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# Albendazole Efficacy and Associated Factors of Hookworm Species Re-Infection Among Schoolchildren in Three Endemic Districts of Amhara Region, Northwest Ethiopia

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COLLEGE OF MEDICINE AND HEALTH SCIENCES
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DEPARTMENT OF MEDICAL LABORATORY SCIENCE

# ALBENDAZOLE EFFICACY AND ASSOCIATED FACTORS OF HOOKWORM SPECIES RE-INFECTION AMONG SCHOOLCHILDREN IN THREE ENDEMIC DISTRICTS OF AMHARA REGION, NORTHWEST ETHIOPIA

BY: SHEGAW BELAY

A THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, SCHOOL OF HEALTH SCIENCES, COLLEGE OF MEDICINE AND HEALTH SCIENCES, BAHIR DAR UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL PARASITOLOGY AND VECTOR CONTROL

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**BAHIR DAR UNIVERSITY**

**COLLEGE OF MEDICINE AND HEALTH SCIENCES**

**SCHOOL OF HEALTH SCIENCES**

**DEPARTMENT OF Medical Laboratory Science**

# Albendazole Efficacy and Associated Factors of Hookworm Species Re-Infection Among Schoolchildren in Three Endemic Districts of Amhara Region, Northwest Ethiopia

**A Thesis Submitted To The Department Of Medical Laboratory Science, School Of Health Sciences, College Of Medicine And Health Sciences, Bahir Dar University In Partial Fulfillment Of The Requirements For The Degree Of Master Of Science In Medical Parasitology And Vector Control**

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# COLLEGE OF MEDICINE AND HEALTH SCIENCES
# SCHOOL OF HEALTH SCIENCES
# DEPARTMENT OF MEDICAL LABORATORY SCIENCE

## Advisors' Approval of Thesis for Defense

I hereby certify that I have supervised, read, and evaluated this Thesis entitled "**Albendazole efficacy and associated factors of hookworm species re-infection among schoolchildren in three endemic districts of Amhara Region, northwest Ethiopia" by Shegaw Belay** prepared under my guidance. I recommend the Thesis be submitted for oral defense,

Advisors:

| Name | Signature | Date |
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| 2. Mr. Getaneh Alemu (PhD candidate, Asst. Prof) | …… | ---------- |

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**DEPARTMENT OF MEDICAL LABORATORY SCIENCE**

## Examiners' Approval of Thesis for Defense Result

We hereby certify that we have examined this Thesis entitled "**Albendazole efficacy and associated factors of hookworm species re-infection among schoolchildren in three endemic districts of Amhara Region, northwest Ethiopia" by Shegaw Belay.** We recommend that the defense is approved for the degree of Master of Sciences in "Medical Parasitology and Vector Control.

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## DECLARATION

I hereby declare that this thesis, entitled "**Albendazole efficacy and associated factors of hookworm species re-infection among schoolchildren in three endemic districts of Amhara Region, northwest Ethiopia"** is my original work and has not been presented for a degree in any other University and all sources of material used for this Thesis have been properly acknowledged.

Name: **Shegaw Belay**

Signature: _____

Date: _____

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## LIST OF ABBREVIATIONS AND ACRONYMS

ALB - Albendazole

AM - Arithmetic Mean

BZ - Benzimidazole

CR - Cure Rate

DALY - Disability-Adjusted Life-Years

EPG - Egg Per Gram

ERR - Egg Reduction Rate

KK - Kato-Katz

MDA - Mass Drug Administration

MM - McMaster

PSAC - Preschool-Age Children

SAC - School-Age Children

SC - Schoolchildren

STH - Soil-Transmitted Helminth

STST - Spontaneous Tube Sedimentation Techniques

WASH - Water, Sanitation, and Hygiene

WHO - World Health Organization

## ABSTRACT

**Background:** Hookworm species are soil-transmitted helminths that dwell in the small intestine. Albendazole is the first-line drug for hookworm species infection, and it has been given as a treatment for a decade. However, its efficacy has been declining with time. Re-infection has also occurred rapidly after treatment in endemic areas. Regular monitoring of albendazole efficacy and re-infection rates are big challenges in endemic areas.

**Objective**: This study aimed to assess albendazole efficacy, re-infection rate, and factors associated with hookworm species re-infection among schoolchildren in endemic districts of Amhara Region, northwest Ethiopia.

**Method**: A school-based open-level single-arm follow-up study was conducted among 403 schoolchildren from May to December 2022. The study participants were selected by a systematic random sampling technique. Stool samples were processed via Kato-Katz, McMaster, and spontaneous tube sedimentation techniques. All hookworm species -infected schoolchildren were treated with single-dose albendazole (400 mg). The re-infection rates of hookworm species were assessed in the $4^{th}$ and $6^{th}$ months of initiation. The data was entered into Epi-data and analyzed using SPSS version 25. The associations of independent variables with the re-infection rate of hookworm species were calculated by bivariate logistic regression. Moreover, all variables with a *P-value* < 0.20 in the bivariate analysis were run in a multivariate logistic analysis and at 95% CI *P-value* < 0.05 were considered to be statistically significant.

**Results:** The baseline prevalence of hookworm species was 28.5% (115/403). Among the 115 hookworm species -infected participants, 101 schoolchildren participated in the drug efficacy analysis. The cure rate of albendazole against hookworm species was 85.1% (95% CI 77.3–91.1) by composite reference methods. The egg reduction rate was 93.4% by McMaster and 95.1% by Kato-Katz methods. The re-infection rates of hookworm species in the $4^{th}$ and $6^{th}$ months were 23.5% (19/81) and 33.3% (27/81), respectively. Living with > 5 family members (AOR = 4.682, 95% CI = 1.322-16.578, P =.017), poor utilization of latrine (AOR = 5.088, 95% CI = 1.517-17.065, P =.008), infrequent shoes wearing (AOR = 3.099, 95% CI = 1.058-9.083, P =.039), and

participating in irrigation (AOR=4.142, 95% CI=1.247-13.753, P=.020) were significantly associated with hookworm species re-infection.

**Conclusions:** Single-dose albendazole was found to be effective against hookworm species infection. The re-infection rates were significant during the $4^{th}$ and $6^{th}$ months after treatment. Participating in irrigation, infrequent shoe wear, poor latrine utilization, and livings with large family sizes were identified as risk factors for hookworm species re-infection. Therefore, albendazole should be administered in conjunction with an improved sanitation and hygiene program to control hookworm.

**Keywords**: Hookworm, Albendazole, Re-infection Rates, Cure Rate, Egg Reduction Rate, Ethiopia

# 1. INTRODUCTION

## 1.1. Background

Hookworm species are soil-transmitted helminths (STHs) that dwell in the small intestine of human beings. The two main species of hookworm that infect humans are *Ancylostoma duodenale* and *Necator americanus*. Hookworm species mainly affect the poorest and most deprived communities with poor nutrition, inadequate sanitation, overcrowding, and barefooted (Asfaw *et al.*, 2020; Sartorius *et al.*, 2021).

Hookworm species were responsible for more than 4 million disability-adjusted life years (DALYs), and more than 500 million people were infected in tropical countries (Bartsch *et al.*, 2016). Of these, nearly 120 million live in sub-Saharan Africa (Pullan *et al.*, 2014). Ethiopia had the third highest sub-Saharan African hookworm species burden (Hotez and Kamath, 2009). The national prevalence of hookworm species were reported at 7.6% (Leta *et al.*, 2020). Reports in Ethiopia also indicated that the prevalence of hookworm varied across the country. For instance, hookworm species prevalence was reported at 15.3% in southern Ethiopia (Leta *et al.*, 2020), 48.2% in west Gojjam, 46.9% in Durbete town, 41.3% in Sebatamit, and (64.2–87.7%) in rural Bahir Dar, Amhara Region (Alelign *et al.*, 2015; Nute *et al.*, 2018a; Hailu *et al.*, 2018; Anegagrie *et al.*, 2021).

Hookworm species are mainly transmitted through skin penetration by the infective filariform (L3) larvae, which is found in contaminated soil. Following skin penetration, the larvae enter the subcutaneous venules and lymphatics to gain accesses to the host's afferent circulation to the right side of the heart and then to the pulmonary vasculature. And then, it enters into the pulmonary capillaries to reach the alveolar spaces, ascends the brachial tree to the trachea, then the pharynx, and is swallowed. Once the larvae reach the lumen, it molts twice to become L5 larvae. In rare cases, *A. duodenale* can be transmitted via ingestion of the filariform larvae *(*Loukas *et al.*, 2016; Jourdan *et al.*, 2018*).* In the small intestine they anchor themselves in position to facilitate feeding and avoid being ejected by gut peristalsis. As they begin to feed on

blood, juvenile worms mature into sexually dioecious adult parasites. Mature adult male and female hookworms mate, and female hookworms produce as many as 10,000-25,000 eggs per day. Eggs are evacuated from the host via the faecal stream, which are diagnostic stages of the parasite (Loukas *et al.*, 2016; Haldeman *et al.*, 2020).

Following hookworm species infection, early clinical symptoms like rash and allergic reactions might be seen in the skin during skin penetration, eosinophilia and pneumonitis in the lung during larval migration, and gastrointestinal discomfort in the intestine by adults (Loukas *et al.*, 2016; Hotez, 2021). Moderate to heavy intestinal infection with hookworm causes mainly gastrointestinal bleeding, which results in iron deficiency anemia and poor intestinal absorption, which results in malnutrition, fatal stunted growth, increased susceptibility to other infections, and low educational achievement in schoolchildren (SC) (Loukas *et al.*, 2016; Grimes *et al.*, 2017; Pabalan *et al.*, 2018).

The diagnosis of hookworm species infection is connived by finding the ova of the parasite in stool through different parasitological diagnostic techniques, including direct wet mount, formol-ether concentration, Kato-Katz (KK), McMaster (MM), and spontaneous tube sedimentation technique (STST) (Ngwese *et al.*, 2020). However, the above methods varied in sensitivity to detect hookworm. The KK and MM are mainly recommended by WHO as diagnostic methods for drug efficacy evaluation (WHO, 2013).

Treatment of hookworm species has been applied primarily using Benzimidazole (BZ) (albendazole (ALB) (400 mg), and mebendazole (MEB) (500 mg) drugs. Mass drug administration (MDA) has been given as preventive chemotherapy (PC) for high-risk population groups (preschool-aged children (PSAC), school-age children (SAC), and women of reproductive age, depending on the prevalence rate (WHO, 2020).

The BZ anthelmintic works by binding to helminth tubulin, causing microtubule depolymerization and selective degeneration of cytoplasmic microtubules in the intestinal and tegmental cells. The metabolite attaches to the B-tubulin subunit of helminth microtubules,

inhibiting microtubule formation. It impairs glucose use and decreases the parasite's glycogen stores. A further decrease in adenine triphosphate production occurs, resulting in energy depletion, immobility, and death of the parasite (Malik and Dua, 2021).

Ethiopia has begun a nationwide MDA program to combat hookworm and other STH by using BZ drugs targeting SC (FMoH, 2016). However, it does not prevent re-infection (Campbell *et al.*, 2016; Mationg *et al.*, 2020). Previous reports showed that there was substantial re-infection after complete deworming of hookworm (Yap *et al.*, 2013b; Speich *et al.*, 2016; Dunn *et al.*, 2019). Socio-demographic, socioeconomic, behavioral, environmental, and reduced BZ therapeutic efficacy factors could be the determinants of hookworm re-infection (Dunn *et al.*, 2019; Tembo *et al.*, 2019). Hence, the prevention of hookworm requires an integrated PC, implementing improved water, sanitation, and hygiene (WASH), and a community-based health education strategy to interrupt the transmission cycle, particularly in high-endemic areas (WHO, 2020).

