo. 20 PSAC had worse lesions at follow up. Among these 20% (4/20) received 20 mg/kg, 25% (5/20) 40 mg/kg, 10% (2/20) 60 mg/kg of praziquantel and 45% (9/20) received placebo.

On the other hand, 23 PSAC had an improvement at sonographic follow-up, of which 21.7% (5/23) received 20 mg/kg of praziquantel, 30.4% (7/23) 40 mg/kg, 34.8% (8/23) 60 mg/kg and 13% (3/23) placebo.

Among SAC who did not experience any improvement of the lesion at 6 months follow up, 22.2% (6/27) received 20 mg/kg of praziquantel, 11.1% (3/27) received 40 mg/kg praziquantel, 25.9% (7/27) received 60 mg/kg praziquantel and 40.7% (11/27) received placebo. 45 SAC had an improvement of the UT at follow-up: 33.3% (15/45) received 20 mg/kg of praziquantel, 24.4% (11/45) 40 mg/kg, 26.7% (12/45) 60 mg/kg and 13.3% (6/45) placebo.

22 SAC had aggravated lesions at follow up: among these 13.6% (3/22) received 20 mg/kg, 18.2% (4/22) 40 mg/kg, 4.5% (1/22) 60 mg/kg of praziquantel and 63.6% (14/22) received placebo.

Residual urine measurements. Residual volume of urines after bladder voiding was measured both at baseline and at follow up. At baseline 82% (133/162) of PSAC had a low post-void residual (<20%) in the bladder, which means that there is no or very light impairment of the detrusor muscle. Only 2 children had a post-void residual higher than 50%. 12% (20/162) of PSAC had an intermediate (50–80%) value.

At the six months follow up, 91% (117/128) of PSAC had a post -void residual lower than 20%. 2 children still had high residual volume in bladder (>50% of the initial).

86% (122/141) of SAC had a good control performance of the detrusor muscle and only 1 child had a urine residual after bladder void higher than 50%. Almost 10% (14/141) of SAC had a post -void residual between 50 and 20%. At the six months follow up 96% (117/122) of SAC had a normal voiding of the bladder and only in one we registered a post-void volume higher than 50% of the initial one. We did not find any statistical difference between the treatment and improvement of bladder voiding.

Discussion

To our knowledge this is the first study that analyses urinary tract morbidity in school-aged and preschool-aged children affected by *S. haematobium* at baseline and six months after treatment with different praziquantel dosages and placebo. In settings where control of morbidity is the main goal of public health interventions, the most widely used criteria to determine it is the measurement of egg counts and urine analyses for hematuria and proteinuria, as indirect signs of UT impairment [3,12]. However, obviously a more accurate and specific evaluation of the organ pathology should be the way to follow [12,21–22]. Ultrasound examination allows to assess the damage of bladder wall and genito-urinary tract, which in combination with parasitological results and urine analyses are good indicators of consequences of chronic infection [4,12,13]. Ultrasonography has been applied since the '70s [21] for schistosomiasis to detect and describe the morphology of lesions. The need to implement diagnostic and monitoring with ultrasound is widely shared [4,10,21], but so far its use is still limited [21].

Since in schistosomiasis UT morbidity often occurs asymptomatic until an advanced grade of pathology [23,24], ultrasound offers the great advantage to spot early complications and progression of pathology in a non-invasive and easy to perform manner.

Our study confirms that early complications and bladder consequences of a *S. haematobium* infection are frequent also in preschool-aged children [5] (Fig 2). We recorded both direct and indirect signs of infection that give a full and detailed picture of UT status in infected children of different ages. In more detail, in our study most children (79%) had low intensity infection but nonetheless of these 54% of children (43% of PSAC and 67% of SAC) presented UT morbidities. As Hatz and colleagues pointed out [4], lesions of the bladder are observed also in absence of excretion of eggs, as these might be stuck and trapped in the wall resulting in an inflammatory reaction, that does not allow their release. Also for other helminthic infections, it has been demonstrated that morbidity (such as anemia, stunting) is mostly triggered by chronicity of infection rather than by its intensity [25,26].

According to our findings, children are not affected by severe morbidity, in fact, the greater part of hydronephrosis resolved immediately after urination. We also did not observe a frequent presence of pseudopolyps or masses in the bladder (6%). Our data are in line with findings by Koukounari and Njaanake [10,27], but in contrast to Elmadani, who described that more than 40% of children had masses in the bladder lumen and 30% had hydronephrosis after urination [13]. The prevalence of UT morbidity is indeed very different from one study to the other. For example, Heutier and colleagues registered a 70% prevalence of bladder lesions in an African village endemic for *S. haematobium* in children [28], whereas Ekwunife and Koukounari reported a lower rate of UT morbidity in infected children (38% and 6% respectively) similar to what we have found [10,12].

As already reported and underlined in several trials on *Schistosoma* morbidity [4,18,29–31], praziquantel treatment is crucial in decreasing morbidity with regard to healing lesions and pathology linked to the infection, especially at early stages of the disease. In the present study we went a step further and studied the effect of different praziquantel doses and placebo on UT morbidity. Strikingly, while in the placebo group almost 40% of children had progression of UT pathology over the 6 months course, this rate decreased with increasing dosages being only 5% in the children treated with 60 mg/kg praziquantel. In addition, all dosages of the drug were correlated with an improvement of the clinical picture. Overall, more than 90% of treated children experienced improvement of lesions, whereas in the placebo group this rate was only 10%.

