Improving the Control of Soil-Transmitted Helminthiasis in School Children in Jimma Town (Ethiopia): Determining Efficacy of Anthelminthic Drugs and Developing Diagnostic Strategies

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<tbody>
<tr>
<td>µg</td>
<td>Microgram</td>
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<tr>
<td>µm</td>
<td>Micrometre</td>
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<tr>
<td>ALB</td>
<td>Albendazole</td>
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<tr>
<td>AchE</td>
<td>Acetyl-cholinesterase</td>
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<tr>
<td>AP</td>
<td>Apparent prevalence</td>
</tr>
<tr>
<td>AR</td>
<td>Anthelminthic resistance</td>
</tr>
<tr>
<td>BZ</td>
<td>Benzimidazoles</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>cm</td>
<td>Centimetre</td>
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<tr>
<td>Coef</td>
<td>Correlation coefficient</td>
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<tr>
<td>CR</td>
<td>Cure rate</td>
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<tr>
<td>DALYs</td>
<td>Disability-adjusted life years</td>
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<tr>
<td>DGDC</td>
<td>Directorate-General for Development Cooperation</td>
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<tr>
<td>EHA</td>
<td>Egg hatch assay</td>
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<tr>
<td>EPG</td>
<td>Eggs per gram of stool</td>
</tr>
<tr>
<td>FEC</td>
<td>Faecal egg counts</td>
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<td>Reduction in faecal egg counts for pools of 60 individual stool samples</td>
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<tr>
<td>FMoH</td>
<td>Federal Ministry of Health of Ethiopia</td>
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<tr>
<td>g</td>
<td>Gram</td>
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<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<tr>
<td>hr</td>
<td>Hr</td>
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<tr>
<td>HDA</td>
<td>Health Development Army</td>
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<td>HSEP</td>
<td>Health Service Extension Programme</td>
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<td>IUC</td>
<td>Institutional University Cooperation</td>
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<td>IVM</td>
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<td>LEV</td>
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<td>MEB</td>
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<td>mg</td>
<td>Milligram</td>
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<td>MDA</td>
<td>Mass drug administration</td>
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<tr>
<td>min</td>
<td>Minute</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
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<tr>
<td>No</td>
<td>Number</td>
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<td>Neglected tropical diseases</td>
</tr>
<tr>
<td>OX</td>
<td>Oxantel</td>
</tr>
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<td>PC</td>
<td>Preventive chemotherapy</td>
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<td>preSAC</td>
<td>Preschool-aged children</td>
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<td>Pyrantel pamoate</td>
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<td>Soil-transmitted helminths</td>
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<td>WASH</td>
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Soil-Transmitted Helminths
I. Introduction

Soil-transmitted helminths (STH) refer to a cluster of four helminths, including *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Ancylostoma duodenale* and *Necator americanus* (hookworms). These four helminths are clustered into one group because they share a number of features: they all (i) are nematodes, (ii) their adult worms dwell exclusively in the gastrointestinal tract of humans (iii), they have a direct lifecycle (no intermediate host is required to complete the lifecycle), and (iv) they produce eggs which are excreted through stool, but which are not immediately infectious. The infectious stages will develop outside the host in the soil (referring to their common name). In addition, they all (v) cause mainly mild chronic, rather than sever acute disorders in the human host, and (vi) can often be treated with the same anthelminthic drug (WHO, 2002a; Utzinger and Keiser, 2004; Bethony *et al.*, 2006; WHO, 2012).

However, despite these common features important differences can be noted in their morphology, fecundity of the adult female worms, route of transmission, survival of infectious stages in the environment, and their location in the gastrointestinal tract. The most important differences are summarized in Table I.
### Table I. Overview of the most important differences between the four soil-transmitted helminth species (*Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale* and *Necator americanus*).

<table>
<thead>
<tr>
<th></th>
<th><em>A. lumbricoides</em></th>
<th><em>T. trichiura</em></th>
<th><em>A. duodenale</em></th>
<th><em>N. americanus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Illustration of lifecycle</strong></td>
<td><img src="https://dpd.cdc.gov/dpdx/HTML/ImageLibrary/Ascariasis_il.htm" alt="Image" /></td>
<td><img src="https://dpd.cdc.gov/dpdx/HTML/ImageLibrary/Trichuriasis_il.htm" alt="Image" /></td>
<td><img src="https://dpd.cdc.gov/dpdx/HTML/ImageLibrary/Hookworm_il.htm" alt="Image" /></td>
<td></td>
</tr>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td>15-35 cm</td>
<td>3-5 cm</td>
<td>+/- 1 cm</td>
<td></td>
</tr>
<tr>
<td>Eggs: Size</td>
<td>40-65 µm</td>
<td>50-54 µm</td>
<td>40-65 µm</td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Oval to round</td>
<td>Barrel shaped</td>
<td>Oval shaped</td>
<td></td>
</tr>
<tr>
<td>Shell thickness</td>
<td>Thick shell</td>
<td>Fairly thick shell</td>
<td>Thin shell</td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>Brown colour</td>
<td>Yellow brown</td>
<td>Pale gray/or Colourless</td>
<td></td>
</tr>
<tr>
<td><strong>Fecundity (eggs/day)</strong></td>
<td>+/- 200,000</td>
<td>2,000-10,000</td>
<td>25,000-30,000</td>
<td>9,000-10,000</td>
</tr>
<tr>
<td><strong>Infectious stage</strong></td>
<td>Egg containing L3 larva</td>
<td>Egg containing L1 larva</td>
<td>L3 larva</td>
<td></td>
</tr>
<tr>
<td><strong>Survival of infectious stage</strong></td>
<td>&gt;10 years in appropriate soil</td>
<td>&gt;10 years in appropriate soil</td>
<td>3-4 weeks in appropriate environment</td>
<td></td>
</tr>
<tr>
<td><strong>Route of transmission</strong></td>
<td>Oral</td>
<td>Oral</td>
<td>Percutaneous</td>
<td>Percutaneous</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Oral</td>
<td></td>
</tr>
<tr>
<td><strong>Extra-intestinal migration</strong></td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td><strong>Location in gastrointestinal tract</strong></td>
<td>Small intestine</td>
<td>Large intestine</td>
<td>Small intestine</td>
<td></td>
</tr>
<tr>
<td><strong>Adult life span</strong></td>
<td>1-2 years</td>
<td>About 1 year</td>
<td>1-3 years</td>
<td></td>
</tr>
</tbody>
</table>

Each of the STH has specific morphological features on which they can be identified, for both adult worms and eggs. Overall, adult worms can be differentiated based on the size. *A. lumbricoides* is the largest of all (up to 35 cm), followed by *T. trichiura* (up to 5 cm). The hookworms are the smallest (+/- 1 cm). In addition to this, adult *T. trichiura* worms are characterized by a thin anterior part and wide posterior part, resembling a whip (referring to its common name). The eggs can be differentiated microscopically based on their size (*A. lumbricoides* and hookworm: 40-65 µm; *T. trichiura*: 50-54 µm), shape (*A. lumbricoides*: oval to round; *T. trichiura*: barrel shaped; hookworm: oval), thickness of the shell (*A. lumbricoides*: thick; *T. trichiura*: fairly thick; hookworm: thin), colour (*A. lumbricoides*: brown; *T. trichiura*: yellow brown; hookworm: pale grey to colourless). Female *A. lumbricoides* worms are the most fecund, excreting approximately 200,000 eggs per day. Fecundity of the remaining STH species varies between 2,000 and 10,000 for *T. trichiura* and from 9,000 to 30,000 eggs per day for hookworms (*Necator americanus* excrete less eggs [9,000 to 10,000] compared to *Ancylostoma duodenale* [25,000 to 30,000]). Both *A. lumbricoides* and *T. trichiura* are transmitted by the oral uptake of eggs containing an L3 (*A. lumbricoides*) L1 (*T. trichiura*) larva, whereas hookworm infections are transmitted by the L3 larva penetrating the skin. The eggs of both *A. lumbricoides* and *T. trichiura* will take 2-3 months to mature in the external environment before becoming infectious. These eggs can survive many years in an appropriate environment and remain infectious throughout (Muller and Wakelin, 2002). This is in contrast to hookworms, for which the infectious stage (L3) can only survive 3-4 weeks under tropical conditions; however, it may remain infective for several months if environmental conditions are optimal (Albonico and Savioli, 1997). The life cycle of both *A. lumbricoides* and hookworm is characterized by an extra-intestinal migration of the immature stadia. Adult worms, however, are found in the small intestine for both STH species. For *T. trichiura*, there is no extra intestinal migration and all stages are found in the large intestine.

**II. Epidemiology and burden of soil-transmitted helminthiasis**

In 2010, it was estimated that 1,450 million people were infected with any STH species, of which *A. lumbricoides* infect 772–892 million, *T. trichiura* 430-508 million, and hookworms 406-480 million people (Pullan et al., 2014).
Although STH infections are distributed worldwide, the highest prevalence's (Figure I) is reported in tropical and subtropical regions from Asia, Sub-Saharan Africa (SSA), the Americas, and East China (WHO, 2002; Hotez and Kamath, 2009; WHO, 2012; Pullan et al., 2014). Their occurrence, transmission and diseases burden largely relate to factors like poor sanitation and hygiene, overcrowding, and limited access to clean water (Stephenson et al., 2000; WHO, 2002b; de Silva et al., 2003; Ukpai et al., 2003; Bethony et al., 2006).

Figure I. Global distribution of any soil-transmitted helminths in 2010. (A) The combined prevalence of any infection, based on geostatistical models for sub-Saharan Africa and available empirical information for all other regions by country. (B) The proportion of the global population covered by the endemic countries (Pullan et al., 2014).
It has been projected that annual deaths from STH infection ranges from 12,000 to 135,000 (WHO, 2002a, 2004; Awasthi and Bundy, 2007). The causes of mortality are mainly due to anaemia associated to hookworm and *T. trichiura* infection, or are linked to intestinal and/or biliary obstruction by *A. lumbricoides* (de Silva *et al.*, 1997; Stephenson *et al.*, 2000; Wani *et al.*, 2010). Given that their consequence is rather disability than death, the worldwide burden of STH infection is typically assessed by disability-adjusted life years (DALYs) lost, that is the number of healthy years lost to premature death or disability. For instance, recent data (2010) on the composition of global DALYs/100,000 shows: 47 (25-83) for hookworms, 19 (10-34) for *A. lumbricoides* and 9 (5-15) for *T. trichiura*, which may appear low in comparison with 1200 (921-1,594) for malaria, 1,184 (1,089-1,283) for HIV/AIDS, 717 (581-814) for tuberculosis, but it is still significant (Brooker, 2010; Murray *et al*., 2012; Pullan *et al*., 2014).

Overall, the severity of the disease caused by STH has consistently been found to depend on the number of worms present per person (Anderson and May 1991; Crompton and Nesheim, 2002; WHO, 2002b). Preschool-aged (preSAC) and school-aged children (SAC), and pregnant women are the three groups of the population at highest risk of the morbidity caused by these infections (Hall *et al*., 2008; Brooker, 2010). SAC harbour the most intense infections and thus they suffer most from the morbidity like malnutrition, growth stunting, intellectual retardation, and cognitive and educational deficits (WHO, 2005).

**III. Soil-transmitted helminth infections in Ethiopia**

It is estimated that *A. lumbricoides, T. trichiura* and hookworms infect 26 million, 21 million and 11 million of the 86 million Ethiopians, respectively. Of all SSA countries, Ethiopia is one of the countries that suffer the most of the burden caused by STH, representing 15% (the 2nd highest), 13% (the 4th highest) and 6.5% (the 3rd highest) of all *A. lumbricoides, T. trichiura* and hookworm infections respectively, in SSA (Hotez and Kamath, 2009; Deribe *et al*., 2012).

Although the national average prevalence is estimated at 37% for *A. lumbricoides*, 30% for *T. trichiura* and at 16% for hookworms, different studies revealed a prevalence range of 0-100% in different parts of the country for all the three STH (Wondimagegnehu *et al*., 1992; Jemaneh 2000; Erosie *et al*., 2002; Erko and Medhin, 2003; Tadesse *et al*., 2008; Fekadu *et al*., 2008; Yami
et al., 2011; Alemu et al., 2011; Pullan and Brooker, 2012; Deribe et al., 2012).

Generally, most regions of Ethiopia are suitable for the transmission of STH, except parts of the Somali and Afar areas where the annual mean temperature is too high for transmission (Figure II). Prevalence is lower in the low and dry areas of the country than in the more humid highlands, where a prevalence ranging 20 – 100% was recorded (Pullan and Brooker, 2012). In addition, there are important differences in methodology such as diagnostic techniques, sample sizes that may contribute to the variation in reported prevalence.

![Figure II. Distribution of soil-transmitted helminths survey data and average district-level prevalence in Ethiopia. Source: Wormy world Project (http://www.thiswormyworld.org/maps/ethiopia)](image)

A report by the Federal Ministry of Health of Ethiopia (FMoH) indicated that STH are a leading cause of morbidity among patients visiting the outpatient
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department of the health institutions (FMoH, 2004). As a result, STH infections cause serious public health problems such as malnutrition, anaemia and growth retardation as well as higher susceptibility to other infections (Tedla, 1986).

Most risk factors associated with STH infections and transmissions (e.g., low socio-economic status, poor personal hygiene and environmental sanitation, lack of access to safe water, faecal contamination of human environment, or inability to use foot wear) also apply for STH infections in Ethiopia (Birrie and Erko, 1995; Assefa et al., 1998; Jemaneh, 1998; Ali et al., 2001; Legesse and Gebere-Silasie, 2007).

Despite the estimated high prevalence of STH and posing enormous disease burdens, in Ethiopia the control measures against STH are at their infancy stage. At the time of this thesis project, disease burden estimations (prevalence and intensity), epidemiological survey and mapping are based on limited studies (not covering all regions). Moreover, these studies are often not up-to-date and scattered. Furthermore, preventive chemotherapy (PC) was not yet fully implemented to SAC. Ethiopia launched a programme entitled ‘Enhanced Outreach Strategy’ in 2004, and since then preSAC are dewormed every 6 months. Accordingly, more than 11 million children by 2009 with a national coverage of 78% (Negash, 2011; WHO, 2012) have received PC. In addition, deworming of SAC started in 2007 and 2008. However, these programmes were restricted to some parts of the country, resulting in a national coverage of only 2% in 2009 (WHO, 2012) (see figure II above for distribution of STH and the PC coverage of (pre-)SAC in Ethiopia). At present, however, there is an immense commitment from Ethiopia’s government to combat NTD including STH. The Federal Ministry of Health (FMoH) has recently inaugurated the National Master Plan to control NTD in June 2013. According to this master plan, mapping of NTD was completed in April-May 2014 and the preliminary data of mapping was released in June 2014. Available and/or donated drugs will be distributed to different regions for PC that need to be followed by regular monitoring and evaluation programmes (National Symposium on NTD in Ethiopia, 12-14 June 2013, Addis Ababa, Ethiopia).
General Introduction: Soil-Transmitted Helminths

References


General Introduction

Chapter 1

Control of Soil-Transmitted Helminthiasis: A Literature Review
1.1 Introduction

Soil-transmitted helminthiasis (STH) refers to a group of parasitic diseases in humans caused by infestations with various species of intestinal worms – collectively called soil-transmitted helminths (STHs). STH infections are now well recognized as a major health issue worldwide. The World Health Organization (WHO) considered control of STH one of the top five health priorities within the global 'Massive Effort Against Poverty' (WHO, 2000). This is because of their global distribution, high prevalence, and effects on the nutritional status and physical and mental development of children.

Strategies have been developed to target morbidity control by means of regular mass anthelmintic drug distribution, and these preventive chemotherapy (PC) programmes remain the mainstay. PC is the cheapest and fastest means compared to other control methods, such as improving environmental sanitation, provision of safe water, and raising standards of living.

Annual or biannual single-dose treatment with benzimidazole (BZ) drugs based on the prevalence of any STH infections leads to significant reduction in the morbidity caused by these parasitic infections (Molyneux, 2004; Molyneux et al., 2005; Fenwick et al., 2005; WHO, 2011). This has had major positive impacts on children’s health, including progress in growth after treatment and replenishment of important nutrients such as iron stores (Hotez et al., 2004, 2009), improvements in childhood intellectual and cognitive development, and decreases in school absenteeism (Bundy et al., 1990; Drake et al., 2000; Savioli et al., 2002; Hotez et al., 2004; Hotez et al., 2005).

Observations of these impacts have led to the implementation of large-scale STH control programmes that target school-aged children (SAC) (Urbani and Palmer, 2001; WHO, 2002, 2006; Hotez et al., 2009; WHO, 2011) through increased pledges of drug donation for PC purposes.

However, as of 2012, only 36.0% of SAC and 24.7% of preschool-age children (preSAC) were being treated for STH (WHO, 2014; Table 1.1). This low coverage was too far below the WHO’s expected coverage of 75% by 2020. As a result, new objectives were set, stating that all the countries where STH was considered a public health problem should start national STH control programmes by 2015, and reach 75% national coverage and 100% geographical coverage by 2020 (WHO, 2012: Figure 1.1)
The objectives of this review are, therefore, to review current (i) control strategies for STH infections, (ii) WHO-recommended anthelminthic drugs for STH control, (iii) ways of monitoring drug efficacies, (iv) factors affecting the monitoring of drug efficacy, and (v) efficacy trials performed on schoolchildren in Africa.

1.2 Current control strategies for soil-transmitted helminth infections

Currently, we have two broad STH control strategies, including (i) periodic administration of anthelminthic drugs, and (ii) improvement of sanitation, personal hygiene and health education.

1.2.1 Administration of anthelminthic drugs

Depending on the purpose (curative vs. preventive) and target (individual vs. group), we can broadly classify drug administration programmes into two categories: (1) selective (individual-based) treatment, and (2) mass treatment (PC). Further, PC can be administered in one of two main ways: (i) mass treatment of targeted groups (i.e. high-risk groups, such as SAC, preSAC, and pregnant women), or (ii) universal (population-based) treatment (WHO, 1996c; Gabrielli et al., 2011).

1.2.1.1 Selective treatment

Selective treatment is based on individual applications of treatment with specific drugs and drug regimens. Selective treatment demands pre-confirmation of infections through various diagnostic methods to guide therapeutic choice, and aims at the relief of symptoms and complaints or a clinical radical cure. The selection is based on the intensity of current or past infection. The choice of drug will be based on specific efficacy irrespective of the mode and length of administration. WHO recommends individual treatment when the prevalence of any STH in a given population is lower than 20%. However, in practice, selective treatment is difficult to achieve. This is mainly because diagnosis is more expensive than treatment, and because of the shortage of health institutions and health professionals proportional to the population in the majority of developing countries where STH are endemic (WHO, 2002).
1.2.1.2 Mass drug administration to targeted groups

Group-based application (PC targeting a specific segment of the community) is one of the most common and widely applied strategies underway in many countries. A specific group can be defined based on age, sex, being at high risk to infection, or social characteristics. Usually, treatment of a particular group through PC aims at interrupting transmission or lowering prevalence to a level below the threshold (prevalence <1%) at which the targeted infections are no longer considered a public health problem and PC becomes unnecessary (WHO, 2012).

Among a range of strategies and target groups for control of STH using PC, the school-based approach has received due attention for the following reasons. First, STH are highly prevalent in children, accounting for the majority of STH infections in the entire population (Bundy et al., 1992). Second, (pre-)SAC tend to comprise more than 30% of the population and school enrolment has increased in recent years (UNESCO, 2011). Third, it is relatively easy to reach SAC, because the school system is usually better established than the health infrastructure in most developing countries, where distant villages without health facilities often have primary schools. Fourth, because of the adverse effects that STH imposes on the physical and mental development of children, it can be taken as a major retarding influence on the economic progress of the developing world.

Thus, the current means of controlling STH infections primarily depends on PC programmes in which single-dose albendazole (ALB) or single-dose mebendazole (MEB) is administered to SAC as the main target group, basically without any prior individual diagnosis. Yet, it is important to note that the frequency of PC depends on the prevalence of any STH in a given population/region.

According to the 2001 World Health Assembly Resolution to eliminate STH as a public health problem in children (WHA54.19), at least 75%, and up to 100%, of all (pre-)SAC at risk of morbidity from STH were targeted to receive PC by 2010 (WHO, 2002). However, recent reports across the world have revealed that coverage has been lagging far below this target. Up to 2012, only about one third (32.6%) of children at risk of STH and requiring PC received an
anthelminthic treatment (Table 1.1), despite all the intensified effort to eliminate STH as a public health problem (WHO, 2014).

Table 1.1 Coverage of preventive chemotherapy among preschool-aged children and school-aged children against soil-transmitted helminths by World Health Organization region, 2012 (WHO, 2014).

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Estimated number in need of PC</th>
<th>Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>300,608,939</td>
<td>25.3</td>
</tr>
<tr>
<td>preSAC</td>
<td>98,193,220</td>
<td>15.5</td>
</tr>
<tr>
<td>SAC</td>
<td>202,415,719</td>
<td>30.1</td>
</tr>
<tr>
<td>Americas</td>
<td>49,312,001</td>
<td>33.1</td>
</tr>
<tr>
<td>preSAC</td>
<td>13,929,732</td>
<td>31.9</td>
</tr>
<tr>
<td>SAC</td>
<td>35,382,269</td>
<td>33.6</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>368,922,141</td>
<td>51.1</td>
</tr>
<tr>
<td>preSAC</td>
<td>105,878,494</td>
<td>35.4</td>
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<tr>
<td>SAC</td>
<td>263,043,647</td>
<td>51.1</td>
</tr>
<tr>
<td>Europe</td>
<td>1,775,391</td>
<td>42.9</td>
</tr>
<tr>
<td>preSAC</td>
<td>602,826</td>
<td>5.2</td>
</tr>
<tr>
<td>SAC</td>
<td>1,172,565</td>
<td>62.3</td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>80,544,663</td>
<td>6.9</td>
</tr>
<tr>
<td>preSAC</td>
<td>25,481,678</td>
<td>17.9</td>
</tr>
<tr>
<td>SAC</td>
<td>55,062,985</td>
<td>1.8</td>
</tr>
<tr>
<td>Western Pacific</td>
<td>74,721,699</td>
<td>19.5</td>
</tr>
<tr>
<td>preSAC</td>
<td>22,330,519</td>
<td>18.2</td>
</tr>
<tr>
<td>SAC</td>
<td>52,391,180</td>
<td>20.1</td>
</tr>
<tr>
<td>Global</td>
<td>875,884,833</td>
<td>32.5</td>
</tr>
<tr>
<td>preSAC</td>
<td>266,416,469</td>
<td>24.7</td>
</tr>
<tr>
<td>SAC</td>
<td>609,468,364</td>
<td>36.0</td>
</tr>
</tbody>
</table>
During the past few years, renewed recognition of the burden imposed by these infections has led to the strengthening of PC programmes for the control and possible elimination of the major human helminths (Molyneux et al., 2005; WHO, 2006; WHO, 2011; Gabrielli et al., 2011; NTD Partner Website, 2012). Moreover, WHO recommendations continue to encourage governments and policy makers to invest in helminth control by strengthening political commitment and coordination, building technical capacity, facilitating sustainability and improving monitoring capacities as assets for national development (WHO, 2005).

Figure 1.1 illustrates the recent and revised WHO strategic plans for eliminating morbidity from STH in children by 2020. The strategic approaches respond to each of the challenges identified in the past, and milestones have been set based on the situation in each WHO region.

**Figure 1.1 Flow chart showing the World Health Organization's (2011-2012) strategic plans to eliminate and control soil-transmitted helminths by 2020 (WHO, 2012).**

In addition, the availability of safe and relatively inexpensive drugs for STH, coupled with significant donations of anthelminthic medicines by GlaxoSmithKline (ALB) and Johnson & Johnson (MEB), and increased interest in
neglected tropical diseases (NTD) by many partners (scientific communities and non-governmental bodies) offer a unique opportunity to control STH in the next 10 years. Altogether, this has made control through PC a potentially affordable option even in resource-poor countries (Handzel et al., 2003; WHO, 2011). Figure 1.2 shows countries where STH is a public health problem and the proportion of children aged 1 to 14 years requiring PC in each country. Furthermore, rather than aiming to achieve eradication, current control programmes are focused on reducing infection intensity and transmission potential, primarily to reduce morbidity and avoid mortality associated with the disease (Albonico et al., 2008).

![Figure 1.2 Proportion of children aged 1-14 years requiring preventive chemotherapy for soil-transmitted helminthiasis, by country, 2009 (WHO, 2011).](image-url)
The current WHO strategy for PC and recommended frequency of PC re-treatment for SAC depends on the prevalence data (Table 1.2).

**Table 1.2 Recommended frequency of re-treatment with preventive chemotherapy for soil-transmitted helminthiasis in school-age children, by category of risk**

<table>
<thead>
<tr>
<th>Category of risk</th>
<th>Prevalence of any STH among SAC</th>
<th>Re-treatment schedule</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk areas</td>
<td>&gt;50%</td>
<td>Twice a year</td>
</tr>
<tr>
<td>Moderate-risk areas</td>
<td>&gt;20% and &lt;50%</td>
<td>Once a year</td>
</tr>
<tr>
<td>Low-risk areas</td>
<td>&lt;20%</td>
<td>None (case-by-case treatment)</td>
</tr>
</tbody>
</table>

Adapted from *Preventive chemotherapy in human helminthiasis (WHO, 2002) Technical report Series N° 912*.

Yet, it might also be important to look into the risk of re-emergence in the event that the parasites maintain transmission capacity despite intense drug pressure—which would be predictive of a rapid return to high prevalence if intervention is interrupted. Thus, WHO has recently suggested (WHO, 2012) a change in frequency of drug administration after five to six years of de-worming (Figure 1.3). In addition, it might be worthwhile to assess the effect of seasonal variation on STH infections to determine the appropriate season to administer available drugs for maximum possible benefit.
Figure 1.3 World Health Organization decision tree to determine frequency of drug administration after five to six years of de-worming (WHO, 2012).