## 1.2. Statement of the problem

Hookworm species infection is an important public health problem in the country. Albendazole is the first-line drug for hookworm species infection, and it has been given as a PC for a decade. Despite previous repeated rounds of deworming using ALB in the country, the prevalence of hookworm remains high in endemic areas, including our study area (Nute *et al.*, 2018b; Hailu *et al.*, 2018). Albendazole efficacy has been declining with time, which might be due to the frequency of MDA, under-dosing, and monotherapy (Soukhathammavong *et al.*, 2012). However, the efficacy of ALB against hookworm species is poorly addressed in the study area.

The high prevalence of hookworm species might be due to the high re-infection rates of hookworm in endemic areas. Its re-infection can occur as early as two months after treatment in areas with high transmission (Yap *et al.*, 2013). But the re-infection rates of hookworm species are not yet determined in our study area. The re-infection rates of hookworm species may be aggravated by different factors, including poor WASH facilities (Appleby *et al.*, 2019; FMoH, 2021), socio-demographic factors, personal habits (direct soil contact with barefoot or bare hands), and environmental factors (soil type) (Pasaribu *et al.*, 2019; Anegagrie *et al.*, 2021), which are poorly addressed in the study area. Therefore, this study aimed to evaluate the efficacy of a single dose of ALB (400 mg), the re-infection rate, and factors associated with hookworm species re-infection among SC in three endemic districts of Amhara Region, northwest Ethiopia.

## 1.3. Significance of the study

This study will help to know the epidemiology of hookworm species infection in the study area to support national and regional treatment, prevention, and control strategies. Besides, this study will also provide information on the efficacy of single-dose ALB (400 mg) for the treatment of hookworm infection for health personnel, community health workers, and policymakers. In addition, this study will also help to provide information on the re-infection rates of hookworm species to community health workers and policymakers to take prevention measures. Moreover, this study will also give information on socio-demographics, personal habits, and environmental risk factors that contribute to the transmission of hookworm species infection among SC in the study area. Furthermore, the findings of the present study can be used as baseline data for researchers for further study on ALB efficacy, and hookworm re-infection.

# 2. LITERATURE REVIEW

## 2.1. Albendazole efficacy against hookworm species infection

Nowadays, the low efficacy level of BZ drugs is going to be the main concern in the developing world. Studies indicated that there is a high risk of reduced effectiveness of BZ drugs. Ample evidence has revealed its inconsistent efficacy status against STH, particularly in *T. trichuria* and hookworm infection, using a single dose of 400 mg ALB (Humphries *et al.*, 2017; Melati., 2019; Keller *et al.*, 2020).

According to a previous study in the Lao People's Democratic Republic among 200 hookworm-infected SC, the therapeutic efficacy of single-dose ALB (400 mg) showed 36% CR and 86.7% ERR against hookworm infection using the quadruplicate KK technique (Soukhathammavong *et al.*, 2012). A similar study conducted in Côte d'Ivoire among 200 SAC indicated that the therapeutic efficacy of a single dose of 400 mg ALB showed a CR of 53.3% and an ERR of 95.8% against hookworm infection (Patel *et al.*, 2021).

Among studies conducted in Ethiopia on the efficacy of ALB against hookworm infection, discrepancy reports were obtained. For instance, in a study conducted in Wondo Genet, southern Ethiopia, among 298 SC, the efficacy of single-dose ALB showed 97.4% CR and 99.8% ERR against hookworm infection using the KK method (Samuel *et al.*, 2014). Similarly, a study conducted at Mendera elementary school showed 93.7% CR and 99.9% ERR against hookworm infection among 326 participants using the MM technique (Tefera *et al.*, 2015).

In other studies that were conducted near our study, a reduced CR of ALB was reported among 70 participants using the MM and Harada-Mori techniques. The therapeutic efficacy of a single dose of ALB (400 mg) against hookworm showed 87% CR and 93% ERR (Bezie *et al.*, 2021). Another cross-sectional study conducted among 409 primary school students in Sebatamet, Ethiopia, also indicated that the therapeutic efficacy of a single dose of 400 mg ALB against hookworm was 76.8% (Hailu *et al.*, 2018).

Generally, the above reports regarding ALB efficacy indicated that there is a controversial report regarding ALB efficacy.

## 2.2. Re-infection rate of hookworm species

Rapid re-infection of hookworm after treatment is another key issue in the hookworm control program. Re-infection can occur starting 2 months after treatment in areas of high transmission (Yap *et al.*, 2013b). A longitudinal study was conducted to determine the pattern of STH re-infection in rural Malaysia among 120 SC. The re-infection rates of hookworm showed 33.3% and 51.8% in the 3$^{rd}$ and 6$^{th}$ months, respectively, using modified cellophane thick smear and Harada Mori techniques (Al-Mekhlafi *et al.*, 2008). Likewise, a cross-sectional study was conducted to assess STH re-infection in the 4$^{th}$ and 6$^{th}$ months after 400 mg single dose of ALB treatment among 523 SC in Myanmar. The re-infection rates of hookworm in the 4$^{th}$ and 6$^{th}$ months by using the KK technique were 18.92% and 28.07%, respectively, after complete treatment (Dunn *et al.*, 2019).

Also, a systematic review and meta-analysis conducted at the 3$^{rd}$, 6$^{th}$, and 12$^{th}$ months post-treatment in different parts of the world showed that the re-infection rates of hookworm were 30%, 55%, and 57% at the 3$^{rd}$, 6$^{th}$, and 12$^{th}$ months, respectively (Jia *et al.*, 2012). The re-infection rates of hookworm after 18 weeks of post-treatment among 405 Tanzanian SAC also showed 25% hookworm re-infection rates with moderate to light intensity (Speich *et al.*, 2016).

Limited studies were also conducted in Ethiopia to determine the re-infection rates of hookworm parasites. For instance, a cross-sectional study was conducted to assess the re-infection rate of STH and associated risk factors in Chencha District, southern Ethiopia, by using the KK thick smear diagnostic technique. Among 408 SAC, the re-infection rate of hookworm after the 3$^{rd}$ month of post-treatment was 1.0% (Zerdo *et al.*, 2016).

Another similar cross-sectional study was also conducted to assess MEB efficacy and re-infection rates of STH in northwest Ethiopia. After one year following 500 mg of MEB

treatment, 7.5% of SC were re-infected by hookworm, and the infections were grouped under "light intensity infection" (Zeleke *et al.*, 2020).

Based on the above-aforementioned scholars' report, we can conclude that re-infection of hookworm infection might be one possible factor for the existence of a high prevalence of hookworm infection in endemic areas.

### 2.3. Associated factors for hookworm species re-infection

Different factors may aggravate the re-infection rates of hookworm in endemic areas. Socio-demographic factors, personal habits (direct soil contact with barefoot or bare hands), and environmental factors (soil type) were some of the factors that increased the re-infection rates of hookworm. A longitudinal study was conducted to determine the pattern and predictors of hookworm re-infection in rural Malaysia among 120 SC. The age ranges between 7 and 12, being female, stunted children, and living in houses without toilets were the identified risk factors for higher re-infection rates of hookworm at 3$^{rd}$ months and maternal employment status at 6$^{th}$ months (Al-Mekhlafi *et al.*, 2008). Similarly, playing with soil or dirt, not having good hand-washing habits, and children who excrete in the open field were the predictors of hookworm infections among SC living in an agricultural area of North Sumatra, Indonesia (Pasaribu *et al.*, 2019). Likewise, being male, working as a farmer, having an educational status below the secondary school, and not having water for personal cleaning after defecation were risk factors for hookworm infection in Timor-Leste (Nery *et al.*, 2019).

A cross-sectional study was also conducted in Kenya among 9,801 SC to assess the associated risk factors of STH infections. Not wearing shoes, and a high number of household members were associated with hookworm infection (Okoyo *et al.*, 2020).

In Ethiopia, some studies were conducted to determine the infection rates of hookworm. Among those studies, a cross-sectional study was done at Goro primary school in southwest Shewa, Ethiopia, to determine the predictors of STH infections among SC. The multivariable analysis

revealed that children with irregular shoe-wearing habits, poor hand-washing habits after the toilet, living within a large family size, drinking water from an unprotected source, and a habit of playing with soil had a significantly higher risk of hookworm infection (Tiruneh *et al.*, 2020). In the same way, a cross-sectional study conducted at Sekela primary school in western Ethiopia showed that being male, being urban dwellers, having large family members, being non-latrine users, playing barefoot, eating raw vegetables, and the absence of hand washing were major factors associated with hookworm infection (Tolera and Dufera, 2020).

Another study conducted in Chencha district, southern Ethiopia, found that children come from families with no regular monthly income; the family's source of income was farming; the mother had no formal education or primary level of education; children who had no latrine for defection; and children who did not always wash their hands before a meal and after going to the toilet were the factors associated with hookworm re-infection (Zerdo *et al.*, 2016).

Generally, there are different socio-demographic, personal habits, and environmental factors associated with hookworm infection.

# 3. OBJECTIVES

## 3.1. General objective

To assess albendazole efficacy and factors associated with hookworm species re-infection among schoolchildren in three endemic districts of Amhara Region, northwest Ethiopia.

## 3.2. Specific objectives

- To evaluate the efficacy of albendazole against hookworm species infection among schoolchildren in the study area.
- To determine the re-infection rates of hookworm species infection among schoolchildren in the study area.
- To identify factors associated with hookworm species re-infections among schoolchildren in the study area.

# 4. MATERIALS AND METHODS

## 4.2. Study design, area, and period

A school-based open-label single-arm follow-up study was conducted in three selected primary schools from Bahir Dar city administration, North Mecha district, and Bahir Dar Zuria district, Amhara National Regional State, from May to December 2022. Bahir Dar city administration is located 565 km from Addis Ababa, the capital city of Ethiopia. The location of Bahir Dar city administration is 1820 m above sea level and at latitude $11^0$ 36'N, and longitude $37^0$ 23'E. It has a mean annual rainfall of 1839 mm and a temperature range of 14–28.1 °C. According to the Bahir Dar city plan 2022 report, the total population of the Bahir Dar city administration was 422,580 people. In the city administration, there were 41 public primary schools and 45,317 public primary school students (source: Bahir Dar city administration education office, 2022). North Mecha district is located 523 km northwest of Addis Ababa (the capital of Ethiopia) and 35 km southwest of Bahir Dar (the capital of the Amhara Regional State). It is located 1,800–2,500 m above sea level at latitude $11^0$ 29'59.99''N and longitude $37^0$ 00'E. The mean annual rainfall and temperature range of the north Mecha district were 1850 mm and 24 °C, respectively. According to the 2022 population estimation, the total population of the district was 366,061. The North Mecha district had 74 public primary schools and 52,128 public primary school students (source: North Mecha district education office, 2022). Bahir Dar Zuria district is located 564 km from Addis Ababa and 1900-2700 m above sea level with latitude $11^0$ 15''N longitudes $37^0$ 10''E. According to the 2022 population estimation, the total population of the district was 196,766. The mean annual rainfall and temperature of the Bahir Dar Zuria district were 1035 mm and 23 °C, respectively. Bahir Dar Zuria district had 66 public primary schools and 42,201 public primary school students (source: Bahir Dar Zuria district education office, 2022). The majority of study area populations were engaged in mixed agricultural activities. The types of soil dominant in the study area are clay, sand, and silt. The residents earn their living as farmers, merchants, daily laborers, and government employees. The inhabitants of the district use water sources such as streams, rivers, wells, pools, and tap water.

## 4.2. Population

### 4.2.1. Source population

All public SC in Bahir Dar city administration, North Mecha, and Bahir Dar Zuria districts.

### 4.2.2. Study population

All SC whose aged 6-14 in the selected public primary schools of the three selected districts during the data collection period.

