ASSESSMENT OF EFFICACY OF ALBENDAZOLE FROM THE LOCAL MARKET AGAINST SOIL-TRANSMITTED HELMINTHS IN SCHOOL CHILDREN IN JIMMA TOWN, JIMMA ZONE, ETHIOPIA

BY

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DEPARTMENT OF PHARMACY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY

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ABSTRACT

Background: Soil-transmitted helminth (STH) infections are a public health problem in many parts of the world, particularly among impoverished communities. Children and pregnant women are the two most vulnerable groups. Benzimidazole (BZ) anthelminthics, particularly albendazole (ALB) and mebendazole (MBZ) are the main stay for large-scale control activity of STH. Low efficacy can lead to treatment failures and may facilitate development of drug resistance. Moreover, some studies reported the reduced efficacy of benzimidazole (BZ) against different species of STH. However, in Ethiopia including the present study site (Jimma), the information on efficacy of different brand of ALB against STH infecting humans is scarce.

Objectives: To evaluate the efficacy of two ALB brands from the local market, Ovis® (Korea, DAEHWA pharmaceutical) and Bendex® (India, Cipla) administered to school-aged children (SAC) against STH infections in Jimma Town, Ethiopia.

Methods: In February, 2014, a two-arm parallel group randomized clinical trial was conducted to evaluate the efficacy of two locally available brands of ALB (Ovis® and Bendex®) among 679 SAC of 5-18 years. Stool samples were examined by the McMaster technique (at base line and 14 days post treatment) and the efficacy of the two drugs were evaluated based on fecal egg count reduction (FECR).

Results: Six hundred seventy nine SAC were recruited in the baseline screening. Of which 418 (61.5%) were positive for one or more STH. The FECR of Bendex® was 98.7% for *A. lumbricoides*, 24.4% for *T. trichiura* and 88.7% for hookworm. While the FECR of Ovis® was 97.8% for *A. lumbricoides*, 20.4% for *T. trichiura* and 98.1% for hookworm. According to WHO guideline based on FECR, the efficacy of both brands showed satisfactory for *A. lumbricoides* and poor efficacy for *T. trichiura*. While the efficacy of Ovis®against hookworm showed satisfactory, while the efficacy of Bendex® was doubtful.

Conclusion and recommendations: The results of this study suggest that those two brands of ALB are therapeutically efficacious for ascariasis, but not for trichuriasis. For hookworm infections, while Ovis® is therapeutically efficacious, Bendex® did not meet the WHO criteria. This could possibly be due to poor *in-vivo* release of Bendex® as indicated by dissolution analysis (manuscript under preparation). Further analysis of *in-vitro/ in-vivo* correlation (*IVIV*C) is required and re- consideration of alternative drug regimens for trichuriasis would be an urgent.

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Acronyms

ALB	Albendazole
AR	Anthelmintic resistance
AM	Arithmetic mean
BZ	Benzimidazole
CR	Cure rate
EPG	Egg per gram of stool
FECR	Faecal egg count reduction
FMHACA	Food, Medicine and Health care Administration and Control Authority of Ethiopia
GMP	Good manufacturing practice
GM	Geometric mean
JUSH	Jimma University specialized hospital
MDA	Mass drug administration
MEB	Mebendazole
NaCl	Sodium chloride
SOP	Standard operating procedure
STH	Soil transmitted helminths
SAC	School aged children
SSA	sub-Saharan Africa
VLIR/IUC	Vlaamse Interuniversitaire Raad/ Institutional University cooperation programme
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1. Background

Infections with soil-transmitted helminths (STH) occur throughout the developing world and remain a major public health problem in the poorest communities. STH infection is caused by four main species of worms commonly known as roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) [1]. It is estimated that more than 1.4 billion people are affected by any of STH worldwide, of which (819 million) with *A. lumbricoides,* (464 million) with *T. trichiura* and (438 million) with hookworm [2].The greatest numbers of STH infections occur in sub-Saharan Africa (SSA) (29%), East Asia (26%) and South Asia (16%), China (16%) and America(10%) [3].

In SSA, the distribution of people infected with *A. lumbricoides* and *T. trichiura* are estimated at 173 million and 162 million, respectively. Of this 36 million school aged children (SAC) infected with *A. lumbricoides* and 44 million with *T. trichiura.* Hookworm is the most widely distributed STH in SSA, it is estimated that almost half of SSA's poorest people, including 40–50 million SAC are infected [3-6]. Ethiopia is one of the countries in SSA also endemic for STH, with national prevalence estimates of *A. lumbricoides* (37%), *T. trichiura* (30%) and hookworm (16%) [7]. According to different previous studies the prevalence and distribution of STH in Ethiopia greatly differ by geographical location that ranges from 0 to 100% in different regions [8-11].

The burden of disease from STH is mainly attributed to their chronic and insidious impact on the health and quality of life of infected people. Morbidity is related to the number of worms harbored, for instance light intensity infections usually have no symptoms. However, heavier intensity infections can cause a range of symptoms including intestinal manifestations (diarrhea, abdominal pain, intestinal obstruction, and rectal prolapse), general weakness, and micronutrient deficiencies including iron-deficiency anemia. Altogether, leading to poor school performance and absenteeism in children, reduced work productivity in adults and adverse pregnancy outcomes such as anemia, significant morbidity with hookworm associated anemia likely

contributing to maternal mortality. Children and pregnant women are the two most vulnerable groups [12-15].