Generally, aiming at SAC has proved to be an extremely acceptable strategy. However, it would be worthwhile to consider focusing on regular de-worming for not only SAC, but also, other risk groups, such as preSAC, pregnant women and occupationally-defined risk groups such as tea pickers or miners (Savioli et al., 2002; Albonico et al., 2008). Hookworm infections in particular are difficult to control when only SAC are targeted, as the prevalence of infection with this parasite increases with age, and adults (or adolescents) contribute the greatest share in propagating transmission (Anderson et al., 2012; Truscott et al., 2014).

1.2.1.3 Mass drug administration to the population

In mass drug administration (MDA) to the population, also known as universal treatment, the whole population of a community is treated irrespective of age, sex, intensity of infection, or social characteristics. MDA depends on the estimated prevalence of particular geographic locations without prior individual diagnosis. With respect to other control efforts, universal treatment (targeting
the whole population) is correspondingly effective and acceptable, although it is more expensive (requires more drugs, human power and other logistics). Furthermore, the delivery channels to reach the whole community may not be easy (WHO, 1996; Albonico et al., 1999), and thus its applicability is questionable. Moreover, it may increase the risk of anthelminthic drug resistance development.

1.2.2 Water, sanitation, and hygiene and health education

Although it has been proven that PC is a key element in reducing the morbidity and spread of STH quickly and cost effectively, re-infection with these diseases will remain a problem if behaviours and the environment remain unchanged (Singer and Castro, 2007; Utzinger et al., 2009; Ziegelbauer et al., 2012). The cycle of STH treatment and re-infection will likely persist until sanitation conditions and hygiene practices are improved. It is widely acknowledged that complementary interventions are indispensable to decrease frequency of re-infection (Bartram and Cairncross, 2010; Spiegel et al., 2010); that is, interventions to promote better living standards and socio-economic development that include improving sanitation and providing health education and information to the community to sustain long-term control of STH.

In particular, exploring ways to integrate water, sanitation, and hygiene (WASH)-through the provision of safe water, latrines, and improved environmental sanitation, as well as the promotion of hygiene and health education-is a vital component of control programmes. This is because access to improved sanitation is a key factor in integrated control programmes aimed at controlling transmission through the reduction of soil and water contamination (Utzinger et al., 2003; Mara et al., 2010). Meanwhile, health education and information campaigns are essential to reduce transmission through the practice of healthy behaviours, and through the augmentation of knowledge and skills regarding infection and re-infection in the public health system at central and peripheral levels (Albonico et al., 1999; WHO, 2002; Ziegelbauer et al., 2012). Additionally, each country should work in parallel to increase the accessibility of health care facilities to their populations as a further control measure.

However, since access to clean water, improved sanitation, and education for the wider population entail high costs, implementing this strategy is difficult.
where resources are limited, and it may take several years before it becomes effective as a primary means of control.

1.2.3. Other approaches

Another complimentary approach to controlling helminth infections could be the development of vaccines – a potential dream for the elimination of STH infections. More members of the scientific community are working on research and development in this area than ever before (Hotez et al., 2003), and existing hookworm vaccine initiatives could be of interest in this regard (http://www.sabin.org). However, despite progress made in recent years, including the discovery of some promising candidate vaccine antigens, there is still no vaccine against hookworm (Hotez et al., 2003, Loukas et al., 2006, Hotez et al., 2010). Yet it is clear that this type of initiative will be encouraged to continue and extend to other parasites as well.

1.3 Anthelminthics recommended by the World Health Organization

According to the WHO Model List of Essential Drugs, four drugs are recommended for wide use in the control of STH (WHO, 1997): the aforementioned two benzimidazoles (BZ) – albendazole (ALB) and mebendazole (MEB), as well as levamisole (LEV) and pyrantel pamoate (PYR). Ivermectin (IVM) and oxantel (OX) also have an effect against human STH, and are considered in MDA for other neglected NTDs such as onchocerciasis, although they are not on the essential drug list recommended for control of STH. All of these anthelminthics were originally registered for use in veterinary medicine and are still used to treat ascarids, hookworms and whipworms in livestock, horses and pets.

1.3.1 Benzimidazoles (ALB and MEB)

1.3.1.1 Description

ALB and MEB are orally administered, broad-spectrum anthelminthic drugs. Figure 1.4 below illustrates the chemical structure of ALB, its initial oxidised metabolite (ALB sulphoxide) and MEB.
As summarized in Table 1.3, both ALB and MEB are available in a pharmaceutical form of chewable tablets (200 or 400 mg ALB and 100 or 500 mg MEB) as well as an oral suspension (100 mg/5 ml). While the initial metabolite of ALB (ALB sulphoxide) is generally considered the active metabolite responsible for the therapeutic activity of ALB, the MEB metabolites have no anthelminthic activity.
### Table 1.3 Characteristics of albendazole and mebendazole: similarities and differences

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Albendazole</th>
<th>Mebendazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>Methyl 5-(propylthio)-2-benzimidazole carbamate</td>
<td>Methyl 5-benzoylbenzimidazole-2-carbamate</td>
</tr>
<tr>
<td>Derivative</td>
<td>BZ-carbamate</td>
<td></td>
</tr>
<tr>
<td>Pharmaceutical form</td>
<td>Chewable tablet (200 or 400 mg) (white to off-white, film-coated); suspension (100 mg/5ml)</td>
<td>Chewable tablet (100 or 500 mg) (white to slightly yellow, powder-coated); suspension (100 mg/5ml)</td>
</tr>
<tr>
<td>First Human use</td>
<td>1982</td>
<td>1995</td>
</tr>
<tr>
<td>Administration</td>
<td>Orally</td>
<td></td>
</tr>
<tr>
<td>Absorption</td>
<td>Poorly absorbed</td>
<td>Increased with fatty meals</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>Relatively=1%</td>
<td>22 %</td>
</tr>
<tr>
<td>Solubility</td>
<td>Relative insolubility (aqueous solutions)</td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td>70% bound to plasma protein</td>
<td>95% bound to plasma protein</td>
</tr>
<tr>
<td>Initial metabolite</td>
<td>Has anthelminthic activity</td>
<td>Devoid of anthelminthic activity</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Extensively metabolized, converted to active form (ALB-sulphoxide)</td>
<td>Extensively metabolized, converted to inactive form</td>
</tr>
<tr>
<td>Excretion</td>
<td>In urine (&lt;1% as active metabolite), or in bile and faeces</td>
<td>In urine and the bile within 24-48 hrs 2% excreted unchanged in urine or faeces</td>
</tr>
<tr>
<td>Maximum plasma concentration</td>
<td>Reach after 3 hrs</td>
<td>Reach within 2-7 hrs</td>
</tr>
<tr>
<td>Plasma half life</td>
<td>4-15 hrs</td>
<td>3-9 hrs</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Inhibition of microtubule polymerization interferes with glucose uptake</td>
<td></td>
</tr>
<tr>
<td>Safety</td>
<td>Well tolerated and safe (teratogenic in pregnancy)</td>
<td></td>
</tr>
</tbody>
</table>

**Sources:** Horton, 2000; Rigter et al., 2004; Friedman et al., 2012; [http://www.medicines.org.uk/emc/medicine/944/spc](http://www.medicines.org.uk/emc/medicine/944/spc); [http://www.accessdata.fda.gov/scripts/cder/drugsatfda/index.cfm?fuseaction=Search.SearchAction&SearchType=BasicSearch&Search_Button=submit&searchTerm=Mebendazole]
1.3.1.2 Pharmacokinetics (absorption, metabolism, distribution)

Generally, both BZ drugs are poorly absorbed from the gastrointestinal tract. The main properties that influence their absorption and behaviour in the body are their low aqueous solubility (relative insolubility in water). In vitro analysis shows that both are poorly soluble in aqueous solutions. ALB is somewhat soluble in methanol, chloroform, ethyl acetate, and acetonitrile, and generally soluble in dimethylsulfoxide, strong acids, and strong bases, while MEB is less than 0.05% soluble in water, dilute mineral acid solutions, alcohol, ether and chloroform, but soluble in formic acid (Gottschall et al., 1990).

Oral absorption of ALB in other animals is higher: about 50% in cattle and 20 to 30% in mice and rats as compared to humans, in whom it is only about 1 to <5%. However, in humans, intake of fatty foods and perhaps bile salts may increase absorption as much as five-fold (Dayan, 2003). Moreover, there have been numerous attempts over the past few years to improve formulations and thereby increase absorption when treating systemic helminth infections. One example of such formulations includes the use of liposomes, which have been shown to be effective experimentally (in animal models) and clinically (in human use), suggesting that improved efficacy could be achieved using absorption enhancement (Hrckova and Velebny, 2001).

After the administration of a single oral dose (200 mg or 400 mg) of ALB, it is variably and intermittently absorbed. It cannot be detected in plasma because the drug is rapidly metabolized in the liver, and possibly, in the intestine, to ALB sulphoxide, which has potent anthelminthic activity (Redondo et al., 1999). It reaches variable maximum plasma concentrations about three hours after a single oral dose, and its plasma half-life is about nine hours on average but highly variable, ranging from 4 to 15 hours (Marriner et al., 1986; Gottschall et al., 1990; Marques et al., 1999). ALB sulphoxide is mostly protein-bound (approximately 70%), distributes well to various tissues, and enters bile and cerebrospinal fluid – where it reaches a concentration of approximately 20% in plasma (Morris et al., 1987; Dayan, 2003).

Similarly, out of the small proportion of MEB that is absorbed (only 10%) approximately 95% is bound to plasma proteins and is extensively metabolized. A combination of poor absorption and rapid first-phase hepatic metabolism is the main reason for the low systemic bioavailability of MEB, which is about 22%
Moreover, MEB is rapidly metabolized, the products (conjugates of MEB and its metabolites) being excreted in urine and the bile within 24 to 48 hours. In humans, approximately 2% of administered MEB is excreted unchanged in urine and the remainder in the faeces as unchanged drug or primary metabolite.

The activity of ALB sulphoxide and the bioavailability of the parent drug explain why ALB is more effective than MEB for the treatment of tissue-dwelling helminths. Generally, the high plasma levels of ALB sulphoxide make it effective against worms in adult and immature stages in various host tissues and organs outside the gastrointestinal tract. ALB metabolites are excreted mainly in the urine (<1% as active metabolite) or bile and faeces.

1.3.1.3 Mechanism of action

As illustrated in Figure 1.5, both drugs have a more or less similar mechanism of action in the sense that they exert their action by selectively binding to the nematode tubulin and inhibiting the tubulin polymerase enzyme, which prevents the formation/assembly of microtubules and so, impedes cell division. The inhibition of microtubule polymerization is accomplished primarily by binding to free β-tubulin (Prichard, 1994; Colbourn et al., 1994). The fact that these two BZ drugs have higher affinity for parasite β-tubulin than for the same target in higher eukaryotes results in the selective toxicity of these agents against helminths. These drugs also impair the uptake of glucose, thereby increasing glycogen depletion, and hampering the formation of adenosine triphosphate (ATP), which is the energy source of the worms (Lacey, 1990). Moreover, inhibition of mitochondrial fumarate reductase (an enzyme involved in the energy management of the worm cells), and uncoupling of oxidative phosphorylation are some of the biochemical changes found in nematodes following exposure to BZ.
1.3.1.4 Safety

Based on comprehensive reviews (Horton, 2000; Urbani and Albonico, 2003) the incidence of side effects in the use of ALB for STH infections is low (around 1%), with only transient, mild gastrointestinal symptoms occurring. Similarly, MEB is also well tolerated, although abdominal discomfort and diarrhoea can occur in cases of severe infection where patients may complain of transient abdominal pain, diarrhoea, slight headache, fever, dizziness,
exanthema, urticaria, and angioedema. Moreover, MEB has low systemic toxic potential although high doses can cause anaemia and liver damage. As both drugs are extremely well tolerated, safe, and easy for paramedical and non-medical personnel to administer in the treatment of both individuals and whole communities, they have an excellent safety profile.

However, it has been shown that BZ drugs are embryotoxic and teratogenic in pregnant rats, and they are not generally recommended for use during pregnancy (Bethony et al., 2006; Teruel et al., 2009a; Longo et al., 2014). Although their safety for pregnant women and young children has not been fully established, due to ongoing and anticipated widespread use of BZ in global control programmes, there is a high level of interest in their use in young children and in the second and third trimesters of pregnancy. However, it is recommended that treatment should be avoided during the first trimester of pregnancy (Urbani and Albonico, 2003).

1.3.1.5 Efficacy

Overall, ALB has broad-spectrum effect against a range of many intestinal nematodes and cestodes in both animals and humans. Because of the systemic bioavailability of its sulphoxide metabolite, it also has an effect against tissue-dwelling nematodes and cestodes such as Echinococcus in animals (Venkatesan, 1998). In general, for humans, a single dose of ALB (400 mg) is used in adults, but in children between the ages of 12 and 24 months, the WHO recommends a reduced dose of 200 mg.

ALB has high efficacy against the adult stages of Ascaris, hookworms and Enterobius. It is also effective against migrating larval stages (larvicidal) of Ascaris, hookworms and even larvae of cestodes – Taenia and Echinococcus (Cline et al., 1984; Horton, 1997; Garcia and Del Brutto, 2000). Finally, ALB has ovicidal effects on eggs of A. lumbricoides, hookworms and T. trichiura (Maisonneuve et al., 1985). In comparison with MEB, ALB is generally superior in curing hookworm infections, whereas MEB is superior to ALB for T. trichiura infections in children, especially when administered as a single-dose (Bennett and Guyatt, 2000; de Silva et al., 2003; Keiser and Utzinger, 2008).

A single-dose of MEB (500 mg) is effective against the adult stage of A. lumbricoides, but has shown low efficacy against hookworms and very low efficacy results against T. trichiura although still better than ALB. However,
administration of 100 mg MEB on at least two consecutive days appears to be as effective as a single dose of ALB (400 mg) for hookworm infection and even better than single doses of either drug (ALB and/or MEB) against *T. trichiura* (Bennett and Guyatt, 2000; Horton, 2000). It has been suggested that heavy *T. trichiura* infection may require up to a three-day course of treatment (WHO, 1995).

Currently, ALB and MEB are the two BZ drugs of choice comprising the mainstay of PC against *A. lumbricoides*, *T. trichiura* and hookworms. The choice rests on several criteria: they are both relatively efficacious, broad-spectrum, typically administered orally in a single dose, easy to administer, safe, and either cheap or available in huge amounts as donations through WHO by dedicated pharmaceutical companies (Montresor *et al*., 2002; WHO, 2008, 2010). During PC, different frequencies of re-treatment are selected based on the category of risk (prevalence) of STH in SAC, with single administration of one tablet of ALB (400mg) or MEB (500 mg) carried once or twice per child per year (see Table 1.2). In short, ALB and MEB have very similar modes of action and very similar efficacies (measured as a reduction in faecal egg counts – FECR) after a single administration. Keiser and Utzinger (2008) have summarized the efficacy result of these two drugs as indicated in table 1.4:

<table>
<thead>
<tr>
<th>STH Species</th>
<th>ALB (FECR%)</th>
<th>MEB (FECR%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>95–100</td>
<td>96–99</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>53–89</td>
<td>81–90</td>
</tr>
<tr>
<td><em>Hookworms</em></td>
<td>64–100</td>
<td>52–100</td>
</tr>
</tbody>
</table>

*FECR = Reduction in faecal egg count*

Sources: Bennett and Guyatt, 2000; Keiser and Utzinger, 2008

1.3.2 Levamisole (LEV)

1.3.2.1 Description

LEV is an anthelmintic compound belonging to the chemical class of the synthetic imidazothiazole derivatives (the laevorotatory isomer of tetramisole). It was first discovered at Janssen Pharmaceutica in Belgium in 1966. The chemical structure is shown in Figure 1.6.
LEV is universally available in pharmaceutical form as chewable tablets (40 mg) and given at a dose of 2.5 mg/kg in a single administration. It is typically used in the form of chlorhydrate salts, and occasionally as a phosphate as well.

1.3.2.2 Pharmacokinetics (absorption, metabolism, distribution)

LEV, being highly soluble in water, can be formulated for many delivery forms (mainly in animals) such as drenches, injectables, feed additives, tablets and pills. It is rapidly and almost completely absorbed from the gastrointestinal tract, and then quickly distributed through the whole body. In humans, peak plasma levels (about 0.7 $\mu$g/ml) are reached within one to two hours after oral administration of a single dose of 150 mg. The drug is quickly metabolized in the liver and has a plasma half-life of about four hours, and the metabolites are eliminated from the body in the urine in about two days (Kouassi et al., 1986). Only about 5% of the administered dose is excreted unchanged and 70% as metabolites through urine, and in small amounts through faeces.

1.3.2.3 Mode of action

LEV acts as an acetyl-cholinesterase (AchE), an enzyme that hydrolyzes acetylcholine. Thus, it exerts its action through binding to the acetylcholine receptors of the autonomic ganglia, causing a spastic contraction followed by a tonic paralysis of the nematode. It thereby plays a key role in eliminating the helminths passively (Martin, 1993). Although it is certain that LEV primarily influences the neuromuscular system of nematodes, it also interferes with the fumarate reduction system in mitochondria, affecting the mitochondrial energy production, which contributes to the anthelminthic efficacy of LEV. It also interferes with the metabolism of carbohydrates in the helminths.
1.3.2.4 Efficacy

LEV is active against a broad spectrum of human and animal nematodes. It has good efficacy against *Ascaris* and hookworms, including *A. duodenale* and other *Ancylostomidae*, although *N. americanus* infection may require a second dose after one week (WHO, 1995). Generally, it is highly effective against adult gastrointestinal and respiratory roundworms and larvae of several species infecting ruminants. Besides anthelmintic activities, it also has a stimulating effect on cellular immune responses in humans. However, although LEV has been studied much less intensively than BZ in humans, the available data suggest that it is not markedly better than the other anthelminthics (Keiser and Utzinger, 2008).

1.3.2.5 Safety

Generally, side effects due to LEV treatment include: nausea, vomiting, diarrhoea, abdominal pain, dizziness and headache, fever, influenza-like syndrome, arthralgia, muscle pain, rash, and cutaneous vasculitis. It is also contraindicated in pregnancy. Due to the occurrence of serious side effects in some people, it has been withdrawn in several countries, including the USA (2000) and Canada (2003) (Source: http://www.drugsupdate.com/generic/view/259 accessed on June 22, 2013).

1.3.3 Pyrantel (PYR) and oxantel (OX)

1.3.3.1 Description

PYR is a narrow-spectrum nematicidal belonging to the tetrahydropyrimidines group of drugs, and is typically a pyrimidine derivative. It is mostly used in the form of salts (e.g., pamoate, citrate and tartrate). PYR pamoate, for instance, is a combination of PYR and pamoic acid. OX is the meta-oxphenyl analogue of PYR (Figure 1.7), also belonging to the tetrahydropyrimidines group.
PYR is available in pharmaceutical form as chewable tablets (250 mg) and is given at a dose of 10 mg/kg in a single administration. OX can be administered at 10 mg/kg, either alone or in combination with other drugs such as PYR.

1.3.3.2 Pharmacokinetics (absorption, metabolism, distribution)

After oral administration, PYR pamoate is poorly absorbed by the gastrointestinal tract (including in the gut in dogs, cats and horses) and PYR is insoluble in water. This allows high concentrations of unchanged drug to reach the large intestine. PYR tartrate is better absorbed into the bloodstream, especially in monogastric animals (e.g. dogs, cats, pigs and horses). This reduces the time it remains in the gastrointestinal system and reduces efficacy against gastrointestinal worms, especially those species in the large intestine (e.g. whipworms). For this reason, in non-ruminants the pamoate salt is preferred, as it is less absorbed and allows higher safety margins. Partially metabolized in the liver, peak plasma levels are reached in one to three hours. A small percentage is excreted in urine and a large amount (over half of the administered dose) is eliminated unchanged in faeces (Webster, 1991). Like PYR pamoate, after oral administration, OX pamoate is poorly absorbed, so that high concentrations are reached in the cecum and colon (Garcia, 1976).

1.3.3.3 Mode of action

PYR is a cholinergic agonist that causes spastic paralysis of helminth muscle. That is, PYR, like any other member of the tetrahydropyrimidines group, acts on the nervous system of the helminths as acetylcholinesterase (AchE) inhibitors. Acetylcholine is a molecule involved in the transmission of nervous signals from nerves to muscles (known as neuromuscular junctions) and between neurons in the brain (known as cholinergic brain synapses). Thus, AchE
inhibition massively disturbs the normal movements of the parasites; it is followed by depolarization-induced paralysis, whereby the worms die more or less quickly or are expelled from the gut because they cannot keep themselves attached to the intestinal wall.

Similarly, OX exerts its anthelminthic action by paralyzing the nematodes (Aubry et al., 1970) and this paralytic effect is due its ability to cause a depolarizing type of neuromuscular block. That is, it acts as a potent agonist at the acetylcholine receptors on the muscle cells of nematodes, causing paralysis by targeting nicotinic acetylcholine receptor molecules.

1.3.3.4 Efficacy

PYR is highly effective against adult gastrointestinal nematodes (particularly, those of dogs, cats and horses). It is also used in human medicines against various gastrointestinal roundworms. It has been shown to be highly effective and safe against human adult ascariasis (a single dose yields cure rates of 85 to 100%) and hookworm infections, but for heavy infections, especially with *N. americanus*, a three-day course might be necessary to reach 90% cure rates (Geerts and Gryseels, 2000; Keiser and Utzinger, 2008).

However, PYR pamoate does not possess any significant efficacy in the treatment of *T. trichiura* despite the fact that high concentrations reach the large intestine (WHO, 1995). On the other hand, the OX analogue of PYR is especially active against *Trichuris* species. The two drugs, when combined, have shown broad-spectrum anthelminthic activity (Rim et al., 1975).

It has also been shown that a combination of PYR/OX (10 mg/kg) as a single dose offers a valuable alternative to mebendazole as a single-dose treatment for the control of intestinal nematodes, including *Trichuris* infections in children in endemic areas of sub-Saharan Africa, due to its comparable efficacy, its low cost and its suitability for use in young children (Albonico et al., 2002).

1.3.3.5 Safety

PYR and/or OX are extremely well-tolerated drugs. In both, adverse effects are minor/infrequent, mild and transient, but include gastrointestinal distress/irritation, vomiting, nausea, headache, fever and dizziness. It is advised that they be used with caution in patients with hepatic dysfunction. They are not
recommended for pregnant women or for children under 12 months old. No adverse events have been reported after use (Albonico et al., 2002).

1.3.4 Ivermectin (IVM)

1.3.4.1 Description

IVM is a semi-synthetic analogue of avermectin B1a (abamectin), an insecticide developed for crop management in the mid 1970s (Figure 1.8). Currently, it is used extensively to control and treat a broad spectrum of infections caused by parasitic nematodes and arthropods (Campbell, 1993) in animals. In humans, it is mainly used in the treatment of onchocerciasis, but also effective against other worm infestations (such as strongyloidiasis, ascariasis, trichuriasis, filariasis, enterobiasis) and some epidermal parasitic skin diseases, including scabies.

![Figure 1.8 Chemical structure of ivermectin](image)

IVM is an odourless off-white powder available in pharmaceutical form as tablets or by injection and is given at a dose of 150 to 200 µg/kg in a single administration.

1.3.4.2 Pharmacokinetics (absorption, metabolism, distribution)

IVM exhibits high lipid solubility but poor solubility in water and can be formulated for delivery either by mouth or injection. It does not readily cross the blood-brain barrier of mammals due to the presence of P-glycoprotein (Borst and Schinkel, 1996), although crossing may still become significant if it is given at high doses, in which case, brain levels peak two to five hours after administration (Guzzo et al., 2002).

1.3.4.3 Mode of action

IVM induces a very long-lasting hyperpolarisation or depolarisation of the neurone or muscle cell of the nematodes through Glutamate-gated chloride
channels (GluCl-channels). IVM-activated channels open very slowly but essentially irreversibly thereby blocking further functions (Wolstenholme and Rogers, 2005). Although it is clear that IVM do have effects on nematode \( \gamma \)-Amino Butyric Acid (GABA) receptors (Feng et al., 2002), and believed to induce tonic paralysis and alter feeding behaviour of nematodes, current attention turned more to alternative GluCl-channels as the most likely molecular target for its action. In onchocerciasis, IVM is microfilaricidal. It does not effectively kill adult worms but blocks the release of microfilariae for some months after therapy (Arena et al., 1995; Cully et al., 1996).

1.3.4.3 Efficacy

IVM, administered as a single oral dose (150 to 200 µg/kg) every 6 to 12 months, is the drug of choice for treatment of onchocerciasis in adults and children five years of age and up (Goa et al., 1991). In lymphatic filariasis, a single annual dose of ivermectin (400 µg/kg) was both effective and safe for mass chemotherapy of infections with *Wuchereria bancrofti* and *Brugia malayi* (Ottesen and Ramachandran, 1995). It is the drug of choice for treatment of human strongyloidiasis at two daily doses of 150 to 200 µg/kg, (Marti et al., 1996).

IVM treatment is also variably effective against other intestinal nematodes. It is more effective in ascariasis and enterobiasis than in trichuriasis or hookworm infection (Keiser and Utzinger, 2008). In the latter two infections, although it is not curative, it significantly reduces the intensity of infection.