### 4.2.3. Study participants

All eligible SC aged 6-14 years, attended the selected public primary schools in the daytime and were selected by chance to participate in the study.

## 4.3. Eligibility criteria

### 4.3.1. Inclusion Criteria

All SC in the selected primary schools, whose ages ranged from 6 to 14 years, permanently lived in the study community and had no plans to move in the next six months after baseline data collection, their parents/guardians volunteered and provided written consent in allowing their children to participate, and children who gave assent and could provide stool samples were included.

### 4.3.2. Exclusion criteria

Children who received anti-helminthic within the last three months before data collection and were taking anti-helminthic, anti-inflammatory, and antibiotic drugs during the data collection time, those who had known allergic reactions to ALB, children who attended other clinical trials during the same study period, mixed infections, diarrheic children, and children who vomited within 4 hours after drug administration took were excluded from the study.

## 4.4. Variable of the study

### 4.4.1. Dependent variables

- Efficacy of ALB (CR and ERR)
- Re-infection rate of hookworm

### 4.4.2. Independent variables for re-infection of hookworm

- ✓ **Socio-demographic factors**: Sex, age, mother's educational level, family size, residence, and family occupation.
- ✓ **Personal habits**: latrine utilization, hand washing before a meal and after a toilet, walking barefoot, frequency of shoes wearing and shoe type, playing with soil, and participation in irrigation.
- ✓ **Environmental factors**: Presence of clean environment, types of soil, toilet floor status, and house floor status.

## 4.5. Operational definition

**Cure rate:** The percentage ratio of baseline prevalence becoming hookworm negative by combinations of KK, MM, and STST after ALB treatment.

**CR** = Number of negative children after treatment who were positive at baseline X 100
number of hookworm-positive children before treatment

E**gg reduction rate:** The change in mean eggs per gram (EPG) counts of hookworm by KK and MM method after ALB (deworming) was given.

$$\left(1 - \frac{\text{Arithmetic mean (AM) egg counts at follow-up}}{\text{Arithmetic mean egg counts at baseline}}\right) \times 100$$

**Re-infection**: Children who were positive for hookworm species infection by at least one method during the baseline survey and negative by all three methods following 14 days of post-treatment and then became positive in the $4^{th}$ or $6^{th}$ month after treatment by at least one method.

$$\text{Re-infection rate} = \frac{\text{Number of infected children after treatment}}{\text{Number of negative children at follow-up who were positive at baseline}} \times 100$$

### 4.6. Sample size determination

The sample size was determined using single population proportion formula to determine ALB efficacy, re-infection rate of hookworm species, and associated factors. The sample size was calculated based on the following assumptions: The prevalence of hookworm infection (P = 41.3%) was taken from a previous study conducted at Sebatamit, Bahir Dar district (Hailu *et al.*, 2018). 95% level of confidence, 5% margin of error.

$$N = (Z\ \alpha/2)^2 \left(\frac{P(1-P)}{d^2}\right) = (1.96)^2 \left(\frac{.413(1-.413)}{0.05*0.05}\right) = 372$$

Where, N=sample size, P=estimated prevalence of hookworm, $d^2$=margin of error, and $Z_{\alpha/2}$ =the value of standard normal distribution corresponding to a significant level of alpha.

The sample size calculated to be a minimum of 372 then by adding 15% lost to the follow-up rate the final sample size was 428 SC.

### 4.7. Sampling technique

The study area was selected by purposive sampling method. The purposive sampling technique was applied for logistic and endemicity reasons. One public primary school in each district was selected by a simple random sampling technique, and the sample size was proportionally allocated in each selected public primary school. Finally, study participants were selected by systematic random sampling technique using a class roster as a sampling frame. The sample size was allocated proportionally to each district and in each class of school based on the total number of students.

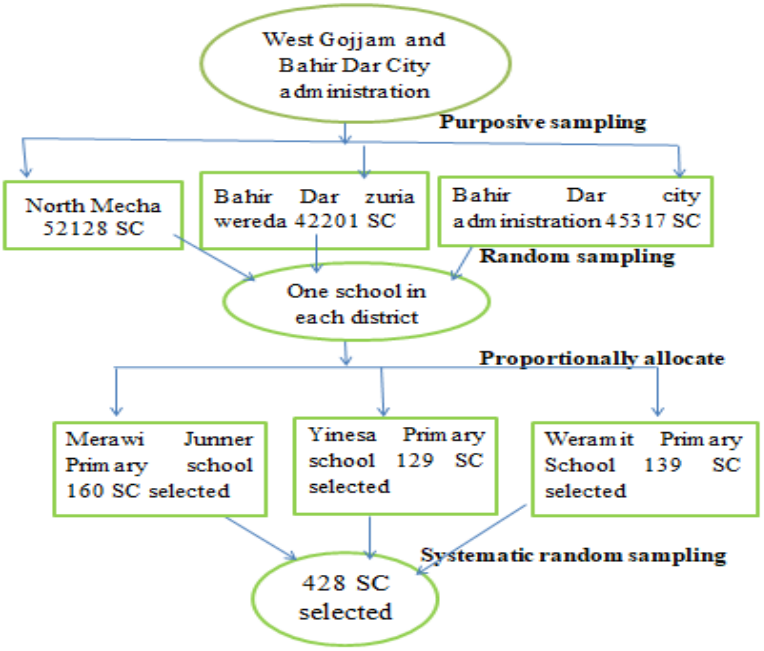


Figure 1: Sampling technique in the selection of study participants

### 4.8. Data collection methods

**Questionnaire data:** Structured questionnaires were used to collect socio-demographic data and WASH indicators at home and school levels that affect the re-infection rate of hookworm. A checklist was used to collect information regarding WASH facilities at the school level (Annex VIII). Trained laboratory professionals were engaged in questionnaire-based data collection, stool sample collection, detection, and ova quantification.

**Laboratory data collection and processing:** After the questionnaire data was collected, a clean and leak-proof stool container labeled with the participant's unique identification number was given to each study participant, and they were asked to provide approximately 10 grams of a fresh stool sample. Participants were instructed to avoid contaminating the stool with soil or urine while collecting the sample. To avoid any possible sample delay, stool samples collected at Merawi junior primary school were transported to the Merawi health center laboratory, whereas stool samples collected at Weramit primary school and Yinesa primary school were transported to Bahir Dar University, College of Medicine and Health Sciences, Medical Laboratory Science

Laboratory within an hour of collection. The stool sample was processed and examined according to the standard operating procedure (Annex V).

**Spontaneous tube sedimentation technique:** Approximately 3 grams of fresh stool were weighed and homogenized in 10 ml of normal saline solution (0.85% w/v). The mixture was filtered through the wire mesh into a 50-ml Falcon tube, then filled with more saline solution up to a 50-ml gauge, plugged, and shaken vigorously. The tube was left to stand for 45 minutes, and then a sample was taken from the sediment, put on a microscope slide, and examined with 10-x followed by 40-x objectives of a microscope to check for the presence of ova of hookworm (Tello *et al.*, 2012) (Annex V).

**Kato-Katz:** About 2–3 grams of a fresh stool sample were pressed through a mesh screen to remove large particles. Approximately 41.7 mg of stool was sieved and transferred to the template, which was placed on a slide until the template hole was filled. Then, the template was removed, and the stool sample was covered and pressed with cellophane, which had been previously immersed overnight in glycerol-malachite green. The KK smears were examined within 30–60 minutes. The EPG of hookworm was calculated by multiplying the fecal egg count by 24 (WHO, 2019) (Annex V).

**McMaster:** Two grams of a fresh stool were suspended in 30 ml of saturated salt (NaCl) solution at room temperature (density 1.2). The fecal suspension was filtered through a wire mesh to remove large debris and mixed ten times using a Pasteur pipette. Then a 0.15-ml aliquot was added to each side of a MM slide chamber. After two minutes of settling, both chambers were examined under a light microscope with 10-x objectives. The EPG of hookworm was calculated by multiplying the fecal egg count by 50 (Vercruysse *et al.*, 2011) (Annex V).

### 4.9. Efficacy of albendazole

Schoolchildren positive for hookworm infections were treated with a single dose of ALB tablet brand (NUBEND, India, Kopran Limited, batch no. K72020010 with a label claim of 400

mg/tablet, which was obtained from Merawi health center) by health officers. Before administration of the drug, the expiry date of the drug was checked, a snack (a biscuit) was given to each child, and finally, ALB was given. After the ALB tablet was chewed and then swallowed, the children were under direct observation by the health officer for 4 hours. Participants were aware of the side effects expected after ALB administration, such as fever, nausea, headache, stomach pain, vomiting, and dizziness. The safety or drug reaction of the ALB was checked after 4 hours and during the follow-up period.

### 4.10. Follow-up survey

Only those SCs that were treated with 400 mg ALB were requested and provided a second specimen at 14 days post-treatment (WHO, 2013). Children who were absent from school on the follow-up day or did not bring a stool were traced 1 or 2 days later. The laboratory methods used in the baseline survey were also applied in the follow-up survey. The ERR and CR, which are the primary outcome measures, were used to assess the therapeutic outcome of ALB against hookworm infection. The drug efficacy status was evaluated using the AM of ERR. According to the WHO guidelines, ALB is satisfactory if ERR≥ 90%, doubtful 80%<ERR<90%, and reduced if ERR<80% (WHO, 2013).

### 4.11. Re-infection rate assessment

Children that had positive for hookworm species infections during the baseline survey and microscopic negative (cures) following 14 days of post-treatment were re-tested in the $4^{th}$ and $6^{th}$ months after the follow-up survey. The same laboratory techniques were utilized as in the baseline and follow-up survey analysis.

$$\text{Re-infection rate} = \frac{\text{Number of infected children after treatment}}{\text{Number of negative children at follow-up who were positive at baseline}} \times 100$$

### 4.12. Data quality control

The structured questionnaire was prepared in English first and then translated to the Amharic language for a better understanding by the data collectors, and then it was translated back to the English language. The reliability of the study finding was ensured by applying the quality control measures to the whole process of the laboratory work (pre-analytical, analytical, and post-analytical quality control steps). The data was reviewed and checked daily for completeness by the principal investigator. The investigator made close follow-ups and was engaged during the process of data collection and laboratory examination. Training was given to data collectors and laboratory examiners on the study's objective and the laboratory diagnosis of hookworm by KK, MM, and STST. The label of the stool cup and the amount of stool samples were checked during stool sample collection. A laboratory technologist and the principal investigator examined smeared slides to eliminate observer bias. The result of our observation was recorded separately for later comparison. All discordant results were re-checked, and the consensus result was taken.

### 4.13. Data management and analysis

All data were entered into Epi Info version 3.1 and analyzed with SPSS version 25 software. The results were summarized using tables, graphs, the standard deviation, and frequency distribution. Descriptive statistics were used to compute the baseline prevalence, re-infection rate, CR, and ERR. The associations of independent variables with the re-infection rate of hookworm were calculated by bivariate logistic regression analysis. All variables with *P-values* < 0.20 in the bivariate analysis were run in a multivariate logistic regression analysis at 95% CI to filter the co-founding effects. Variables with a *P-value* < 0.05 were considered to be statistically significant.

### 4.14. Ethical consideration

Ethical clearance was obtained from the College of Medicine and Health Sciences Institutional Review Board, Bahir Dar University (R. No. 384/2022). A permission letter was secured from Amhara Public Health Institute (R. No. 03/1334). Supporting letters were also obtained from the

Bahir Dar City administration, North Mecha district, and Bahir Dar Zuria district health and education offices. Furthermore, written informed consent from the parent and assent was obtained from all study participants before involvement in the study. Confidentiality of the collected information and laboratory test results were maintained. Individual test results were communicated with each study participant, hookworm-positive cases were treated with a single-dose ALB (400 mg), and appropriate counseling was given. Finally, those who tested positive for intestinal parasitosis were referred to nearby healthcare facilities for treatment.