In our study 74% of children had no residual urine after bladder void and 12% had a residual volume greater than 50%. We did not perform an uroflowrimetry to confirm pathological voiding, but children were asked about symptoms linked to urination discomfort and reported urge of voiding even if the bladder was almost empty and a feeling of incomplete voiding was present. Voiding impairment is difficult to assess and confirm, especially in children and in conditions of stress such as ultrasound performance and clinical examination. Nonetheless, we observed an improvement at follow up both in bladder filling and discomfort in urination, though this was not properly validated. Akpata stated that the above mentioned symptoms are better indicators of schistosomiasis than residual volume calculation [21]. Stiffness of detrusor muscle, polyps and hydronephrosis are signs of severe stage of the pathology, which fortunately was rare in our study cohort. This suggests that the annual drug administration that takes place in the area is a good strategy to fight morbidity and decrease UT impairment [4,27].

Urinary tract infections (UTI) are common in childhood, accounting for 6% of infection in this age range [32]. In our study leucocyturia was often documented (57%), especially among SAC (72% *versus* 48% in PSAC), whereas nitrituria was more frequent among PSAC (15 vs 3%) [33,34]. According to our data, lower urinary tract morbidity was correlated with a general worsening of the UT, revealing a higher rate of nitrates and proteins and blood cells in urines, which is an evident sign of mucosa damage. As in previous trials, we also found hematuria and

proteinuria to be good indicators of UT pathology (66% and 56% respectively) [10,15]. The prevalence of UTI in our study was higher than earlier findings for same age group [32], but this is not surprising given the fact that almost half of the children had chronic infections with *S. haematobium*. Chronic infections with *S. haematobium* are the main cause of mucosa damage and hence more likely to develop subsequent bacterial infections [35]. After treatment we observed an improvement of microhematuria in treated children compared to placebo treated children in both age groups.

On the other hand, clinical symptoms documented, such as fever and cough, were not found to be related to an *S. haematobium* infection, but are likely triggered by other diseases. For instance, at physical examination splenomegaly and hepatomegaly was observed in 30% of children, which might be correlated to *S. mansoni* or to other co-infections (e.g. malaria, leishmaniasis).

In conclusion, we have demonstrated that extending treatment (40 or 60 mg/kg praziquantel) from school-aged to preschool-aged children is crucial, in order to prevent morbidity to a *S. haematobium* infection. We have shown that already a high percentage of PSAC present bladder inflammation and mucosa thickening due to *S. haematobium* infection, which could be decreased by including this age group in treatment programs. We observed a high re-infection rate with *S. haematobium*, therefore preventive chemotherapy must be conducted at least once a year in PSAC and SAC in order to decrease morbidity.

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Chapter 8

General discussion

The most recent estimates suggest that between 600 and 800 million people are infected with at least one of the common soil-transmitted helminths (STH) [1,2]. Recently, the burden due to STHs has been estimated at 1.2-10 million DALYs for *A. lumbricoides,* 1.8-22.2 million DALYs for *T. trichiura* and 1.6-6.4 million DALYs for hookworm [3].

For *A. lumbricoides* and *T. trichiura,* only infections of moderate and heavy intensity are mainly linked to morbidity, while for hookworm any intensity of infection may cause morbidity, depending on the iron status of the host [3]. Infections are typically most intense and debilitating in SAC and adolescents and chronically infected children might suffer from malnutrition, physical and cognitive retardation, and reduced work performance in adulthood [4]. The backbone of the preventive chemotherapy (PC) strategy to control STH relies on the distribution of a single dose of albendazole or mebendazole, to PSAC, SAC and women of childbearing age [5]. In 2016 637.5 million children (PSAC and SAC) received PC; reaching a coverage of 50% in PSAC and 69% in SAC. WHO aims at a global coverage of 75% of SAC at risk of morbidity from both STH and schistosomiasis by 2020. Pharmaceutical companies support the process by donating the benzimidazoles [5]. A meta-analysis recently conducted on the impact of PC on morbidity, linked to STH infections, shows that, for all species, the average proportion of moderate-heavy intensities decreases by 73% after 12 months and by 95% after 130 months of 2 or more PC rounds [3]. Despite the declining trend of morbidity and mortality [3,6,7], there is still evidence that linked morbidity is a huge problem [8,9]. Also from an economical point of view, estimates have shown that in settings aiming at the control of morbidity, PC is highly cost-effective even where the prevalence of STH is below the WHO threshold [10]. These considerations drive attention to the need of an attentive re-evaluation of WHO guidelines in terms of broadening the coverage spectrum by including low endemicity settings that would also benefit from this strategy [10,11].

The main topic of my PhD project was the evaluation of new drugs and drug combinations against the more prevalent intestinal helminth infections. In this context, we selected moxidectin as a good alternative to the available drugs. In the first trial, we tested moxidectin in comparison with Synriam against *S. mansoni* and S.

haematobium. Synriam is a new drug on the market for malaria, which might have had efficacy against schistosome infection.

Secondly, in a different study setting, we evaluated the non-inferiority of moxidectin to ivermectin in treating *S. stercoralis* infections. Having a common chemical background, moxidectin was considered a potential good weapon against *S. stercoralis*.

The third research question was to test the efficacy of moxidectin alone and in co-administration with albendazole and tribendimidine vs albendazole plus oxantel-pamoate against *T. trichiura* and concomitant STH infections. This trial was designed to target an important research topic, which are the drawbacks of benzimidazoles in treating *T. trichiura* infections. Tribendimidine and albendazole have been added to broaden the spectrum of activity, since moxidectin is known to be scarcely effective against hookworm [12].

Another objective was driven by the low sensitivity of current diagnostic methods towards intestinal helminthic infections and the rising interest in molecular techniques. We therefore, compared direct microscopy-based methods with PCR.

As already mentioned, one of the targets of PC is to reduce morbidity linked to intestinal parasitic infections [5,10,11]; thus, we designed a trial to evaluate the effect of different doses of praziquantel on urinary tract morbidity in PSAC and SAC infected with *S. haematobium*.