The benzimidazole (BZ) drugs, mebendazole (MEB) and albendzole (ALB) are commonly used to reduce the morbidity caused by these infections, as well remain as the drugs of choice in mass drug administration (MDA) programmes, the main strategy for STH control [13,16,17].

However, different studies reported reduced efficacy of BZ against some of helminths. For instance, in Pemba Island, Zanzibar, the efficacy of MEB against hookworms in SAC appeared to have fallen over a period of five years, during which time the children were regularly treated with MEB. Similarly, a report in Mali shows reduced efficacy of MEB against hookworms [18,19]. Another multinational study on clinical trial conducted in seven countries including Ethiopia (Jimma) in SAC showed also low efficacy of ALB against *T. trichiura* [10]. Furthermore, in Wondo-Genet, Southern Ethiopia, significant differences were observed among the cure rate (CR) and fecal egg count reduction (FECR) of the three brands of MEB (Janssen, Unibios and East African Pharmaceuticals) and ALB (Smith Kline Beecham) in the treatment of *T. trichiura* [20]. In the same way, the study conducted in Hawassa, Southern Ethiopia evaluating the efficacy of seven brands of ALB against gastrointestinal nematodes in sheep showed that only five of the seven brands were satisfactory [21]. Moreover, a systematic review and meta-analysis reported that the efficacy of single-dose oral ALB and MEB against *T. trichiura* was generally unsatisfactory [22, 23].

There are a number of contributing factors that may affect efficacy of anthelmintic drugs. For example, variations between batches in the quantity of the active ingredient, degradation during storage and transport as well as the frequency and continuous use of single drugs for the mass drug administration (MDA). The later raises some concerns about a sustained drug efficacy issues as it exposes parasites to selective pressure and the potential risk of development of anthelmintic drugs resistance (AR). The major mechanism of BZ drug resistance in nematodes involves selection of a naturally occurring "resistant" genotype involving a Pheline →Tyrosine change at position 200 of β-tubulin, which is already a serious problem in veterinary medicine but not demonstrated for human STH yet [24, 25]. However, as we rely only on BZ and ever increasing use of BZ in parasite control programmes, emergence of AR are expected sooner or later and therefore periodic evaluations of efficacy of those drugs remain critical.

1.2. Statement of the problem

STH infections are of major public health importance in developing countries. The morbidity due to STH infections is greatest in SAC who typically have the highest intensity of helminth infections. Growth stunting, iron-deficiency anemia, rectal prolapse and chronic dysentery are features of STH infections. These parasitic infections can also adversely affect cognitive development in childhood [1,12,15]. The highest prevalence occurs in areas where sanitation is inadequate and water supplies are unsafe. Almost all cases occur in areas of poverty in low-income countries in the tropics and subtropics. It is being increasingly recognized as a significant public health problem, especially in poor populations [13,16].

The highest prevalence of STH and intensity of infection occurs in SSA [3]. On top of that Ethiopia is one of the countries in SSA endemic for STH including the present study area (Jimma Town) with prevalence of more than 60% STH in SAC [10]. This implicate that STH are a significant public health problem in Jimma Town as well.

Mass drug administration of BZ is the recommended strategy to control the morbidity of these infections at the population level, with the ultimate goal of treating 75% pre-school aged and SAC in all countries by 2020 [16, 26].

Among many factors influencing the efficacy of anthelmintic drugs, quality of drugs is most important [27]. Currently a wide variety of ALB and MEB drugs are manufactured by many pharmaceutical factories with various brands, imported and distributed by several agents for individual and mass drug administrations. However, little is known about their quality and efficacy. The study in Kenya showed the concentration of active ingredient in anthelmintic drugs varied of their claimed composition in different brands [28]. Moreover, a recent study in Ethiopia (Jimma) reported that *in-vitro* quality of BZ varies with country of origin and/or brands [29]. So the quality variations in different brands might have impact on *in-vivo* efficacy results. Furthermore, different studies reported reduced efficacy of BZ against some helminths [10, 20, 22,23]. Indeed, determining the quality of all imported drugs requires specialized laboratories and it is costly for those poor countries like Ethiopia. But on the other hand, the efficacy of a given drug can be evaluated through parasitological investigation and the results can be interpreted based on parasitological indicator either cure rate (CR) or fecal egg count reduction (FECR). Nevertheless, in Ethiopia as well as the present study area (Jimma) there is a scarcity of

studies evaluating efficacyof different brands of ALB against STH, even though different brands of ALB tablets are circulating in drug retail. Therefore, the present study aimed at evaluating the efficacy of two brands of ALB available in local markets against STH among school children in Jimma Town.

1.3. Literature review

Under this review, the epidemiology and burden, control of STH and efficacy results of BZ anthelminthics to control STH were described.

1.3.1 Epidemiology and burden of STH

STH are included in the list of the world's neglected tropical diseases [30]. They are distributed throughout the world with high prevalence rates. The morbidity caused by STH is commonly associated with heavy infection intensities. Compared with any other age group, children are the most vulnerable group and they harbor the greatest numbers of intestinal worms.The geographical disparities in prevalence of intestinal helminth infections can be depicted by results of several studies [2,3].