# 5. RESULTS

## 5.1. Socio-demographic characteristics of study participants

### 5.1.1. Socio-demographic characteristics of participants at baseline

Among the 428 SC in this study, 403 gave complete data and provided stool samples in the baseline survey with a response rate of 94.2%. Sixteen SC refused to participate in the study, and 9 SC could not provide a stool sample. Among the 403 participants, 223 (55.3%) were males. The mean age of participants was 10.84 ± 2.04 SD years. The study participants were selected from three districts in Amhara Region: 131 (32.5%) participants from Bahir Dar city administration, 155 (38.5%) participants from North Mecha district, and 117 (29%) participants were selected from Bahir Dar Zuria district. Two hundred ten (52.1%) SC lived from families with < 5 family members. Three hundred sixteen (78.4%) of the study participants were from families with farmer occupations, and more than three-quarters (76.4%) were rural dwellers. Two hundred seventy-two (67.5%) SC mothers have no formal education. Two hundred forty-six (61%) participants had toilets. Most (56.9%) of their toilet floors were made from mud, and only (49.6%) of SC regularly used toilets (Table 1).

Table 1: Socio-demographic characteristics of schoolchildren attending selected schools in Amhara Region, northwest Ethiopia, from May to December 2022 (N=403)

| Variables | | Frequency n (%) | IP n (%) |
|---|---|---|---|
| Sex | Male | 223 (55.3) | 104 (46.6) |
| | Female | 180 (44.7) | 64 (35.6) |
| Age | 6-10 | 156 (38.7) | 45 (28.8) |
| | 11-14 | 247 (61.3) | 123 (49.8) |
| Residence | Rural | 308 (76.4) | 134 (43.5) |
| | Urban | 95 (23.6) | 34 (35.8) |
| The study area of the participant | North Mecha | 155 (38.5) | 53 (34.2) |
| | Bahir Dar city | 131 (32.5) | 51 (38.9) |

| | | | |
|---|---|---|---|
| | Bahir Dar Zuria | 117(29.0) | 64 (54.7) |
| Educational status of mothers | No formal education | 272 (67.5) | 119 (43.8) |
| | Primary school | 102 (25.3) | 39 (38.2) |
| | Secondary school and above | 29 (7.2) | 10 (34.5) |
| Occupational status of household head | Farmer | 316 (78.4) | 138 (43.7) |
| | Merchant | 70 (17.4) | 24 (34.3) |
| | Daily laborer | 9 (2.2) | 3 (33.3) |
| | Government employee | 8(2.0) | 3 (37.5) |
| Family size | ≥ 5 | 193 (47.9) | 87 (45.1) |
| | < 5 | 210 (52.1) | 81 (38.6) |

*IP=intestinal parasite

### 5.1.2. Prevalence of intestinal parasites at baseline

The overall baseline prevalence of intestinal parasite infection among SC was 41.7% (168/403). Parasites identified were hookworm species, *A. lumbricoides*, *S. mansoni*, *E. histolytica/dispar*, *T. trichuria*, *E. vermicularis*, and *H. nana*, with the respective prevalence rates of 28.5%, 3.7%, 3.5%, 4%, 0.5%, 0.7%, and 0.7%. The prevalence of hookworm was 25.1% by KK, 26.3% by STST, 27.8% by MM, and 28.5% by composite reference (Table 2). The hookworm prevalence was 25.9% (95% CI 18.7–34.3), 27.7% (95% CI 20.9–35.5), and 32.5% (95% CI 24.1-41.8) in Bahir Dar city administration, north Mecha district, and Bahir Dar Zuria district, respectively. The AM infection intensity of hookworm was 232.5 EPG and 373 EPG by KK and MM, respectively. The majority of hookworm infection intensity was light.

Table 2*:* Parasites detected by KK, MM, STST, and Composite reference method among SC attending selected schools in the Amhara Region, northwest Ethiopia, from May to December 2022 (N = 403)

| **Parasite identified** | **Diagnostic Method** | | | |
|---|---|---|---|---|
| | **Kato-Katz** | **McMaster** | **STST** | **Composite Reference** |
| | **n (%)** | **n (%)** | **n (%)** | **n (%)** |
| Hookworm species | 101 (25.1) | 112 (27.8) | 106 (26.3) | 115 (28.5) |
| *A. lumbricoides* | 13 (3.2) | 8 (2) | 15 (3.7) | 15 (3.7) |
| *T .trichuria* | 1 (0.2) | 1 (0.2) | 2 (0.5) | 2 (0.5) |
| Total STHs | 115 (28.5) | 121 (30) | 123 (30.5) | 132 (32.8) |
| *S.mansoni* | 9 (2.2) | - | 11 (2.7) | 14 (3.5) |
| *E.histolytica/dispar* | - | - | 16 (4) | 16 (4) |
| *E. vermicularis* | 1 (0.2) | - | 3 (0.7) | 3 (0.7) |
| *H.nana* | 1 (0.2) | 2 (0.5) | 3 (0.7) | 3 (0.7) |
| Total parasite | 126 (31.3 ) | 123 (30.5) | 156 (38.7) | 168(41.7) |

*STST=spontaneous tube sedimentation technique, n=number of positive children

### 5.1.3. Socio-demographic characteristics of the treatment group

Of the 115 SC that tested positive for hookworm species infection by combined reference methods, six participants did not fulfill the required eligibility criteria (five participants had mixed infections, and one participant was taking an anti-protozoan drug). One hundred nine SC fulfilled the required eligibility criteria of the efficacy study. Of 109 participants, 8 refused to take the drug and only provided a single stool. Finally, 101 SC participated in the final drug efficacy analysis. Of these, (62.4%) were males, and most study participants (80.2%) in the efficacy study were rural dwellers. The participants in the efficacy study had an average age of 11.27±1.94 SD. The enrolled participants were from the Bahir Dar city administration 31

(30.7%), the Bahir Dar Zuria district 33 (32.7%), and the north Mecha district 37 (36.6%) (Table 3).

Table 3: The socio-demographic characteristics of study participants of the treatment group attending selected schools in the Amhara Region, northwest Ethiopia, from May to December 2022 (N = 101)

| Socio-demography | Variables | Frequency (n) | Percent (%) |
|---|---|---|---|
| Sex | Male | 63 | 62.4 |
| | Female | 38 | 37.6 |
| Age | 6-10 | 21 | 20.8 |
| | 11-14 | 80 | 79.2 |
| Residence | Rural | 81 | 80.2 |
| | Urban | 20 | 19.8 |
| The study area of participants | North Mecha | 37 | 36.6 |
| | Bahir Dar city | 31 | 30.7 |
| | Bahir Dar Zuria | 33 | 32.7 |

### 5.2. Efficacy of single-dose albendazole (400 mg) against hookworm infection

After we finished the baseline survey, a school meeting was held, and training was given about the aim of the study, the benefits of deworming, how to swallow the drug, and the side effects of the drug. Finally, a total of 101 (63 male and 38 female) participants received treatment and completed follow-up examinations, hence being included in the final efficacy analysis. After 14 days of post-treatment, the CR of ALB against hookworm was 85% (95% CI 77–92%), 85.6% (95% CI 77.8–92.2), 84.4% (95% CI 76.1–90.6), and 85.1% (95% CI 77.3-91.1%) by MM, KK, STST, and composite reference, respectively. The ERR of ALB against hookworm was 93.4% by MM and 95.1% by KK. Using KK as a diagnostic technique, the AM EPG of hookworm infection at baseline was 232.5 EPG (95% CI 171.5–321.1) with a minimum of 48 EPG and a maximum of 2568 EPG. The AM intensity of hookworm infections was reduced to 11.5 EPG (95% CI 5.3–18.4), with a minimum of 0 EPG and a maximum of 192 EPG following two weeks

of post-treatment. But when we used MM as a diagnostic technique, the AM EPG of hookworm infection at baseline was 373 EPG (95% CI 297.5–478.0), with a minimum of 100 EPG and a maximum of 3350 EPG. The AM intensity of hookworm infections was reduced to 24.5 EPG (95% CI 12.5–38.0) after two weeks of treatment, with a minimum of 0 EPG and a maximum of 350 EPG (Table 4).

Table 4: Infection intensity, ERR, and CR measured by Kato-Katz, McMaster, Spontaneous tube sedimentation, and composite reference methods among schoolchildren attending selected schools in the Amhara Region, northwest Ethiopia, from May to December 2022 (n = 101)

| Hookworm Method | Mean intensity of infection at baseline | Mean intensity of infection at follow-up | ERR (%) | Cured n (%) | Non-cured n (%) | Total n (%) |
|---|---|---|---|---|---|---|
| McMaster | 373.0 | 24.5 | 93.4 | 85 (85.0) | 15 (15) | 100 (100) |
| Kato-Katz | 232.5 | 11.5 | 95.1 | 77 (85.6) | 13 (14.4) | 90 (100) |
| STST | NA | NA | NA | 81 (84.4) | 15 (15.6) | 96 (100) |
| Composite reference | | | | 86 (85.1) | 15 (14.9) | 101 (100) |

* ERR=egg reduction rate, NA=not applicable, STST=spontaneous tube sedimentation technique

**Safety of Albendazole**: The safety of ALB was also monitored for 4 hours after drug administration and during the follow-up period. The result revealed that no drug-related adverse events were observed. Hence, single-dose 400 mg ALB treatments were safe for SC and well tolerated in the study area.

### 5.3. Re-infection rate of hookworm species

Eighty-six (33 female and 53 male) participants who were hookworm species positive at baseline and tested negative after 14 days of post-treatment were followed for 4 months to determine their re-infection rate status. Five students were absent from school in the 4$^{th}$ month of the re-infection rate data collection period. Eighty-one (28 female and 53 male) participants gave a stool sample

and participated in the re-infection rate assessment. The overall re-infection rate of hookworm species among SC in the study area was 23.5% (95% CI 14.8–34.2) in the 4th month and 33.3% (95% CI 23.2-44.7) in the 6th month by the composite reference method. The re-infection rate of hookworm was 21% by KK, 23.5% by STST, and 23.5% by MM in the 4th month. In the 6th month, the re-infection rate was 29.6% by KK, 33.3% by STST, and 33.3% by MM (Figure 3). In the six months, 3 (3.7%) *S. mansoni*, 3 (3.7%) *A. lumbricoides*, and 1 (1.2%) *T. trichuria* were detected.

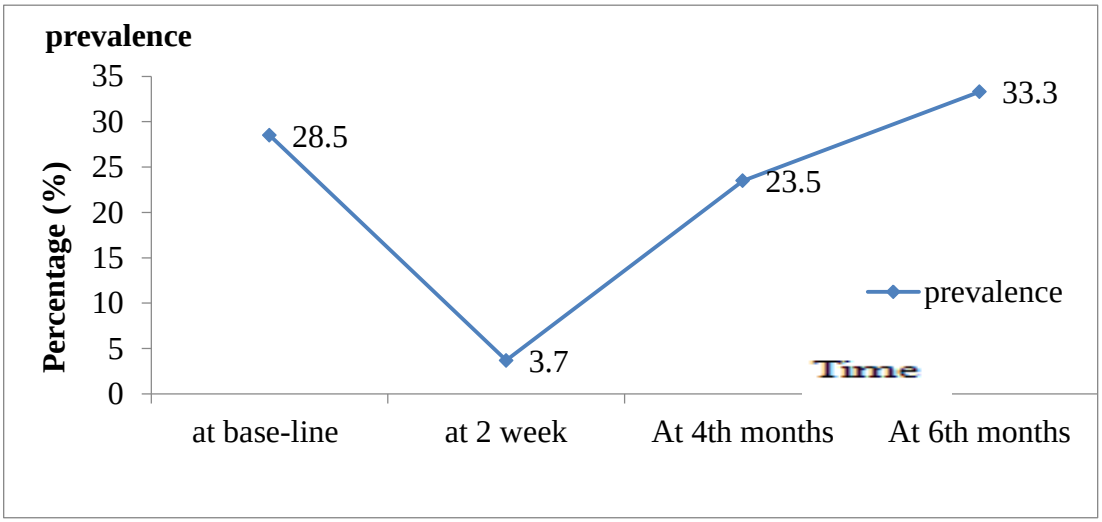


Figure 2: Prevalence of hookworm at baseline, 2 weeks, and 4th and 6th months after deworming by composite reference method

**The intensity of infection:** The AM intensity of hookworm re-infections in the 4th month was 53.7 EPG and 139.9 EPG by KK and MM methods, respectively. In the 6th month, the AM intensity of infection was increased to 92.6 EPG and 207.1 EPG by the KK and MM methods, respectively. The AM intensity of hookworm re-infections was grouped under "light infection intensity" in the 4th and 6th month follow-up periods based on WHO thresholds for determining infection intensity (Figure 4).