The results of the individual studies have already been discussed within the separate manuscripts, thus, in this section of my thesis I would like to focus on three main research themes which I have explored in the past three years:

1) Efficacy of the tested drugs and drug combinations and future steps
2) Molecular diagnosis: its application in parasitology and future prospective
3) Evaluation of urinary tract morbidity linked to *S. haematobium* infection and its response to different doses of effective treatment versus placebo

8.1 Efficacy of the tested drugs and drug combinations and future steps

Recently, WHO updated the guidelines on PC strategies against STH and schistosome infections [5]. This guide should regulate the distribution of treatment to in-need and at-risk population groups. This approach is tailored to the specific needs of different settings: from aiming at control of morbidities, to their elimination. Large scale distribution of drugs is not based on diagnosis of helminthic infections, but targets specific groups such as young children (12-23 months), PSAC (24-59 months of age), SAC (5-12 years of age) and pregnant women after the first trimester [5]. The recommended drugs are the benzimidazoles (albendazole 400 mg and mebendazole 500 mg) against STH and praziquantel (40 mg/kg) against schistosomes. However, a recent meta- analysis, however, confirmed the low efficacy of the benzimidazoles especially against *T. trichiura* and hookworm infections [13].

Efficacy is measured using the cure rate (CR) and the egg reduction rate (ERR) after treatment; the former is based on the proportion of cured individuals who were infected before treatment; the latter is the proportional reduction in egg counts after treatment, but the way it is calculated is still under debate. In fact, ERR varies depending on whether it is calculated using arithmetic or geometric mean [14]. Both methods have limitations mainly due to the fact that egg output is not normally distributed, even after logarithmic transformation.

Geometric mean is used to overcome the dispersion that "outliers" would produce on the mean; geometric mean normalizes the variance of the data because it is more interested in the whole population, and usually generates an overestimation of the ERR. However, "outliers" in helminthic infections are important, since those are the ones who will suffer the most from the morbidity caused by these infections [14–17]. On the other side, arithmetic mean is not the ideal indicator either, because the untransformed data cannot be analysed with the parametric tests (such as ANOVA) [15]. The two means are only interchangeable for ERR>95% [14]. In conclusion, although both means are used in clinical trials and efficacy assessments, both parameters are suboptimal measurements [14,17–19]. In fact, for high intensity of infections, geometric mean tends to overestimate ERR, the opposite happens for low intensity of infections; therefore, the best option to evaluate drug efficacy would be to stratify the analysis by class of intensity of infection [15].

Both albendazole and mebendazole are highly active against *A. lumbricoides* (CR 92-98% and ERR 94-100% for both drugs), albendazole has good activity against hookworm (CR 70-85% and ERR 82-97%), but mebendazole does not (CR 20-47% and ERR 50-70%). What is most alarming, though, is the confirmed low performance of both drugs against *T. trichiura* with a maximum CR of 60% and ERR of 77% for mebendazole and CR of 42% and ERR of 61% for albendazole [13]. Of great interest, is the trend of reduced efficacy of the benzimidazoles in the past 20 years against both hookworm and *T. trichiura*, which may be a sign of occurrence of drug resistance in the worm populations [20,21]. Reduced efficacy of praziquantel has also been reported in literature in the schistosome population, for *S. haematobium* ERR ranging from 50% to 99% and CRs from 65 to 89% have been reported For *S. mansoni* ERRs from 83 to 99% and CRs ranging from 26 to

89% were found [14,22]. An assessment conducted in Uganda, reported a lower efficacy of praziquantel in SAC that received higher number of PC rounds than in SAC who received fewer or no rounds of PC, even though not statistically significant [23]; ERR in schools with higher PC exposure was 91.5% compared to 9897% in schools with lower exposure.

In the framework of my PhD, moxidectin has been evaluated as a potential alternative drug against intestinal helminths. The first study setting was Côte d'Ivoire, endemic for both *S. mansoni* and *S. haematobium.* The trial design was a proof of concept aiming at 30 participants per treatment arm [chapter 4]. Synriam, a combination of arterolane and mefloquine, alone and in co-administration with praziquantel was also evaluated; praziquantel alone was used as comparator [24]. Previous studies have shown a good performance of moxidectin against *S. mansoni* with a CR of around 60% [12]. Also artemisinin derivatives have potential antischistosomal activity known since the '80s [25]. *In vitro* and *in vivo* trioxolanes have shown high activity both against juvenile *S. mansoni* and *S. japonicum* infections and against the adult worm stage in the hamster model [26]. In particular, Synriam, after being licensed in 2011, has been studied for its excellent activity against *S. mansoni* [27].

Disappointingly, in our trial, these promising data were not confirmed; we observed low efficacy of both moxidectin and Synriam against *S. haematobium,* despite intensity of infections being low. The highest CR

and ERR was reached with the co-administration of Synriam and praziquantel (60% and 96%, respectively), which suggests an interaction between the two mechanisms of action. Of note, both new drugs tested alone performed better against *S. mansoni* than against *S. haematobium* in terms of ERR, whereas CRs were consistent in both cohorts. In our study the ERR ranged from 87.5% against *S. mansoni* to 94% against *S. haematobium* with praziquantel alone. These results are consistent considering that the study area is highly exposed to PC [23,28]. Until now there is no clear evidence of wide range resistance to the treatment with praziquantel [29]. Few isolates of *S. mansoni* were reported as being resistant [30], but no resistance was reported for *S. haematobium* yet [31,32]. Nonetheless, in experimental models it has been shown that *Schistosoma* spp can develop some level of resistance to praziquantel [32]. The topic of praziquantel resistance remains an open field for research, since neither the precise mode of action of the drug, nor the genetic bases of schistosomes are completely understood [23,28,33].