1.3.4.5 Safety

In general IVM is well tolerated by uninfected humans, but infrequent adverse effects due to its treatment may include fatigue, dizziness, nausea, vomiting, abdominal pain, and rashes. Moreover, IVM is not approved for use in children under five years of age or in pregnant women. Lactating women taking the drug secrete low levels in their milk; the consequences for nursing infants are unknown. Currently, the WHO recommends against IVM treatments in MDA campaigns for pregnant women, lactating women in the first week after birth, children less than 90 cm in height/15 kg in body weight, and the severely ill (WHO, 2006).
1.4 Anthelminthic resistance in humans and other challenges of continuous use of preventive chemotherapy

There are a number of concerns and challenges directly or indirectly related to the continuous implementation of PC programmes. One such concern is the possibility of emergence of anthelminthic resistance due to increasing use of only BZ. Others relate to safety, poor understanding of the pharmacology of BZ, frequency of re-treatment, low coverage of PC so far, lack of integration of PC with other control measures, and failure to target key risk groups. These issues are discussed in more detail below.

1.4.1 Anthelminthic resistance in humans

The most pressing concern for the future in the era of PC programmes is the possible emergence of resistance against the administered anthelminthic drugs. Since anthelminthic drug resistance is already a severe problem in veterinary medicine, it is only a matter of time before it becomes a worldwide problem in human medicine.

It has been suggested that the major mechanism of BZ drug resistance in nematodes involves the selection of a naturally occurring ‘resistant’ genotype involving a Phenylalanine→Tyrosine change at position 200 of β-tubulin (Sangster and Gill, 1999). Although some previous studies have shown low efficacy (treatment failures) of single-dose ALB and MEB for the treatment of hookworm and Trichuris infections (Albonico et al., 1994; Adams et al., 2004; Flohr et al., 2007), drug resistance has not been fully demonstrated for human STH. Evidence for a selection pressure on the β-tubulin gene at position 200 is indicated for T. trichiura possibly explaining the reduced efficacy of ALB against this parasite (Diawara et al., 2009). The availability of even such minimal evidence of widespread dissemination of resistant forms is already a warning signal. However, the empirical evidence of drug resistance in human STH is still poor (Vercruysse et al., 2011).

In addition to the issue of anthelminthic resistance; the fact that BZ efficacy is not optimal for all species of STH, the contraindication of BZ in early pregnancy, and the paucity of comprehensive data on the pharmacology of both drugs in humans (Dayan, 2003) are reasons enough to seek possible
alternatives, either to optimize the available anthelminthic drugs or discover new ones.

One such alternative could be the use of a combination of OX with either ALB or MEB. Recent work has confirmed the excellent trichuricidal properties of OX pamoate, although it has no effect on hookworms. On the other hand, OX-ALB combination has shown a promising result, which calls for further in-depth studies on drug combinations such as OX-MEB, OX-ALB or others as alternatives, including dosing and regimens (Keiser et al., 2013; Speich et al., 2014). Earlier studies have also shown and recommended that a single-dose mixture of OX and PYR pamoate is a highly effective and acceptable treatment for multiple infections with STH (Rim et al., 1975; Albonico et al., 2002).

Moreover, taking advantage of the geographic overlap of STH with other NTDs, it is possible to target these conditions simultaneously by combining drugs in an integrated ‘rapid-impact package’ (Molyneux et al., 2005; Hotez et al., 2006). It is also equally important to ensure access to good quality anthelminthic drugs at all levels of the health care system in endemic areas.

1.4.2 Other issues regarding the continuous use of preventive chemotherapy

Apart from fear of the emergence of anthelminthic resistance, there are a number of other concerns and challenges related to the continuous use of PC, as follows:

(i) Safety: The introduction of drug distribution to target groups or populations raises the issue of drug safety. When treatment is not under health-sector supervision, the community needs full assurance that treatment will be safe and provided to meet the intended use and level of acceptance.

(ii) Poor understanding: Many gaps remain in our understanding of the pharmacology of the BZ drugs, despite remarkable efforts of some researchers in reviewing the existing knowledge and forwarding their urgent recommendations (de Silva et al., 1997; Albonico et al., 1999; Horton, 2000; Dayan, 2003; Albonico et al., 2004; Utzinger and Keiser, 2004). These gaps, which have been noted in the review of the uses, effects and modes of action of the drugs in section 1.3 as well as in 1.4.1 above, represent a challenge to the continued successful use of these drugs, as they leave us poorly equipped to
understand or monitor the emergence of drug resistance, and constrain attempts to look for new treatment options.

(iii) Timing/frequency of re-treatment: There is a lack of practical modelling to resolve key questions about how often drugs should be administered in mass PC programmes, and preferred seasons for administration to optimize effectiveness, so these issues remain a challenge.

(iv) Low coverage: As previously noted in this review, only about 36.0% of SAC and 24.7% of preSAC in areas affected by STH have been receiving regular treatments with either ALB or MEB (WHO, 2014), indicating very low coverage until now.

(v) Lack of integration with other control measures: PC must be supported by political commitment and synergized with socio-economic development, since long-term success depends on the integration of other control measures such as WASH together with chemotherapy.

(vi) Failure to target key risk groups: The targeting of SAC only is another challenge, particularly when it comes to hookworm treatment, as infection prevalence increases with age and the consequences of this infection are also serious in other risk groups. Hence, it is of paramount importance to extend PC to other segments of the population at high risk to hookworm infection, or if possible, to target the whole community (Brooker et al., 2004; Christian et al., 2004; Albonico et al., 2008).

1.5 The monitoring of drug efficacy in preventive chemotherapy programmes

It is vital to monitor drug efficacy in large-scale interventions to reduce the incidence of infection and associated morbidity and mortality due to STH, not only to demonstrate health benefits, but also to assess cost-effectiveness and show that drugs and other logistics from donor agencies have been used wisely.

From the health standpoint, there is now ample evidence clearly demonstrating that regular treatment of STH infections produces immediate as well as long-term benefits, significantly contributing to the development of affected individuals, particularly children (Savioli et al., 1992; Crompton and Nesheim, 2002; WHO, 2005).

However, given the scarcity of suitable alternative anthelminthics it is crucial for monitoring programmes to be introduced to assess the progress and
effectiveness of interventions and to detect any changes in therapeutic efficacy that may arise from a parasite's potential to develop resistance (Albonico et al., 2004).

1.5.1. How to monitor drug efficacy

Monitoring the efficacy of drugs is not straightforward, as it is subject to many factors, either inherent or external. This may lead to significant variation in efficacy monitoring results. Hence, the factors at different levels should be considered well in advance to help exercise more caution in the interpretation of results and distinguish between reduced efficacy and drug resistance.

1.5.1.1 Indicators of drug efficacy

Efficacy is determined by measuring the change in faecal helminth egg output in an individual pre- and post-treatment with a given drug at an optimal interval of time. In STH infections of humans, this can be done either through determination of the cure rate (CR), expressed as the percentage of subjects excreting no eggs after drug administration, or by measuring faecal egg count reductions (FECR). These are the two most commonly used efficacy indicators.

However, the meaning and interpretation of these indicators varies according to different health viewpoints. Some researchers prefer the use of CR to FECR, while others suggest the use of FECR is better. Yet in the latter case, the application of different formulas to calculate FECR by different researchers produces different FECR results, making it difficult to use FECR as an accurate and good indicator of drug efficacy (Kopp et al., 2008; Montresor, 2011).

Furthermore, there is some controversy regarding the use of CR and FECR as indicators in monitoring drug efficacy against human STH. Although Bennett and Guyatt (2000) reported both CR and FECR in their analysis of published ALB and MEB efficacy monitoring data, they observed a noticeable variation when the CR is the analysed endpoint. Whereas Keiser and Utzinger (2008) were only able to use CR as the primary outcome measure in their systematic review and meta-analysis of the efficacy of single-dose oral ALB and MEB, as there was an insufficient number of valid studies reporting FECR.

Generally, wide variations in efficacy results are found, even in trials in which the same drug is given at the same dosage. Different co-morbidities (such as gastrointestinal diseases, immunodeficiency, malnutrition, etc.) and several
other factors may influence the efficacy of anthelminthic drugs, thus hindering the comparison of efficacy results by different investigators. Further exacerbating the problem is that methods for detecting eggs and analysing data are not fully synchronized. Additionally, much of the CR and FECR data investigated by different studies cannot be directly compared as many of these studies are confounded by differing methodological considerations and study designs (Keiser and Utzinger, 2008). Those and other factors are described in more detail in the next section.

1.5.1.2 Factors affecting drug efficacies

Among the many factors, the following are thought to be most important in accounting for the variations in efficacy and should be considered well in advance and minimized if it is not possible to avoid them: (i) lack of use of standardized parasitological techniques, (ii) different intervals of post-treatment follow-up, (iii) different intensity levels at baseline, (iv) sample size, (v) age group of study subjects, (vi) quality of the drug, (vii) treatment regimens and dosing, (viii) diverse statistical measurements of intensity, and (ix) possible presence of drug resistant parasites/variable parasite strains (Albonico et al., 1999; Bennet and Guyatt, 2000).

i. Lack of use of standardized parasitological techniques: It is generally agreed that there is significant day-to-day and intra-specimen variation in helminth egg output. Parasitological techniques used to examine the eggs also vary, mainly in their sensitivity, particularly for low-infection intensities (Utzinger, 2003; Goodman et al., 2007). The majority of investigators have overlooked the effect of the sensitivity of the methods they used as one factor. Moreover, with regard to diagnostic approach, different trials have evaluated drug efficacy based on either a single stool sample per individual, examined before and after treatment employing only one diagnostic test, or two samples over consecutive days, resulting in different efficacy results based on CR (Keiser and Utzinger, 2008). Therefore, we need to adopt standard operating procedures so that monitoring systems yield comparable results and avoid confounding variables that may affect drug efficacy arising from different parasitological techniques.

ii. Interval of post-treatment follow-up: When evaluating drug efficacy, data for calculating this indicator should be collected no later than three weeks
after treatment. The optimal period is 7 to 14 days, as all dead worms and remaining eggs are eliminated during this interval. A follow-up during the pre-patent period may also be indicative for efficacy against both adult and immature stages. However, the interval between pre- and post-treatment should be adapted to each parasite species and to the drugs used. For instance, for hookworms, a period of about two weeks is appropriate. A longer period would allow immature or even new infections to become patent, while a shorter one may overestimate efficacy, since some drugs temporarily suppress egg production without killing the worms (Geerts and Gryseels, 2000). Thus, monitoring after the pre-patent period is not recommended to avoid counting any possible cases of re-infection that may have occurred, and results are not indicative for efficacy.

**iii. Baseline intensity levels:** This is one of the parasite-related factors, whereby infection intensity and worm fecundity vary and may affect the efficacy of an anthelminthic drug. For instance, at a given dose, when the intensity of the infection is high, the indicator of efficacy might be reduced, affecting the final interpretation of the efficacy results. Drops in CR and FECR at high intensities of *T. trichiura* and hookworm infections have been demonstrated when the drug efficacy data were separately analysed by class of intensity (Bennett and Guyatt, 2000). The WHO classification of intensity is shown in (Table 1.5).

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Light-intensity infection</th>
<th>Moderate-intensity infection</th>
<th>Heavy-intensity infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. lumbricoides</em></td>
<td>1-4,999 EPG</td>
<td>5,000-49,999 EPG</td>
<td>&gt;50,000 EPG</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>1-999 EPG</td>
<td>1,000-9,999 EPG</td>
<td>&gt;10,000 EPG</td>
</tr>
<tr>
<td>Hookworms</td>
<td>1-1,999 EPG</td>
<td>2,000-3,999 EPG</td>
<td>&gt;4,000 EPG</td>
</tr>
</tbody>
</table>

*EPG = eggs per gram of stools*

Moreover, efficacy variation is further affected by the fact that egg counts are an inadequate measure of adult worm abundance in veterinary, and probably in human parasitic infections (Kopp *et al*., 2008; Kotze and Kopp, 2008). For instance, in hookworms there is an inverse correlation between adult worm number and the fecundity of female worms. The fecundity of the remaining worms after drug administration increases, and hence thwarts the
interpretation of drug efficacy summarized by means of FECR (Kopp et al., 2008).

iv. Sample size: The different sample sizes employed by different investigators might have an influence on the power of the analysis, hence confounding the results. Keizer and Utzinger (2008) indicated in their systematic review and meta-analyses that the sample sizes in several of the trials they reviewed consisted of fewer than 50 individuals infected with a specific STH and treated with an anthelminthic drug. They concluded that such trials were likely underpowered, impeding comparison with efficacy trials in studies that used larger sample sizes.

v. Age group enrolled: The underlying distribution of parasite numbers per host (intensity) is central and it exhibits heterogeneity in different age groups. Moreover, changes in behaviour, exposure, immune responsiveness and/or physiology may cause a child’s worm burden to change markedly with age and therefore, from one year to the next. This will influence the baseline infection level; the proportional reduction in infection after chemotherapy; the rate of re-infection; and the estimated impact of treatment. Moreover, the efficacy of BZ may depend on the main mechanism of action, and on their ability to reach high and sustained concentrations at the sites where parasites are located. This, in turn, depends on host-related pharmacokinetic, metabolic and tissue distribution factors (Lanusse and Prichard, 1993), which can all be affected by age. Thus, monitoring only in one age group/class may not provide robust measures of drug efficacy results.

vi. Quality of the drugs: Drugs must be manufactured according to good manufacturing practice and be of sufficient quality to perform as required in control programmes. Nevertheless, variations between batches in the quantity of the active ingredient in a pharmaceutical formulation, its bioavailability, and degradation during storage/transport may result in inconsistent efficacy results. It is also acknowledged that there are many counterfeit drug products available on the market and that these may be used unconsciously in control programmes (Albonico et al., 1999). Thus, it is necessary to verify that the product is not counterfeit or sub-standard and actually contains the stated active compound. As well, it must meet the International Pharmacopoeia standard of dissolution and disintegration times, which may affect drug efficacy (Albonico et al., 2007).
vii. Different treatment and dosing regimens: Efficacy results may vary depending on the treatment regimens and dosing regimens. For example, ALB is effective in a single dose against *A. lumbricoides*, and it is also active against both species of hookworm, in contrast to MEB, which is principally active only against *A. duodenale* at single dose. There is, however, some evidence suggesting that very heavy infections with *N. americanus* may require more than one dose to achieve a complete cure. Moreover, dosing over three days may be required to cure heavier *T. trichiura* infections. Apart from affecting efficacy results, under-dosing selects for the development of anthelmintic resistance in target parasites, which may represent a major concern in human medicine (Horton, 2000).

viii. Diverse statistical measurements of intensity: Different investigators used either geometric or arithmetic means during the calculation of FECR to assess drug efficacy. However, there is evidence that geometric means provide a biased efficacy result when conducting a FECR (Dobson *et al.*, 2009). Hence, lack of uniformity in the use of statistical measurements will present variable (either overestimating or underestimating) efficacy results.

ix. Other possible factors contributing to variable efficacy results may include: the possible presence of drug-resistant parasites, previous PC history, geographic differences among STH species, diet, health status of the child and level of immunity, rate of intestinal transit, episodes of vomiting during treatment, and faecal consistency.

The scale-up of chemotherapy programmes with BZ currently underway in various parts of Africa, Asia, South America will likely exert a drug pressure on the parasites, and this has the potential to select for parasite genotypes that can resist anthelmintics. However, it is important that reduced efficacy be differentiated from anthelmintic resistance, as many potential factors may affect the efficacy of an anthelmintic, and should first be excluded before anthelmintic resistance is assumed. Amusingly, many investigators have not seriously looked into the possible factors that might have contributed to and affected their efficacy trial endpoints. It is also important to note that all the aforementioned factors are not an exhaustive list, and many factors remain to be identified.
1.6 Benzimidazole drug efficacy studies in school-aged children in Africa

The following section summarizes a number of efficacy studies published from 1980 to 2011 on trials conducted to assess the efficacy of single-dose ALB and MEB against STH in children in Africa. For each trial the following aspects are reported (if available): the country in which the study was done, the year, the number of study subjects, the post-intervention time, and the CR and FECR for each STH covered by the study concerned.

The studies are summarized in Tables 1.6, 1.7 and 1.8 for ALB and Tables 1.9, 1.10, and 1.11 for MEB. In general, most anthelminthic drug efficacy studies reported in Africa in the last three decades are limited to certain geographical areas/countries. Hence, the results are neither comprehensive nor representative of the whole continent, as the prevalence/intensity of STH infections may diverge strongly in different regions of Africa. Moreover, there is variation among the studies in terms of the duration of follow-up after treatment, statistical measurements, and to some extent, dosage and treatment regimens. Consequently, it is difficult to compare these studies, as they may be confounded in many ways.

1.6.1 Albendazole

As indicated below (Tables 1.6, 1.7 and 1.8), very few studies have been conducted to evaluate the efficacy of ALB against the three main STHs in SAC in Africa: *A. lumbricoides* (7), *T. trichiura* (10), hookworm (7).

1.6.1.1 Ascaris lumbricoides

According to the seven studies identified, ALB has shown fair to excellent efficacy results against *A. lumbricoides*, both in terms of CR (91.3 to 100%) when the Kato-Katz diagnostic method was used, and FECR (>97%), regardless of the variation in length of post-treatment follow-up, except for one study that reported a very low FECR (52.2%) as evaluated 90 days post-treatment (Nkengazong *et al.*, 2010). Since most of the studies used Kato-Katz, diagnostic method is not considered as factor explaining the variation in efficacy results in the present review. These findings– showing that ALB is efficacious against *A. lumbricoides* provided that post-treatment evaluation is done within three weeks–are consistent with those reported in the rest of the world, as
summarized in many published studies (Olsen, 2007; Reddy et al., 2007; Keiser and Utzinger, 2008).

Table 1.6 Albendazole efficacy trials against *Ascaris lumbricoides* in school-aged children in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1992</td>
<td>1,174</td>
<td>6-12</td>
<td>400</td>
<td>21</td>
<td>98.9</td>
<td>99.6</td>
<td>Albonico et al (1994)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>377</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>430</td>
<td>_</td>
<td>400</td>
<td>14</td>
<td>98.2</td>
<td>97.0</td>
<td>Albonico et al (2011)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>240</td>
<td>_</td>
<td>400</td>
<td>21-35</td>
<td>91.3</td>
<td>99.9</td>
<td>Knopp et al (2011)</td>
</tr>
<tr>
<td>Kenya</td>
<td>2004</td>
<td>1,942</td>
<td>4-18</td>
<td>400</td>
<td>60</td>
<td>100.0</td>
<td>100.0</td>
<td>Kihara et al (2007)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>2007</td>
<td>82</td>
<td>1-20</td>
<td>400</td>
<td>90</td>
<td>82.0</td>
<td>52.2</td>
<td>Nkengazong et al (2010)</td>
</tr>
</tbody>
</table>

*CR: Cure rate; FECR: reduction in faecal egg count; ‘FECR is based on geometric mean

1.6.1.2 Trichuris trichiura

Of the ten studies done to evaluate the efficacy of ALB against *T. trichiura*, six are from the same country (Tanzania), representing only one confined geographical area. Nine studies used 400 mg ALB as a single dose, while one used 400 mg twice at an eight-hour interval (Namwanje et al., 2011). However, the length of post-treatment duration varies extremely (ranging from 7 to 120 days). Equally, there was a huge variation in the size of the samples analysed among the trials and across the different countries, ranging from 66 to 1,942 individuals.
Table 1.7 Albendazole efficacy trials against *Trichuris trichiura* in school-aged children in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)</th>
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<td>1174</td>
<td>6-12</td>
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<td>73.3</td>
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<td>Albonico et al (1994)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>430</td>
<td>4-18</td>
<td>400</td>
<td>14</td>
<td></td>
<td></td>
<td>Albonico et al (2011)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>140</td>
<td>&gt;5</td>
<td>400</td>
<td>22-29</td>
<td>10.0</td>
<td></td>
<td>Knopp et al (2010)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2003</td>
<td>130</td>
<td>6-19</td>
<td>400</td>
<td>60</td>
<td>17.0</td>
<td></td>
<td>Legesse et al (2004)</td>
</tr>
<tr>
<td>Uganda</td>
<td>2005</td>
<td>66</td>
<td>6-15</td>
<td>400</td>
<td>7</td>
<td>8.0</td>
<td>89.0</td>
<td>Olsen et al (2009)</td>
</tr>
<tr>
<td>Uganda</td>
<td>2009</td>
<td>101</td>
<td>5-14</td>
<td>400</td>
<td>14</td>
<td>36.2</td>
<td></td>
<td>Namwanje et al (2011)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>2007</td>
<td>82</td>
<td>1-20</td>
<td>400</td>
<td>90</td>
<td>84.6</td>
<td></td>
<td>Nkengazong et al (2010)</td>
</tr>
</tbody>
</table>

**CR**: Cure rate; **FECR**: reduction in faecal egg count; *400 mg albendazole used two times at eight-hour interval. Note: most of the studies used Kato-Katz as a diagnostic method.

Overall, the CRs observed were below 20% for the majority of the studies when the efficacy evaluation was made between 14 and 21 days post-treatment, but the FECRs varied considerably among the trials even when evaluated during the same interval period.

1.6.1.3 Hookworms

Of the seven studies done to evaluate the efficacy of ALB against hookworms, five indicated FECRs of >90% regardless of post-treatment interval
variation (between 21 and 90 days). This is surprising if one considers the post-treatment interval a possible factor affecting the efficacy results. Seven of the studies evaluated the CR as well, and results varied from 56.8 to 97.8% as evaluated between 14 and 21 days post-treatment (Table 1.8). The lowest CR (56.8%) observed (Albonico et al., 1994) might be due to one or more of the factors identified above. One study evaluated the efficacy of using a dosage of 200 mg ALB for three consecutive days and reported both a CR and FECR of 100% (Nkengazong et al., 2010).

Table 1.8 Albendazole efficacy trials against hookworms in school-aged children in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample Size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1992</td>
<td>1,174</td>
<td>6-12</td>
<td>400</td>
<td>21</td>
<td>56.8</td>
<td>97.7</td>
<td>Albonico et al (1994)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>430</td>
<td></td>
<td>400</td>
<td>7</td>
<td></td>
<td></td>
<td>Albonico et al (2011)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>240</td>
<td></td>
<td>400</td>
<td>14</td>
<td>97.8</td>
<td></td>
<td>Knopp et al (2011)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2009</td>
<td>240</td>
<td></td>
<td>400</td>
<td>21-35</td>
<td>61.2</td>
<td>89.9</td>
<td></td>
</tr>
<tr>
<td>Kenya</td>
<td>2004</td>
<td>1,942</td>
<td>4-18</td>
<td>400</td>
<td>60</td>
<td>95.1</td>
<td>96.0</td>
<td>Kihara et al (2007)</td>
</tr>
<tr>
<td>Cameroon</td>
<td>2007</td>
<td>82</td>
<td>1-20</td>
<td>200*</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>Nkengazong et al (2010)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CR: Cure rate; FECR: reduction in faecal egg count; * 200 mg albendazole used for three days; Note: majority of the studies used Kato-Katz as diagnostic methods.

In general, it has been shown that repeated doses of 400 mg ALB, but not higher single doses, achieve much better efficacy against hookworms than the single 400 mg dose, whereas both increased single doses (such as 600 mg) and repeated doses of ALB 400 mg improve efficacy against T. trichiura (Horton, 2000). This is also reflected in the African studies covered in this review, suggesting that a broader range of studies comparing different doses and regimens are still needed to identify optimal treatment for PC application strategies in Africa.
1.6.2. Mebendazole

As indicated below (Tables 1.9, 1.10 and 1.11), even fewer studies have been done to evaluate the efficacy of MEB against the three main STH in SAC in Africa: *A. lumbricoides* (7), *T. trichiura* (10), hookworm (4).

1.6.2.1 *Ascaris lumbricoides*

The seven studies to evaluate MEB efficacy against *A. lumbricoides* were all conducted at 21 days post-treatment. Four out of the seven studies used 500 mg MEB whereas three studies used 100 mg doses of three different brands of MEB twice a day for three consecutive days (Legesse *et al*., 2004). As indicated in Table 1.9, MEB has shown very good efficacy against *A. lumbricoides*: six studies showed CRs and FECRs of >96%; and one study indicated a relatively low CR of 93.0% (Legesse *et al*., 2004) and the authors concluded this could be due to the variation in quality among the different brands of MEB they used from different companies.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample Size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
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<td>6-12</td>
<td>500</td>
<td>21</td>
<td>99.9</td>
<td>97.7</td>
<td>Albonico <em>et al</em> (1994)</td>
</tr>
<tr>
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<td>1999</td>
<td>236</td>
<td>6-12</td>
<td>500</td>
<td>21</td>
<td>96.5</td>
<td>99.9</td>
<td>Albonico <em>et al</em> (2003)</td>
</tr>
<tr>
<td>Tanzania</td>
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<td>448</td>
<td>6-9</td>
<td>500</td>
<td>21</td>
<td>98.0</td>
<td>96.1</td>
<td>Albonico <em>et al</em> (2002)</td>
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<td>143</td>
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<td>100***</td>
<td>21</td>
<td>93.0</td>
<td>99.9</td>
<td></td>
</tr>
</tbody>
</table>

**CR:** Cure rate; **FECR:** reduction in faecal egg count; *100 mg MEB twice a day for three days (Vermox, J & J); **100 mg MEB twice a day for three days (Unibios, India); ***100 mg MEB twice a day for three days (East African Pharmaceutical)**

1.6.2.2 *Trichuris trichiura*

Eight of the ten studies evaluating the efficacy of MEB against *T. trichiura* were done in Tanzania. MEB 500 mg was used except in one study (100 mg);
one study used 500 mg per day for three consecutive days and the rest, 500 mg as single one-time dose. When efficacy was evaluated at an interval of 14 to 21 days post-treatment, the CR ranged from 10 to 20% and the FECR, from 45 to 85%, regardless of single or repeated doses of 500 mg (Table 1.10).