Globally more than 1.4 billion people are infected with STH and the highest prevalence and intensity of infection occurs in SSA (29%) [2,3]. The distribution of people infected with *A. lumbricoides* and *T. trichiura* in SSA are estimated 173 million and 162 million respectively, of this 36 million SAC infected with *A. lumbricoides* and 44 million with *T. trichiura* [3,4]. For both infections the largest number of cases occurs in Nigeria, where co-infections with hookworm are common. Tens of millions of cases are also found in Ethiopia, Democratic Republic Congo, and South Africa. Approximately one-third of the world's hookworm today occurs in SSA with the greatest number of cases occurring in Nigeria (38 million) and the Democratic Republic of Congo (31 million), followed by Angola, Ethiopia, and Cote d'Ivoire (10–11 million) [3,5,6].

Different epidemiological studies indicated that STH are widespread in Ethiopia but, the prevalence and distribution of STH differ by geographical location. A study conducted in Bahirdar special zone indicated prevalence in SAC is as high as 46.4% and hookworm was the most prevalent [8]; while in South Gondar zone the prevalence of STH among SAC was 19.5% where *A. lumbricoides* and hookworm being the most rampant [9]. The study conducted in SAC of Jimma Town showed that more than 60% of them infected at least with one of the three STH. The prevalence of *A. lumbricoides*, hookworms and *T. trichiura* was 36.8%, 22.2% and 25.6%, respectively [10]. In the same Town another study showed that half of SAC (49.5%) were

infected with some form of intestinal helminths, among which *T. trichiura* was the most prevalent [11].

1.3.2 Control of STH infections

STH can be controlled by use of anthelmintic drugs. Current efforts to control STH infections focus on the SAC. Because most intense helminth infections and related illnesses occur among SAC, who represents 25 - 35% of population, and SAC suffers from significant consequences health from these infections [16]. Removal of STH in children by a single-oral dose of one of the two BZ improves their health, as evidenced by post-treatment catch-up growth improved physical fitness, appetite and replenishment of iron stores. Besides, improve in childhood intellectual and cognitive development [12-15].

These observations have led to advocacy for the implementation of large-scale STH control programs that target SAC. So global control strategies recommended drugs for use in public health programmes to control STH infections are the two BZ anthihelmintic drugs (ALB and MEB); even though older drugs including pyrantel pamoate and levamisole are also occasionally used in some developing countries [16,31].

Currently, BZ are drugs of choice because they are relatively efficacious, effective against a wide range of helminths, typically administered orally as single dose, easy to administer, safe, and either cheap or available as donation through WHO by dedicated global pharmaceutical companies in huge amount [32,33].

1.3.3 Efficacy of BZ to control STH

BZ causes selective degeneration of cytoplasmic microtubules in intestinal and tegmental cells of intestinal helminths and larvae; glycogen is depleted, glucose uptake and cholinesterase secretion are impaired, and desecratory substances accumulate intracellulary. ATP production decreases causing energy depletion, immobilization, and worm death [34].

The Pharmacokinetic of ALB; is administered orally and it’s poorly absorbed from the intestine but the absorption increased with a fatty meal. So when used ALB to treat intraluminal parasites used on empty stomach but with a fatty meal when used against tissue parasites.it metabolized in the liver to the active metabolite albendazole sulfoxide,the plasma half life 8-12 hours, its

metabolites sulfoxide is mostly protein bound and distributes well to tissues and enters bile, CSF and hydatid cysts, excreted in urine. In short terms use (1-3 days) no significant adverse effects but in long term use might causes (abdominal pain, headache, fever, fatigue, alopecia, increased liver enzymes, and pancytopenia). And it is in the category of pregnancy risk factor C [34].

There is a huge body of evidence that periodic treatment against STH reduces moderate and severe morbidity and prevents chronic sequel in high risk groups even with continuous transmission and re-infection [13,16,17]. But this large-scale periodic distribution of BZ promoted by MDA programme increases drug pressure for possible occurrence of resistance against single dose anthelminthics. As evidenced in veterinary practice, frequent treatment of sheep and other domestic animals has led to a serious problem of front-line anthelmintic drugs resistance which is now largely irreversible. Although there are as yet no convincing reports of drug resistance in human STH [24,25], there are signs of reduced efficacy results and development of anthelminthics resistance may occur in the due course of large-scale use.

One of the methods to sustain treatment successes is close monitoring of drug efficacy. So monitoring the efficacy of anthelmintic drugs is needed to detect any possible treatment failure that may indicate emergence of resistance. The two parasitological indicators to monitor efficacy of anthelmintic drugs are done, either through determination of the cure rate (CR) which is qualitative or through the fecal egg count reduction (FECR) which is quantitative. Both indicators can be evaluated, by measuring the change in helminths faecal egg output in humans stool at pre- and post-treatment with a given drugs at optimal interval of time [27,35,36,37]. Most commonly used parasitological examination methods include Kato Katz, mini-FLOTAC and McMaster as recommended by WHO. Of these methods, McMaster egg counting technique thought to be more rapid and slides are cleaner, easier to read and robust, and slides can be reused several times, making it is more feasible in resource limited settings [38].