More promising data were collected during my second randomized, phase 2 exploratory trial in which the efficacy of moxidectin was compared to that of ivermectin against *S. stercoralis* infections [chapter 5a]. The study was designed as a non-inferiority single blind study. Moxidectin has a similar structure to ivermectin and it has been used in macaques against *S. fuelleborni* infections [34]. As expected, moxidectin showed good efficacy in curing *S. stercoralis* infections, confirming non-inferiority to ivermectin (94 vs. 95%, respectively). Despite the great efficacy of ivermectin against *S. stercoralis*, it is currently the only available and highly efficacious drug against this parasite, thus, it is crucial to find alternatives. In fact, in veterinary medicine, resistance to ivermectin is a major global problem in the control of gastrointestinal roundworms of sheep, cattle and horses. Additionally, there is recent evidence of ivermectin resistance in canine heartworm [35]. Concurrently, a reduced effect of ivermectin on human *O. volvulus* has raised concerns that ivermectin resistance might occur also against other helminth species. It is becoming increasingly evident that basing control strategies on one single drug (i.e. ivermectin for lymphatic filariasis) is risky and no longer sustainable; this also highlights the importance of new, integrated approaches [35]. Resistance has also been described towards moxidectin in veterinary medicine, but the molecular patterns are different. Prichard *et al.* have thoroughly described the diversity of the two molecules in pharmacokinetic,

this includes a larger volume of distribution, longer elimination half-life and slower clearance rate of moxidectin compared with ivermectin [36]. This evidence also hints that the mechanism of action and resistance patterns might differ between in ivermectin and moxidectin. Further studies in *Caenorhabditis elegans* on efflux pumps components involved in drug elimination, have clarified the mode of action of both drugs. The transporter pumps are ATP-binding cassettes (ABC) which consist of transmembrane proteins that use ATP hydrolysis as energy source for the active extrusion of a variety of drugs, carcinogens and toxins across the cellular plasma membrane. Pglycoprotein (PgP), Multidrug Resistance associated Proteins (MRPs of the ABCC family) and Breast Cancer Resistance Protein (BCRP) are among the best characterized drug transporters. These pumps are located in the apical side of the cells of barrier epithelia (intestine, placenta, mammary gland and the blood-brain barrier), and participate in the active efflux of different toxic compounds out of the cell [36]. Ivermectin and moxidectin, although sharing a similar structure, are not identical, with moxidectin showing a weaker affinity for the PgP, having a stronger affinity for BCRP and slower efflux out of the organism. These differences allow moxidectin to be effective against ivermectin-resistant strains [37,38].

During the above-mentioned trial, we had the opportunity to test the efficacy of moxidectin against concomitant *O. viverrinii* and hookworm infections, being the study site endemic for these parasites.

Unfortunately, we did not experience any ancillary benefit on the co-infection with *O. viverrinii*: the CR and ERR of moxidectin were 18% and 13%, respectively. Although the reported activity of ivermectin against hookworm is low (CR between 11 and 33%) [39–41], we registered a moderate efficacy for moxidectin (CR of 57%).

In the framework of my third clinical trial [Chapter 6], efficacy of moxidectin alone and in co-administration with albendazole and tribendimidine against STH was assessed. The first objective of this trial was to evaluate the efficacy of those treatments versus the co-administration of albendazole and oxantel-pamoate against *T. trichuris* infection. Until now, the latter formulation is the one that has proved to have the highest efficacy against *T. trichiura* [42]. Our results confirmed the non-inferiority of the co-administration of moxidectin/albendazole to albendazole/oxantel pamoate in terms of ERR (98.5% vs 99.8%, respectively),

but CR was lower (51% vs 83%, respectively). The co-administration moxidectin/albendazole showed very good efficacy against *A. lumbricoides* infections (both ERR and CR>95%) and against hookworm co-infections (ERR 93% and CR 77%). This study confirmed that this co-administration is a good alternative to the albendazole/oxantel pamoate regimen against STH infection. As for the other co-administration (moxidectin/tribendimidine), we reported a fairly good efficacy against *T. trichiura* in terms of ERR (92%) but not in terms of CR (23%). Being tribendimidine a good alternative to benzimidazole for treating hookworm, we were not surprised by its good efficacy against hookworm infection both in terms of ERR and CR (93% and 88%, respectively). The monotherapy with moxidectin was the least effective drug against all STH; it showed high efficacy only against *A. lumbricoides.* This observation hints for synergistic action between moxidectin and the partner drugs. The increased efficacy of moxidectin when administered with either albendazole or tribendimidine, suggests a synergistic effect. This synergisms may be explained by the pathways of efflux of these drugs [36,37,43] This synergy might be partially explained by looking at the mechanism that regulates the excretion of benzimidazoles and macrocyclic lactones. Both groups are actively transported by the efflux pump. The PgP is largely expressed in different organs and tissues both of humans and parasites and it is involved in the defence of the organisms against toxic compounds, therefore, the higher the affinity the faster is a toxin effluxed [36]. Ivermectin has a stronger affinity for the PgP than moxidectin [37]. Macrocyclic lactones and the metabolites of benzimidazoles are good substrates and inhibitors of the ABC transporters which control the concentration of these drugs by affecting their *in vivo* absorption, distribution, and elimination. Pharmacokinetics studies have shown that drugs have different affinity for the different ABC transporters: ivermectin is transported by the PgP[44] and albendazole sulfoxide and moxidectin by the BCRP[45]. It has been reported that the inhibition of the ABC transporters increases moxidectin bioavailability, raising its plasmatic levels[45]. Since albendazole metabolites and moxidectin are transported by the same transporters, it is likely that the competition and consequent inhibition of the BRCP transported from one of the two drugs, enhances the bioavailability of the other. This mechanism would explain the synergistic action of albendazole and moxidectin when administered together and therefore, the better efficacy of the co-administration compared the

monotherapy. What we observed, confirms what has been reported in previous clinical trials, on the better efficacy of co-administration of drugs than monotherapy, especially when macrocyclic lactones are combined with benzimidazoles [43,46,47]. This data supports additive or synergistic behaviour which involves different drug classes: tribendimidine co-administered with ivermectin has shown some synergism on STH infection [46] and albendazole/oxantel pamoate performed better than each drug in monotherapy against *T. trichiura* infection [42]. In my first clinical trial on *S. haematobium* we also experienced a better performance of co-administration of Synriam and praziquantel than the monotherapy, which could also suggest an interaction between the two drugs; however, this effect was not confirmed for *S. mansoni.*