Table 1.10 Mebendazole efficacy trials against *Trichuris trichiura* in school-aged children in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample Size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)</th>
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<td>21</td>
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<td>81.6</td>
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</tr>
<tr>
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<td>354</td>
<td>6-12</td>
<td>500</td>
<td>21</td>
<td>77.2</td>
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<td>21</td>
<td>25.2</td>
<td>83.6</td>
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<td>21</td>
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<td>Tanzania</td>
<td>2009</td>
<td>138</td>
<td>&gt;5</td>
<td>500</td>
<td>22-29</td>
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<td>67.0</td>
<td>Knopp et al. (2010)</td>
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<td>138</td>
<td>6-19</td>
<td>100</td>
<td>21</td>
<td>90.0</td>
<td>90.0</td>
<td>Legesse et al. (2004)</td>
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<td>100</td>
<td>21</td>
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<td>105</td>
<td>5-14</td>
<td>500</td>
<td>7</td>
<td>25.6</td>
<td>84.7</td>
<td>Namwanje et al. (2011)</td>
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<td>66.7</td>
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<td>21</td>
<td>10.6</td>
<td>57.2</td>
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<td>21</td>
<td>41.9</td>
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<td></td>
</tr>
</tbody>
</table>

CR: Cure rate; FECR: reduction in faecal egg count; *500 mg twice a day for three days** 100 mg MEB twice a day for three days (Vermox, J &J); *** 100 mg MEB twice a day for three days (Unibios, India); **** 100 mg MEB twice a day for three days (East African Pharmaceutical)

The trials in which a dose of 100 mg twice per day for three consecutive days was used showed a significant overall increase in CR (28.0 to 90.0%) and in FECR of over 88% (Albonico et al., 2002; Legesse et al., 2004). The most striking result in Legesse et al., 2004 study is the huge variation in CR (53.5 to 90%) despite the fact that all other confounding factors seemed to be the same
(study site, study time, age group, parasitological technique, length of interval, statistical analysis, etc.) except the difference in the brands of the MEB used. Thus, one might attribute such variation to the quality of the drugs.

1.6.2.3 Hookworms

The four studies evaluating the efficacy of MEB against hookworms were all done in Tanzania and involved a single 500 mg dose of MEB. The follow-up interval was at 21 days post-treatment in all except one case where it was 120 days post-treatment (Table 1.11). There was a considerable variation both in CR, ranging from 7.6 to 85%, and in FECR, ranging from 52.1 to 87.6%.

Table 1.11 Mebendazole efficacy trials against hookworms in school-aged children in Africa

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Sample Size</th>
<th>Age-range</th>
<th>Dose (mg)</th>
<th>Follow-up Interval (days)</th>
<th>CR (%)</th>
<th>FECR (%)</th>
<th>Reference</th>
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<td></td>
<td></td>
<td></td>
<td>120</td>
<td>87.6</td>
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<tr>
<td>Tanzania</td>
<td>1992</td>
<td>1120</td>
<td>6-12</td>
<td>500</td>
<td>21</td>
<td>22.4</td>
<td>82.4</td>
<td>Albonico et al (1994)</td>
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<tr>
<td>Tanzania</td>
<td>1999</td>
<td>236</td>
<td>6-9</td>
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<td>21</td>
<td>7.6</td>
<td>52.1</td>
<td>Albonico et al (2003)</td>
</tr>
<tr>
<td>Tanzania</td>
<td>2000</td>
<td>448</td>
<td>6-9</td>
<td>500</td>
<td>21</td>
<td>85.0</td>
<td>67.0</td>
<td>Albonico et al (2002)</td>
</tr>
</tbody>
</table>

CR: Cure rate; FECR: reduction in faecal egg count

1.6.3 Discussion

This review of BZ drug efficacy studies in school-aged children in Africa shows that there is a lack of consensus on the definition of efficacy or standardization of multiple indicators of morbidity in the evaluation of the impact of anthelminthics in PC programmes. It points to the need for standardized methods and tools developed for use in large-scale chemotherapy-based control programmes to better define and monitor the negative health consequences of helminth infections that may be reversed by treatment.

Overall, this review has noted the variation in the length of time between treatment and post-treatment follow-up as a major factor that vary across studies done so far in Africa. The other possible factors are: (i) differences in the brands of the drugs (might be a quality issue), (ii) lack of wide coverage (limited to very few geographical areas/countries) (iii) use of different sampling
techniques (either two slides per sample or two samples per single subject) (iv) differences in FECR calculations (while most used a geometric mean, a few used an arithmetic mean), and (v) to some extent, differences in dose and treatment regimens.

All these variations in one way or another influence the endpoint, i.e., the CR and FECR, and in turn lead to the variable conclusions drawn from such studies. It should also be noted that a rigorous evaluation of the geographic variation in efficacy of these drugs against STH is lacking, and the available data may not reflect the real picture in Africa. Hence, the possible differences in allele frequency in populations across the continent that could contribute to differences in efficacy are totally unknown.

1.7 Overall conclusions

Currently, drug administration in the form of PC is the stronghold strategy to control STH. BZ drugs are presently the drug of choice because of their relatively good efficacy for most STH species, safety, dosing (as they do not require weight-based dosing) and ease of administration in the treatment of individuals and/or whole communities. However, the present strategy of STH control through PC is not as straightforward and effective as might at first be anticipated. As revealed by this review of the literature, there are a number of gaps that need to be filled.

First, the frequency of PC is based on the prevalence of STH in a given affected community, yet still little is known about the demographic, socio-economic and environmental determinants that explain variations in STH infections both within and across populations. This, in turn, might limit how national governments and international organizations define, and target resources to combat, the disease burden due to STH infection.

Second, the efficacy of the two commonly-used BZ drugs (ALB and MEB) varies across STH species. Factors accounting for such variations remain poorly defined. Among other factors under consideration is the contribution of baseline infection intensity, which requires in-depth investigation under varying epidemiological settings and infection-intensity levels.

Third, despite the global expansion of PC, standard operating procedures to monitor anthelminthic drug efficacy are lacking. Hence, standardizing clinical
trials, determining therapeutic efficacy thresholds, and identifying appropriate indicators of efficacy are crucial for future improvement of control programmes.

Fourth, there are a number of factors that affect and complicate the assessment of drug efficacy (such as age group, drug resistant parasites, quality of drugs, previous history of PC, diet, etc). Those factors need to be further studied.

Fifth, healthcare decision-makers have a limited repertoire of strategies for rapid assessment of infection intensity and for checking drug-resistance development. Hence, searching for simplified fieldwork strategies for, and cost-effective approaches to, screening a large population remains an important research topic in this era of PC programmes.

Overall, there are a limited number of alternative anthelminthic drugs available today, and this underscores the need for a well-designed research protocol that might address the concerns raised in the course of control programme implementation and drug efficacy monitoring. The present PhD thesis was formulated based on the paucity of the body of knowledge regarding these concerns, and is devoted to generating scientific evidence to allow us to close the existing gaps in our knowledge.
References


Bundy DA; Hall A; Medley GF; Savioli L (1992) Evaluating measures to control intestinal parasitic infections. World Health stat Q;45:168-179
Dobson RJ, Sangster NC, Besier RB, Woodgate RG (2009) Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test. Vet Parasitol 161:162-167
Dobson RJ, Sangster NC, Besier RB, Woodgate RG (2009) Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test. Vet Parasitol 161:162-167


Objectives
The general objective of this PhD is to improve the control of soil-transmitted helminthiasis in school-aged children in Jimma Town.

Jimma is the capital of Jimma zone (one of the seventeen zones) in Oromia regional state of Ethiopia. It is situated about 350 kilometers (southwest) away from Addis Ababa. The zone has a total area of 19,293.5 square kilometers (details of the study area is described in chapter 2).

The four specific objectives are:

1. To determine the seasonal prevalence and intensity of soil-transmitted helminth infections among school-aged children in schools in Jimma Town.

2. To determine the efficacy of single dose albendazole and mebendazole against soil-transmitted helminths in school-aged children in Jimma Town.

3. To determine the repeated doses of albendazole and mebendazole against *Trichuris trichiura* in school-aged children in Jimma Town.

4. To develop a rapid and cost effective diagnostic strategy to assess the infection intensity and efficacy of anthelminthics.
Chapter 2

Assessment of Seasonality in Soil-Transmitted Helminth Infections across 14 Schools in Jimma Town, Ethiopia: a Meta-Analysis

Based on:
2.1 Introduction

Up to date, the mass drug administration (MDA) is the only strategy in control programmes targeting soil-transmitted helminths (STH). The frequency of these MDA is based on the overall STH prevalence, drugs being administered annually when the prevalence is at least 20% but less than 50% or bi-annually when the prevalence exceeds 50% (WHO, 2002, 2006; Montresor et al., 2012; see Table 1.2 of chapter 1). When the prevalence is less than 20%, it is recommended to provide a case-by-case (individual-based) treatment. However, the overall STH prevalence may be affected by climatic and/or seasonal changes. Adequate moisture and warm temperature are essential for egg/larval development in the soil (Brooker et al., 2006; Chen et al., 2008; Pullan and Brooker, 2012). For example, the eggs of of *A. lumbricoides* and *T. trichiura* will not embryonate at low humidity, whereas higher humidity is associated with faster development of eggs (Lorcan and Holland, 2000; Crompton, 2001), and hence influencing the transmission and eventually the prevalence estimates of STH. Furthermore, there is evidence that in rainy seasons, pre-parasitic stages of worms might survive in the environment, and subsequently increasing disease transmission. This is in contrast with dry climatic conditions, which negatively affect the infective stages on the soil surface, resulting in a drop in transmission (Weaver et al., 2010).

Overall, many epidemiology surveys revealed a correlation between the prevalence of parasitic infections, including STH, and the seasons of the year (Amaechi et al., 2013). It has been described that understanding of the seasonal changes of STH is essential to support public health decision-making to launch MDA programmes (Brooker et al., 2002; Weaver et al., 2010). For instance determining prevalence at times of the year when there is a low transmission of STH might underestimate the actual prevalence of infections. In addition, it is important to note that, administration of drugs at that time point when prevalence and/or infection intensity is at its highest might have a bigger impact on the morbidity caused by these worms.

In Ethiopia, and in Jimma Town (our study area) in particular, there is lack of up to date prevalence data that is required to determine the frequency of MDA. Moreover, season in Jimma Town are meteorologically different, and hence impact of MDA may vary across seasons. Therefore, the main objective of this
chapter was to describe the prevalence and infection intensity of any STH and the three species separately in two different seasons (dry vs. rainy season) in school across 14 primary schools in Jimma Town.

2.2 Materials and methods

2.2.1 Study sites and population

The study was conducted in Jimma Town (capital of Jimma Zone, Ethiopia), which is located approximately 350 km southwest of the capital Addis Ababa. The town has currently 174,000 inhabitants, and is situated at a latitude and longitude of 7° 40’ N 36° 50’ E, and at 1,720-2,010 m above sea level. It lies in a climatic zone locally known as Woina-dega, which is considered ideal for agriculture as well as human settlement. Like most of the subtropical (Woina-dega) regions of Ethiopia, the Jimma Zone is known to have the four seasons in a year: spring [September-November (end of rainy season)], winter [December-February (dry season)], autumn [March-May (beginning of rainy season)] and summer (June-August (rainy season)]. Of note, the term winter does not represent the characteristics of winter weather elsewhere. In average, the daily temperature equals 19 °C, but ranges from 12 to 30 °C. Maximum precipitation and heavy rain occurs between June and September (56.3% of the total rainfall), with minimum rainfall between October and February (17.5% of the total rainfall). In this study, for the sake of simplicity, the seasons were clustered into a rainy (June-November) and a dry season (December-May).

This study focused on school-aged children (SAC) from 5 to 18 years of age, covering all eight grades of primary school. In total, there were 24 primary schools hosting a total of 23,492 children. Figure 2.1a shows the map of Jimma Zone, and Figure 2.1b shows the schools studied in Jimma Town. The female/male ratio across the different schools was approximately 1:1 (Report Document 2011/2012 of Jimma Town Education Bureau). STH infections have been documented in Jimma Town, but at present, no control measures for STH in SAC have been implemented. The study was conducted in 14 out of 24 schools, as they were hosting all eight grades.
Figure 2.1 Map of Jimma Zone (a) and map of Jimma Town indicating the 14 schools included in the survey (b).
2.2.2 Study design

The assessment of prevalence and infection intensity of STH was carried out during the dry season (February-March, 2012) and at the end of rainy season (September-October, 2012) among 14 schools in Jimma, Ethiopia. For this study, a total of 1,680 schoolchildren (840 in each season) were included. The sample size was estimated based on varying epidemiological scenarios that at least 120 subjects per school (60 per season per school) were required for a cost-effective identification of regions in need of MDA (Sturrock et al., 2010).

To this end, all primary schools in Jimma Town hosting all eight grades of students were invited to participate. In each school, subjects were stratified into three age groups (5-9 years, 10-13 years, and 14-18 years). For each age group at least 20 subjects were selected on a voluntary basis, resulting in a total of at least 60 subjects per school. The subjects were asked to provide at least 3 g of stool. This quantity of stool was required to process and examine the samples individually using the McMaster egg counting method for detection and enumeration of STH eggs. Figure 2.2 illustrates the number of primary schools eligible, recruited, and included in the statistical analysis.
2.2.3 Parasitological examination

All stool samples were processed by the McMaster egg counting method. McMaster is a flotation technique that is commonly used in veterinary parasitology both to assess the intensity of gastro-intestinal parasite infections and to evaluate drug efficacy against these parasites. For the diagnosis and enumeration of STH in public health, it has been found to be user-friendly (vs. FLOTAC (Levecke et al., 2009) robust (vs. Kato-Katz thick smear (Levecke et al., 2011) and accurate for enumeration of STH, but less sensitive when intensity of
infection is low (vs. Kato-Katz and FLOTAC (Levecke et al., 2009; Levecke et al., 2011). The procedure for this method was done as previously described by Levecke et al., 2011. In short, two grams of stool was suspended in 30 ml of saturated salt solution (NaCl). The faecal suspension was poured three times through a wire mesh to remove large debris. Then, two chambers of a McMaster slide (Figure 2.3) were filled with this suspension. Both chambers were examined under a light microscope using a 100x magnification and faecal egg count (FEC) as eggs per gram of stool (EPG) for each helminth species were obtained by multiplying the total number of eggs by 50. A tutorial on how to perform the McMaster egg counting method can be found at http://www.youtube.com/watch?v=UZ8tzswA3tc, a tutorial on how to organize the staff in the laboratory to process a large number of stool samples can be found at: https://www.youtube.com/watch?v=q_yfdtE3TSE

Figure 2.3 McMaster slide used to process and quantify the eggs of any soil-transmitted helminths.

2.2.4 Statistical analysis

The apparent prevalence (% of children excreting eggs) and the infection intensity (mean FEC) were calculated on STH species for the different schools, both sexes, three age groups (5-9 years, 10-13 years and 14-18 years) and two seasons (dry vs. rainy). A meta-analysis was performed to assess any differences in prevalence and infection intensity between the two seasons. To this end, random effect models were built at the school level based on the odds ratio and the difference in mean FEC (mean FEC in the rainy season-mean FEC in the dry season), respectively. The statistical analysis was performed in the statistical software R (The R Foundation for Statistical Computing, version 3.0). The meta-analysis was carried out using the ‘metafor’ package of the statistical software R. The level of significance was set at $p < 0.05$. 
2.3 Results

2.3.1 Overall prevalence and infection intensity

Infections with any STH were observed in 824 out of the 1,680 subjects screened (49.0%). *T. trichiura* was the most prevalent (n=596, 35.5%), followed by *A. lumbricoides* (n = 394, 23.4%). Hookworm was observed in 166 subjects (9.9%). STH infections were observed in all 14 schools, except for hookworms for which infections were absent in 2 schools. Overall, the mean FEC was 1,938 EPG, 207 EPG and 29 EPG for *A. lumbricoides*, *T. trichiura* and hookworm, respectively. The prevalence and infection intensity varied across schools and age groups, but not between sexes. Appendix 1.1 reports the prevalence and infection intensity of any STH and the three STH species separately for the 14 schools, 2 sexes, and three age groups, but these results will not be further discussed in detail.

2.3.2 Seasonal differences in prevalence and infection intensity

The prevalence and infection intensity of any STH and the three STH species across the two seasons is reported in Tables 2.1 and 2.2 respectively. STH infections were more prevalent in the dry season (52.4%) than in the rainy season (45.7%). This seasonality in prevalence was most pronounced for *T. trichiura* (39.2% vs. 31.8%), followed by hookworms (11.4% vs. 8.3%). For *A. lumbricoides*, the proportion of infections across seasons was comparable (23.9% vs. 23.0%). Intensity of all three STH infections was higher in the dry season. This seasonality in infection intensity was most pronounced for *A. lumbricoides* (2,411 EPG vs. 1,465 EPG), followed by *T. trichiura* (295 EPG vs. 119 EPG) and hookworms (35 EPG vs. 23 EPG).
Table 2.1. The prevalence of any soil-transmitted helminth, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections at the school level for the rainy and the dry season.

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<td></td>
<td></td>
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<td>Dry (%)</td>
<td>Rainy (%)</td>
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<td>70.0</td>
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<td>31.8</td>
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Table 2.2. The intensity of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections at the school level for the rainy and the dry season.

<table>
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<th>School</th>
<th>Sample size (rainy/dry)</th>
<th><em>A. Lumbricoides</em></th>
<th><em>T. Trichiura</em></th>
<th>Hookworm</th>
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</tr>
<tr>
<td>11</td>
<td>60/60</td>
<td>1,343</td>
<td>2,442</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>60/60</td>
<td>610</td>
<td>799</td>
<td>128</td>
</tr>
<tr>
<td>13</td>
<td>61/60</td>
<td>413</td>
<td>2,707</td>
<td>42</td>
</tr>
<tr>
<td>14</td>
<td>60/60</td>
<td>317</td>
<td>907</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>840/840</strong></td>
<td><strong>1,465</strong></td>
<td><strong>2,411</strong></td>
<td><strong>119</strong></td>
</tr>
</tbody>
</table>

The intensity of infections is measured by the mean of individual faecal egg counts (expressed in eggs per gram of stool (EPG)).

Overall, any STH infection was less prevalent in the rainy season compared to the dry season, the odds of any STH infection is 0.74 (95% confidence interval [0.57-0.97], \( z = -2.19, p = 0.03 \)) times smaller than the odds of any STH infection in the dry season (Figure 2.4).
Chapter 2: Assessment of Seasonality in STH Infections in SAC in Jimma Town

Figure 2.4 the odds ratio of any soil-transmitted helminth infection across seasons (95% confidence interval)

The odds ratio of infection and difference in infection intensity are reported separately for *A. lumbricoides*, *T. trichiura* and hookworm in Figures 2.4, 2.5, and 2.6, respectively. An odds ratio significantly different from 1 was only observed for *T. trichiura* infections (Figure 2.5).
Chapter 2

Figure 2.5 The odds ratio of infection and the difference in infection intensity for *Trichuris trichiura* across seasons

For this STH, the odds of infection in the rainy season were 0.71 ([0.53-0.96], \( z = -2.26, p = 0.02 \)) times smaller than those in the dry season. A comparable odds ratio (0.68 [0.68-1.06]) was observed for hookworms, however this ratio was not significantly different from 1 (\( z = -1.71, p = 0.06 \)) (Figure 2.6).

For *A. lumbricoides* infections, the odds ratio equalled 1.00 [0.68-1.48] (\( z = 0.02, p = 0.98 \)) (Figure 2.7).
A significant difference in infection intensity across seasons was observed for *A. lumbricoides* (Figure 2.7) and *T. trichiura* (Figure 2.5). For both STH species, infection intensities were lower in the rainy season compared to those in the dry season. The estimated difference in infection intensity equalled 569 EPG ([35-1104], $z = -2.09$, $p = 0.04$) for *A. lumbricoides* and 60 EPG ([1-120], $z = -1.98$, $p = 0.05$) for *T. trichiura*. For hookworms, there was no significant difference in infection intensity across seasons (= 10 EPG [-8-28], $z = -1.11$, $p = 0.27$).

Table 2.3. summarizes the heterogeneity in effect size between the different schools for any STH and for the three STH species separately. With the exception for the model describing the odds ratios across seasons for hookworms, there was a statistical evidence of heterogeneity in effect size between schools (Q-statistic: 21.5-27.0, $p$: 0.01-0.03). The heterogeneity in effect size between schools accounted for at least 45% of the total variation in effect size.
Table 2.3. The heterogeneity in effect size for the random effect models describing the odds ratio and the difference in intensity of any STH, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections.

<table>
<thead>
<tr>
<th></th>
<th>Odds ratio</th>
<th>Difference in Infection Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q statistic</td>
<td>Total heterogeneity / total variability (%)</td>
</tr>
<tr>
<td>Any STH</td>
<td>23.2 0.04</td>
<td>40.5 NA</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>25.1 0.02</td>
<td>60.3 25.6 0.02</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>27.0 0.01</td>
<td>48.5 25.9 0.02</td>
</tr>
<tr>
<td>Hookworm</td>
<td>14.9 0.19</td>
<td>34.4 21.5 0.03</td>
</tr>
</tbody>
</table>

2.4 Discussion

Overall, the prevalence and intensity of any STH infection using the McMaster was high among SAC in Jimma Town. Although the overall prevalence in Jimma did not exceed the 50%, it is suggested to administer drugs twice a year. This is because the diagnostic method applied lacks sensitivity, and hence the true prevalence is underestimated.

This study indicates that, even within a well-defined geographic area located in less than 10 km radius (Figure 2.1), STH infections are not equally distributed. This finding supports results of recent studies revealing considerable and distinct spatial heterogeneity in STH infections (Stothard *et al.*, 2008; Knopp *et al.*, 2008). However, it remains unclear which demographic, socio-economic and environmental factors are causing these distributions. Although it has been documented that unhygienic sanitation, and inadequate water supply are contributing to disease transmission (Bethony *et al.*, 2006; Ohta and Waikagul, 2007), these are less likely to be the cause of the observed STH distribution. Aspects that need further attention are soil types of the specific study locations, as it has been shown that this may play a significant role in the transmission of STH (Brooker *et al.*, 2003; 2004; Saathoff *et al.*, 2005a, 2005).

School children were marginally more infected with *T. trichiura* and hookworms during dry season. The occurrence of *A. lumbricoides* infections was equally distributed across seasons. A significant difference in infection intensity across seasons was observed for both *A. lumbricoides* and *T. trichiura*, the
intensity of infections being significantly more pronounced in the dry season. For hookworms no significant difference was observed. This seasonality in STH infection can be explained by the washing effect during the rainy season, eggs being washed away from the soil after heavy rains. This hypothesis is supported by the findings of Nwoke and colleagues (2013). In this study, the highest number of eggs recovered from the soil were observed in the dry season (18.7%), whereas this was 12.0% in the rainy season. Nevertheless, it has been argued that although seasonal dynamics in transmission may occur, such fluctuations may be of little significance to the overall parasite equilibrium within communities (Anderson and May, 1991).

We conclude that STH infections are a public health problem in Jimma Town, and a biannually MDA is recommended. Given the seasonality in STH infections it is recommended to treat at least once in the dry season. The study also highlights once more the considerably variation among schools, even within small geographical area, which needs further attention.

2.5 Ethics Statement

Ethical approval was obtained from Jimma University Institutional Review Board. The school authorities, teachers, parents, and the children were informed about the purpose and procedures of the study. The written consent form was prepared in two commonly used local languages (Afaan Oromo and Amharic) and handed over to the children’s parents/guardians. Only those children (i) who were willing to participate and (ii) whose parents or guardians signed the written informed consent form were included in the study. Following submission of stool sample, a single-oral dose of 400 mg albendazole was administered to all subjects.

2.6 Acknowledgements

The authors are grateful to the schoolteachers, study subjects, and parents who allowed their children to participate. Additionally, we would like to thank the staff of Medical Laboratory Sciences, Jimma University (Mio Ayana, Ahmed Zeynudin, Dereje Atomisa, Mitiku Bajaro, Dereje Jirata, Nuredin Abduselam, Tesfaye Deme, and Mestawet Getachew) for processing stool sample
samples and/or technical/medical assistance in the field. Finally, we would like to thank the VLIR-IUC/JU for supporting financially this study.
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References


Appendix 2.1. The prevalence and infection intensity by means of faecal egg counts across 14 schools in Jimma Town, Ethiopia, 2 sexes, 3 age groups and 2 seasons.