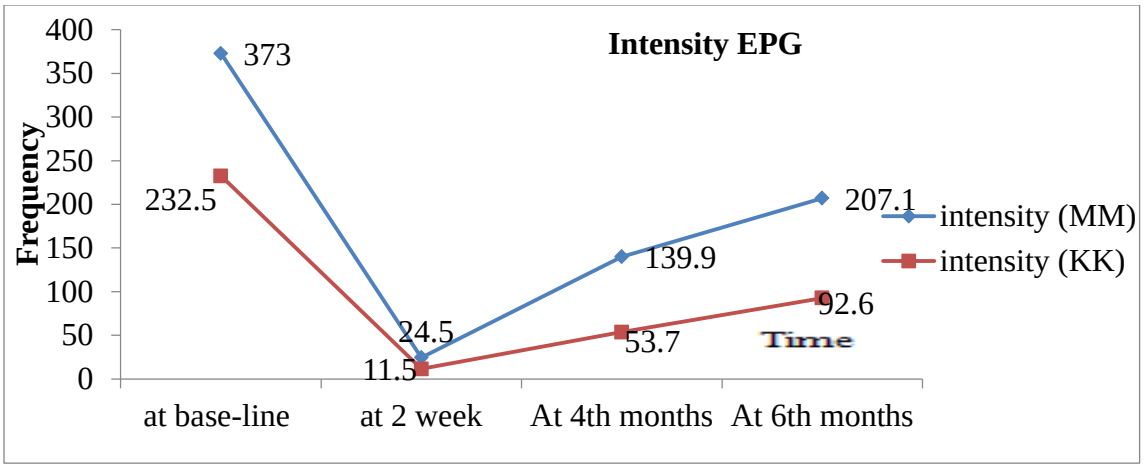


Figure 3: Arithmetic mean intensity of hookworm (EPG) at baseline, at 2 weeks, 4th, and 6th months after deworming by KK and MM diagnostic method

The re-infection rates of hookworm were 19.4% (95% CI 7.5-37.5), 20.8% (95% CI 7.1-42.2), and 30.8% (95% CI 14.3-51.8) at North Mecha district, Bahir Dar city administration, and Bahir Dar Zuria district, respectively, in the 4th month. In the 6th month, the re-infection rate was 29% (95% CI 14.2-48.0) in the North Mecha district, 42.3% (95% CI 23.4-63.1) in the Bahir Dar Zuria district, and 29.2% (95% CI 12.6-51.1) in the Bahir Dar city administration (figure 5).

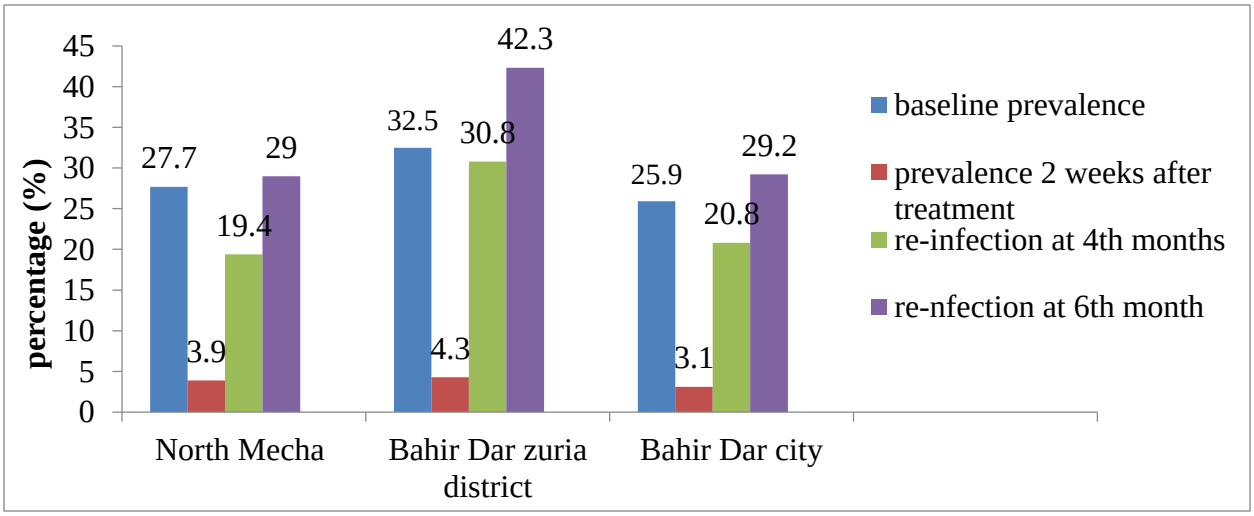


Figure 4: Baseline prevalence, two weeks after treatment, in 4th, and 6th -month re-infection rates of hookworm after deworming in the study area by composite reference method

### 5.4. Factors associated with hookworm species re-infection

The potential predictors of hookworm re-infection were investigated using bivariate and multivariate analyses. The results of the bivariate analysis showed that statistically significant associations were shown between the re-infection rate of hookworm and the risk factors, including latrine availability and utilization, hand washing after using the toilet, shoe-wearing frequency, living with large family size, and child participation in irrigation.

In the 4$^{th}$ month, bivariate analyses showed that SC who had open defecation practice (COR=3.186 CI=1.023-9.927, p=.046), children with poor hand washing practice after using a toilet (COR=2.941 CI=1.020-8.484, p=.046), not wearing shoe consistently (COR=3.877 CI=1.241-12.109), p=.02), and SC who participate in irrigation (COR= 3.400 CI=1.091-10.60, p=.035) had significantly higher re-infection rates of hookworm species infection than the other group.

In the multivariate analysis, children who did not have consistent shoe-wearing habits were 3.4 times more likely to be re-infected by hookworm (AOR =3.359 CI=1.024-11.026, p=.020). Similarly, SC who participated in irrigation activity were 3.7 times more likely to be re-infected by hookworm in the 4$^{th}$ month (AOR= 3.704 CI=1.092-12.562, p=.036) than SC who did not participate in irrigation (Table 5).

Table 5: Bivariate and multivariate analysis of socio-demographic and WASH risk factors associated with hookworm re-infection in the 4th month among schoolchildren attending selected schools in the Amhara Region, northwest Ethiopia, from May to December 2022 (n = 81)

| **Variables** | **Re-infection rates of hookworm species in 4th month** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Categories** | **Pos. n (%)** | **Neg. n (%)** | **COR (95% CI)** | ***p*-value** | **AOR (95% CI)** | ***p.* value** |
| Sex | Female | 5 (17.9) | 23 (82.1) | 1 | | | |
| | Male | 14 (26.4) | 39 (73.6) | 1.651 (.397-3.574) | .754 | | |
| Age | 6-10 | 3 (21.4) | 11 (78.6) | 1 | | | |
| | 11-14 | 16 (23.9) | 51 (76.1) | 1.150 (.285-4.640) | .844 | | |
| Family size | =<5 | 5 (14.7) | 29 (85.3) | 1 | | | |
| | >5 | 14 (19.8) | 33 (70.2) | 2.461(.790-7.667) | .120 | 3.010(.874-10.369) | .081 |
| Residence | Urban | 2 (10) | 18 (90) | 1 | | 1 | |
| | Rural | 17 (27.9) | 44 (72.1) | 3.477(.727-16.621) | .118 | 1.603(.286-8.978) | .591 |
| Latrine | Yes | 5 (11)2 | 33 (88.8) | 1 | | | |
| utilization | No | 14 (32.6) | 29 (67.4) | 3.186 (1.023-9.927) | .046 | 2.746 (.789-9.555) | .112 |
| Hand washing | Yes | 9 (16.7) | 45 (83.3) | 1 | | 1 | |
| after the toilet | No | 10 (37) | 17 (63) | 2.941 (1.020-8.484) | .046 | 1.585 (.472-5.326) | .456 |
| Participation | No | 5 (17.3) | 34 (82.7) | 1 | | 1 | |
| in irrigation | Yes | 14 (33.3) | 28 (66.7) | 3.400 (1.091-10.60) | .035 | 3.704 (1.092-12.56) | .036* |
| Frequency of | Always | 5 (12.2) | 36 (87.8) | 1 | | 1 | |
| shoe wearing | Sometimes | 14 (35) | 26 (65) | 3.877(1.241-12.109) | .020 | 3.359 (1.024-11.026) | .046* |

***** Pos=positive, Neg=negative, CI=confidence interval, COR=crude odds ratio, AOR=adjusted odds ratio, *=significant association

In the 6th month, multivariate analysis revealed that SC who lived with more than five family members (AOR= 4.682 95% CI=1.322-16.578, P=.017), poor utilization of latrines (AOR=5.088 95%CI=1.517-17.065, P=.008), not wearing shoe consistently (AOR=3.099 95% CI=1.058-9.083, P=.039), and actively participated in agricultural practices (AOR=4.142 95% CI=1.247-13.753, P=.020) had significantly higher re-infection rates than their counterparts (Table 6).

Table 6: Bivariate and multivariate analysis of socio-demographic and WASH risk factors associated with hookworm re-infection in the 6th month among schoolchildren attending selected schools in the Amhara Region, northwest Ethiopia, from May to December 2022 (n = 81)

| **Variable** | **Re-infection rates of hookworm species in 6th month** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Categories** | **Pos. n (%)** | **Neg. n (%)** | **COR (95%CI)** | ***p-value*** | **AOR 95% CI** | ***p. value*** |
| Family size | <5 | 7 (20.6) | 27 (79.4) | 1 | | 1 | |
| | >5 | 20 (42.6) | 27 (57.4) | 2.857 (1.038-7.865) | .042 | 4.682 (1.322-16.578) | .017* |
| Residence | Urban | 3 (15) | 17 (85) | 1 | | 1 | |
| | Rural | 24 (39.3) | 37 (60.7) | 3.676 (.972-13.905) | .055 | 1.535 (.328-7.178) | .586 |
| Latrine | Yes | 6 (16.2) | 31 (83.8) | 1 | | 1 | |
| availability | No | 21 (47.7) | 23 (52.3) | 4.717 (1.642-13.55) | .004 | 1.402 (.263-7.484) | .692 |
| Latrine | Yes | 6 (15.8) | 32 (84.2) | 1 | | 1 | |
| utilization | No | 21 (48.8) | 22 (51.2) | 5.091(1.769-14.65) | .003 | 5.088 (1.517-17.065) | .008* |
| Hand wash | Yes | 15 (27.8) | 39 (72.2) | 1 | | 1 | |
| after toilet | No | 12 (44.4) | 15 (55.6) | 2.080 (.793-5.458) | .137 | .770 (231-2.564) | .671 |
| Participation | No | 8 (20.5) | 31 (79.5) | 1 | | 1 | |
| in irrigation | Yes | 19 (45.2) | 23 (54.8) | 3.201 (1.194-8.585) | .021 | 4.142 (1.247-13.753) | .020* |
| Frequency of | Always | 8 (19.5) | 33 (80.5) | 1 | | 1 | |
| shoe wearing | Sometimes | 19 (47.5) | 21 (52.5) | 3.732 (1.386-10.052) | .009 | 3.099 (1.058-9.083) | .039* |

COR=crude odds ratio, AOR=adjusted odds ratio, *=significant association

# 6. DISCUSSION

Hookworm infection is a treatable and preventable disease with high public health importance in endemic countries like Ethiopia. The national control program was designed to eliminate this parasitic disease by 2025 and achieve less than 2% prevalence using a single dose of ALB (400 mg) or MEB (500 mg), WASH, health education, and advocacy (FMOH, 2021). Albendazole is the treatment of choice against hookworm infections in PC control programs. Yet it reveals inconsistent efficacy (Humphries *et al.*, 2017). Reports showed that re-infections of hookworm occur quickly after treatment and significantly affect the success of PC (Yap *et al.*, 2013b; Speich *et al.*, 2016; Dunn *et al.*, 2019).