In all trials conducted, moxidectin has proved to be a safe and well tolerated drug. In the first two trials [Chapter 4-5a] moxidectin was used as syrup formulation, as tablets were not yet available. A sweetener was added to the syrup in order to mask the bitter taste of the drug. Whereas, for the last trial [Chapter 6], tablets were manufactured in Basel, Switzerland. In all trials we experienced a good compliance of participants, with no episodes of vomiting or refusing to swallow the drug. No severe side effects were registered, some mild symptoms (such as headaches or abdominal cramps) were reported, but they resolved without intervention. Despite the vast number of trials and research conducted in the recent years on intestinal helminthic infection at different level, many aspects remain unclear and need further research. The body of evidence accumulated from data collected in the past three years of my research studies, concur to demonstrate that moxidectin is a promising drug for treatment of several infections. It is about to be registered for the treatment of *Onchocerca volvulus* in humans and data have shown that it has a good potential against different parasites, other than filariasis. Lately, more attention has been drawn to the co-administration of drugs not only to broaden the spectrum of activity, but also to avoid resistance to spread. Therefore, the coadministration of moxidectin with albendazole has shown a good efficacy and could be used as alternative to the current treatment options. Of course, more trials are needed to further explore and confirm the efficacy we obtained.

During my PhD the safety and efficacy of moxidectin were assessed through trials that, highlighted interesting aspects of this drug. The finding of its low efficacy against schistosomes is an important scientific

outcome, although disappointing; on the other hand, moxidectin has resulted in a very good efficacy against *S. stercoralis,* which is worthly of further investigations.

Another interesting aspect of moxidectin is the dose-dependent effect; we only used 8 mg (or 8 ml) as standard dose, but a higher dosage could elicit better efficacy. For example, despite the excellent efficacy profile reported by Speich *et al.* [42] for oxantel pamoate, the co-administration with albendazole reached high levels of CR after conducting a dose-finding study [48]. Dose-finding studies are important to fine tune the treatment regimen once the drug has proved to be efficacious and safe. Thus, it could be that moxidectin would also benefit from a higher dose regimen, especially against STH infections.

Another interesting aspect is the synergism of moxidectin with the benzimidazoles that, in our study, we could only verify at a clinical and drug efficacy level. It would be interesting to deepen the knowledge at laboratory level, with pharmacokinetic studies, that could better elucidate the interaction of different drugs with efflux pumps and metabolic enzymes.

8.2 Molecular diagnosis: its application in parasitology and future prospective

S. stercoralis is known to be the neglected of the neglected tropical diseases, which is mainly due to the scarcity of data on its prevalence and morbidity [49,50]. Despite being always considered a tropical disease, recent migration flows and increased travels to endemic areas, have changed the geographical distribution on many parasites, including *S. stercoralis* [51,52]. As mentioned before, an accurate diagnosis, is highly relevant for all intestinal helmintic infections, however it is particularly relevant for *S. stercoralis* as it is the only intestinal helminth that replicates within the human host prolonging the infection for years, if not properly cured. This peculiarity allows the parasite to persist in old people, in areas where it is no longer present, but was once endemic. The diagnosis is burdened by many issues: infections are often asymptomatic or lightly symptomatic, diagnostic techniques used for STH fail to detect *S. stercoralis,* and the few methods that detect the larvae have low sensitivity [52–57]. Direct methods, such those recommended by WHO (Baermann and Koga Agar culture), are the only techniques used in endemic countries, yet they are timeconsuming and show scarce to moderate sensitivity [58,59]. Furthermore, their

performance requires resources (specific incubator, stable source of power, funnels, tubes and enough space in the laboratory for incubation), which are crucial for the good outcome of the results [60–62].

Serology has been proved to be a good alternative, but it is not applicable in high endemicity settings due to the long time needed to decrease the antibody titers after treatment [63].

Molecular techniques are starting to be considered for diagnosis also in parasitology, even if their application is still limited to high-resource settings [59,60]. The detection of *S. stercoralis* might greatly benefit from PCR methods, as larvae output is not regular, and up to seven samples are needed in order to reach a sensitivity of 100% with microscopy [64]. Moreover, often the intensity of infections is low and microscopy fails to detect it. PCR for *S. stercoralis* is still under development [58,65] and results are discordant on PCR performance [65,66]. The main limitation of this method, is related to the DNA extraction protocol which is still not standardized; different research groups are working to find the most suitable protocol [66].

In my trial on efficacy of moxidectin against *S. stercoralis* infection, subsamples of the first collected stool sample were preserved in ethanol and shipped to the Swiss TPH for PCR analysis [Chapter 5b]. We compared two extraction methods: QIAamp Stool Mini kit (Qiagen; Hilden, Germany) following the manufacturer protocol and the QIAamp DNA Mini Kit with a modified protocol according to Polley *et al.* [67]. To avoid adding too many variables in the study, we used the same primers for both extraction protocols to perform PCR, although different primers are reported in literature [60,68,69].

When analyzing the results, the major problem encountered, was that, in the absence of a "true gold standard", it was difficult to estimate the sensitivity of the techniques. Therefore, we assessed sensitivity and specificity of the methods comparing results of the two extraction methods and one Bearmann to the results of 2 days of Baermann (being the WHO recommended technique) and all techniques to any positive sample found by any of the three methods. Surprisingly the two DNA extraction methods did not perform similarly; in fact, the standard QIA method showed a sensitivity of 27% compared to Baermann and 31% to any positive found. As mentioned above, controversial results on the performance of QIA Stool Mini kit

protocol were reported in published literature, with either higher and lower sensitivity when compared to microscopy [58,59,68,70].