Evidence of reduced drug efficacy of some ALB has been suggested by different studies (**Table1**).Great variations of BZ efficacies were reported even in trials in which the same drug is given at the same dosages, indicating that there are many confounding factors affecting efficacy results. Thus, those confounding factors must be taken into account when assessing the effectiveness of BZ drugs in general. Some of the confounding factors includes: quality of the drug (poor quality of drug can affect both pharmacokinetic and pharmacodynamic of the drug);

heavy intensity of infection; the lack of use of a standardized parasitological technique; quality control of diagnostic procedures, the different time spans of follow-up after treatment, diverse statistical measurements of drug efficacy (arithmetic or geometric means),the sample size and possible presence of drug resistance making the results of effectiveness trials more difficult to compare and interpret among different studies and in different geographical locations [27,39,40]. Table 1 summarizes efficacy trials done using ALB against STH in different part of the world.

Table 1: Summary of albendazole efficacy studies against STH

Source(Study Design , Location, Year Trial was Implemented) Reference no	Calculation of egg count	Diagnostic egg counting Technique	Interval of follow-up days	STH species	Drug product	N	CR %	FECR %
Randomized, single-blind trial In Nepal, south Asia, (2007), Albonico. M., *et al* [41]	*GM	Kato-Katz Technique	After 21 days interval	*A. lumbricoides*	GSK[a]	429	97.0	92.6
					Royal[b]	419	95.0	93.8
					Curex[c]	429	82.6	91.9
				T. trichiura	GSK[a]	429	28.6	71.7
					Royal[b]	419	26.6	71.4
					Curex[c]	429	28.0	63.2
				Hookworm	GSK[a]	429	74.3	87.1
					Royal[b]	419	53.3	80.8
					Curex[c]	429	50.7	73.1
Malaysia, (1983), Ramalingam S, *et al* [42]	*NA	*NA	After 21 days interval	*A. lumbricoides*	Zentel®400 mg[d]	91	100	100
					Zentel ®600mg[d]		100	100
					Zentel® 800mg[d]		100	100
				T. trichiura	Zentel®400 mg[d]	152	27.3	39.2
					Zentel®600 mg[d]		60.9	85.1
					Zentel®800 mg[d]		48.0	72.8
				Hookworm	Zentel®400 mg[d]	188	68.8	94.5
					Zentel®600 mg[d]		100	100
					Zentel®800 mg[d]		84	96.1

Randomized, open label trial China,(2011) Steinmann P., *et al*. [43]	*GM	Kato-Katz Technique	Between 21–30 days interval	*A. lumbricoides*	Zentel® 400mg tablet (GlaxoSmith Kline Plc)	82	96.1	99.9
				T. trichiura			33.8	76.7
				Hookworm			69 .1	97.3
Randomized trial Pattani Province, Thailand,(1993) Jongsuksuntigul P., *et al*. [44]	*NA	Kato-Katz Technique	After 14days interval	*A. lumbricoides*	Albendazole ® 400mg tablet (original)	49	100	100
				Hookworm			84.3	96.0
				T. trichiura			67.4	87.0
A randomized, open-label trial LaoPDR, Southeast Asian (2012),Soukhat hammavong P., *et al* [45]	*GM	Kato-Katz Technique	Between 21-23 days interval	*A. lumbricoides*	Albendazole ® 400 mg tablet (South Korea)	100	90	100
				T. trichiura			33.3	67.0
				Hookworm			36.0	86.7
Randomized controlled trial Zanzibar Island, Tanzania, (2010) Knopp S.,*et al* [46]	*GM	Kato-Katz Technique	22–39 days interval	*T. trichiura*	Albendazole ® 400mg tablet (Laboratoria Wolfs)	132	9.8	40.3
Kabale ,Uganda, (2009) Olsen A .,*et al* [47]	*GM	Kato-Katz Technique	After 14days interval	*T. trichiura*	Albendazole ® 400 mg tablet, (GlaxoSmith Kline Beecham, Brentford, UK)	70	8	57
Randomized trial. Wondo-Genet, southern Ethiopia, (2004) Legesse M.,*et al* [20]	*GM	Kato-Katz Technique	After 21 days interval	*A. lumbricoides*	Albendazole ® 400 mg tablet (Smith Kline Beecham**)**	130	92.5	99.9
				T. trichiura			17.1	69.8
Northwest, Ethiopia(2007) Adugna S., *et al.* [48]	*NA	*NA	*NA	*A. lumbricoides*	Albendazole ® 400mg tablet (Smith Kline Beecham)	NA *	83.9	96.3
				Hookworm			84.2	95

Seven countries trials, (2011) Vercruysse J., *et al* [10]	*AM	McMaster technique	Between 14 to 30 days interval	*A. lumbricoides*	Zentel ® 400mg tablet (Glaxo Smith Kline Pharmaceuticals Limited, India)	952	98.2	99.5
				T. trichiura		1046	46.6	50.8
				Hookworm		912	87.8	94.8
A randomized multi-arm efficacy trial Jimma Town, Ethiopia, (2013), Mekonnen Z.,*et al*. [49]	*AM	McMaster technique	After 14 days interval	*T. trichiura*	Zentel® 400mg tablet (Glaxo Smith Kline Pharmaceuticals Limited, India)	107	-----	29.3