The baseline prevalence of hookworm infection among SC was 28.5% (95% CI 24.2–33.2) by the composite reference method. This result is in line with the study in South Sumatra, Indonesia (25.9%) (Rahmi *et al.*, 2021) and North Gondar (26.2%) (Nute *et al.*, 2018). However, the current result is lower than the studies in Debre Elias and Sanja districts, Amhara Region (63.2%) (Zeleke *et al*., 2021), and rural communities in Bahir Dar, Amhara Region (64.2–87.8%) (Anegagrie *et al*., 2021). The variation could be attributed to differences in study participant age (the previous study included adults), parasite endemicity (level of soil contamination), diagnostic methods, and health status of participants (Zeleke used five methods, and the study participants were symptomatic adults). On the contrary, the current finding is higher than the study in Central Kenya (1%) (Sartorius *et al*., 2021) and in Bibugn, Amhara Region (7.5%) (Goshu *et al*., 2021). This variation could be attributed to the diagnostic method (only the KK method was used in Kenya, but three methods were used in this study), temperature, and altitude (in Bibugn, there is cold weather at 9-24 °C, which is not comfortable for hookworm larvae survival, development, and transmission). In addition, *A. lumbricoides* was more prevalent in the previous study, unlike hookworm.

The present study showed that a single dose of 400 mg ALB had an ERR of 93.4% and 95.1% by the MM and KK methods, respectively. The efficacy status of ALB against hookworm in the

current study is considered a satisfactory therapeutic outcome according to the WHO efficacy evaluation standard (WHO, 2013). This result is consistent with previous studies conducted in different countries, with an ERR of 96,6% in Cambodia, 91.9% in Cameroon, 92.6% in Tanzania (Vercruysse *et al.*, 2011), 95.8% ERR in Côte d'Ivoire (Patel *et al.*, 2021), and 93% ERR in Adet Hospital, Amhara Region (Bezie *et al.*, 2021). However, this result is higher than a study report (67.3%) in Vietnam study (Dyer *et al.*, 2022). This study is also higher than the earlier study reported in Nigeria (69.2%) (Sam-Wobo *et al.*, 2021). This variation might be due to the diagnostic method (in Vietnam, polymerase chain reaction (PCR) was used, whereas KK and MM were used in the current study), the day-to-day variation of the egg released by the female adult worm, treatment history, and geographic location (the differences in strain and species susceptibility or resistance).

The CR of ALB against hookworm species in this study was 85% (95% CI 77–92%), 85.6% (95% CI 77.8–92.2), 84.4% (95% CI 76.1–90.6), and 85.1 (95% CI 77.3-91.1%) by MM, KK, STST, and composite reference method, respectively. This is in line with studies conducted in Brazil (88.5%), in Cameron (87.1%), in Tanzania (86.8%) (Vercruysse *et al.*, 2011), and in Debre Elias District, Amhara Region (81.7%) (Aschale *et al.*, 2022). However, the current CR result is higher than a study conducted in Vietnam (68.4%) (Dyer *et al.*, 2022). The difference might be due to the diagnostic method (the previous study used PCR, which has a higher diagnostic sensitivity than microscopy-based approaches and can detect even very light hookworm infections that microscopy would miss), the baseline egg load (15.5%) of hookworm infection were moderate to high intensity, treatment history, and drug quality.

The CR of this study is also higher than the studies in Côte d'Ivoire (55.3%) (Patel *et al.*, 2021) and Lao People's Democratic Republic (36%) (Soukhathammavong *et al.*, 2012). The difference could be due to the intensity of infection: nearly 20% of infections in the People's Democratic Republic were high intensity, whereas only 1.7% of infections in this study were moderate intensity. The treatment history, geographic location of the parasite (thus differences in strain and species susceptibility or resistance), and the number of eggs released by the female adult

worm between days could also be the cause of the discrepancy. However, the current result is lower than previous studies report conducted in Wendo Genet, southern Ethiopia (97.4%) (Samuel *et al.*, 2014), and Jimma, southwest Ethiopia (93.7%) (Tefera *et al.*, 2015). This discrepancy might be due to drug quality (variations in the amount of active ingredient in a drug formulation), degradation during storage or transportation, and study time difference (there were many rounds of MDA between this study and the previous study; due to this repeated MDA drug exposure, it may be associated with an increase in the selection of the proportions of the drug-resistant allele).

The post-treatment re-infection patterns of hookworm species largely depend on the degree of endemicity within the community and the level of soil contamination. In the current endemic districts of the Amhara region, follow-ups in the $4^{th}$ and $6^{th}$ months after the administration of single-dose 400 mg ALB revealed that re-infection with hookworm was rapid in terms of prevalence. However, the intensity was light. Indeed, the re-infection rate of hookworm reached 23.5% and 33.3% in the $4^{th}$ and $6^{th}$ months after treatment, respectively. This increase in the hookworm re-infection in the $4^{th}$ and $6^{th}$ months of follow-up was probably attributable to the convenient soil temperatures and humidity in the summer season (June–October), the soil type in the study area, and the level of soil contamination (wide distribution of the infective stages of hookworm). This condition favors the survival, development, and transmission of hookworm larvae. Furthermore, the school was closed in the summer season, and children spent most of their time in the field (they defecate in an open field at a farm, which is conducive to parasite survival and distribution). Moreover, there is also a habit of walking barefoot in the agricultural area in the summer; this could be the cause of the high hookworm re-infection rate during the study period. `

The re-infection rate of hookworm in the $4^{th}$ month among the SC was 23.5% (95% CI 14.8–34.2%). The current study's re-infection rate is consistent with previous studies in Tanzania (25.0%) (Speich *et al.*, 2016) and Debre Elias District, Amhara Region (21.4%) (Aschale *et al.*, 2022). However, this result is higher than that of the Chinese study (2%) (Yap *et al.*, 2013a). The

difference might be due to seasonal differences. In China, the study was conducted from December–March, which is a cold and dry season that is unsuitable for the development and survival of hookworm larvae), the baseline prevalence, the level of soil contamination, and the dose of ALB (the previous study used a triple dose). This result is also higher than the study in Chencha district, southern Ethiopia (1%) (Zerdo *et al.*, 2016). The difference might be due to follow-up time, STH species difference, and diagnostic method differences. In Chencha, re-infection was assessed at the 3rd month and used only the KK method, but there is one extra month in this study that allows more time for SC to become newly re-infected and for surviving infections to re-establish and release eggs, and *A. lumbricoides* was the dominant species, unlike hookworm in Chencha.

In the 6th month, the re-infection rate of hookworm was 33.3% (95% CI 23.2–44.7). This result is in line with the study in Myanmar (28.07%) (Dunn *et al.*, 2019). However, this result was lower than the study in Malaysia (51.8%) (Al-Mekhlafi *et al.*, 2008) and systematic review and meta-analysis (55%) (Jia *et al.*, 2012). The difference could be due to study time variation, during which many intervention activities have been done in the country. This decreases hookworm prevalence through implementing the MDA program among SAC, creating an open defecation-free environment, and house-to-house education by health extension workers. On the contrary, this result is higher than the study in Yunnan, China (5.1%) (Yap *et al.*, 2013a). The difference might be due to the study season difference; the previous study was done in the winter season (December–March), which was a cold and dry condition known to be unsuitable for the development and survival of hookworm larvae. Similarly, the current finding is higher than the study in northwest Ethiopia, at 7.5% after one year (Zeleke *et al.*, 2020). The difference could be due to the diagnostic methods and species differences in Zeleke's study, in which direct wet mount and KK methods were used. In addition, hookworm was less prevalent in the area, unlike *A. lumbricoides*.

In both the 4th and 6th months, there was a slightly higher rate of re-infection in the Bahir Dar Zuria district than in the north Mecha district and Bahir Dar city administration. In the 4th month,

Bahir Dar Zuria district participants were nearly 1.7 and 1.9 times more likely to be re-infected by hookworm than participants from Bahir Dar city administration and north Mecha district, respectively. At the same time, Bahir Dar Zuria district participants were 1.8 times more likely to be re-infected than Bahir Dar city administration and north Mecha district participants in the 6$^{th}$ month. But the difference was not statistically significant. A slight increase in Bahir Dar Zuria might be due to the initial prevalence and level of environmental sanitation in the school compound, where there was open defecation. During the visits to the study areas, we observed that the school's hygiene was poor; the class floor was covered in mud and dust, many of the students were barefooted, and there was no water for drinking or hand washing after using the toilet in the school compound.

The present study also assessed factors for hookworm re-infection and revealed that the frequency of shoe wearing was significantly associated with an increased re-infection rate of hookworm. Study participants who do not always wear shoes were 3.4 times more likely to be re-infected by hookworm in the 4$^{th}$ month than those who always wear shoes. This finding agrees with study findings in Kenya (Okoyo *et al.*, 2020), Shewa, Ethiopia (Tiruneh *et al.*, 2020), and western Ethiopia (Tolera and Dufera, 2020). This is because wearing shoes minimizes hookworm transmission by providing a physical barrier and preventing larval penetration through the exposed skin of the feet, thus reducing the risk of infection. Similarly, participants who participated in farming were 3.7 times more likely to be re-infected by hookworm than those who did not participate in agriculture. This result agrees with the study in Timor-Leste (Nery *et al.*, 2019). The reason could be due to the defecation behavior of people; most adults deposit their faces in the agricultural fields (an agricultural field is ideal for parasite survival and distribution).

In the 6$^{th}$ month, living with more than five family members and poor utilization of the latrine emerged as risk factors for hookworm re-infection. Study participants with a family size greater than five were 4.7 times more likely to be re-infected by hookworm than individuals with five family members or fewer. This finding is consistent with the studies in Kenya (Okoyo *et al.*,

2020) and western Ethiopia (Tolera and Dufera, 2020). The reason could be the difficulty of buying shoes for the entire family as the family size increases, and the children will be barefooted; this increases hookworm re-infection, and also, if one family member is infected, the transmission will be probable. Similarly, children who defecated in the open field were five times more likely to be re-infected with hookworm than those who used latrines. This finding agrees with the study conducted in Chencha, southern Ethiopia (Zerdo *et al.*, 2016). This is because latrine use minimizes hookworm transmission from open defecation, which is conducive to parasite distribution.

**Limitations of the study**

Microscopy-based approaches have limited diagnostic ability to detect very light hookworm infections and would likely result in an overestimation of cure. The light-surviving infections may re-establish and be detected in the $4^{th}$ and $6^{th}$ months. Due to this, the observed re-infection may not be a true re-infection.