The modified protocol performed much better with a sensitivity of 80%; still, the Baermann method on two samples collected over two days was the best method and reached the highest score in terms of sensitivity. Although our study was not quantitative, we have seen a later positivisation of PCR for Baermann negative samples, which hints to a correlation between larvae quantity and PCR detection.

We confirmed that PCR protocols are still to be improved, as many of the published data rely on in-house protocols, that are not yet standardized [66]. In contrast with Repetto *et al.* [66], but in line with other studies, we did not experience a striking improvement in sensitivity of PCR compared to coprological methods, and we can confirm that, at present, PCR is still insufficiently sensitive to replace microscopy [58,59,68].

Of note, the combination of the modified extraction method and a single Baermann increases the sensitivity to almost 100%. This allows the detection of all positive samples in one day of collection. This would significantly simplify field work, especially when working with adolescents and/or adults, who often fail to provide more than one sample. Moreover, it has already been reported that PCR performs well on a single sample and no consecutive samples are needed to increase the accuracy of the technique [59,71,72]. As suggested elsewhere, a combination of PCR and microscopy would be ideal to increase sensitivity [59,68,69]. This combined approach would, nonetheless, have important drawbacks (i.e. time-consuming, specific and sometimes expensive equipment) that still limit its application on the field and would fail to simplify and fasten its performance.

Molecular techniques are already widely applied in many fields of infection diseases [73–75] and their extension to parasitology is needed. Currently, protocols are not standardized and fail to meet the expectancies in term of better sensitivity compared to copro-microscopic techniques. As demonstrated also with my study, the path to a standardized, sensitive PCR protocol is still long; debates and incongruities show up early on even during the very first step of DNA extraction. We can conclude that further research in the diagnostic field is needed and a standardized procedure would allow useful comparison among trials.

8.3 Evaluation of urinary tract morbidity linked to *S. haematobium* infection and its response to different doses of effective treatment vs placebo

One of my PhD projects focused on the ultrasonographical evaluation of urinary tract morbidity in PSAC and SAC infected with *S. haematobium* before and after treatment. *S. haematobium* eggs trapped in the tissue trigger a constant inflammatory reaction and lead to the formation of granulomas. This results in collagen deposition and laminated fibrous tissue replacement of the healthy tissues of the urinary tract, causing morbidity [76]. Indeed, schistosomiasis is linked to systemic morbidity, such as anaemia, malnutrition and organ complications. The most severe cases of *S. haematobium* lead to bladder and ureteral dysfunction, such as ureteral reflux, hydronephrosis, renal impairment and bladder cancer [77,78].

Urinary schistosomiasis is widely spread in Africa, affecting mainly children [79]. The goal of PC programs is to control morbidity in childhood, in order to prevent organ failure and growth impairment [5]. Even in low intensity and chronic infections, sequelae may still be found; most bladder pathology heals after treatment, but urinary tract dilatation frequently persists even in successfully treated patients [80]. In recent years there have been improvements in the detection of urinary tract morbidity in older children and adult population [81]. However, diagnosis of the target population is not commonly performed and relies mostly on indirect signs of organ impairment or questionnaire administered to the older population [79]. Measuring the burden of infection in endemic populations and the impact of treatment, would be of great importance in order to assess the effectiveness of control programmes and, thus, formulate future evidence-based control policies. Point-of-care ultrasonographic evaluation, is becoming a widely used diagnostic tool which has been recognized and standardized for fast diagnosis, as well as an integrated part of diagnostic algorithm in emergency medicine [82]. Ultrasonography for evaluation of *S. haematobium* morbidity has been reported to be a “simple-to-learn” examination [83].

In the framework of a dose-finding clinical trial for praziquantel, in which PSAC and SAC were randomly assigned to different doses of praziquantel (20, 40, 60 mg/kg) or placebo, participants underwent an ultrasonographic evaluation of the urinary tract. The same children were re-examined 6 months after

treatment. Concomitantly, a urine filtration for the diagnosis of *S. haematobium* and urine dipstick test was performed for each child, to look for infection and linked morbidity detectable at point-of-care level. Of the infected children, the vast majority (60% of PSAC and 76% of SAC) had microhematuria. Proteinuria was present in 40% of PSAC and in 75% of SAC. These results, reveal a high level of organ impairment already in younger children. As expected, after treatment, we observed a high prevalence of infection in the placebo group (64% in PSAC and 80% in SAC). Also, in the different dose-groups, CRs were quite low with no clear indication on which dose has the highest efficacy, especially among SAC. However, all treatment arms showed good efficacy in terms of ERR, with almost all high intensity infections cleared or at least reduced after treatment; we also experienced an improvement in proteinuria, which dropped below 10% in both PSAC and SAC.

The main focus of my trial was the ultrasonography assessment, which revealed alarming data on the prevalence of urinary tract morbidity even in PSAC. Almost half of PSAC (43%) had light to moderate bladder morbidity, characterized mainly by focal wall thickening or bladder heterogeneously echoic. Only a few of the PSAC had polyps or masses in the bladder (2%) and hydronephrosis (7%). The picture of SAC was even more alarming, with 67% of children presenting bladder morbidity. Yet, similarly to PSAC; in SAC the pathology was not severe, as only 4% had masses or polyps. A significant correlation ($p<0.005$) with urine analysis was reported for haematuria, leukocyturia and intensity of infection.