*AM - Arithmetic mean

*GM - Geometric mean

*NA- not applicable

[a]GSK 400mg originator product GlaxoSmithKline

[b]Royal 400mg locally manufactured generic

[c]Curex 400mg locally manufactured generic

[d] Smith Kline Beecham

To minimize or if possible to avoid, the different confounding factor that affect the drug efficacy, it has been suggested by WHO to use a standard protocols, and usually WHO attempt to set a guidelines for this purpose. Moreover, it has been recommended to use FECR as best indicator of monitoring drug efficacy over CR as CR is more prone to variation due to confounding factors compared to FECR. In addition, WHO has recently developed a revised protocol as benchmarks to be used as a guide in assessing drug efficacies. Moreover, a new threshold was suggested to evaluate the efficacy of ALB for each of the STH based on FECR levels. Accordingly, FECR below 95% for *A. lumbricoides*, below 50% for *T. trichiura* and below 90% for hookworm should be taken as a warning sign for reduced efficacy and trigger the need for further investigation. Thus, this research work was conducted with the aim of assessing the efficacy of two brands of ALB anthelmintic drugs against STH based on the newly adopted WHO guideline for efficacy assessment [40].

1.4. Significance of the study

Control of morbidity associated with STH infections depend mainly on the delivery of anthelmintic drugs. Literatures indicate that the efficacy of single-dose regimen with ALB can be confounded by many factors like quality of drugs, sample size, diagnostic methods, and statistical analysis, etc.

In principle, drugs must be manufactured according to good manufacturing practice (GMP) and be of sufficient quality to perform as required in the 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. Thus, deworming of these parasites using appropriate anthelmintic drugs that have good quality, efficacy and safety is important in improving the health condition and productivity of patients and minimizing the rate of transmission.

Hence, the result from this study will fill the gap by generating data on efficacy of different brands of ALB, add an input to the well being of people by forwarding the efficacious brand of ALB currently found in use for therapeutic purpose, used to inform the policy makers (local regulatory agent), the results will be used in future monitoring and evaluation studies of MDA programmes and finally serve as baseline for further in-depth researches.

CHAPTER TWO: OBJECTIVE OF THE STUDY

2.1. General objective

The main objective of this study is to evaluate the efficacy of two ALB brands purchased from the local market: Ovis® (Korea, DAEHWA pharmaceutical) and Bendex® (India, Cipla) administered to SAC against STH infections in Jimma Town, Ethiopia.

2.2. Specific objectives

- ✓ To determine the FECR of Ovis® against *A. lumbricoides*, *T. trichiura* and hookworm after treatment.
- ✓ To determine the FECR of Bendex® against *A. lumbricoides*, *T. trichiura* and hookworm after treatment.

CHAPTER THREE: METHODS AND MATERIALS

3.1. Study area and period

This study was conducted in Hamle-19 and Jiren N° 1 elementary schools, found in Jimma Town, southwestern Ethiopia, in February 2014. Those two schools were purposively selected based on previous study results showing high prevalence of STH in those schools [10]. Jimma Town is located 352 km southwest of Addis Ababa having a latitude and longitude of $7^0$40'N$36^0$50'E, with an area of 50.52 square kilometers. It is characterized by a semi-arid type climate with an average annual rainfall of 800–2,500 mm. The mean daily temperature is 19 °C, and ranges from 12 to 30°C. It is located 1,720–2,010 m above sea level. The Town has a population density of 174,000, according to the data from the Towns' municipality. Public health facilities in Jimma Town include Jimma University Specialized Hospital (JUSH), Shenen Gibe Hospital, Jimma Health Center, Higher two Health Center and Mendera Kochi Health Center and private clinics.

3.2. Study design

A two-arm parallel group randomized clinical trial was conducted to evaluate the efficacy of two brands of ALB (Ovis® and Bendex®) against STH among SAC of 5 to 18 years in Hamle-19 and Jiren N° 1elementary schools.

3.3. Populations

3.3.1. Source population

All SAC attending Hamle-19 and Jiren N° 1 elementary schools in 2013/ 2014 academic year.

3.3.2. Study population

Selected SAC within age range of 5 to 18 years old, attending the aforementioned elementary schools, volunteered to participate in the study, and fulfilled the inclusion criteria.

3.4. Sample size

Sample size calculation was based on WHO-2013 guideline, which states that the minimum sample size required to conduct the efficacy trial needs at least 50 infected subjects for each STH

(i.e., 50 positive for *A. lumbricoides*, 50 positive for hookworm and 50 positive for *T. trichiura)* for each treatment arm making the total minimum sample size of 300.

Hence, we estimated that 625 subjects were needed to identify 300 infected individuals. As indicated below, the calculation is based on a sample size of at least 50 infected subjects for each STH in each treatment arm, taking an apparent prevalence of 60% for STH species and a drop out of 20% [40].

Accordingly;

$$\text{No of children to be screened} = \frac{\text{No of infected children required}}{\text{Compliance rate X prevalence rate}}$$

$$300/0.8 \times 0.6$$

$$n = 625$$

Where;

Number of study subjects to be screened at base line (n = 625)

Number of infected subjects required (50 x 3 x 2 =300)

Compliance rate of 80 %

Apparent prevalence rate in the study area for any STH (60%)

However, in the due course of the study, the actual sample size had increased to 388. This is because; it was not possible to get the minimum required sample size especially for hookworm, i.e., (100 infected study subjects with hookworm for the two arm-trials) within the above anticipated calculations. Thus, enrollment of study participants continued until the minimum number was attained.

3.5. Variables

3.5.1. Dependent Variable

- Fecal egg count reduction

3.5.2. Independent Variable

- Brands/ origin of products.