# 7. CONCLUSIONS

Single-dose ALB (400 mg) was found to be effective against hookworm. The re-infection rate was high during the $4^{th}$ and $6^{th}$ months after treatment. The intensity of infection during the $4^{th}$ and $6^{th}$ month's detection was light. Participating in irrigation and irregular shoe wear were predictors of hookworm re-infection in the $4^{th}$ month. In addition, participating in irrigation, irregular shoe wear, poor latrine utilization, and livings with large family sizes were also risk factors for hookworm re-infection in the $6^{th}$ month.

## 8. RECOMMENDATIONS

- ✓ Single-dose ALB can be used as an MDA in the study areas.
- ✓ The Ministry of Health and Education should facilitate sustained mass administration of ALB, WASH implementation, and health education in schools and communities to prevent re-infections of hookworm.
- ✓ Shoes-wearing among SC should be strongly advocated in schools by a trained teacher.
- ✓ The wereda health offices should continue administering ALB biannually and redouble their efforts to increase WASH coverage in the study areas.
- ✓ The health extension workers and the health development army should conduct health education on footwear campaigns and behavioral change interventions to promote good hygiene practices to reduce the risk of hookworm re-infections.
- ✓ Research that covers a large area should be conducted using more sensitive diagnostic techniques, such as quantitative polymerase chain reaction, to assess the effectiveness of the MDA campaign, the resistant gene, the true hookworm re-infection, and potential risk factors that influence hookworm transmission.

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# ANNEXES

## Annex I: Participant information sheet (English Version)

Good morning / good afternoon my name is _____post-graduate Medical Parasitology and vector control student, from the department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Bahir Dar University.

**Title of the research project**: albendazole efficacy and re-infection rates of hookworm among primary school children in Bahir Dar city administration, North Mecha and Bahir Dar Zuria district, northwest Ethiopia.

**Purpose:** To assess albendazole efficacy, re-infection rates, and associated risk factors of hookworm re-infections among primary schoolchildren in Bahir Dar city administration, North Mecha and Bahir Dar Zuria district, northwest Ethiopia, from May to December 2022

**Procedure:** To perform the above-mentioned study at Bahir Dar city administration, North Mecha, and Bahir Dar Zuria district primary school students, your children are invited to take part in this project. If you are willing for your children to participate they will be asked to provide one stool sample at the beginning of the study and these will then be checked for the presence of worm eggs. Infected individuals will then receive ALB principal investigator. Another stool sample will be collected and analyzed on day 14 to determine fecal ERR and CR. Finally, the child positive at baseline and those negative after ALB treatment give another stool sample after 3rd and 6th months of the follow-up survey.

**Risk:** For this survey, your child is asked to provide their faces only, which is a non-invasive procedure. Therefore, we do not expect any harm to occur on your child. But if your child is positive for hookworm; the drug that will be given will have some side effects including mild abdominal pain, nausea, vomiting, diarrhea, and fatigue, and do not normally require medical treatment.

**Benefits:** If your children participate in this study and are found to have Hookworm, based on the result he/she able to receive ALB treatment. Your children will not receive any payment for his/her

participation in this research study. And also we will facilitate diagnosis and treatment if your child is positive for other parasites; the result of the study will benefit giving appropriate intervention.

**Confidentiality:** All information collected from your children will be kept confidential. It will be stored in a file using codes. Any student's personal information will not be transmitted and revealed to anyone or any organization. Only the principal investigator had access to it. Also, it will be used only for this particular research but not for other purposes.

**Right to refuse or withdraw:** Your children's participation in the study is voluntary; you have full right to refuse from participating in this research.

## Annex II: Consent form

Study code number _____Date_____

I am informed to my satisfaction, of the purpose of this study and the nature of laboratory investigations. I am also aware of my right to drop out of the study at any time during the study without having to give reasons for doing so.

This consent form has been read out to me in my language. Therefore, with a full understanding of the situation, I agree my child give the entire necessary information and stool sample voluntarily.

Family/guardian Name----------Signature----------Date----------

Data collector name---------- ---Signature----------Date----------

**Contact Address of the Principal Investigator:** if you need any additional information contact me at any time at the address below

**Investigator name:** Shegaw Belay Phone no**:** +251 924275902

E-Mail**:** **shagawbelay35@gmail.com**

**Annex III: ለጥናቱ ተሳታፊወች የተዘጋጀ የሚጃ ቅጽ (በአማርኛ)**

ሰላም እንዴት አደራችሁ/ዋላችሁ ስሜ _____ እባላለሁ የጥገኛ ትላትል እና የአስተላላፊ ነፍሳት ድህረ ምረቃ ተማሪ ነኝ የመጣሁት ከ ባህርዳር ዩኒቨርሲቲ ፣ ህክምናና ጠፍ ሳይንስ ኮሌጅ ፣ የህክምና ላቦራቶሪ ትምህርት ክፍል ነዉ።

**የጥናቱ ርእስ**: የአልቤንዳዞል የተባለዉ ፀረ-ትላትል መዳሃኒት የሜዳን ቸሎታ እና እንደገና በትላትል የመያዝ ምጣኔ ፡ ፡

**የጥናቱ ዓላማ**የዚህ ጥናት አላማ 400 ሚግ የአልቤንዳዞል ስሪት የሆድ ትላትል መድሃኒት የሜዳን ቸሎታ ፣ በመንጠቆ ትላትል እንደገና የመያዝ ምጣኔን እና እንደገና ለመያዝ አጋላጭምክንያት ለማወቅ፡ ፡

**የአሰራር ሂደት፡** ከላይ የተጠቀሰዉን ጥናት በባህር ዳር ከተማ አስተዳደር፣ በሰሜን ሜጫእና በባህር ዙሪያ ወረዳ አንደኛ ደረጃ ትምህርት ቤት እድሜያቸዉ ከ6-14 ከሆኑ ተማሪወች ላይ ለማረት ልጅወት የጥናቱ ተሳታፊ እንዲሆን ተጋብዘዋል፡ ፡ ለማተፍ ፍቃደኛ ከሆኑ የጥናቱን አላማ በሚዳት ና ፍቃደኝነትዎን መግለጽ ይጠበቃል፡ ፡ እንዲሁም በተጨማሪ ማህበራዊ ነክ እና የጤንነት ሁኔታን የሚያሳዩ ጥያቄዎች ከያንዳንዱ የጥናቱ ተሳታፊዎች በማዉይቁ ማረት ይሰበሰባል ፡ ፡ በዚህ ጥናት የሚተፍ 10 ግራም የሚከል የሰገራ ናሙና ይስጥና ምርመራ ይደረግለታል/ላታል በዉጩም ማረት አንድ ጊዜ ብቻ የሚወሰደዉ 400mg አልቤንዳዞል ይሰጠዋል፡ ፡ በ 14ኛ ቀን የሰገራ ናሙና ለሁለተኛ ጊዜ በመሰድ ምን ያክል ከሆድ ትላትል ድኗል/ለች የሚለዉን ለማወቅ ምርመራ ይደረግለታል፡ ፡ በስተመጨረሻ በ 4 እና 6 ወር በምን ያህል ምጣኔ እንደገና በትላትል ተይዘዋል እና በትላትል እንደገና ለመያዝ ዋና ምክንያት ምንድን ናቸዉ የሚለዉን ለማወቅ የሠገራ ምርመራ ይደረግለ/ላታል፡ ፡

**ከጥናቱ ጋር ተያይዞ ሊከሰቱ የሚችሉ ጉዳቶች፡-** ለዚህ ጥናት ልጅወት የሰገራ ናሙና እንዲሰጥ /እንድትሰጥ ብቻ ይጠየቃል/ለች። የሰገራ ናሙና ማጠት ምንም አይነት ጉዳት የለወም። ሰገራዉ ተመርምሮ የሆድ ትላትል ከተገኘበት አልቤንዳዞል በጠፍ ባለሞያ ይሰጠዋል/ታል። መድሀኒቱ የጎላ የጎንዮሽ የለወም ሆኖም በአንዳንድ ሰወች ላይ የቅለሽለሽ እና ትወኪያ ሊያመጣይችላል፡ ፡

**ጥቅሞች፡** በዚህ ጥናት ልጅዎት ተሳታፊ ከሆነች በሽታው በነጻ ይመረመራል/ትመረመራለች እንጅ የሚሰጥ ክፍያ የለወም። ፡ በሽታው ከተለየ በምርመራ ወጩት መሠረት አስፈላጊወን መድሃኒት እንዲወስዱ ይረዳዋል/ታል፡ ፡

በተጨማሪም የእራስዎ ልጅ በጥናቱ ላይ መሳተፍ ከጥናቱ የሚገኘዉ ወጪት በትላትል የሚመጣ በሽታ ለመቀነስ የሚዳ ይሆናል። ወጪቱን መሸረት በሚድረግ ሌላ ጥናት እንዲካሄድ ግባት በመሆን ከፍተኛ ሜፔ ይጫወታል::

**የጥናቱ መረጃ ሚስጥራዊነት፡** ከሁሉም ተሳታፊዎች የሚሰበሰበዉ መረጃ ሚስጥራዊነቱ የተጠበቀ ነዉ። ለዚህ ጥናት የሚሰበሰበዉ መረጃ በማህደር የማቃመጥ ሲሆን ማህደሩም በኮድ የማቃመጥ ሲሆን ከዋናዉ ተመራማሪ በስተቀር ለማንም አይገለጽም/ ተላልፎ አይሰጥም። እንዲሁም ደግሞ ለዚህ ጥናት ብቻ እንጅ ለሌላ ጥቅም አይዉልም። ።

**የ ማቋረጥ ወይንም የ መዉጣት መበት፡** በዚህ ጥናት ላይ መሳተፍዎ በሙሉ ፍቃደኝነት ላይ የተመሰረተነዉ። ናማ ያለመሳጠት፤ ማንኛዉንም ያልፈለጉትን ጥያቄ ወይም ሁሉንም መመለስ ከአልፈለጉ እንዲመልሱ አይገደዱም። ።

**Annex IV:** □□□□□□□ □□□□□ □□ □□ን□□□□/□□□□/ □□□□□ □□□□

ቀን .....መለያ ቁጥር .....

ስሜ ከታች የተጠቀሰዉ/ችዉ የጥናቱ ተሳታፊ ስሆን የጥናቱን አላማ እና የላበራቶሪ አሰራር ሂደቱን በሚገባ ተረድቻለሁ እንዲሁም ካልተመቸኝ ጥናቱን በማንኛዉም ሰአት ምክንያት መግለጽ ሳይኖርብኝ የ ማቋረጥ መበት እንዳለኝ እወቅናዉ ተፈጥሮልኛል። ይህ የስምማት ቅጽ በራሴ ቋንቋ አንብቤዋልሁ/ተነቦልኛል በመሆኑም ሁኔታዉን ሙሉ በሙሉ በሚዳት ልጀ አስፈላጊዉን መረጃ እንዲሁም የሰገራ ናማ እነዲሰጥ በፍቃደኝነት ተስማምቻልሁ።

የ ወላጅ/አሳዳጊ ስም.....ፊርማ.....

የ መረጃ ሰብሳቢዉ ስም.....ፊርማ.....