The most interesting finding of the trial, was a significantly positive ($p<0.005$) correlation between treatment and the reversal of *S. haematobium* induced morbidity. Surprisingly, however, we did not experience a better performance of higher doses of praziquantel which suggests a non-linear correlation between increase of drug dose and reverse of morbidity. In treated groups, we observed an improvement of the clinical picture in 90% of children; in both PSAC and SAC the progression of morbidity was inversely proportional to treatment dose. On the other hand, in the placebo group, we reported a small proportion (13%, both in PSAC and SAC) of spontaneous reversal of morbidity and a big proportion (40% in PSAC and 60% in SAC) of progression of urinary tract morbidity.

These data highlight the importance of regular treatment. In fact, early complications of the infection are quantifiable with ultranosography, and, if not treated, this infection can lead to severe organ failure [76,78,84,85]. Moreover, we reported an improvement in urine analysis in treated children, which mirrors a general improvement of urinary tract morbidity consequent to praziquantel.

Based on published literature and our results, we can conclude that the extension of PC to PSAC is crucial to prevent early urinary tract morbidity in *S. haematobium* infected children, especially in areas where endemicity and reinfection rates are high.

Chapter 9

Conclusions

In the frame of this PhD thesis, we analysed few innovative issues within the control of intestinal helminthic infections. More specifically, we conducted four clinical trials in which we had the opportunity to explore several aspects of intestinal helminthiases, such as diagnosis, morbidity linked to infection and different treatment options.

We have found moxidectin to be a good alternative option for the treatment of *S. stercoralis* in endemic setting and confirmed its good safety profile. Regarding schistosomiasis, the previously described antischistosomal effect of moxidectin, was not confirmed in our study. On the other hand, we proved the efficacy of the co-administration of moxidectin and albendazole against *T. trichiura* and its non-inferiority against the best available option (albendazole/oxantel pamoate). Moxidectin with albendazole has also proved to be effective against hookworm and *A. lumbricoides*; these results indicate that this combination may find application in areas endemic for STH and *S. stercoralis.* Therefore, moxidectin in co-administration with albendazole is worth of further evaluations with dose-finding and pharmacokinetic studies to be conducted, which will hopefully clarify the mechanism of action of the two drugs.

On the diagnostic side, we have highlighted difficulties in *S. stercoralis* detection; copro-microscopic techniques, although time-consuming and cumbersome have revealed a better performance than novel molecular tools. Therefore, further research is needed to improve DNA extraction to optimize PCR application for the diagnosis of this parasite.

Finally, we confirmed the early onset of urinary tract morbidity linked to *S. haematobium* infection, which already affects young children. The ultra-sonographic evaluation before and after treatment with praziquantel allowed us to highlight the importance of regular treatment for the regression of urinary tract morbidity, and consequently also highlighting the relevance of ultrasound as monitoring tool as well as the crucial impact of PC on control of morbidity in *S. haematobium* infected children.

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Beatrice Barda

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WORK EXPERIENCE

Agosto 2018 > up to date

Internal medicine

MD in internal medicine

At **Clinica Luganese Moncucco, Lugano**, Switzerland - Department of Internal Medicine

2014 > June 2018

Clinical trials in drug development

Clinical trials in drug development for intestinal parasitic infections

At **Swiss Tropical and Public Health Institute, Basel**, Switzerland - Department of infection biology and drug development

2014 > December 2017

PhD in Epidemiology

Clinical trials in drug development for intestinal parasitic infections and comorbidities due to *Schistosoma haematobium* infection. Experiences as principal investigator in several clinical trials on neglected tropical diseases. The role covered the main aspects of clinical trials, from protocol development to field team organization, baseline screening, diagnosis, treatment, follow-up and data management and analyses, to the final scientific paper and report.

Field work in developing countries:

- Principal investigator of the clinical trial phase 2 on the assessment of efficacy and safety of moxidectin and Synriam® vs praziquantel against *Schistosoma mansoni* and *S. haematobium* infections in adolescents, Côte d'Ivoire Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS)
- Principal investigator of the clinical trial phase 2 on the assessment of efficacy and safety of moxidectin vs ivermectin against *Strongyloides stercoralis* infection, a community-based trial in Lao People's Democratic Republic
- Principal investigator of the clinical trial phase 2 on the assessment of efficacy and safety of moxidectin alone and in combination with albendazole and with tribendimidine vs albendazole/oxantel pamoate against *Trichuris trichiura* infection in adolescents, Pemba Island, Tanzania, Public Health Laboratoy, WHO-collaborating centre
- Ultrasound evaluation of *Schistosoma haematobium* related bladder morbidity in infected pre-school and school-aged children before and after treatment with different doses of praziquantel vs placebo, Côte d'Ivoire d'Ivoire Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS)
- Evaluation and comparison of different diagnostic methods (Baermann method, Kato-Katz, CCA, PCR) for the diagnosis of *Strongyloides stercorali, Schistosoma spp* and Soil-transmitted helminths

At **Swiss Tropical and Public Health Institute, Basel**, Switzerland - Department of infection biology and drug development

2011 > 2017

Teaching parasitology at Master in Tropical Diseases

University of Florence, Italy

2011 > 2016

Teaching parasitology at residency in clinical Microbiology and Virology

University of Vita e Salute San Raffaele, Milan
University, Turino

2009 > 2014

Resident in Clinical Microbiology and Virology

University of Vita e Salute San Raffaele, Milan
Project on
Evaluation of Mini-FLOTAC: impact of 5% formalin preservation on egg count of soil-transmitted helminths overtime. Public Health Laboratory IdC Pemba Island, Tanzania October - November 2013
Impact of multiple Mass Drug Administration with DEC and albendazole on intestinal parasites infections in Leogane. Hospital Saint Croix. Haiti February - June 2013
Evaluation and analysis of transferability of innovative diagnostic techniques (Mini-FLOTAC) for intestinal parasites infections: multicentric study. Delek Hospital, Himachal Pradesh, India; Bukumbi Hospital, Mwanza region, Tanzania; Laboratory of tropical diseases, university of Salta, Argentina. January-November 2012