3.6. Inclusion and exclusion criteria

3.6.1. Inclusion criteria

- ✓ Age: 5–18 years old,
- ✓ Sex: males and females,
- ✓ Signed written informed consent sheet by parents/or guardians and assent by the child,
- ✓ Volunteered to comply with study procedures (stool submission, drug treatment).

3.6.2. Exclusion criteria

- ✓ Late exclusion had vomit within 4 hours after drug administration,
- ✓ Had diarrhea at time of the first sampling,
- ✓ Subjects who were unable to provide a stool sample at follow-up,
- ✓ Subjects who experienced a severe medical condition,
- ✓ Subjects with known history of allergic reaction to ALB,
- ✓ Females: claimed to be pregnant (as verbally assessed by clinician upon enrolled to treatment).

3.7. Data Collection and Processing

3.7.1. Data collection tools/instruments

A consent form was distributed to all parents/guardians of the study subjects. The data collectors explained the procedures and purpose of the study. Once informed consent was obtained, the initial name, age, sex, and school grade of each child were recorded. Then, after giving adequate instruction on how to provide stool samples, plastic stool containers with unique identification numbers were distributed to the study subjects to provide sizeable fresh stool specimens.

3.7.2. Data collectors

Three qualified laboratory technicians were recruited and the purpose of the study was explained before the commencement of the data collection. Refreshment training was given on how to collect, transport and process the stool samples by senior expertise. One experienced clinical nurse was employed as demographic data collector as well as administrator of the drug to the study participants.

3.8. Baseline parasitological examination

The collected stool samples were transferred to Jimma University STH lab, and processed the same day by using the McMaster egg counting technique (analytic sensitivity of 50 EPG) for the detection and the enumeration of infections of *A. lumbricoides, T. trichiura* and hookworms [38]. A total of 679 children were recruited at base line and provided stool samples.

3.9. Brands of albendazole

For this particular study purpose two brands of ALB, Ovis® (Korea, DAEHWA pharmaceutical; batch number 2020, manufactured date 12/2012 and expiry date11/2011) and Bendex®400 (India, CIPLA LTD; batch number X21253 manufactured date 16/11/12 and expiry date15/11/15) were purchased from the same place of local pharmacy in Jimma Town and used for the efficacy studies among SAC against STH.

3.10. Treatment Allocation

Children were positive for at least one of the three helminth at baseline were randomized **(Figure 1**) on daily bases, stratifying for both mono and mixed infections. To this end the 'rand' function in excel was used. Following randomization eligible children were checked for exclusion criteria (experiencing a severe medical condition; had diarrhea at time of the first sampling; had known history of allergic reaction to BZ; or were pregnant) and excluded from the study before drug administration. More over the intervention days was on the following day of each stool examination and the drug was administered under the supervision of a nurse. Monitoring of children was within 4 hours after drug administration.

3.11. Follow-up

Two weeks after treatment, study participants were asked to provide a second stool samples and processed and examined with similar McMaster egg counting technique as baseline. **Figure 1** illustrates number of subjects recruited, enrolled, lost at follow-up, and included in the statistical analysis in a two armed clinical efficacy trial.