<table>
<thead>
<tr>
<th>School</th>
<th>N</th>
<th>STH (%)</th>
<th>A. lumbricoides</th>
<th>T. trichiura</th>
<th>Hookworms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Prevalence (%)</td>
<td>Mean FEC (EPG)</td>
<td>Prevalence (%)</td>
</tr>
<tr>
<td>1</td>
<td>120</td>
<td>29.2</td>
<td>12.5</td>
<td>555</td>
<td>20.0</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>16.7</td>
<td>6.7</td>
<td>436</td>
<td>10.8</td>
</tr>
<tr>
<td>3</td>
<td>120</td>
<td>57.5</td>
<td>30.0</td>
<td>1,983</td>
<td>40.8</td>
</tr>
<tr>
<td>4</td>
<td>119</td>
<td>26.9</td>
<td>6.7</td>
<td>317</td>
<td>26.1</td>
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<tr>
<td>5</td>
<td>120</td>
<td>62.5</td>
<td>35.8</td>
<td>4,138</td>
<td>42.5</td>
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<tr>
<td>6</td>
<td>120</td>
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<td>27.5</td>
<td>2,055</td>
<td>52.5</td>
</tr>
<tr>
<td>7</td>
<td>120</td>
<td>54.2</td>
<td>22.5</td>
<td>2,840</td>
<td>37.5</td>
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<tr>
<td>8</td>
<td>120</td>
<td>68.3</td>
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<td>3,242</td>
<td>55.0</td>
</tr>
<tr>
<td>9</td>
<td>120</td>
<td>60.0</td>
<td>33.3</td>
<td>1,875</td>
<td>34.2</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>60.0</td>
<td>33.3</td>
<td>4,926</td>
<td>41.7</td>
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<td>11</td>
<td>120</td>
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<td>1,892</td>
<td>38.3</td>
</tr>
<tr>
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<td>120</td>
<td>55.0</td>
<td>17.5</td>
<td>705</td>
<td>41.7</td>
</tr>
<tr>
<td>13</td>
<td>121</td>
<td>55.4</td>
<td>28.9</td>
<td>1,550</td>
<td>34.7</td>
</tr>
<tr>
<td>14</td>
<td>120</td>
<td>24.2</td>
<td>7.5</td>
<td>612</td>
<td>20.8</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>782</td>
<td>50.0</td>
<td>23.3</td>
<td>1,908</td>
<td>34.5</td>
</tr>
<tr>
<td>Male</td>
<td>898</td>
<td>48.2</td>
<td>23.6</td>
<td>1,968</td>
<td>36.3</td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-9 yrs</td>
<td>539</td>
<td>47.7</td>
<td>26.0</td>
<td>2,884</td>
<td>37.1</td>
</tr>
<tr>
<td>10-13 yrs</td>
<td>603</td>
<td>47.6</td>
<td>21.2</td>
<td>1,727</td>
<td>35.2</td>
</tr>
<tr>
<td>14-18 yrs</td>
<td>538</td>
<td>52.0</td>
<td>23.4</td>
<td>1,228</td>
<td>34.2</td>
</tr>
<tr>
<td>Total</td>
<td>1,680</td>
<td>49.0</td>
<td>23.5</td>
<td>1,938</td>
<td>35.5</td>
</tr>
</tbody>
</table>
Chapter 3

Efficacy of Single Oral Dose Albendazole and Mebendazole against Soil-Transmitted-Helminths in School Children in Jimma Town, Ethiopia

Based On:


Note: MZ was the principal investigator of the trial in Ethiopia.
3.1 Introduction

To control the burden of soil-transmitted helminths (STH) on public health the World Health Organization (WHO) recommends the implementation of preventive chemotherapy (PC) programmes, in which a single oral dose of albendazole (ALB, 400 mg) or mebendazole (MEB, 500 mg) are periodically administered to preschool-aged (preSAC) and school-aged children (SAC) (WHO, 2006, 2010, 2011). These PC programmes have recently received increased political and scientific attention (WHO, 2012; Gabrielli et al., 2011). The WHO has devised a roadmap to guide implementation of the policies and strategies set out in a global plan to combat neglected tropical diseases (NTD, period 2008-2015). More than 70 pharmaceutical companies, governments, and global health organizations committed to support this roadmap (WHO, 2011) in the London Declaration on NTD in January 2012 by sustaining or expanding drug donation programmes (Uniting to Combat NTDs, 2013).

These pledges of drug donations are now in place. However, we are relying only on two drugs with the same mode of action, and hence the emergence of anthelminthic resistance (AR) as drug donations expand, as substantiated in veterinary medicine, is likely (Geerts and Gryseels, 2001; van Wyk, 2001; Wolstenholme et al., 2004). This would particularly become a problem when there is a paucity of alternative drugs (Awasthi et al., 2003; Olliaro et al., 2011). As a consequence of this, it is imperative that monitoring systems are designed to detect any change in drug efficacy due to emerging resistance of the parasites against these benzimidazole (BZ) drugs (Geerts and Gryseels, 2001; Albonico M, 2004).

Currently, the assessment of eggs in stool following drug administration by means of cure rate (CR) and reduction in faecal egg counts (FECR) is the most commonly applied assay for monitoring the efficacy of anthelminthic drugs against STH (Keiser and Utzinger, 2008; see Chapter 1). In contrast to other available assays, it allows for the assessment of drug efficacy against all three STH (vs. in vitro assays and molecular assays) with a minimum of laboratory equipment (vs. molecular assays) (Smout et al., 2010; Kotze et al., 2011; Diawara et al., 2013). However, interpretation of the results from the stool assay remains difficult, since reliable reference efficacy values of BZ drugs against each of the STH are still lacking. In a systematic review and meta-
analysis of published efficacy trials targeting STH, Keiser and Utzinger (2008) highlighted recently that there is a lack of high quality trials to determine these reference values. Available efficacy data are obtained through a variety of study protocols, including variation in diagnostic methods, follow-up periods, origin of drugs and statistical analyses (see also Chapter 1).

The objective of the present work was therefore to assess the efficacy of a single oral dose ALB (400 mg) and a single oral dose MEB (500 mg) against STH infections in SAC based on a protocol standardized in terms of diagnostic method (McMaster egg counting method), follow-up period (approximately 14 days), origin of the drugs (ALB: Zentel, GlaxoKlineSmith, batch N° L298; MEB: Vermox, Janssen-Cilag, Latina, Italy, batch N° BCL2F00) and statistical analysis of CR and different formulae of FECR. To give the study wide relevance, the study was conducted in seven populations of SAC in Brazil, Cameroon, Cambodia, Ethiopia, India, Tanzania and Vietnam. In the present Chapter, we will present the results obtained in Ethiopia. In the discussion, we will put these results obtained in Ethiopia more in perspective by comparing the results with results obtained in other countries.

3.2 Materials and methods

3.2.1 Study Area and Study Population

Both the ALB and the MEB trial were conducted in Jimma Town. The ALB trial was conducted in school 9 in May 2009. The MEB trial was conducted in school 6 and school 9 in December 2011. For details on the epidemiology of STH in these two schools, we refer the reader to Chapter 2.

3.2.2 Study design

A standardized protocol was applied for both trials. In short, during the baseline survey, school children aged 5 to 18 at the different study sites (Brazil, Cameroon, Cambodia, Ethiopia, India, Tanzania and Vietnam) were asked to provide a stool sample. For the initial sampling, the aim was to enrol at least 250 infected children with a minimum of 150 eggs per gram of stool (EPG) for at least one of the STH.
This sample size was selected based on statistical analysis of study power, using random simulations of correlated over-dispersed faecal egg count (FEC) data reflecting the variance-covariance structure in a selection of real FEC data sets. This analysis suggested that a sample size of up to 200 individuals (alpha = 0.05, power = 80%) was required to detect a 10 percentage point drop from a null efficacy of 80% (mean percentage FEC difference per individual) over a wide range of infection scenarios. Standard power analyses for proportions also indicated that the detection of a 10% point drop from a null CR required sample sizes up to 200 (the largest samples being required to detect departures from null efficacies of around 50%). Given an anticipated non-compliance rate of 25%, a sample of 250 individuals with 150 EPG pre-treatment was therefore considered necessary.

All children providing stool samples were treated under vision with a single oral dose ALB (400 mg, Zentel, GlaxoKlineSmith, batch N° L298) or a single oral dose MEB (500 mg, Vermox, Janssen-Cilag, Latina, Italy, batch N° BCL2F00) under supervision (chewing + water). No placebo control subjects were included in the trial for ethical and operational reasons. Approximately 7 to 15 days (but exactly 14 days for the site of Ethiopia) after the baseline survey, stool samples were collected from the treated subjects. All samples were processed using the McMaster egg counting method within the same day of collection (see Chapter 2). None of the samples were preserved. Subjects who were unable to provide a stool sample at follow-up, or were experiencing a severe concurrent medical condition or had diarrhoea at time of the first sampling, were excluded from the study.

The participation, the occurrence of STH and sample submission compliance at baseline and follow-up surveys in the ALB and MEB trial conducted in Ethiopia are summarized in Figure 3.1. The participation, the occurrence of STH and sample submission compliance for baseline and follow-up surveys for the remaining sites involved are reported elsewhere (Vercruysse et al., 2011 for ALB and Levecke et al., 2014 for MEB).
Chapter 3: Efficacy of ALB and MEB against STH in School Children

Figure 3.1 The enrolment of school-aged children, occurrence of soil-transmitted helminths and sample submission compliance at baseline and follow-up surveys. Subjects who were not able to provide a sample for the follow-up, or who were experiencing a severe current medical condition or had diarrhoea at the time of the first sampling were excluded from the trial.

3.2.3 Statistical analysis

The efficacy of the treatment for each of the three STH was evaluated qualitatively based on the reduction in infected children (CR) and quantitatively based on the FECR. The outcome of the FECR was calculated using three formulae. The first two formulae were based on the mean (arithmetic/geometric) of the baseline and follow-up intervention FEC largely ignoring the individual
variability, whereas the third formula represented the mean of the reduction in the FEC per subject.

\[
\text{FECR (1)} = 100 \times \left( 1 - \frac{\sum_{i=1}^{n} \text{FEC at follow up}_i}{\sum_{i=1}^{n} \text{FEC at baseline}_i} \right)
\]

\[
\text{FECR (2)} = 100 \times \left( 1 - \frac{\sum_{i=1}^{n} \ln \left( \frac{\text{FEC at follow up}_i + 1}{\text{FEC at baseline}_i + 1} \right)}{n} \right)
\]

\[
\text{FECR (3)} = 100 \times \left( 1 - \frac{\sum_{i=1}^{n} \text{FEC at follow up}_i}{\sum_{i=1}^{n} \text{FEC at baseline}_i} \right)
\]

3.3 Results

3.3.1 Cure rate

Table 3.1 summarizes the CR for ALB and MEB. Overall, the CR of ALB was the highest for *A. lumbricoides* (99.3%), followed by hookworm (98.9%). The lowest CR was observed for *T. trichiura* (85.7%). For MEB, a similar trend was observed, highest CR results observed for *A. lumbricoides* and lowest for *T. trichiura*, however MEB resulted in lower CR results compared to ALB. Overall, the efficacy of MEB by means of CR was 90.3% for *A. lumbricoides*, 58.0% for hookworm and 50.9% for *T. trichiura*. 


Table 3.1 Efficacy measured by means of cure rate and reduction in faecal egg counts of a single oral dose albendazole (400 mg) and a single oral dose mebendazole (500 mg) against *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm infections in school children in Jimma Town, Ethiopia

<table>
<thead>
<tr>
<th></th>
<th>ALB</th>
<th>MEB</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>School 9</td>
<td>School 6</td>
<td>School 9</td>
<td>School 6+9</td>
</tr>
<tr>
<td><em>A. lumbricoides</em></td>
<td>N = 151</td>
<td>N = 133</td>
<td>N = 146</td>
<td>N = 279</td>
</tr>
<tr>
<td>CR (%)</td>
<td>99.3</td>
<td>87.2</td>
<td>93.1</td>
<td>90.3</td>
</tr>
<tr>
<td>FECR (1) (%)</td>
<td>&gt;99.9</td>
<td>97.6</td>
<td>99.4</td>
<td>98.6</td>
</tr>
<tr>
<td>FECR (2) (%)</td>
<td>&gt;99.9</td>
<td>&gt;99.9</td>
<td>&gt;99.9</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td>FECR (3) (%)</td>
<td>&gt;99.9</td>
<td>92.5</td>
<td>93.0</td>
<td>92.7</td>
</tr>
<tr>
<td><em>T. trichiura</em></td>
<td>N = 105</td>
<td>N = 197</td>
<td>N = 129</td>
<td>N = 326</td>
</tr>
<tr>
<td>CR (%)</td>
<td>85.7</td>
<td>47.2</td>
<td>56.6</td>
<td>50.9</td>
</tr>
<tr>
<td>FECR (1) (%)</td>
<td>92.4</td>
<td>67.7</td>
<td>60.5</td>
<td>65.9</td>
</tr>
<tr>
<td>FECR (2) (%)</td>
<td>99.5</td>
<td>95.1</td>
<td>95.3</td>
<td>95.1</td>
</tr>
<tr>
<td>FECR (3) (%)</td>
<td>93.0</td>
<td>57.6</td>
<td>13.2</td>
<td>40.0</td>
</tr>
<tr>
<td>Hookworm</td>
<td>N = 91</td>
<td>N = 18</td>
<td>N = 82</td>
<td>N = 100</td>
</tr>
<tr>
<td>CR (%)</td>
<td>98.9</td>
<td>61.1</td>
<td>57.3</td>
<td>58.0</td>
</tr>
<tr>
<td>FECR (1) (%)</td>
<td>99.7</td>
<td>51.9</td>
<td>67.7</td>
<td>65.4</td>
</tr>
<tr>
<td>FECR (2) (%)</td>
<td>&gt;99.9</td>
<td>94.9</td>
<td>95.3</td>
<td>95.3</td>
</tr>
<tr>
<td>FECR (3) (%)</td>
<td>99.9</td>
<td>68.9</td>
<td>66.1</td>
<td>66.6</td>
</tr>
</tbody>
</table>

3.3.2 Faecal egg count reduction rate

Figure 3.2 indicates the pre-intervention FEC for *A. lumbricoides*, *T. trichiura* and hookworm for the ALB and MEB trial, respectively. The pre-intervention FEC in the ALB trial ranged from 50 to 62,500 EPG for *A. lumbricoides* (arithmetic mean = 3,443 EPG), from 50 to 5,200 EPG for *T. trichiura* (arithmetic mean = 420 EPG) and from 50 to 1,650 EPG for hookworm (arithmetic mean = 326 EPG). In the MEB trial the pre-intervention FEC ranged from 50 to 60,950 EPG for *A. lumbricoides* (arithmetic mean = 6,497 EPG), from 50 to 11,750 EPG for *T. trichiura* (arithmetic mean = 653 EPG) and from 50 to 1,450 EPG for hookworm (arithmetic mean = 266 EPG). As illustrated in Figures 3.2 pre-intervention FEC were highly aggregated, majority of the eggs being excreted by a minority of the subjects.
Figure 3.2. The distribution of pre-intervention faecal egg counts for *Ascaris lumbricoides* (top row graphs), *Trichuris trichiura* (middle row graphs) and hookworm (bottom row graphs) for the albendazole (left column) and mebendazole trial (right column).
The FECR calculated using all three formulae (based on FECR (1)-(3)) for both ALB and MEB in turn for *A. lumbricoides*, *T. trichiura* and hookworms are summarized in Table 3.1. Overall, FECR (1) for ALB was the highest for *A. lumbricoides* (>99.9%), followed by hookworm (99.7%) and *T. trichiura* (92.4%). Compared to ALB, MEB resulted in lower FECR (1) results for both hookworm (65.4%) and *T. trichiura* (65.4%). Efficacy results for *A. lumbricoides* for MEB (98.6%) were comparable to that of ALB.

Compared to FECR (1), FECR (2) gave higher efficacy results. Of the 12 estimates of drug efficacy based on FECR (1) summarized in Table 3.1, FECR (2) provided higher efficacy results in 11 cases. The most pronounced differences between FECR formulae were observed in the MEB trial. In this trial the difference ranged from 27.4 (school 6) to 34.8% (school 9) for *T. trichiura* and from 27.6% (school 9) to 43.0% (school 6) for hookworm. For the remaining estimates, the difference did not exceed 7.1% (*T. trichiura* ALB trial). Overall, FECR (3) resulted in lower efficacy results in 6 out of 12 cases, with a difference ranging from 5.1% (MEB, school 6, *A. lumbricoides*) to 43.1% (MEB trial, school 9, *T. trichiura*). In 5 cases, the difference between efficacy summarized by FECR (1) and FECR (3) was less than <2%. In the remaining cases (MEB, school 6, hookworm), the FECR (3) resulted in higher efficacy results (68.9% vs. 61.1%).

### 3.4 Discussions

Overall, the results in Ethiopia support previous reports indicating (i) that a single dose of both ALB and MEB is most effective for infection with *A. lumbricoides*, (ii) that ALB is more effective against hookworm infections compared to MEB and (iii) that MEB has a poor efficacy for *T. trichiura* infections (Bennet and Guyatt, 2000; Keiser and Utzinger 2008, Chapter 1). However, the results did not confirm a poor efficacy of ALB against *T. trichiura* for which a 3-day dose schedule of ALB has been shown to be necessary to achieve acceptable therapeutic efficacy (Bennet and Guyatt, 2000). In addition to this, the results highlight that different metrics of drug efficacy (CR and FECR formulae) rarely provide comparable results, and hence impede readily interpretation/comparison of drug efficacy results.

To put the efficacy results of both ALB and MEB obtained in Ethiopia in perspective, this section will now discuss the findings obtained from the
combined data sets. Further analysis on the combined data set indicated that CR should not be the recommended parameter, as it is sensitive to variation in the intensity of infection before treatment. In addition, it can be affected by the sensitivity of the diagnostic methods used. The CR of ALB declined in all three STH with increasing intensity of infection (FEC) at the pre-intervention survey (Figure 3.3), including Ethiopia. Hence, comparison between populations (e.g., countries, villages, schools) differing in pre-intervention FEC are guaranteed to arrive at different conclusions about drug efficacy.

Differences in the outputs of calculations based on processing quantitative data in different ways also showed variation that requires careful review if standard operating procedures for data processing are to be adopted. The observation that therapeutic efficacies based on arithmetic means were mostly lower than those based on geometric means is in agreement with another study (Dobson et al., 2009), and arises because the arithmetic means captures the variation more effectively, while the geometric means compress the data such that efficacies are highly overestimated.

![Figure 3.3](image_url) *Figure 3.3 The factors affecting the cure rate of Ascaris lumbricoides (A), Trichuris trichiura (B) and hookworms (C).* Generalized linear models (binomial error) were built with the test result (infected/uninfected) as the outcome, ‘trial’ (7 levels: trials in Brazil, Cambodia, Cameroon, Ethiopia, India, Tanzania and Vietnam) and ‘sex’ (2 levels: female and male) as factors, and ‘age’ and the log transformed pre-intervention FEC as covariates. Full factorial models were evaluated by the backward selection procedure using the likelihood ratio test of $\chi^2$. The level of significance was set at $p<0.05$. Finally, the CR for each of the observed values of the covariate and factor was calculated based on these models (The R Foundation for Statistical Computing,
version 2.10.0). Only 3 ages (9, 10 and 11) were considered in the graph A, as they represent 50% of the total study population.

An additional exploratory analysis of different statistical approaches for analyzing data also indicates that FECR based on individuals was highly affected by excluding subjects with pre-intervention FEC below 150 EPG (Table 3.2). Therefore, this finding conclude that the group-based formula using an arithmetic mean is the best summary statistic to employ in analysis of therapeutic efficacy in future large-scale drug administration trials, since it represents a robust indicator that is sensitive to changes in drug efficacy.

Although it can be argued that the application of a diagnostic method with poor sensitivity, such as the McMaster (Levecke et al., 2011), may result in biased efficacy results, there is a growing literature suggesting that the sensitivity of the diagnostic method has little impact on assessment of drug efficacy estimates as it is summarized using FECR (1) (Albonico et al., 2012, Levecke et al., 2014).

Table 3.2 Faecal egg count reduction (FECR (1)) for Trichuris trichiura across 7 countries based on all subjects excreting eggs at pre-intervention and subjects that are at least excreting less 150 eggs per gram of stool.

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>FECR (1) (%)</th>
<th>FECR (2) (%)</th>
<th>FECR (3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>1*</td>
<td>100.0</td>
<td>98.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Cambodia</td>
<td>2*</td>
<td>100.0</td>
<td>92.2</td>
<td>100.0</td>
</tr>
<tr>
<td>Cameroon</td>
<td>386</td>
<td>39.2</td>
<td>93.0</td>
<td>34.7</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>105</td>
<td>92.4</td>
<td>99.1</td>
<td>93.1</td>
</tr>
<tr>
<td>India</td>
<td>18*</td>
<td>74.5</td>
<td>98.9</td>
<td>92.1</td>
</tr>
<tr>
<td>Tanzania</td>
<td>396</td>
<td>52.0</td>
<td>82.6</td>
<td>-36.2</td>
</tr>
<tr>
<td>Vietnam</td>
<td>138</td>
<td>92.3</td>
<td>98.8</td>
<td>86.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Country</th>
<th>N</th>
<th>FECR (1) (%)</th>
<th>FECR (2) (%)</th>
<th>FECR (3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>0*</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cambodia</td>
<td>1</td>
<td>100.0</td>
<td>99.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Cameroon</td>
<td>233</td>
<td>39.9</td>
<td>93.4</td>
<td>50.4</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>72</td>
<td>92.3</td>
<td>99.2</td>
<td>92.6</td>
</tr>
<tr>
<td>India</td>
<td>11*</td>
<td>72.0</td>
<td>99.1</td>
<td>87.0</td>
</tr>
<tr>
<td>Tanzania</td>
<td>325</td>
<td>58.3</td>
<td>86.6</td>
<td>36.4</td>
</tr>
<tr>
<td>Vietnam</td>
<td>71</td>
<td>93.1</td>
<td>99.2</td>
<td>88.0</td>
</tr>
</tbody>
</table>

FEC: faecal egg count, EPG: eggs per gram of stool; FECR: reduction in faecal egg counts; FECR (1): group based and arithmetic mean; FECR (2): group based and geometric mean; FECR (3): individual based and arithmetic.

*Due to the low number of infected subjects (<50), the trials conducted in these countries were excluded from further analysis.
A meta-analysis of the outcome of the different trials showed that the estimated efficacy [95% confidence interval] of MEB was highest for *A. lumbricoides* (97.6% [95.8; 99.5]), followed by hookworms (79.6% [71.0; 88.3]). For *T. trichiura*, the estimated efficacy was 63.1% [51.6; 74.6]. Compared to MEB, ALB was significantly more effective against hookworm (96.2% [91.1; 101.2], \( p = 0.0001 \)) and to a lesser extent against *A. lumbricoides* infections (99.9% [99.0; 100]), but equally effective for *T. trichiura* infections (64.5% [44.4; 84.7]). In addition to this, the efficacies of both ALB and MEB were dependent on infection intensity in both *A. lumbricoides* and *T. trichiura*, decreasing in both cases with increasing infection intensity (Figure 3.4). However, the magnitude of this loss of efficacy as a function of increasing infection intensity differed between both BZ drugs and the STH. Between BZ drugs, the change in drug efficacy was more pronounced for MEB with *A. lumbricoides*, whereas for *T. trichiura* the decrease was more pronounced for ALB. Between STH, the overall impact of infection intensity on treatment with BZ was pronounced for *T. trichiura* (1.2-7.8% per 100 EPG), but almost negligible for *A. lumbricoides* (0.4% per 1000 EPG).

For hookworm the efficacy did not depend on the infection intensity. This could be explained by either a true constant efficacy across infection intensity or a low number of moderate and high infection intensities in these trials. The bases of these differences in efficacy between the BZ drugs and their effects on STH remain unclear, mainly due to the paucity of detailed pharmacokinetic and pharmacodynamics studies in pediatric populations in STH endemic countries (Horton, 2000; Dayan, 2003; Utzinger and Keiser, 2004).
Figure 3.4 The efficacy of a single oral dose albendazole (400 mg) and a single oral dose of mebendazole (500 mg) as a function of infection intensity at pre-intervention survey. The estimated efficacy (straight line) and 95% confidence intervals (dashed line) of a single oral dose of 400 mg ALB (black) and a single oral dose of 500 mg MEB (red) as a function of infection intensity at the pre-intervention survey (arithmetic mean of FEC) for Ascaris lumbricoides and Trichuris trichiura. To assess the impact of infection intensity on drug efficacy, generalized mixed effect models were fitted for each of the three STH species with FECR at the trial level as the dependent variable, and BZ drug (two levels: ALB and MEB) and mean FEC at the pre-intervention survey as covariates, and the interaction between these covariates. The meta-analysis was carried out using the ‘metafor’ package of the statistical software R. The level of significance was set at $p < 0.05$.

These findings emphasize a need to adhere to strict standard operating procedures (SOP) and methodologies, and to change the WHO recommended threshold levels for the efficacy of BZ (WHO, 1999) where a FEC reduction rate below 70% in the case of A. lumbricoides or below 50% for the hookworms are the currently accepted thresholds. Thus, it is recommended that in future monitoring of single-dose ALB-dependent control programmes a minimum FEC reduction rate (based on arithmetic means) of 95% for A. lumbricoides and 90% for hookworms and 50% for T. trichiura are appropriate thresholds, and that
efficacy levels below this should raise concern. For a single oral dose MEB, we propose 95%, 70% and 50%, respectively.

In conclusion, the present study is the first to evaluate drug efficacy of a single oral dose of ALB (400 mg) and MEB (500 mg) in Jimma Town (Ethiopia) as part of a multi-national trial representing such a large scale across three continents. The results confirm the therapeutic efficacy of these treatments against *A. lumbricoides*, the superior efficacy of ALB against hookworm infections, and the low efficacy against *T. trichiura*. The efficacy widely varied across the seven different trials, and infection intensity is thought to be an important confounding factor. The FEC reduction rate based on arithmetic means is the best available indicator of drug efficacy, and should be adopted in future monitoring and evaluation studies of PC programmes. Finally, these findings emphasize the need to revise the WHO recommended efficacy thresholds for a single oral dose ALB treatments.

### 3.5 Ethics Statement

The overall protocol of the study was approved by the Ethics committee of the Faculty of Medicine, Ghent University (Nr B67020084254 and 2011/374), Belgium and followed by the Ethical Review Board of Jimma University (RPGC/09/2011), Ethiopia. These clinical trials were registered under the Clinical-Trials.gov Identifier NCT01087099 and Clinical Trials.gov identifier B670201111554.

### 3.6 Acknowledgements

We would like to thank the schoolteachers, study subjects, and parents who allowed their children to participate. Additionally, we would like to thank the staff of Medical Laboratory Sciences, Jimma University. Finally, we would like to thank the WHO and VLIR-IUC/JU for supporting financially this study.
References


Dobson RJ, Sangster NC, Besier RB, Woodgate RG (2009) Geometric means provide a biased efficacy result when conducting a faecal egg count reduction test (FECRT). Vet Parasitol 161:162-167


Efficacy of Different Albendazole and Mebendazole Regimens against Heavy-Intensity *Trichuris trichiura* Infections in School Children in Jimma Town, Ethiopia

**Based On:**

4.1 Introduction

As illustrated in Chapter 3, a single dose of benzimidazole (BZ) drugs shows poor efficacy against *Trichuris trichiura*. For this, multiple dose regimens are likely to be more efficacious (Bennett and Guyatt, 2000; Keiser and Utzinger, 2008). Moreover, the results from Chapter 3 also indicate that the efficacy of BZ drugs may vary across infection intensity, the efficacy of a single oral dose albendazole (ALB) decreasing against *T. trichiura* when the mean faecal egg counts (FEC) at baseline increases (Levecke *et al.*, 2012; Chapter 3). Hence, the infection intensity should be considered as an important determinant of drug efficacy.