2009 > 2011

Infectious diseases ward as clinician

San Raffaele hospital, Milan

Microbiology laboratory: studies on HIV mutations and drug resistance

San Raffaele hospital, Milan

2008 > 2009

MD consultant and on-call activity at care home for the elderly

RSA San Francesco, Milano
RSA Golgi-Redaelli, Milano

2006 > 2009

Internship in Infectious Diseases ward

Ospedale San Raffaele, Milano

2002 > 2006

Internship in Internal Medicine

Ospedale San Raffaele, Milano

2002 > 2003

Internship in dermatology and day hospital surgery

Ospedale San Raffaele, Milano

Education

Residency in Clinical Microbiology and Virology, 2014

University Vita e Salute San Raffaele, Milan. Grade: 70 cum laude/70

Thesis on: Impact of multiple Mass Drug Administration with DEC and albendazole on intestinal parasites infections in Leogane, Haiti

Degree in Medicine and Surgery, 2008

University Vita e Salute San Raffaele, Milan. Grade: 110 cum laude/110

Thesis on: impact of 20 years of HIV infection and treatment on glucose metabolism

Italian registration: N 41309. Swiss registration N 7601007560578

Classical high school diploma, 2001

Liceo classico G. Parini, Milan. Grade 100/100

Personal skills

Languages

Italian: mother tongue

English: Advanced. Cambridge **CPE**: Certificate of Proficiency in English (1999). Cambridge **CAE**: Certificate in Advanced English (1999). Cambridge **FCE**: First Certificate in English (1997).

French: Very good understanding and good speaking and writing

German and ***Spanish***: basic

Communication skills

Very good communication skills acquired during my wide experience abroad

Publications

Efficacy and tolerability of moxidectin alone and in co-administration with albendazole and tribendimidine versus albendazole plus oxantel pamoate against *Trichuris trichiura* infections: a randomised, noninferiority, single-blind trial. THELANCETID-D-18-00136R2 S1473-3099(18)30233-0

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Ultrasonographic evaluation of urinary tract morbidity in school-aged and preschool-aged children infected with *Schistosoma haematobium* and its evolution after praziquantel treatment: A randomized controlled trial PLoS Negl Trop Dis 11(2): e0005400. doi:10.1371/journal.pntd.0005400

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Efficacy and safety of moxidectin, Synriam, Synriam + Praziquantel versus praziquantel against *Schistosoma haematobium* and *S. mansoni* infections: a randomizd exploratory phase 2 trial Barda B, Coulibaly JT, Puchkov M, Huwyler J, Hattendorf J, Keiser J.

Plos Negl Trop Dis 10(9): e 0005008; 2016

Human trichuriasis: diagnostic update Current tropical medicine report vol2 issue4 pp 201-208; 2015 Barda B, Keiser J, Albonico M

How long can stool samples be fixed for an accurate diagnosis of soil-transmitted helminth infection using Mini-FLOTAC? Plos Neg Trop dis 9(A) e0003698; 2015

Barda B, Albonico M, Ianniello D, Shaali MA, Keiser J, Speich B, Rinaldi L, Cringoli G, Burioni R, Montresor A, Utzinger J

Mini-FLOTAC, Kato-Katz and McMaster: three methods. One goal; highlights from north Argentina. Parasites and Vectors; 7:271; 2014

Barda B, Vilagran E, Cimino R, Juarez M, Cajal P, Krolewiecki A, Rinaldi L, Burioni R, Albonico M **Parasitic infections on the shore of Lake Victoria (east Africa) detected by mini-FLOTAC and standard techniques**. Acta Tropica; 137:149-6; 2014

Barda B, Ianniello D, Zepheryne H, Rinaldi L, Cringoli G, Burioni R, Albonico M

"Freezing" parasites in pre-Himalayan region, Himachal Pradesh: experience with mini-FLOTAC. Acta Tropica; 130: 11-6; 2014

Barda B, Ianniello D, Salvo F, Sadutshang T, Rinaldi L, Cringoli G, Burioni R, Albonico M

Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. Plos Negl Trop Dis; 7(8): e2344; 2013

Barda B, Rinaldi L, Ianniello D, Zepherine H, Salvo F, Sadutshang T, Cringoli G, Clementi M, Albonico M

Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victoria. Parasites and Vectors; 6(1)_220; 2013

Barda B, Zepherine H, Rinaldi L, Cringoli G, Burioni R, Clementi M, Albonico M

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Residual viraemia does not influence 1 year virological rebound in HIV-infective patients with HIV RNA persistently below 50 copies/ml. J Antimicrob Chemother; 67(1):213-7; 2012
Gianotti N, Galli L, Racca S, Salpietro S, Cossarini F, Spagnuolo V, Barda B, Canducci F, Clementi M, Lazzarin A, Castagna A

Paraparesis caused by vertebral canal leishmaniotic granuloma in a dog. J Vet Intern Med; 25(2): 398-9; 2011
Cauduro A, Favole P, Lorenzo V, Simonetto L, Barda B, Cantile C Asperio R

Evolution patterns of raltegravir-resistant mutations after integrase inhibitor interruption. Clin Microbiol Infect. 2011 Jun;17(6): 928-34; 2011
Canducci F, Barda B, Ceresola E, Spagnuolo V, Sampaolo M, Boeri E, Nozza S, Cossarin F, Galli A, Gianotti N, Castagna A, Lazzarin A, Clementi M

Detecting impaired glucose tolerance or type 2 diabetes mellitus by means of an oral glucose tolerance test in HIV-infected patients. HIV Med. 2011 Feb;12(2): 109-17
Gianotti N, Visco F, Galli L, Barda B, Piatti P, Salpietro S, Bigoloni A, Vinci C, Nozza S, Gallotta G, Lazzarin A, Castagna A

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