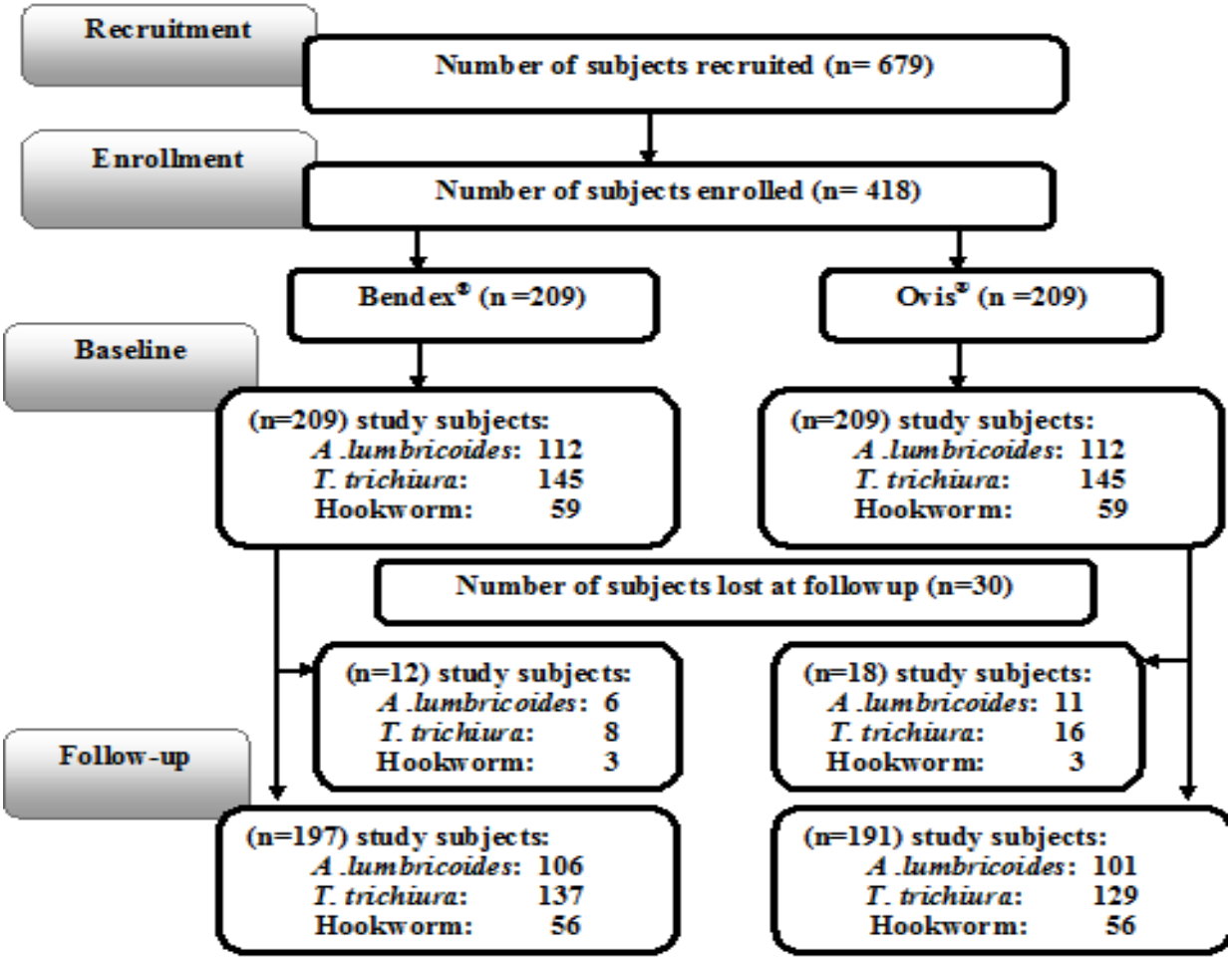

Figure 1 Flowchart summarizing number of subjects recruited, enrolled, lost at follow-up, and included in the statistical analysis in a two armed clinical efficacy trial to assess the efficacy of two brands of 400 mg ALB (Bendex® and Ovis®) against STH (*A .lumbricoides*, *T. trichiura* and hookworm) in school children of Jimma Town, Ethiopia.

3.12. Data analysis

The outcome of the FECR was calculated using formulae:

$$FECR = 100\% \times \frac{\text{Arithmetic mean (FEC at baseline)} - \text{Arithmetic mean (FEC at followup)}}{\text{Arithmetic mean (FEC at baseline)}}$$

Based on the result of FECR, the efficacy of the drugs was classified into 'satisfactory', 'doubtful' and 'reduced' using the criteria summarized in **(Table 2)** [40].

Table 2: The criteria based on FECR: 'satisfactory', 'doubtful' and 'reduced'

	A. lumbricoides	***T. trichiura***	**Hookworm**
Satisfactory	FECR ≥ 95%	FECR ≥ 50%	FECR ≥ 90%
Doubtful	95%> FECR ≥85%	50%> FECR ≥40%	90%> FECR ≥80%
Reduced	FECR <85%	FECR <40%	FECR <80%

As well, permutation test (10,000 iterations) was performed to do a pair wise comparison of age, sex ratio, and FECR in the two treatment arms. The level of significance was set *P*-value<0.05.

Finally, the analysis was performed on a total of 388 children who were positive and completed the study, both base line and follow-up **(Figure 1).**

3.13. Data quality management

The specimens were checked for label, quantity and procedures of collection. The chemicals/reagents, and materials for laboratory examination were obtained and purchased from reputable companies and known standard suppliers. The quality of reagents and instruments were checked before starting the actual work. For the purposes of quality assessment, qualified technician have examined all samples and the laboratory personnel were also kept blind about randomization. In order to verify the consistency of the microscopic readings during the baseline and follow-up surveys, a random sample of approximately 10% of the positive samples and all the negative samples were re-examined by senior supervisor/ independent technician for quality control purposes.

3.14. Operational definitions

Fecal egg count reduction (FECR): is a quantitative evaluation of the efficacy of the drug, literally defined the percentage reduction in egg intensity as measured by eggs per gram of feces after drug treatment.

WHO -2013 threshold for *A. lumbricoides:* FECR≥ 95% for a single dose of ALB
WHO -2013 threshold for *T. trichiura:* FECR ≥50% for a single dose of ALB
WHO -2013 threshold for hookworms*:* FECR ≥90% for a single dose of ALB [40].

Light intensity of infection for *A. lumbricoides* 1–4,999 egg count per gram
Light intensity of infection for *T. trichiura* 1–999 egg count per gram
Light intensity of infection for hookworm 1–1,999 egg count per gram
Heavy intensity of infection for *A. lumbricoides* ≥ 50,000 egg count per gram
Heavy intensity of infection for *T. trichiura* ≥ 10,000 egg count per gram
Heavy intensity of infection for hookworm ≥ 4,000 egg count per gram [33].

3.15. Ethical consideration

This study protocol was approved by the Ethical Committee of Jimma University, Ethiopia (Ref. No RPGC/282/2014) and Ethics Committees of Ghent University, Belgium (2013/1114). The school administrators, parents, and the children were informed about the nature and purpose of the study. Only those children, who were willing, as well as whose parents/guardians consented by signing, participated in the study. Finally, all students who remained positive for any STH after post-intervention had received a single oral dose Zentel® (GlaxoSmithKline).

3.16. Dissemination plan

The research report and findings will be primarily submitted to department of Pharmacy, Jimma University postgraduate school as MSc thesis document. The result will be disseminated to larger audience during the public defense of the thesis. Moreover, the result will be communicated to all stakeholders and other concerned bodies for further appropriate interventions. Lastly, an effort will made to publish the findings either on international or national scientific journals.