The objective of this study was to determine the efficacy of different regimens of ALB and mebendazole (MEB) administered to school children in Jimma Town, Ethiopia with heavy-intensity of *T. trichiura* infections.

4.2 Materials and methods

4.2.1 Study area and study populations

This study was conducted in school 6 in Jimma Town, Ethiopia (see Chapter 2).

4.2.2 Study design

The study was designed as a randomized multi-arm efficacy trial utilizing four arms for the treatment of *T. trichiura*. In total, 605 school children (age 5 to 18 years) attending grade 1 to 8 were recruited at baseline and asked to provide one stool sample. All together, 425 school children excreting eggs of *T. trichiura* were randomly assigned to one of the four treatment arms stratifying for baseline FEC. To this end, baseline FEC were stratified into three strata based on the 33rd and 66th percentile. Subsequently, subjects within each stratum were randomly assigned to one of the four treatment arms using the ‘rand’ function in xls. The treatment arms included were (i) a single dose of ALB 400 mg (Zentel, GlaxoSmithKline Pharmaceuticals Ltd, India) for one day (1xALB), (ii) a single dose ALB 400 mg for two consecutive days (2xALB), (iii) a single dose MEB 500 mg (Vermox, Johnson & Johnson) for one day (1xMEB), and (iv) a single
dose of 500 mg MEB for two consecutive days (2xMEB). The study subjects enrolled, randomized, followed-up and analyzed were summarized in Figure 4.1.

Fourteen days after the first treatment, a single stool sample of 385 was collected and screened for the presence of STH eggs. All stool samples (both at baseline and at follow-up) were processed with the McMaster egg counting method as described by (Levecke et al., 2011; see Chapter 2).
Subjects who were unable to provide a stool sample at baseline, experiencing a severe concurrent medical condition, had diarrhoea at time of the first sampling, had known history of allergic reaction to BZ drugs or were pregnant were excluded from the study.

4.2.3 Statistical Analysis

The efficacy of the different treatment arms were reported by means of reduction in faecal egg counts (FECR), using the formula below:

\[
\text{FECR} = 100\% \times \frac{\text{arithmetic mean (FEC at baseline)} - \text{arithmetic mean (FEC at follow up)}}{\text{arithmetic mean (FEC at baseline)}}
\]

The 95% confidence intervals (95% CI) for age, sex ratio (females/males) and FECR were determined by bootstrap analysis (10,000 iterations). Permutation tests and Bonferonni post-hoc corrections were performed to do a pairwise comparison of age, sex ratio and FECR in the different treatment arms (6 pair-wise comparisons). For each pair-wise comparison, the permutations test consisted of 2 consecutive steps. First, the permutation distribution under the null hypothesis that in average there was no difference in age, sex ratio, baseline FEC and FECR between two treatment arms was generated. To this end, all individuals of both trials were randomly re-assigned to one of the treatment arms (the number of individuals in each treatment arm remained unchanged). The new mean age, sex ratio, baseline FEC and FECR for each of the two treatment arms was determined, and the absolute value of the difference in age, sex ratio, baseline FEC and FECR between treatment arms was calculated. Next, this procedure was repeated 10,000 times. The distribution of these 10,000 permutated age, sex ratio, baseline FEC and FECR differences represented the permutation distribution when the null hypothesis is true. Second, the probability of finding a value at least equal to the absolute value of the observed difference in age, sex ratio, baseline FEC and FECR between the two treatment arms in this permutation distribution was determined (= p-value). The level of significance was set at \( p < 0.05 \).

4.3 Results

In total, 385 subjects received the assigned treatment regimen and provided a stool sample at follow-up (compliance rate of 90.6%) who had
completed the trial. The baseline characteristics for each of the four treatment arms are reported in Table 4.1. Overall the mean age and proportion of males of the subjects included in the final analysis ranged from 10.8 years (MEBx2) to 11.1 years (MEBx1) and from 36.7% (ALBx2) to 48.9 (MEBx2). The mean FEC of *T. trichiura* ranged from 1,075 EPG in MEBx1 to 1,262 EPG in ALBx2. These parameters did not significantly differ between the four treatment arms.

Table 4.1 Baseline characteristics of the children who completed the efficacy trial against *Trichuris trichiura* in school 6 of Jimma Town (Ethiopia)

<table>
<thead>
<tr>
<th>Parameter (95% CI)</th>
<th>1xALB n = 102</th>
<th>2xALB n = 90</th>
<th>1xMEB n = 103</th>
<th>2xMEB n = 90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>11.0 (10.6-11.3)</td>
<td>11.1 (10.9-11.4)</td>
<td>11.1 (10.9-11.4)</td>
<td>10.8 (10.5-11.1)</td>
</tr>
<tr>
<td>Proportion of males (%)</td>
<td>45.1 (38.3-51.7)</td>
<td>36.7 (29.8-43.3)</td>
<td>40.0 (32.8-46.8)</td>
<td>48.9 (41.8-55.7)</td>
</tr>
<tr>
<td>Mean FEC of <em>T. trichiura</em> (EPG)</td>
<td>1193 (867-1590)</td>
<td>1262 (977-1578)</td>
<td>1075 (863-1305)</td>
<td>1122 (899-1371)</td>
</tr>
</tbody>
</table>

1xALB: a single dose albendazole 400 mg; 2xALB: a repeated single dose albendazole 400 mg across 2 consecutive days; 1xMEB: a single dose mebendazole 500 mg; 2xMEB: a repeated single dose mebendazole 500 mg across 2 consecutive days; 95% CI: 95% confidence interval; EPG: eggs per gram stool; n: number of subjects.

The efficacy of the four treatment regimens is summarized in Table 4.2. In general, MEB treatment arms were more efficacious than ALB arms (MEBx1: 60.0% vs. ALBx1: 29.3% and MEBx2: 87.1% vs. ALBx2: 73.5%) and repeated doses were more efficacious than single dose arms (ALBx1: 29.3% vs. ALBx2: 73.5% and MEBx1: 60.0% vs. 87.1%). However, this difference in efficacy between treatment arms was not significant for pairwise comparison of 1xALB-1xMEB (p = 0.21) and ALBx2-MEBx1 (p = 0.29).
Table 4.2 Reduction in faecal egg counts of four treatment arms against *Trichuris trichiura* in School 6 of Jimma Town (Ethiopia)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>FECR (%) (95% CI)</th>
<th>Significant pairwise comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALBx1</td>
<td>102</td>
<td>29.3 (-9.9-56.2)</td>
<td>2xALB, 2xMEB</td>
</tr>
<tr>
<td>MEBx1</td>
<td>103</td>
<td>60.0 (48.5-70.9)</td>
<td>2xMEB</td>
</tr>
<tr>
<td>ALBx2</td>
<td>90</td>
<td>73.5 (64.2-81.3)</td>
<td>1xALB 2xMEB</td>
</tr>
<tr>
<td>MEBx2</td>
<td>90</td>
<td>87.1 (81.4-91.2)</td>
<td>1xALB, 1xMEB</td>
</tr>
</tbody>
</table>

1xALB: a single dose albendazole 400 mg; 2xALB: a repeated single dose albendazole 400 mg across 2 consecutive days; 1xMEB: a single dose mebendazole 500 mg; 2xMEB: a repeated single dose mebendazole 500 mg across 2 consecutive days; 95% CI: 95% confidence interval; EPG: eggs per gram stool; n: number of subjects

4.4 Discussions

In agreement with a previous review (Bennett and Guyatt, 2000), both drugs show limited efficacy against *T. trichiura* and repeated doses of MEB are the most efficacious. However, administering multiple doses has some important logistic implications in PC programmes. Compared to a previous study which was conducted by our group under comparable demographic (mean (95% CI) age = 11.1 years (10.7-11.5); sex ratio = 1.1 (0.75-1.63)), environmental (Jimma Town, December, 2009) and research conditions (McMaster egg counting method, same lot no of ALB), but including light-intensity *T. trichiura* infection (mean baseline FEC = 420 EPG) (Chapter 3, Vercruysse et al., 2011), a single dose ALB was significantly less efficacious (92.4% (88.1;95.9) vs. 29.3% (-9.9; 56.2)).

This finding (a negative correlation between infection intensity and drug efficacy) may be attributed to different factors, including (i) increased intestinal transit (due to heavy infections that cause irritation of the intestines and will subsequently increase the excretion of ALB), (ii) density dependent fecundity (following chemotherapy may not always yield a reduction in FEC, due to an increase in fecundity among the small residual worm population) and (iii) the bioavailability of single-dose ALB per adult worm may decrease with increasing infection levels (Albonico, 2003). Although these are all credible hypotheses, it remains unclear why such a large variation in efficacy was not observed for A.
*lumbricoides* and hookworms, despite the large variation in baseline FEC (see Chapter 2). These observations warranted a further research to test these hypotheses. More importantly, it is essential existing or new compounds against *T. trichiura* should be assessed under varying levels of infection intensity.

### 4.5 Ethics Statement

This trial was registered under Clinical trials.gov identifier B67020109355 and approved by the Ethical Committee of Jimma University (RPGC/11/2003) Ethiopia, Ghent University (2010/517) and Antwerp University (A10-55), Belgium. The school administrators, parents, and the children were informed about the nature and purpose of the study. Only those children, who were willing, and where the parents consented by signing, participated in the study.

### 4.6 Acknowledgments

We are grateful to study subjects, and parents who allowed their children to participate. Additionally, we would like to thank the staff of the Medical Laboratory Sciences, Jimma University. Finally, VLIR-IUC-JU project for financing the study.
References


Chapter 5

Comparison of Individual and Pooled Stool Samples for the Assessment of both Infection Intensity of Soil-Transmitted Helminths and Drug Efficacy

Based On:
5.1 Introduction

Over the past few decades, significant progress has been made in the control of soil-transmitted helminths (STH) through preventive chemotherapy (PC), and a worldwide scale-up of these large-scale programmes is underway in various parts of Africa, Asia and South-America (Neglected Tropical Diseases (NTD) Partner Website, 2012). Although these pledges of drug donations are in place, there are two factors that have received little attention but which might affect the success of these programmes. First, the therapeutic efficacy of the two benzimidazoles (BZ) differs across STH species (Chapter 3 and 4). Both are highly efficacious against A. lumbricoides, but ALB is more efficacious against hookworm, whereas both drugs are unsatisfactory when used as single dose against T. trichiura infections (Chapter 3 and 4). Moreover, therapeutic efficacy may change across the level of infection intensity (Chapter 3 and 4). Second, we only rely on two drugs with the same mode of action, and hence the emergence of anthelmintic resistance (AR) as drug donations are expanding, as substantiated in veterinary medicine, is likely (Geerts and Gryseels, 2001; van Wyk, 2001; Wolstenholme et al., 2004). Hence, it is important to seek for alternative strategies (i) to ensure appropriate choice of drug and regimen, (ii) to monitor AR, and (iii) to assess the long-term impact of MDA programmes.

Traditionally, the assessment of both infection intensity and drug efficacy is based on the examination of individual stool samples. However, this strategy impedes an up-scale of epidemiological surveys that are required to support health care decision makers to further maximize the efficiency of PC at the level of the countries. An alternative to individual stool examination could be the examination of pooled stool samples. The validity of pooling samples (e.g., stool, serum, and urine) of the same individual has been found valuable for diagnosis of various pathogens, including Giardia (Wahlquist et al., 1991), HIV (Verstraeten et al., 1998), Salmonella (Randall et al., 2006) and Chlamydia (Shipitsyna et al., 2007). Studies validating a pooling strategy for human STH are lacking. In animal health it has been shown that pooling stool samples allows for a rapid assessment of both infection intensity and drug efficacy. Pools of up to 10 animals provided comparable estimates of intensity of helminth infections by means of faecal egg counts (FEC) (Morgan et al., 2005; Eysker et al., 2008). They also highlight that the accuracy of these estimates may be thwarted as
infections become more aggregated. The effect of the number of samples to pool has not yet been examined.

The main objective of the present study was therefore to develop and to evaluate a sampling strategy based on pooling of stool samples. To this end, we assessed intensity of STH infections across varying epidemiological settings and drug efficacy of a single dose mebendazole (MEB, 500 mg) on both individual samples and pooled samples (pool sizes of 10, 20, and 60 individual stool samples).

5.2 Materials and Methods

5.2.1 Study Area and study population

The study was conducted in Jimma Town (see Chapter 2 for the details of the study site). Our study focused on school children from age 5 (grade 1) to age 18 (grade 8), and the study was conducted in 14 schools (Chapter 2).

5.2.2 Study Design

5.2.2.1 Assessing Infection Intensity

The assessment of infection intensity of STH was part of an epidemiological survey in Jimma Town (Chapter 2) that aimed to assess (i) STH prevalence in order to determine frequency of PC, and (ii) to assess seasonal differences in STH prevalence and infection intensity (rainy vs. dry season). The present study assessed the intensity of STH infections in the dry season between February and March 2012.

To this end, all primary schools in Jimma Town hosting grade 1 to grade 8 students were invited to participate. In each school subjects were stratified according to three age groups (age 5-9 years, age 10-13 years, and age 14-18 years). For each age class at least 20 subjects were selected on a voluntary basis, resulting in a total of at least 60 subjects per school. The subjects were asked to provide at least 5 g of stool. This quantity of stool was required to examine the samples individually (2 g) and to pool individual stool samples (1 g). All samples were processed with the McMaster egg counting method within the same day of collection (see Chapter 2; Levecke et al., 2011) for detection and enumeration of STH eggs. Figure 5.1 illustrates the number of primary schools eligible, recruited, and included in the statistical analysis.
5.2.2.2 Monitoring Drug Efficacy

In December 2011, the efficacy of a single dose of MEB (500 mg) against STH based on a standard protocol for ALB (Chapter 3, Vercruysse et al., 2011) was evaluated both on individual and pooled samples. This study was part of a multi-country trial designed to assess the efficacy of a single dose of MEB (see Chapter 3). In short, schoolchildren aged 5 to 18 years at different study sites were asked to provide a stool sample during the pre-intervention survey. A
single dose of MEB 500 mg was administered to all subjects, regardless of the infection status of STH. Stool samples were processed using the McMaster egg counting method for the detection and the enumeration of STH infections. Fourteen days post-treatment, stool samples were again collected and processed by the McMaster egg counting method (Chapter 2).

This Ethiopian trial was conducted in two schools (school 6 and 9) in Jimma Town. Subjects who were unable to provide a stool sample at baseline, experienced a severe concurrent medical condition, had diarrhoea at time of the first sampling, had a known history of allergic reaction to MEB, or were pregnant were excluded from the study. Pregnancy was ruled out based on the following criteria: (i) date of last menstrual period, (ii) sexual intercourse after the last menstrual period, and (iii) correct use of a reliable contraceptive method. Figure 5.2 summarizes the study subjects enrolled, and followed-up, and the sample submission compliance and the number of pooled samples (both at baseline and follow-up) included in the analysis.

5.2.3 Parasitological Examination

All stool samples were individually processed with the McMaster egg counting method, as described in Chapter 2.
Figure 5.2 Participation and compliance for assessing drug efficacy against soil-transmitted helminths in school children in Jimma Town (Ethiopia). At follow-up pooling of exactly up to 10, 20, or 60 stool samples were not always possible: * pool$_{10}$ includes: pools of 9 (n = 12) and pools of 10 (n = 36) samples; ** pool$_{20}$ includes: pools of 18 (n = 5), pools of 19 (n = 2) and pools of 20 (n = 17) samples; and *** pool$_{60}$ includes: pools of 55 (n = 1), 56 (n = 1), 58 (n = 1) and 59 (n = 1) and pools of 60 (n = 4) samples.

In addition, a subset of the stool samples was pooled in pools of 10, 20, and 60 individual stool samples, and this in both studies. We considered pooling as a rapid alternative for individual stool examination if at least 10 samples were pooled. Pools of 60 individual samples allowed for pooling all stool samples of one school in the study assessing infection intensity. For uniformity across the two studies, pooling of 60 stool samples was also applied for the evaluation of
the drug efficacy. Pools of 20 samples were considered as an intermediate of pools of 10 and 60. The procedure to pool individual samples is illustrated in Figure 5.3, and will be discussed more in detail below.

**Figure 5.3 Procedure to obtain pools of 10, 20, and 60 individual stool samples.**

Sixty individual stool samples were arranged in 6 rows with each row consisting of 10 individual samples, subsequently 6 pools of 10; 3 pools of 20; and 1 pool of 60 individual stool samples, resulting in total of 10 pooled stool samples per school.

At first, 60 individual samples were randomly organized in 6 rows of 10 individual stool samples. From each row 1 g of each of the 10 individual stool samples was transferred into a new pre-labelled plastic beaker (resulting in a total of 6 pools of 10 individual stool samples). After homogenization, 5 g from 2 plastic beakers representing pools of 10 individual samples were transferred into another new pre-labelled plastic beaker, resulting in a total of 3 pools of 20 individual samples. Next, 3.33 g was transferred from the 3 beakers of pools 20 into new pre-labelled plastic beaker, resulting in 1 pool of 60 individual stool samples. Finally, each of the pools was processed using the McMaster egg counting method as done for individual samples. A detailed tutorial on how to pool stool samples can be found on http://www.youtube.com/watch?v=UZ8tzswA3tc.

This pooling procedure has two important advantages. First, the cascade procedure applied (e.g. we pooled pools of 10 to make pools of 20) allowed for
pooling samples into different pool sizes with only 1 g per individual sample. Second, it avoids the homogenization of too large quantities of stool. For example, for pools of 60 we only had to homogenize 10 g of stools (3.33 g of three pools of 20 individual stool samples), whereas this would have been 60 g if we had pooled 60 times 1 g of individual samples.

For the assessment of infection intensity, samples were randomized according age group (2 rows of 10 samples per age group). For the efficacy trial, small deviations from the aforementioned procedure should be noted. Samples were randomly pooled both at baseline and at follow-up. However, due to unforeseen dropout, pools at baseline did not always match pools at follow-up and at follow-up pooling of exactly 10, 20, or 60 samples was not always possible. In addition, not all subjects were included at both baseline and follow-up.

Quality of the parasitological examination was ensured by (i) analyzing the samples within an average of 4 hr, (ii) verification of density of the sodium chloride solution (NaCl), (iii) sensitivity of the scale to weigh stool, (iv) supervision of the procedure of the McMaster and pooling, and (v) re-examination of 10% of the McMaster slides by a senior researcher. The total numbers of the individual samples and pooled samples across the assessment of the infection intensity and the efficacy trial are provided in Figures 5.1 and 5.2, respectively.

5.2.4 Statistical Analysis

5.2.4.1 Assessing Infection Intensity

The infection intensity was determined for *A. lumbricoides*, *T. trichiura* and hookworm, and expressed as eggs per gram of stool (EPG) for each individual and pooled sample. A total of 140 pools (84 pools of 10, 42 pools of 20, and 14 pools of 60) consisting a total of 840 individual samples were pooled. Subsequently, the agreement in mean FEC based on the examination of individual samples and the FEC based on the examination of the pooled sample was evaluated by the Spearman rank correlation coefficient (SAS 9.3 SAS Institute Inc.; Cary, NC, USA). In addition, a permutation test was applied to test for differences in mean FEC between examination of individual and pooled samples. This permutation test consisted of 2 consecutive steps. First, the
permutation distribution under the null hypothesis that in average there was no difference between the mean FEC of individual stool samples and its corresponding FEC of pooled stool sample was generated. To this end, the sign of the observed difference in mean FEC of the individual stool sample and its corresponding FEC of the pooled sample was randomly changed with a probability of 0.5, and the absolute value of the mean of this new permutated difference in FEC was calculated. Next, this procedure was repeated 10,000 times. The distribution of these 10,000 permutated differences represents the permutation distribution when the null hypothesis is true. Second, the probability of finding a value at least equal to the absolute value of the observed mean difference in FEC between individual and pooled stool samples in this permutation distribution was determined (= p-value). The level of significance was set at $p < 0.05$.

5.2.4.2 Monitoring Drug Efficacy

The efficacy of single dose of MEB (500 mg) treatment regimen was evaluated quantitatively based on the faecal egg count reduction (FECR), using the following formula:

$$\text{FECR} = 100\% \times \frac{\text{arithmetic mean (FEC at baseline)} - \text{arithmetic mean (FEC at follow up)}}{\text{arithmetic mean (FEC at baseline)}}$$

At baseline a total of 600 individual samples were pooled into 60 pools of 10, 30 of 20 and 10 of 60 individual samples. Whereas at follow-up 468 individual samples were pooled into 80 pools. As highlighted above, the pool size did not always include the anticipated number of individual stool samples (see also Figure 5.2). FECR was calculated for the three STH separately for each of the three pool sizes. A permutation test was applied to test for differences in FECR based on individual and on pooled samples. This permutation test consisted of 2 consecutive steps. First, the permutation distribution under the null hypothesis that in average there was no difference between the FECR based on the examination of individual stool samples with the FECR based on examination of pooled stool samples. To this end, the observed mean FEC of the individual FEC at baseline and follow-up were swapped with the FEC of the corresponding pooled sample at baseline and follow-up with a probability of 0.5.
The new permutated FECR for both the examination of individual and pooled samples was calculated, and the absolute value of difference in FECR was calculated. Next, this procedure was repeated 10,000 times. The distribution of these 10,000 permutated FECR differences represents the permutation distribution when the null hypothesis is true. Second, the probability of finding a value at least equal to the absolute value of the observed difference in FECR between individual and pooled stool samples in this permutation distribution was determined (= p-value). The level of significance was set at $p < 0.05$. The level of significance was set at $p < 0.05$.

5.3 Results

5.3.1 Correlation in infection intensity

Overall, there was a significant positive correlation between mean FEC of individual samples and the FEC of the pooled samples for each of the three STH species ($R_{A. lumbricoides} = 0.91$, $p < 0.001$; $R_{T. trichiura} = 0.82$, $p < 0.001$; $R_{hookworm} = 0.68$, $p < 0.001$). As illustrated in Figure 5.4, these correlation coefficients were more or less consistent across the different pools of individual samples ($A. lumbricoides$: $R_s = 0.91-0.98$, $p < 0.001$; $T. trichiura$: $0.75-0.85$, $p < 0.001$; hookworm: $R_s = 0.62-0.92$, $p < 0.001$).
Figure 5.4 The agreement in faecal egg counts of soil-transmitted helminths between individual and pooled samples. Each of the 9 scatter plots represents the agreement in mean individual FEC and pooled FEC of stool samples. The plots in column A, B and C represent *A. lumbricoides*, *T. trichiura*, and hookworm, respectively. The plots in top, middle and bottom row represent pool sizes of 10, 20 and 60, respectively. The magnitude of correlation for each plot is based on the Spearmann correlation coefficient (Rs).
5.3.2 Difference in infection intensity

Table 5.1 summarizes the mean FEC for both individual and pooled samples. Overall, there were no significant differences in FEC between individual and pooled samples across the three STH. Only for *A. lumbricoides* a significant difference in FEC was observed when pool size increased up to 60 samples, resulting in higher FECs (FEC$_{60} = 3,321$ EPG vs. FEC$_{\text{individual}} = 2,411$ EPG, $p = 0.007$). For the remaining two STH, no significant difference across pool sizes was observed ($p > 0.05$).

### Table 5.1 Mean faecal egg counts for soil-transmitted helminths based on individual and pooled samples.

<table>
<thead>
<tr>
<th>Pool size</th>
<th>Sample size</th>
<th><em>A. lumbricoides</em></th>
<th><em>T. trichiura</em></th>
<th>Hookworm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean FEC (EPG)</td>
<td>p value</td>
<td>Mean FEC (EPG)</td>
</tr>
<tr>
<td>1</td>
<td>840</td>
<td>2,411</td>
<td>0.295</td>
<td>295</td>
</tr>
<tr>
<td>10</td>
<td>84</td>
<td>2,604</td>
<td>0.19</td>
<td>289</td>
</tr>
<tr>
<td>20</td>
<td>42</td>
<td>2,842</td>
<td>0.17</td>
<td>312</td>
</tr>
<tr>
<td>60</td>
<td>14</td>
<td>3,321</td>
<td>0.006</td>
<td>428</td>
</tr>
</tbody>
</table>

FEC, faecal egg count; EPG, eggs per gram of stool

5.3.3 Monitoring Drug Efficacy

The mean FEC and FECR for each of the STH based on examination of individual and pooled samples are described in Table 5.2. Based on individual samples, FECR was high for *A. lumbricoides* (97.2%), but only moderate for *T. trichiura* (60.9%) and low for hookworm (44.2%). Pooled samples provided comparable FECR results for *A. lumbricoides* and *T. trichiura*, however, for hookworm, a significant statistical difference was found for pools of 10 ($p = 0.027$) and 60 individual samples ($p = 0.015$).
Table 5.2 Faecal egg count reduction of mebendazole for soil-transmitted helminths assessed by individual and pooled samples.

At follow-up pooling of exactly up to 10, 20, or 60 samples were not always possible: * pool10 includes: pools of 9 (n = 12) and pools of 10 (n = 36) samples; ** pool20 includes: pools of 18 (n = 5), pools of 19 (n = 2) and pools of 20 (n = 17) samples; and*** pool60 includes: pools of 55 (n = 1), 56 (n = 1), 58 (n = 1) and pools of 60 (n = 4) samples. FECR, faecal egg count reduction; FEC, faecal egg count; EPG, eggs per gram of stool.