**ማግኘት (መመከር) ከፈለጉ ፡** ማንኛዉንም ተጨማሪ መረጃ ከፈለጉ ከዚህ በታች የተጠቀሰዉን አድራሻ መጠቀም ይችላሉ፡ ፡

-ሸጋዉበላይ ስ.ቁ +251 924275902 ኢ.ሜል-**shagawbelay35@gmail.com**

## Annex V: Information letter for schools

Evaluation of the drug efficacy, re-infection rate of hookworm, and associated factors of hookworm re-infection in the school children

Schoolmaster of _____Primary School, address_____ in the context of the title, an evaluation will be conducted in your school. A graduate student from the Bahir Dar University, Colleague of Medicine and Health Science, School of Health Sciences, Department of Medical Laboratory Sciences will visit the school on; _____dd/mm/yy and will invite a group of children in the school to answer some questions and to provide a stool specimen. The children recruited will receive a dose of ALB. The medicine is recommended by WHO and FMOH for use in school control programs and is considered safe. Some children may experience some minor and temporary side effects as the worms are destroyed by the drug. This includes mild abdominal pain, nausea, vomiting, diarrhea, and fatigue and does not normally require medical treatment. Three additional specimens of feces will be collected again after 2 weeks, 4, and 6 months i.e. on:

- _____dd/mm/yy
- _____dd/mm/yy
- _____dd/mm/yy

Children participating in the evaluation and positive for hookworm will receive a single dose of 400mg ALB. The individual results of any investigation will remain confidential. You are invited to contact the organizer of the evaluation in case you need any additional information.

Thank you in advance for your collaboration,

The principal investigator Mr. Shegaw Belay

Department of Medical Laboratory Science, Bahir Dar University

Contacts Email: shagawbelay35@gmail.com:

Mobile phone: +251924275902

## Annex VI: Procedures for laboratory methods

Materials and reagents for spontaneous tube sedimentation, Kato Katz and McMaster technique

- Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)
- Newspaper or glazed tile
- Microscope slides
- Cover slide
- Cellophane as coverslip soaked in Glycerol-malachite green
- Gloves
- Microscope
- Conical plastic tube
- 60-ml containers
- Digital scales (precise to 0.01 g)
- Stirring device (fork, spatula, tongue depressor, spoon)
- Measuring cylinder
- Pasteur pipettes and rubber teats
- Saturated NaCl solution is to be prepared at least 1 day before use and kept at room temperature (specific density = 1.2)
- McMaster slides
- 5 L distilled water
- 3 kg NaCI

### A. Kato–Katz thick smear method procedure

1. Put the cellophane strip in the container with having 50% glycerol solution of malachite green for overnight
2. Take a scrap or newspaper, put a small amount of feces onto it

3. Press the screen on top of the fecal sample
4. Use a flat applicator stick, and scrape the screen thoroughly on the upper surface of the screen to sieve the fecal sample
5. Take a clean microscope slide and place a template on
6. Using the applicator stick, scoop a little amount of sieved fecal material into the template hole, filling it to level with the applicator stick
7. Carefully remove the template ensuring all the fecal material is left on the slide with none sticking to the template
8. Cover the fecal sample on the slide with a glycerol–soaked cellophane strip
9. Spillover of glycerol if present on the upper surface of the cellophane should be wiped off using absorbent tissue or toilet paper
10. By inverting the microscope slide, press the fecal sample on a smooth surface against the cellophane onto spread the sample evenly
11. Hold the cellophane and gently turn the microscope slide side
12. Using a microscope examine the slide between 30-60 min and multiply by 24

**B. McMaster egg counting method**

1. Homogenize the stool with a wooden spatula
2. Weigh exactly 2 g of stool on the scale
3. add 30 ml of saturated NaCI

4. Homogenize and pour the fecal suspension three times through a tea strainer to withholding large debris. During the last sieving step, the filtrate must be squeezed dry
5. Rinse the McMaster slide and tap it on a hard surface
6. Homogenize the suspension filtrate by pouring it 10 times from one beaker to another, and fill one chamber of a regular McMaster slide using a Pasteur pipette. Repeat for the other side. Minimize the time between taking the suspension up in the pipette and transferring it into one of the chambers of the McMaster slide
7. Allow the McMaster slide to stand for 2 min, place it under a light microscope and examine it with 100x magnification. Count all the eggs under the two separate grids (representing a volume of 2 x 0.15 ml). If the slides are read before 2 min, the eggs will not have reached the surface of the slide
8. Calculate the number of eggs per gram of faeces by multiplying the total number of eggs under the two grids by 50

**C. Procedure for spontaneous tube sedimentation technique**

1. Measure 3g of a fresh stool sample
2. Homogenize into 10 ml of normal saline solution
3. Filter the mixture through surgical gauze into a 50 ml plastic tube then fill with more saline solution up to 50 ml gauge, plug, and shake vigorously
4. Left the tube to stand for 45 minutes, and then discard the supernatant
5. Take the sample from the bottom and put it on a microscope slide and see the presence of ova of hookworm

## Annex VII: Form for collecting personal data

Individual data collection form

Date [dd/mm/yy]___/___/_____

Drug tested: albendazole

I. Personal data

Wereda_____ Kebele _____School _____

Age_____ (years) Sex M ☐ F ☐ Id number_____

II. Exclusion

- Does the child have diarrhea yes ☐ no ☐
- Did the child take ALB in the past 3 months yes ☐ no ☐ I do not know
- Are you taking any drugs currently?
- Episodes of vomiting occurred after drug administration Yes ☐ No ☐

## Annex VIII: Questionnaire in the English version

Bahir Dar University, College of Medicine and Health Sciences, School of Health Sciences, Department Of Medical Laboratory Science: Questionnaire for the socio-demographic and WASH variables on re-infection rate of hookworm and ALB efficacy against hookworm infection at Bahir Dar city administration, North Mecha district, and Bahir Dar Zuria district selected primary school, Amhara region, northern Ethiopia.

**Participant Identification**

School name _____ Year _____ Participant code _____

Participant's address: (Sub City/Keble) _____ Telephone _____

Data collectors' name _____date _____signature_____

| No. | Question | Answer | Remark |
|---|---|---|---|
| Socio-demographic variables | | | |
| 101 | Age | _____years | |
| 102 | Sex | 1. Male 2 Female | |
| 103 | No. of family size | _____ | |
| 4 | Residence | 1 Rural 2 Urban | |
| 105 | Educational status of the mother | 1 Illiterate 2 Primary school 3-Secondary school 4 Higher education | |

| | | | |
|---|---|---|---|
| | | 5 other | |
| 106 | Family occupation | 1 Farmer 2 Merchant 3 Government employers 4 daily laborers 5 other | |
| Personal and environmental variables | | | |
| 201 | Do you have a latrine? | 1 Yes 2 No | If no go to Q204 |
| 202 | Status of the toilet floor | 1 Ceramic 2-Cement 3-Mud 4 other | |
| 203 | Utilization of latrine | 1 Always 2 Sometimes 3 never use | |
| 204 | Washes hands after toilet | 1-yes 2- no | |
| 205 | Status of your house floor | 1 Ceramic 2-Cement 3-Mud 4 other | |
| 206 | Washes hands before a meal | 1- yes 2- no | |
| 207 | Do you participate in irrigation? | 1-Yes 2- No | |
| 208 | Do you play in the soil/mud | 1- Yes 2 No | |
| 209 | Do you walk barefoot? | 1-Yes 2- No | |
| 210 | Frequency of shoes wearing | 1 Always 2 Sometimes 3 Never | |
| 211 | Shoe type | 1 Closed 2 Open | |
| 212 | Do you go to the toilet barefoot? | 1-Yes 2- No | |
| 213 | Do you eat unwashed vegetation? | 1-Yes 2- No | |

## Annex IX: School WASH and environmental condition observation checklist

Date of visit: DD/MM/YY_____

Name of school: _____

Total number of SC _____Male _____Female____

1. Do the children walk by barefoot? Yes □ No □
2. Are there water sources close to the school? Yes □ No □
3. Water available for drinking today.....Yes □ No □
4. What is the main source of water for drinking for students in this school? (Piped/tap water, Borehole(well), Rainwater, Stream, lake, or river)
5. Are there latrines in the school ……Yes □ No □
6. If yes how many for Boys……How many for Girls…….
7. Unlocked and accessible separate toilets for boys and girls……Yes □ No □
8. Latrine with roof and concrete slab……Yes □ No □
9. Latrine without roof earth /wooden slab/……Yes □ No □
10. Is there an open defecation in the school compound ……Yes □ No □
11. Hand washing facilities near the toilets.....Yes □ No□
12. Are water and soap available at the hand washing facilities.....Yes □ No □

**Annex X: የቃለ-ጣጠይቁ የአሚርኛ ቅጅ**

ባህርዳር ዩኒቨርሲቲ ፣ ህክምና ጠ/ሳይንስ ኮሌጅ ፣ ጠ/ሳይንስ ትምህርት ክፍል ፣ ህክምና ላቦራቶሪ ዩኒት

የአልቤንዳዞል ስሪት የሆድ ትላትል መድሃኒት የማዳን ችሎታ ፣ በመንጠቆ ትላትል እንደገና የመያዝ ምጣኔን እና አጋላጭምክንያቶችን ለማወቅ የጣጠየቂያ ቅፅ ፡ ፡

የትምህርት ቤቱ ስም _____ የተሳታፊውመለያ ኮድ_____

የተሳታፊውአድራሻ ጎጥ _____ ስልክ ቁጥር_____

የሚጃ ሰብሳቢውስም_____ቀን _____ፊርማ_____

| ተ.ቁ | ጥያቄ | መልስ | አስተያየት |
|---|---|---|---|
| | I. ማህበራዊ ነክ ጥያቄ | | |
| 101 | እድሜ | _____ | |
| 102 | ጾታ | 1-ወንድ 2-ሴት | |
| 103 | የቤተሰብ አባላት ብዛት | _____ | |
| 104 | የእናት የትምህርት ደረጃ | 1 ያልተማረች 2 1ኛ ደረጃ 3 2ኛ ደረጃ 4 ከፍተኛ ትምህርት 5 ሌላ | |
| 105 | የመኖርያ አድራሻ | 1 ገጠር 2 ከተማ | |
| 106 | የቤተሰብየስራ ሁኔታ | 1 አርሶአደር 2 ነጋዴ 3 የመንግስትሰራተኛ 4 የቀን ሰራተኛ 5 ሌላ | |
| | II. የግልና የአካባቢንጽህና ሁኔታ | | |
| 201 | ሽንት ቤት አላችሁ | 1 አዎ 2 የለም | የለምከሆነ ወደ204 |
| 202 | የሽንት ቤት ወለል ሁኔታ | 1 ሴራሚክ 2 ሊሾ 3 አፈር 4 ሌላ | |
| 203 | ሽንት ቤቱን ምንያህል ይጠቀማሉታል | 1 ሁልጊዜ 2 አልፎአልፎ 3 አልጠቀምም | |
| 204 | ከመፀዳጃ ቤት መልስ እጅህን ትታጠባለህ | 1 አዎ 2 የለም | |
| 205 | የመኖሪያ ቤት ወለል ሁኔታ | 1 ሴራሚክ 2 ሊሾ 3 አፈር | |
| 206 | ከምግብ በፊት እጅህን ትታጠባለህ | 1 አዎ 2 የለም | |
| 207 | የእርሻ ስራ ትሰራለህ | 1 አዎ 2 የለም | |
| 208 | አፈር ላይ ትጫወታለህ | 1 አዎ 2 የለም | |
| 209 | በባዶ እግር ትሄዳለህ | 1 አዎ 2 የለም | |

| | | | |
|---|---|---|---|
| 210 | ጫማምንያህልያደርጋሉ | 1 ሁልጊዜ 2 አልፎአልፎ<br>3 ፈጽሞአላድርግም | |
| 211 | ምን አይነት ጫማነ ዉየ ምታደርገ ዉ | 1 ሽፍን 2 ክፍት | |
| 212 | ሽንት ቤት በባዶ እግር ሄዳህ ታዉቃለህ | 1 አዎ 2 የለም | |
| 213 | ያልታጠበ እፅዋትን ትበላለህ/ሽ | 1 አዎ 2 የለም | |