<table>
<thead>
<tr>
<th>Pool size</th>
<th>Sample size at baseline/follow-up</th>
<th>A. lumbricoides</th>
<th></th>
<th>T. trichiura</th>
<th></th>
<th>Hookworm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean FEC at baseline (EPG)</td>
<td>FECR (%)</td>
<td>p-value</td>
<td>Mean FEC at baseline (EPG)</td>
<td>FECR (%)</td>
<td>p-value</td>
</tr>
<tr>
<td>1</td>
<td>600/468</td>
<td>3,091</td>
<td>97.2</td>
<td>320</td>
<td>60.9</td>
<td>43</td>
<td>44.2</td>
</tr>
<tr>
<td>10</td>
<td>60/48*</td>
<td>3,354</td>
<td>96.8</td>
<td>0.74</td>
<td>338</td>
<td>53.2</td>
<td>60</td>
</tr>
<tr>
<td>20</td>
<td>30/24***</td>
<td>4,965</td>
<td>96.8</td>
<td>0.80</td>
<td>607</td>
<td>77.7</td>
<td>67</td>
</tr>
<tr>
<td>60</td>
<td>10/8***</td>
<td>4,020</td>
<td>96.1</td>
<td>0.24</td>
<td>305</td>
<td>57.7</td>
<td>50</td>
</tr>
</tbody>
</table>

FECR, faecal egg count reduction; FEC, faecal egg count; EPG, eggs per gram of stool.
5.4 Discussion

Given the upcoming pledges of drug donations, and hence prospects of an increasing drug pressure on parasite populations, cost-effective tools to guide health care decision makers on how to optimize treatment strategies and on how to monitor the control of STH are urgently needed. In analogy with studies conducted in animal health, our results show that pooling stool samples, also holds promise as a rapid strategy in public health (i) to assess infection intensity, (ii) to ensure appropriate choice of drug and regimen, (iii) to monitor AR, and (iv) to assess the long-term impact of the upcoming PC programmes to control STH.

However, before we can provide specific recommendations further research is required to gain more insights on how and when to apply this pooling strategy. First, it is essential to assess the effect of pool size, sample size (number of pools), detection limit of the FEC method, and level of aggregation and intensity of infections on the precision and the accuracy of FEC and FECR results. This is in particular when level of infection and aggregation of STH infections change across different rounds of PC, and hence demanding a different pool and sample size and FEC method. This became already apparent in the present study where pooling samples to assess drug efficacy worked for *A. lumbricoides* and *T. trichiura*, but not for hookworm for which the level of FEC was low and FEC were highly aggregated. Because it is impossible to thoroughly evaluate the impact of each of these 5 aforementioned factors by field or laboratory experiments, a simulation study, as recently initiated in both animal (Morgan *et al.*, 2005) and public health (Levecke *et al.*, 2011), is at place.

Second, a detailed cost-effectiveness analysis is highly recommended (Speich *et al.*, 2010). Examination strategies resulting in a comparable level of accuracy or precision on FEC or FECR may still require a different level of technical and financial support. The present study was not designed to verify the cost-effectiveness of our pooling strategy. However, from the current results conducted in a region endemic to STH; pooling samples is most likely cost-effective. For example, in the present study we are able to reduce the samples examined with a tenfold without a significant loss in accuracy of the FEC results (only for *A. lumbricoides* a significant difference in FEC was observed between examination of individual and pools of 60). We estimate that processing and
reading a McMaster requires approximately 5 min (Levecke et al., 2009). With pooling 10 individual samples we would have won 270 min per day (= 60 individual samples * 5 min – 6 pools of 10 * 5 min). Of course, pooling samples requires some additional time, and pooling will be hard to justify as being cost-effective when the pooling procedure requires more than 270 min. In the current strategy we had to weigh a certain quantity of stool 60 times (60 individual samples to make 6 pools of 10) and to homogenize 6 pools. If we assume that homogenization of 1 pool demands 5 min (this way too much), the pooling strategy will still be cost-effective when the quantity of stools could be measured within 240 minutes (270 – 6 pools of 10 x 5 min) or 4 min per step of measuring stool (240/60). Given that McMaster can be applied in 5 min and comprises weighing of 2 grams of stool, homogenization in a flotation solution, filling and reading of the McMaster slide, it is clear that the 4 min available to transfer a fixed quantity too is quite conservative.

Third, various strategies to pool stool samples should be evaluated. In the present study, we pooled samples in a cascade: pools of 10 were made by pooling individual samples, but rather than repeating this procedure of pooling individual samples for the other pool sizes, we used the pools of 10 to make pools of 20, subsequently the pools of 20 to make the pools of 60. This procedure provided an equal amount of stool pooled for each pool, in casu 10 g, and an elegant way to assess different pool sizes without too much additional work. However, it remains uncertain whether this procedure itself does not introduce any bias, particularly when the contribution of each subject decreased over pool size. For pools of 10, each individual contributed 1 g, whereas this was 0.5 g and 0.15 g for pools of 20 and 60, respectively. Moreover, pools were homogenized by simple stirring. Homogenization in a liquid phase prior to examination, however, should be recommended, as it facilitates homogenization of pools. This is in particular when eggs are not equally distributed among stool samples. Therefore, homogenization of stool remains a crucial step in the most important FEC methods applied in veterinary parasitology, including McMaster, FECPAK (www.fecpak.com) and (mini-)FLOTAC (Cringoli et al., 2010)). In addition to this, homogenization will prevent drying of the stool while pooling samples. Due to our cascade procedure larger pool sizes were made at the end. However, this probably resulted in an increase of evaporation of water from the
stool samples. As a consequence of this the mass of the stool decreased over time whereas the number of eggs in the stool remained unchanged, and hence resulting in the observed trend of increasing FEC over pool sizes. This could have been overcome by homogenizing the pools immediately in the flotation solution. Finally, to simplify procedures under field conditions, it would be worth to evaluate pooling based on a fixed volume rather than pooling a fixed amount of faeces.

Fourth, assessing drug efficacy based on pooled samples remains delicate, and this despite the comparable FECR results between individual and pooled stool samples for *A. lumbricoides* and *T. trichiura*. Subjects who are not infected (truly or apparent) at baseline cannot be excluded from the analysis and there is no perfect match of pools before and after drug administration due to drop out. Therefore, both analysis need to be validated independently.

Fifth, we only focused on STH infections, but since the advocacy to integrate NTD control measures, this pooling strategy should also be validated for the other NTD, such as *Schistosoma*. Finally, although pooling samples does not provide prevalence data, various models have been developed for other pathogens to estimate prevalence based on pooled samples. Validation of these models for STH is required (Speybroeck et al., 2012).

In conclusion, this study highlights that pooling stool samples is a rapid strategy that holds promise as a cost-effective strategy to assess intensity of STH infection and to monitor PC programmes. However, further research is required (i) to gain more insights into the impact of pool size, sample size, detection limit of the FEC method, intensity and aggregation of infections on the validity of pooling stool samples, (ii) to verify the cost-effectiveness of pooling, (iii) to optimize the methodology of pooling stool samples, and (iv) to validate models to estimate prevalence based on pooled samples.

### 5.5 Ethics Statement

Ethical approval was obtained from Ghent University (2011/374), Belgium, and Jimma University (RPGC/09/2011), Ethiopia. The efficacy trial was registered under Clinical Trials.gov identifier B670201111554. The school authorities, teachers, parents, and the children were informed about the purpose
and procedures of the study. The written consent form was prepared in two commonly spoken local languages (Afaan Oromo and Amharic) and handed-over to the children parents/guardians. Only those children (i) who were willing to participate and (ii) from whom the parents or guardian signed written informed consent form were included in the study. Moreover, an additional separate written informed consent form for children older than 12 years was prepared, read, and handed-over to them and obtained their additional written informed consent.

5.6 Acknowledgments

We are grateful to the schoolteachers, study subjects, and parents who allowed their children to participate. Additionally, we would like to thank the staff of the Medical Laboratory Sciences, Jimma University (Ahmed Zeynudin, Dereje Atomisa, Mitiku Bajaro, Shiferaw Bekele, Dereje Jirata, Nuredin Abduselam, Tesfaye Deme and Mestawet Getachew) for processing the stool samples and/or technical/medical assistance at the field.
References


Chapter 5: Comparison of Individual and Pooled Stool Samples


Chapter 6

General Discussion:
Control of Soil-Transmitted Helminthiasis in Children of Jimma Town
6.1 Introduction

The overall objective of this thesis was to improve the control of soil-transmitted helminths (STH) in school-aged children (SAC) in Jimma Town. To do this, we assessed STH infections in SAC in 14 primary schools across seasons, assessed the efficacy of different drugs and drug regimens against STH and validated new diagnostic strategies to reduce both financial and technical resources to assess infection intensity and to monitor the efficacy of the drugs administered. The studies performed indicated that STH are highly prevalent in children going to primary schools, but that STH infections in terms of prevalence and intensity vary both among schools and between seasons (Chapter 2). The assessment of efficacy of a single oral dose of albendazole (ALB) and mebendazole (MEB) pointed out that both drugs are highly efficacious against *A. lumbricoides* infections, but show poor efficacy against *T. trichiura* infections. For hookworms, ALB was more efficacious. In addition, the expected efficacy values for each drug and STH species were established. Any efficacy below these values should warrant further investigation for the potential development of anthelmintic resistance (AR) (Chapter 3). To further improve the control of *T. trichiura* a single oral dose needs to be administered over consecutive days (Chapter 4). Finally, the findings emphasize that the pooling of stool samples holds promise as a rapid diagnostic tool to assess both infection intensity of STH and drug efficacy (Chapter 5).

Overall, these results provide insight into different aspects related to the control and monitoring of mass drug administration (MDA) programmes. The objective of this chapter is to combine all insights and propose a plan on how to further improve the control of STH in children and how to monitor MDA programmes in Jimma Town (Ethiopia). To this end, we will first describe the current control measures for STH in Jimma Town. Second, we will provide concrete suggestions on how to improve current control measures through MDA, WASH (water, sanitation and hygiene) and health education. Third, we will outline a concrete plan to monitor MDA programmes. Finally, we will make some suggestions for future research.

6.2 Current control measures in Jimma Town

Current measures to control STH in Jimma Town are based on MDA only. However, these measures remain limited and scattered. Since 2008, a single
oral dose of ALB has biannually been administered to preschool-aged children (preSAC, <5 years of age) in Jimma Zone (Jimma Town is the capital of Jimma Zone). However, other population groups who are also at risk of morbidity caused by STH, such as SAC and women of childbearing age, have not been included. For preSAC, although it is claimed that MDA has been applied twice a year for the last 5 years, there is no complete report that indicates (i) in which months of the year it was applied, (ii) how the MDA was organized (e.g., whether drugs were distributed at kindergartens or health institutions or from house-to-house), and (iii) how many preSAC were covered. Moreover, there is no regular supply of drugs. Drugs were donated by United Nations Children’s Fund (UNICEF) and channelled through the Pharmaceuticals Fund and Supply Agency (PFSA) of Ethiopia. Subsequently, the drugs were forwarded to the Oromia Regional Health Bureau, which sent it to Jimma Zone Health Bureau.

Although the Health Bureau estimated that about 80% of the preSAC were covered at zonal and national level, the data from Jimma Town shows a coverage of about 70% of the total population of preSAC. As highlighted in Chapter 2, besides the claimed relatively high coverage of MDA the prevalence of any STH remains high in the study area. These measures seem to have little impact on the re-infections of STH unless MDA is supplemented with other control measures like health education, improving environmental sanitation and personal hygiene to break the transmission cycle in the long run. In this Figure 6.1, we report the distribution of STH infections in children (1 to 18 years) as a function of age. The data for children aged 6 to 18 (SAC) are derived from the survey presented in Chapter 2. The data from children aged 1 to 4 were obtained during an additional survey that was designed to assess STH infections in preSAC in 12 kindergartens in Jimma Town (Dana et al., 2014). The data for children aged 5 were obtained from both surveys. The prevalence of any STH in SAC does not drop below 40% at any point in their childhood, and hence suggesting that, although the morbidity caused by STH will be reduced in preSAC, children remain exposed to infections and re-infections and vulnerable to the morbidity caused by these worms during the majority of their childhood. Moreover, this Figure also illustrates the high prevalence of any STH in preSAC, and this despite the ongoing MDA for the last 5 years. The estimated prevalence of any STH in preSAC was 46.6%. We did not observe the high coverage of
preSAC reported by the Health Bureau. Less than a third of the preSAC screened received a tablet during the last round of MDA.

![Graph showing distribution of any soil-transmitted helminth infection in children aged 1 to 18 in Jimma Town.](image)

**Figure 6.1** Distribution of any soil-transmitted helminth infection in children aged 1 to 18 in Jimma Town.

### 6.3. How to improve the control of soil-transmitted helminths in Jimma Town

Control of STH in Jimma Town will have to rely on three pillars, namely MDA, WASH and health education. We will discuss their applicability and feasibility in Jimma Town separately, with the ultimate goal that the results of this thesis will be of relevance for the STH control programme in Jimma Town and might find application at a broader level in Ethiopia.

#### 6.3.1 Mass drug administration

Although it is generally recommended to include both preSAC and SAC, MDA programmes are mainly focussing on SAC (Awasthi et al., 2003; Hotez et al., 2005; Bundy et al., 2005). In contrast to SAC who can be largely reached through schools, preSAC usually stay at home, and hence are difficult to reach. This difference in accessibility may have contributed to the increasing MDA coverage in SAC worldwide over the last three years (from 28% in 2010 to 36%
in 2012), but the decreasing coverage in preSAC over the same period (from 37% in 2010 to 25% in 2012) (WHO, 2014). We will include both preSAC and SAC in our plan for MDA in Jimma Town. Our plan for MDA in Jimma Town is graphically illustrated in Figure 6.2.

Figure 6.2 Flow chart indicating the proposed framework of implementing mass drug administration in Jimma Town.
6.3.1.1. Coverage

Clearly, the coverage of children in Jimma Town is currently insufficient for preSAC and nil for SAC, despite the well organized health and education system in Jimma Town, allowing increasing the coverage while minimizing both technical and financial resources. In Jimma Town there are 24 registered primary schools (either public or private), hosting a total of 23,492 children. In addition, the Ethiopian Federal Ministry of Education is increasingly encouraging parents to send their preSAC to kindergartens to prepare them for formal schooling. Consequently, the number of kindergartens has tripled in the last 10 years. There are currently 44 (either public or private) kindergartens in Jimma Town, in which 5,491 preSAC are enrolled (= 20.2% of the total population of preSAC, Jimma Town Education Bureau, August 2014). Moreover, the majority of these kindergartens, especially the public ones, are often included in the compounds of the primary schools, and hence this allows reaching both preSAC and SAC with a minimum of additional efforts. Nevertheless, it has to be kept in mind that if we succeed to cover all preSAC and SAC attending kindergartens and schools, we will only cover 51.8% of all children in Jimma Town (see Table 6.1). This coverage is still about 25% below the 75%-target proposed by WHO to be reached by 2020.

Table 6.1. The proportion of the total population of preschool-aged and school-aged children who are attending kindergartens or schools in Jimma Town.

<table>
<thead>
<tr>
<th>Number of children attending kindergarten/school</th>
<th>Proportion of the total population (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PreSAC</td>
<td>5,491</td>
</tr>
<tr>
<td>SAC</td>
<td>23,492</td>
</tr>
<tr>
<td>Total</td>
<td>28,983</td>
</tr>
</tbody>
</table>

In response to these challenges, we will first look for an alternative strategy to reach the preSAC and SAC that do not attend kindergartens or schools. Such means include strengthening the collaborative work with different stakeholders and utilizing the existing Health Service Extension Programmes and Health Extension Workers (details will be found in the subsequent sections). In addition, we will practise the philosophy of Jimma University, which has as a
motto ‘we are in the community’. For instance, the postgraduate students of the university (clinical pharmacists, medical parasitologists and public health students) are taking community-based training courses as part of their curriculum. During these courses, they spend at least 2 months in the community, forming an additional platform to give health education, to trace children who are not enrolled in kindergartens or schools, and even to distribute drugs during rounds of MDA. This, we will not only improve the control of STH, it will also decrease the burden involving MDA on the Jimma Town Health Bureau. Finally, in collaboration with the Jimma Town Health Bureau and political administrations, we will propose a special day that will be named 'Deworming Day', which will be publically announced (the communication with the concerned governmental bodies has already been initiated). This 'Deworming Day' will be taken as an excellent opportunity to treat all children at a specified place, which could be health centres or a stadium or school compounds, and to to give health education through postgraduate students and schoolteachers.

6.3.1.2 Choice of frequency of mass drug administration, period, drug, and drug regimen

Although the overall prevalence in Jimma did not exceed the 50% in both preSAC (46.6%; Dana et al., 2014) and SAC (49.0%, Chapter 2), we suggest administering drugs twice a year (we used a diagnostic method that lacks sensitivity, and hence the true prevalence is underestimated). Due to seasonal variation in STH infections, we would administer drugs in well-chosen periods (Chapter 2). Although this may be scientifically grounded, the implementation of such a timed MDA is bound to important logistic issues in the field (e.g. school holidays and term exam schedules). We therefore suggest starting the first round of MDA in September (towards the end of the rainy season), and a second one 6 months later in March (towards the end of the dry season). September is probably the most ideal time of the year to initiate MDA. In this month, another school year starts, and hence MDA can easily be promoted as a boost of school performance. For this, it is important that the MDA receives the necessary media attention to further leverage the coverage and to increase the sustainability of the MDA programme (see also 'Deworming Day’ and the sections below).
Given the relatively high prevalence of *T. trichiura* compared to hookworms, we would opt for a single oral dose of mebendazole (MEB, 500 mg). To further improve the control of *T. trichiura*, we would apply single-dose of MEB at two consecutive days (Chapter 2 and 3) in kindergartens/schools where the intensity of infection is high (mean FEC >800 EPG, see Chapter 3 for proposed thresholds). Although it can be argued that multiple doses are impractical in a MDA application, there are various opportunities that may facilitate the administration of drugs over consecutive days, including the philosophy of Jimma University, the availability of postgraduate students and the presence of the Health Service Extension Programme. However, it is worth to note that our plan might not fit perfectly into the plan at the national level. When this thesis was forwarded for publication, it was communicated that Ethiopia will soon start a nationwide MDA programme to control STH. It is anticipated that both drugs will be distributed, and that the frequency of MDA will vary among and within regional states depending on the recent nationwide STH prevalence results.

### 6.3.1.3 Delivery of the drugs

To effectively deliver the planned MDA to preSAC and SAC, we will use the advantage of the unique health service system of Ethiopia. The main features of this service include a focus on decentralization and encouraging partnerships as well as the participation of the whole community in health activities. It expands healthcare service at the grass roots level through the implementation of the Health Service Extension Programme (HSEP). HSEP is an innovative, community-based (‘kebele’-based) programme that was first introduced in Ethiopia in 2003. The primary aim of HSEP is to bring health service to the rural community at household level. This programme receives due political commitment from the government that has led to the construction of more than 14,000 Health Posts (the smallest health service unit within the health service system hierarchy, Figure 6.3), the employment and training of 30,000 Health Extension Workers (HEW, 2 HEW per Health Post), and the organizational structure of Health Development Arms (HDA).
HEW are females, trained for one year with special training emphasizing on disease prevention measures (see Box 6.1). By means of basic curative and preventive health services, they help the community in acquiring the knowledge and skills required to ensure health in the community.

**Box 6.1 Elements of Primary Health Care where Health Extension Workers play a crucial role**

1. Education on health problems and how to prevent and control them.
2. Development of effective food supply and proper nutrition.
3. Maternal and child healthcare, including family planning.
4. Adequate and safe water supply and basic sanitation.
5. Immunization against major infectious diseases.
6. Local endemic diseases control.
7. Appropriate treatment of common diseases and injuries.
8. Provision of essential basic medication.
Currently, like any part of Ethiopia, every household in Jimma Town is organized into what is called HDA. These HDA consists of representatives of 5 neighbouring households (one representative per household) who meet on a regular basis to discuss various issues at the household level, including health. One of these household representatives will take responsibility to organize these meetings. He/she will also be the contact person for the HEW to identify those children that are not enrolled in kindergartens or schools. Thus, for the delivery of drug we will closely work with both HEW and HAD, as they will facilitate the administration of drugs to children and health education.

6.3.1.4 Training

As described in the above section, HEW and HDA will be our primary working partners to administer drugs, to trace children who are not enrolled in kindergartens or schools and to give health education. Hence, considering their limited knowledge on STH, we will organize a series of trainings. These trainings will focus on (i) STH and their burden on the community, (ii) the role of MDA as a measure to control STH, (iii) the importance of WASH and health education in the fight against STH, (iv) how to organize rounds of MDA, including how to administer drugs and how to trace children not attending kindergartens or schools. Moreover, this training will not only be limited to HEW or HAD, depending on the necessity they can also be extended to schoolteachers and concerned stakeholders depending on necessities.

6.3.1.5 Increase the participation of the community

Participation of the community is of vital importance for the success of any control programme. During this PhD thesis we paid attention on how we could increase the participation of the community. We therefore presented our data from each of the different chapters to the schools and the communities involved, and invited school representatives to the laboratory to demonstrate the diagnosis of STH. To further disseminate our findings we discussed the magnitude of STH problem in the school community, the potential risk factors and the way forward to the control of STH in Jimma Town on the local radio station. Finally, we also wrote policy letters to the Educational and Health Bureau of Jimma Town, FMoH and other stakeholders. Although it is difficult to measure the impact of these measures on the participation of the communities, we experienced an improved cooperation of
both schools and the parents/guardians to take part in our studies, an increased rate of SAC taking the drugs, and a positive feedback of stakeholders. Figure 6.4 illustrates some of awareness creation sessions at schools.

Figure 6.4 An example of an awareness creation session at school 9 in Jimma Town, Jimma, Ethiopia.

6.3.2 Water, sanitation and hygiene

MDA alone will not be effective to eliminate STH infections on the long-term in the absence of clean water, sanitation and hygiene (Ziegelbauer et al., 2012). Thus, whenever resources allow we shall supplement the MDA programme with improved access to clean water together with adequate sanitation and hygiene (WHO, 2010; Ziegelbauer et al., 2012). However, in our opinion, this will not be possible in a short time of period in Jimma Town. This will require a long-term commitment of the different partners, such as politicians, city administrators and health sectors, to change the overall living standard of the community, which in our opinion is not present and not within our reach. Alternatively, as outlined in the following section we will focus on health education.
6.3.3 Health education

Health education is the principal component of most of the control and elimination programmes. Health education and information campaigns aim to enhance knowledge on how the disease can be treated and prevented, and hence reducing the transmission of STH (Albonico et al., 1999; WHO 2002). Health education and information can be given in different ways, such as formal trainings, production of different educational tools (e.g., posters, games, leaflets) or via mass media in form of news, policy letters or songs.

Besides our ongoing efforts to increase the participation of the community through e.g. seminars, workshops, and radio sessions (see section above), we have started working on some of these educational materials. For example, at the time this thesis was send for publication, we finalized a 14 page (script and illustrations) for a drama at the level of SAC (Figure 6.5). In this script, we discuss details of the life cycle of STH, the role and the effect of MDA, importance of WASH through the daily life of a boy called ‘Chuchu’ (the name of the boy who plays a key role in the drama). At this stage, we have transformed the script into an audio-drama, which we would like to make radio-born at the first round of MDA in Jimma Town. Eventually, we would like to film this drama, and broadcast it through the national TV (Figure 6.5). In this play, the key actors will be selected from SAC, teachers, HEWs, community leaders and representatives of the scientific community. It will be our plan to produce different educational materials (e.g., leaflets and posters) to further improve STH control in Jimma Town and making it as a model for the rest of the regions, and Ethiopia at large.
6.4 How to monitor mass drug administration programmes in Jimma Town

Monitoring MDA programmes is of vital importance to detect emerging AR (short term impact) and to determine whether the MDA programme progresses as anticipated (long term impact). Therefore, a monitoring system will be set up
in Jimma Town to verify whether the drugs administered are still efficacious and whether the programme has an impact on prevalence/infection intensity (\(\sim\) morbidity). In addition to this, we will give special attention to standardization of protocols and capacity building / strengthening of the laboratories. Figure 6.7 illustrates the proposed monitoring system.

6.4.1 Detection of emerging drug efficacy

6.4.1.1 Assessment of drug efficacy

In accordance with WHO guidelines (WHO, 2013), we will evaluate the efficacy of the administered drugs every 4 years. To evaluate this we will select 25% of the schools (= 6 schools out of 24). The selection will be based on the prevalence and intensity of STH infections, with special attention for *T. trichiura*. Schools that we consider to include are Schools 3, 5, 7, 8, 12 and 13 (see also Figure 2.1 in Chapter 2). At each school, at least 60 infected children (aged 5-18) per STH species will be requited (WHO recommends a minimum of 50). Subsequently, we will compare the efficacy results with the thresholds recently set by WHO (Chapter 3).
Figure 6.7 Flow chart indicating the proposed framework of monitoring mass drug administration in Jimma Town.
6.4.1.2 Monitoring emergence of drug resistance

It has been well argued that frequent administration of drugs will exert pressure on the parasites, and might give rise to resistant parasites (Albonico et al., 2004; Humphries et al., 2012). Thus, as it is clearly indicated in Figure 6.7, it is our plan to preserve a portion of the positive stool samples (10%) for further molecular analysis each time we assess drug efficacy. If at some point during the course of monitoring, the drug efficacy becomes suspected or reduced we will further analyse the preserved samples for possible anthelminthic resistance gene (for instance for any change in mutations in the β-tubulin gene).

6.4.1.3 Assessment of prevalence and infection intensity

We will re-assess the prevalence and the intensity of STH infections in the 6 schools selected for assessment of drug efficacy. The obtained prevalence data will allow us to accordingly adapt the frequency of MDA (Jia et al., 2012; see Chapter 1 Figure 1.3).

6.4.2 Application / development of standardized operating procedures

Despite the global expansion of MDA programmes, standard operating procedures (SOP) to monitor anthelminthic drug efficacy are lacking. As shown in Chapter 3, the research conducted by the STH research team at Jimma University (as part of the seven countries study all over the world) has been instrumental in generating new SOP to monitor anthelminthic drug efficacy (Chapter 3). These SOP have contributed significantly in the standardization and adaptation of existing monitoring drug efficacy tools and development of new therapeutic efficacy thresholds. Moreover, the use of such SOP will be of paramount importance in enormously minimizing the confounding factors listed in the literature such as the use of different diagnostic techniques, the time interval for follow-up, treatment and dosing of drug, and statistical approach) thought to affect the drug efficacy results (Keiser and Utzinger, 2008; Chapter 1). We will effectively apply those SOP during the launch of MDA and subsequent monitoring efforts (Figure 6.8 and 6.9).
Chapter 6: Control of STH in Children Jimma Town

6.4.3 Capacity building/strengthening of research institutions

6.4.3.1 training

Experience gained in the early days of this PhD work made the STH research team of Jimma University realize that capacity building for health research is a prerequisite for developing a lasting research tradition and for ensuring that the research outcomes are translated into practical recommendations for the health decision makers. During this PhD staff from the laboratory have been trained in a wide range of areas such as how to perform laboratory techniques applied for the detection and quantification of STH, how to organize the laboratory and field, how to preserve stool samples and how to manage data (Figure 6.9).

In addition to these onsite trainings, we have developed video clips of the different operational procedures. These clips, seven in total, have been made publically available on YouTube (https://www.youtube.com/).
watch?v=q_yfdtE3TSE, Figure 6.8 above), hoping they will serve not only for local people but also for a wider scientific community. Since we have put them on YouTube in October 2013, they have been viewed more than 4,500 times. Currently, they have also been embedded in different internationally recognized websites (Global Atlas of Helminth Infection, Schistosomiasis Control Initiatives, and Children without worms).

![Image of training](image)

**Figure 6.9** Some pictures illustrating training given, field and laboratory organizations, in the Jimma Neglected Tropical Diseases (NTD) Laboratory, Jimma University, Ethiopia.

6.4.3.2 **Capacity building/strengthening of the laboratories**

We have established both the NTD Laboratory and the Molecular Biology Laboratory. These two laboratories are the result of the capacity building efforts within the frame of this PhD work in collaboration with our partners (Laboratory of Prof. Dr. Jozef Vercruysse, Ghent University). Taking the experience and expertise of our partners, we have managed to transfer different coprological techniques to the Jimma University-NTD laboratory (e.g., McMaster method, FLOTAC/mini-FLOTAC, and FECPACK$^{(52)}$), and different molecular techniques in the Molecular Biology Laboratory. At present our laboratories have the expertise on all diagnostics methods.
In addition, we have agreed with the Ethiopian Public Health Institute (EPHI) to make these laboratories one of the reference centres in south-western region of Ethiopia. We will be involved in organizing workshops on a wide range of operational procedures involved in MDA. For example, we have proposed a national workshop on STH and schistosomiasis to be hosted in Jimma in summer 2015. During this workshop we expect participation of national and international stakeholders and experts. Moreover, we are striving to maximize and standardize these laboratories with the ultimate goal of making them one of the reference laboratories for monitoring MDA programmes against STH in Africa.

6.5 Implications for future research

Our group would like to continue working on the following areas:

1. **Re-infection status studies:** This study indicated that STH are highly prevalent in children (Chapter 2, Dana et al., 2014). Although MDA remains the global strategy to control STH, MDA must be repeated regularly because of the high re-infection rates. However, it is important to elucidate how frequently the re-infection occurs and to identify factors that contribute most to re-infection.
Thus, our future work will focus on the role of local risk factors, such as soil-composition and moisture, as well as socioeconomic discrepancies and differences in sanitation, hygiene and health seeking behaviour, which are thought to sustain helminth transmission.

2. **Genetic and/or molecular studies:** Firstly, the molecular characterization of the STH species will be optimized. Secondly, we will explore the genetic variability among STH species (both from human and animal sources) to rule out the possibility of zoonotic transmissions. We will extend this genetic variability studies to other zoonotic parasites such as *Giardia*, *Cryptosporidium* and *Entamoeba*. Finally, genetic/molecular studies will be carried out to detect the possible emergence of drug resistance.

3. **Efficacy trials on the combination of different drugs against *T. trichiura***: a single-dose of ALB and MEB have poor efficacy against this parasite. Currently there is a paucity of new drugs in the pipeline. Therefore, it would be a wise approach to look for possible new combinations of 'old' well-known drugs. For example, the combination of pyrantel (PYR) and oxantel (OX) has shown broad-spectrum anthelminthic activity (Rim et al., 1975) and might offer a valuable alternative to ALB and MEB treatments for the control of intestinal nematode including *Trichuris* infections (Albonico et al., 2002; keiser et al., 2013). Moreover, this drug combination has a different mode of action, and hence providing a back-up plan would AR against ALB and MEB arise. Thus, we plan to evaluate the efficacy of PYR and OX combination.

6.6 Conclusions

MDA-based STH control programmes have been implemented in different parts of the world since the mid-1990s. However, the control of STH in Ethiopia is yet to start. Ethiopia has launched a "National Master Plan for Integrated Control of NTD" in June 2013 and completed the mapping of NTD. Preliminary result of this mapping was released June 2014. (Figure 6.11). It is anticipated that the first nationwide round of MDA will take place early next year. We strongly believe that the existing health service system will play a key role in rolling out and sustaining this MDA, but that the expertise and universities and research institutions, such as Jimma University, should also be duly consulted.
Figure 6.11 Flow chart indicating the summary of proposed roadmap and available human resources in implementing mass drug administration in Jimma.
References


Summary
Soil-transmitted helminths (STH), including *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *Ancylostoma duodenale* and *Necator americanus* (hookworms) pose the highest burden of all neglected tropical diseases. They affect the most socio-economically deprived populations in both subtropical and tropical countries, children and women being at the highest risk of morbidity caused by these worms. To fight these worms mass drug administration (MDA) programmes are implemented. However, despite the high prevalence of STH, and its enormous burden on public health, the measures to control STH in Ethiopia are limited. The overall objective of this thesis was to improve the control of STH in children in Jimma Town, Ethiopia.

In **chapter 1**, we reviewed (i) the current available and recommended control strategies for STH infections, (ii) the World Health Organization (WHO) recommended anthelminthic drugs, (iii) the ways of monitoring drug efficacy, (iv) the factors affecting drug efficacy, and (v) the efficacy trials performed in the last three decades in Africa. This review indicates that MDA programmes remain the stronghold strategy to control STH, the benzimidazole drugs (albendazole (ALB) and mebendazole (MEB)) being the drugs of choice. They are relative efficacious, safe, and easy to administer (dosing is not based on bodyweight). However, the present strategy of STH control through MDA may not be optimal and can be further improved. First, the frequency of MDA is based on the prevalence of STH, but little is known about the demographic, socio-economic and environmental factors that contribute to these infections. Second, efficacy of ALB and MEB varies across the different STH species, and hence the choice of drug may depend on the geographical distribution of STH. Third, despite the global expansion of MDA, standard operating procedures to monitor anthelminthic drug efficacy are lacking. Fourth, there are a number of factors that may affect the efficacy of drugs. Fifth, there is a lack of strategies that allow health decision makers to rapidly assess the infection intensity and monitor the development of drug resistance.

In **chapter 2**, the infection intensity and prevalence of STH was assessed during the dry season (February-March) and end of the rainy season (September-October) across 14 primary schools in Jimma Town, Ethiopia. A total of 1,680 school children (840 in each season; and 60 children/school) were included. All stool samples were processed using the McMaster egg counting method. A meta-analysis was performed to assess any differences in prevalence
and infection intensity between the two seasons. Infections with any STH were observed in 49.0% (824/1,680) of the subjects. *T. trichiura* was the most prevalent (35.5%), followed by *A. lumbricoides* (23.4%) and hookworm (9.9%). Among the schools there was a considerable variation in prevalence, ranging from 16.7% to 68.3% for any STH, 6.7% to 39.2% for *A. lumbricoides*, 10.8% to 55.0% for *T. trichiura* and 0% to 28.3% for hookworms. Overall, any STH infection was significantly less prevalent in the rainy season compared to the dry season. A significant difference in prevalence across seasons for the three STH species separately was only observed for *T. trichiura* infections. A significant difference in infection intensity across seasons was observed for *A. lumbricoides* and *T. trichiura*. For both STH species, infection intensities were lower in the rainy season compared to those in the dry season. Our results indicate that STH infections are highly prevalent in Jimma Town and that biannually MDA is recommended to control the morbidity caused by these worms. In addition, they suggest that MDA in the dry season may have a higher impact compared to MDA in the rainy season, as prevalence and intensity of STH infections is highest in the dry season. Finally, the large variation in both prevalence and intensity of infections observed in Jimma Town warrant further research to identify factors that explain the observed variation.

In **chapter 3**, we report the results of a drug efficacy trial in Jimma Town. This trial was part of a multi-national efficacy trial that was performed in different STH endemic countries in Asia, Africa and Latin-America to assess the efficacy of a single oral dose of ALB (400 mg) and MEB (500 mg) against STH infections in school-aged children. In each country the same protocol was applied. This protocol was standardized in terms of diagnostic method (McMaster egg counting method), follow-up period (approximately 14 days), origin of the drugs and statistical analysis (cure rate (CR) and different formulae for faecal egg reduction rate (FECR; (1), (2), (3)). The first two formulae were based on the mean (arithmetic/geometric) of the pre- and post-intervention faecal egg counts (FEC) largely ignoring the individual variability, whereas the third formula represented the mean of the reduction in the FEC per subject). In Ethiopia, the CR of ALB was the highest for *A. lumbricoides* (99.3%), followed by hookworm (98.9%). The lowest CR was observed for *T. trichiura* (85.7%). For MEB, a similar trend was observed, highest CR results observed for *A. lumbricoides* and
lowest for *T. trichiura*, however MEB resulted in lower CR results compared to ALB. Overall, the efficacy of MEB by means of CR was 90.3% for *A. lumbricoides*, 58.0% for hookworm and 50.9% for *T. trichiura*. The FECR (1) for ALB was the highest for *A. lumbricoides* (>99.9%), followed by hookworm (99.7%) and *T. trichiura* (92.4%). Compared to ALB, MEB resulted in lower FECR (1) results for both hookworm (65.4%) and *T. trichiura* (65.4%). Efficacy results for *A. lumbricoides* for MEB (98.6%) were comparable to that of ALB. Compared to FECR (1), FECR (2) gave higher efficacy results. The combined results obtained in the different countries involved confirmed the therapeutic efficacy of these treatments against *A. lumbricoides*, the superior efficacy of ALB against hookworm infections, and the low efficacy against *T. trichiura*. Efficacy varied widely across the seven different trials, and infection intensity was an important confounding factor. The FECR rate based on arithmetic means (FECR (1)) was the best available indicator of drug efficacy, and should be adopted in future monitoring and evaluation studies of MDA programmes. Finally, our findings emphasize the need to revise the WHO recommended efficacy thresholds for a single oral dose ALB treatments.

In chapter 4, a randomized multi-arm efficacy trial was conducted to assess the efficacy of ALB and MEB administered for 1 (ALBx1 and MEBx1) or 2 consecutive days (ALBx2 and MEBx2) against heavy-intensity trichuriasis (mean FEC >1000 eggs per gram of stool (EPG)) in 385 school-aged children in Jimma Town. The efficacy (95% confidence intervals) by means of FECR (1) was 29.3% (29.9–56.2), 60.0% (48.5–70.9), 73.5% (64.2–81.3), and 87.1% (81.4–91.2) for ALBx1, MEBx1, ALBx2, and MEBx2, respectively. MEB treatment arms were more efficacious than ALB arms and repeated doses were more efficacious than single dose arms. These observations highlight that assessment of the anthelmintic efficacy of existing or new compounds against *T. trichiura* should be assessed under varying levels of infection intensity.

In chapter 5, we developed and evaluated a pooling strategy to assess the intensity of STH infections and drug efficacy. Stool samples from 840 children attending 14 primary schools in Jimma Town were pooled into different pool sizes (pool sizes of 10, 20, and 60 individual stool samples) to evaluate the infection intensity of STH. In addition, the efficacy of a single dose of MEB (500 mg) by means of FECR (1) was evaluated in 600 children from two of the 14 schools. Individual and pooled samples were examined with the McMaster egg
counting method. For each of the three STH, we found a significant positive correlation between the mean FEC of individual stool samples and the FEC of pooled stool samples, ranging from 0.62 to 0.98. Only for *A. lumbricoides* was any significant difference in mean FEC of the individual and pooled samples found. For this STH species, pools of 60 samples resulted in significantly higher FECs. FECR for the different number of samples pooled was comparable in all pool sizes, except for hookworm. For this parasite, pools of 10 and 60 samples provided significantly higher FECR results. This study highlights that pooling stool samples holds promise as a strategy for rapidly assessing infection intensity and the efficacy of administered drugs in programmes to control human STH. However, further research is required to determine when and how pooling of stool samples can be cost-effectively applied along a control programme, and to verify whether this approach is also applicable to other neglected tropical diseases.

In chapter 6, we combined all insights from the previous chapters and propose a plan on how to further improve the control of STH in children and how to monitor MDA programmes in Jimma Town. To this end, we describe the current control measures for STH in Jimma Town. Second, we provide concrete suggestions on how to improve current control measures through MDA, WASH (water, sanitation and hygiene) and education. Third, we outline a concrete plan to monitor MDA programmes. Finally, we make some suggestions for future research topics.
Samenvatting
Worminfecties veroorzaakt door *Ascaris lumbricoides* (rondworm), *Trichuris trichiura* (zweepworm), en *Ancylostoma duodenale* en *Necator americanus* (haakwormen) beïnvloeden in grote mate de volksgezondheid in zowel subtropische als tropische landen. De impact van deze worminfecties is vooral uitgesproken bij kinderen en vrouwen. Om deze wormen te bestrijden werden grootschalige ontwormingsprogramma’s ingevoerd. Maar ondanks de hoge prevalentie van worminfecties in Ethiopië, staan maatregelen om deze wormen te bestrijden in de kinderschoenen. De algemene doelstelling van dit proefschrift is daarom het verbeteren van de controle van worminfecties bij kinderen in Jimma, Ethiopië.

In **hoofdstuk 1** geven we een overzicht van de literatuur over (i) de huidige beschikbare en aanbevolen controlestrategieën voor worminfecties, (ii) de ontwormingsmiddelen aanbevolen door de Wereldgezondheidsorganisatie, (iii) de manieren om de effectiviteit van de ontwormingsmiddelen op te volgen, (iv) de factoren die de effectiviteit van geneesmiddelen kunnen beïnvloeden, en (v) de studies die de effectiviteit van ontwormingsmiddelen evalueerden in Afrika in de laatste 3 decennia. Dit overzicht toont aan dat grootschalige toediening van ontwormingsmiddelen de belangrijkste strategie is, en *albendazole* (ALB) en *mebendazole* (MEB) zijn hierbij de eerste keus ontwormingsmiddelen. Ze zijn relatief effectief, veilig en gemakkelijk toe te dienen (dosering is niet gebaseerd op lichaamsgewicht). De huidige strategie kan echter verbeterd worden. Allereerst is de frequentie van het toedienen van ontwormingsmiddelen gebaseerd op prevalentie, maar het is niet geweten welke demografische, sociale-economische en ecologische factoren bijdragen tot infectie. Ten tweede, de werkzaamheid van ALB en MEB is verschillend voor de verschillende wormsoorten, dus de keuze van ontwormingsmiddel zal afhangen van de lokale epidemiologie van de individuele wormsoorten. Ten derde, ondanks de wereldwijde uitbreiding van de ontwormingsprogramma’s, zijn er geen richtlijnen om de effectiviteit van de ontwormingsmiddelen te evalueren. Ten vierde, zijn er een aantal factoren die de effectiviteit van ontwormingsmiddelen beïnvloeden. Ten vijfde, er is een tekort aan strategieën voor een snelle beoordeling van de intensiteit van infecties en voor het opsporen van resistentie-ontwikkeling in de wormen.

In **hoofdstuk 2** werd de intensiteit en prevalentie van worminfecties nagegaan in 14 scholen in Jimma (Ethiopië) tijdens het droog- (februari-maart)
en regenseizoen (september-oktober). Een totaal van 1.680 schoolgaande kinderen werden onderzocht (840 in elk seizoen, 60 kinderen / school). Iedere stoelgang werd onderzocht met de McMaster techniek. Een meta-analyse werd uitgevoerd om verschillen in prevalentie en infectie-intensiteit tussen de twee seizoenen te evalueren. In 49.0% (824/1680) van de kinderen werd minstens 1 van de wormsoorten opgespoord. Infecties met *T. trichiura* kwamen het meest voor (35,5%), gevolgd door *A. lumbricoides* (23,4%) en haakwormen (9,9%). Tussen de scholen was er een enorme variatie in prevalentie, variërend van 16,7% tot 68,3% voor worminfecties, 6,7% tot 39,2% voor *A. lumbricoides*, 10,8% tot 55,0% voor *T. trichiura* en 0% tot 28,3% voor haakwormen. Worminfecties kwamen significant minder voor in het regenseizoen. Een significant verschil in prevalentie tussen seizoenen voor de drie wormsoorten afzonderlijk werd alleen waargenomen voor *T. trichiura*. Een significant verschil in infectie-intensiteit tussen seizoenen werd waargenomen voor *A. lumbricoides* en *T. trichiura*. Voor beide wormen werd een lagere graad van intensiteit waargenomen in het regenseizoen. Onze resultaten geven aan dat worminfecties wijd verspreid zijn in Jimma en dat tweejaarlijks toediening van een ontwormingsmiddel aanbevolen is voor de controle van deze wormen. Ze suggereren ook dat ontworming in het droog seizoen een grotere impact zal hebben op worminfecties dan wanneer ontworming in het regenseizoen gebeurt. De grote waargenomen variatie in zowel prevalentie en infectie-intensiteit in de scholen in Jimma rechtvaardigen verder onderzoek om na te gaan welke factoren deze variatie verklaren.

In *hoofdstuk 3* beschrijven we de effectiviteit van geneesmiddelen tegen worminfecties in schoolkinderen in Jimma (Ethiopië). Dit onderzoek maakte deel uit van een studie waarin de effectiviteit van een enkelvoudige orale toediening van ALB (400 mg) en MEB (500 mg) tegen wominfecties in schoolkinderen werd onderzocht in Azië, Afrika en Latijns-Amerika. Deze multinationale studie werd gestandaardiseerd op het gebied van diagnostische techniek (McMaster), opvolgperiode (ongeveer 14 dagen), de herkomst van de ontwormingsmiddelen en statistische analyse (percentage van kinderen dat geneest (CR) en 3 formules voor het bepalen van de reductie in ei-uitscheiding (FECR (1), (2) en (3); de eerste twee formules zijn gebaseerd op het groepsgemiddelde (rekenkundig / geometrisch) van de ei-uitscheiding vóór en na behandeling, de derde formule
Samenvatting

geeft het gemiddelde van de reductie in ei-uitscheiding per kind weer). De CR van ALB was het hoogst voor *A. lumbricoides* (99,3%), gevolgd door haakwormen (98,9%). De laagste CR werd waargenomen voor *T. trichiura* (85,7%). Voor MEB werd een gelijkaardige trend waargenomen: de hoogste CR resultaten werden waargenomen voor *A. lumbricoides* en de laagste voor *T. trichiura*. MEB resulteerde echter in lagere CR resultaten vergeleken met ALB. Wanneer de effectiviteit van MEB werd samengevat in CR was de effectiviteit 90,3% voor *A. lumbricoides*, 58,0% voor haakwormen en 50,9% voor *T. trichiura*. De FECR (1) voor ALB was het hoogst voor *A. lumbricoides* (>99,9%). De FECR (1) voor haakwormen was 99,7%, en 92,4% voor *T. trichiura*. Vergeleken met ALB, resulteerde MEB in lagere FECR (1) resultaten voor zowel haakwormen (65,4%) en *T. trichiura* (65,4%). De werkzaamheid voor MEB tegen *A. lumbricoides* (98,6%) was vergelijkbaar met die van ALB. Vergeleken met FECR (1), gaf FECR (2) hogere waarden. De gecombineerde resultaten bekomen in de verschillende landen die betrokken waren in deze studie bevestigden de therapeutische werkzaamheid van deze behandelingen tegen *A. lumbricoides*, de superieure werkzaamheid van ALB tegen haakworminfecties, en de lage werkzaamheid tegen *T. trichiura*. FECR (1) varieerde sterk tussen de verschillende landen, en intensiteit van de infecties was een belangrijke verklarende factor. De reductie in ei-uitscheiding op basis van het rekenkundig groepsgemiddelde (FECR (1)) was de beste indicator voor het evalueren van de effectiviteit van ontwormingsmiddelen, en moet de standaard statistiek worden voor studies die effectiviteit van geneesmiddelen in ontwormingsprogramma's evalueren. Tot slot, de resultaten benadrukken de noodzaak om de door Wereldgezondheidsorganisatie vooropgestelde minima voor effectiviteit van ALB en MEB moeten worden herzien.

In hoofdstuk 4 evalueerden we de effectiviteit van ALB en MEB toegediend gedurende 1 dag (ALBx1 en MEBx1) of 2 opeenvolgende dagen (ALBx2 en MEBx2) tegen *T. trichiura* infecties van hoge intensiteit (gemiddelde ei-uitscheiding >1000 eieren per gram stoelgang) in schoolgaande kinderen in Jimma. De effectiviteit (95% betrouwbaarheidsinterval) gemeten aan de hand van FECR(1) was respectievelijk 29,3% (29,9-56,2), 60,0% (48,5-70,9), 73,5% (64,2-81,3), en 87,1% (81,4- 91,2) voor ALBx1, MEBx1, ALBx2 en MEBx2. MEB was effectiever dan ALB, en een herhaalde toediening was effectiever dan een enkelvoudige toediening. Deze resultaten benadrukken dat de beoordeling van
de efficiëntie van bestaande of nieuwe ontwormingsmiddelen tegen *T. trichiura* onder verschillende niveaus van infectie-intensiteit moeten worden beoordeeld.

In **hoofdstuk 5** ontwikkelden en evalueerden we een strategie om stoelgang te mengen voor het bepalen van de intensiteit van worminfecties en de effectiviteit van ontwormingsmiddelen. De intensiteit van worminfecties werd bepaald voor 840 kinderen in 14 basisscholen in Jimma. De effectiviteit van een enkelvoudige dosis MEB (500 mg) werd bepaald bij 600 kinderen in 2 scholen en dit door middel van FECR (1). Stoelgang van 10, 20 en 60 kinderen werd gemengd tot 1 mengstaal. Individuele en mengstalen werden onderzocht met de McMaster techniek. Voor elk van de drie wormsoorten vonden we een significante positieve correlatie tussen de gemiddelde individuele ei-uitscheiding (FEC) en de FEC van het mengstaal, variërend van 0,62 tot 0,98. Alleen voor *A. lumbricoides* was er een significant verschil in ei-uitscheiding: mengstalen van 60 individuen gaven een significant hogere FEC. FECR (1) resultaten waren vergelijkbaar, behalve voor haakwormen. Voor deze wormen gaven mengstalen van 10 en 60 individuen een significant hoger FECR (1) resulat. Deze studie benadrukt dat het mengen van stoelgang een potentiële strategie is voor het snel beoordelen van de infectie-intensiteit van worminfecties en de effectiviteit van de toegediende ontwormingsmiddelen. Er is echter meer onderzoek nodig om te bepalen wanneer en hoe het mengen van stoelgang kosteneffectief is, en om na te gaan of deze aanpak ook toepasbaar is op andere tropische ziekten.

In **hoofdstuk 6**, combineren we alle inzichten uit de voorgaande hoofdstukken en stellen op basis van de huidige controle maatregelen een plan op om de controle van worminfecties in kinderen van Jimma te verbeteren. We bieden ook concrete suggesties aan voor het opvolgen van deze ontwormingsprogramma’s. Tot slot geven we enkele suggesties voor toekomstige onderzoeksonderwerpen.
Curriculum Vitae
1. Personal information

Name: Zeleke MEKONNEN KURMANE
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**September 2003-July 2005**
Degree: MSc degree in Molecular and Cellular Biology
Institution: Heidelberg University, Heidelberg, Germany

**September 1995-March 1998**
Degree: BSc degree in Medical Laboratory Technology
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**September 1987-March 1990**
Degree: Diploma in Medical Laboratory Technology
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3. Professional carrier

**April 2010-up to date**
Position: Associate professor (Since July 2014)
            Assistant professor (April 2010-June 2014)
Institution: Department of Medical Laboratory and Pathology, Faculty of Public
            Health and Medical Sciences, Jimma University, Jimma, Ethiopia
Duties: Teaching, research and public services in laboratory diagnosis

**September 2005-September 2006**
Position: Associate researcher
Institution: Laboratory of Prof. Dr. Herman Bujard, ZMBH, Heidelberg Germany

**March 1998-August 2003**
Position: Assistant lecturer and later as Lecturer
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            Health and Medical Sciences, Jimma University, Jimma, Ethiopia
Duties: Teaching, assisting research and conducting practical sessions for students

**March 1990-August 1995**
Position: Technical Assistant
Institution: Department of Medical Laboratory Sciences, Jimma Institute of
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4. Posts held

**2011 - present**
Post: Project leader of infectious diseases and epidemiology project,
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**2006-2007**
Post: Head, School of medical laboratory sciences and pathology, Jimma
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5. Selected peer reviewed publications


Curriculum vitae

4 years at Jimma Hospital, southwest Ethiopia. Tropical Medicine and International Health; 15:890-893


6. Selected poster and oral presentations