CHAPTER FOUR: RESULTS

Six hundred seventy nine SAC (n = 204 from Hamle-19 and n = 475 from Jiren N° 1 elementary schools) were recruited at the baseline screening. Overall 418 (61.5%) were positive for one or more STH, of which 211 (50.5%) boys and 207 (49.5%) girls. The most prevalent was *T. trichiura* (69.4%) followed by *A .lumbricoides* (53.6%) and then hookworm (28.2%) as presented in **(Figure 2)** below**.**

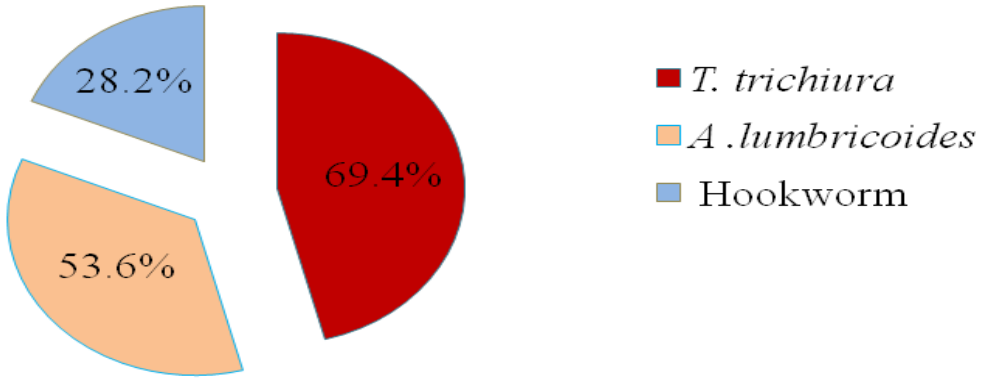

Figure 2: The prevalence of *A .lumbricoides, T. trichiura* and hookworm among elementary school-aged children in Jimma Town, Ethiopia.

Out of these 418 positive children, 30 (7.2%) children were excluded from the study (12 from the Bendex® treated arm and 18 from Ovis® treated arm) because they were either lost on treatment day or on follow-up day (during post-treatment stool sample collection). Hence the analysis was performed on a total of 388 children who were positive for one or more STH. The distribution of STH in two treatments arms were: 197 children in Bendex® treated group; of which *A .lumbricoides* (n=106), *T. trichiura* (n=137) and hookworm (n=56); and 191children in Ovis® treated group; of which *A .lumbricoides* (n=101), *T. trichiura* (n=129) and hookworm (n=56) as presented in **(Figure 1).**

At baseline, the mean age of the study participants in both treatment groups was 10.3 years (95% CI: 9.9; 10.6). The proportion of females to males in Bendex® treated arm was 1.07 (95% CI: 0.81; 1.43) while in Ovis® treated arm, it was 0.87 (95% CI: 0.65; 1.15). There were no significant statistical difference (*P-value*>0.05) in both sex ratio and age at baseline which is presented in **(Table 3)**. In addition there is no statistical significant difference (*P-value*>0.05) in infection intensity of *A. lumbricoides*, *T. trichiura*, and hookworm with respect to mean fecal egg counts egg per gram of stool (EPG) at baseline in both treatment groups as indicated in **(Table4).**

Table 3. A pair wise comparison of the mean age, sex ratio (number of females/ number of males) at baseline between two brands of 400 mg ALB, Bendex® and Ovis® in school children of Jimma Town, Ethiopia.

	Bendex® (n=197)	**Ovis® (n=191)**	***P*-value of pairwise comparison**
Mean age	10.3	10.3	1
(95%CI)	(9.9; 10.6)	(10.0; 10.6)	
Sex ratio	1.07	0.87	1
(95%CI)	(0.81; 1.43)	(0.65; 1.15)	

Fecal egg counts (FEC) for each of *A. lumbricoides*, *T. trichiura*, and hookworm and the arithmetic mean fecal egg counts (EPG) at both baseline and follow-up and the overall percentage of FECR for both brands of ALB are shown in (**Table 4)**.

Our study revealed that for *A. lumbricoides* infections, Bendex® (n = 106) the FECR was 98.7%, while for Ovis® (n = 101), the FECR was 97.8%. Similarly, for *T. trichiura* infections Bendex® (n = 137), the FECR was 24.4%; while for Ovis ® (n = 129), the FECR was 20.4%. Based on the newly adopted WHO guideline **(Table 2)**, both brands were highly efficacious (categorized as satisfactory) against *A. lumbricoides.* However, for *T. trichiura*, both brands revealed poor efficacy (categorized as reduced). Whereas, against hookworm infections Ovis ® (n = 56, FECR = 98.1%) appears to be more efficacious compared to Bendex ® (n = 56, FECR = 88.7 %) [40].

Table 4 The mean FEC at baseline and follow up, and the corresponding FECR for *A. lumbricoides*, *T. trichiura* and hookworm for two brands of 400 mg ALB, Bendex® and Ovis® in school children of Jimma Town, Ethiopia.

	Bendex® (n=197)	**Ovis® (n=191)**	***P*-value of pairwise comparison**
A. lumbricoides			
N	106	101	
Mean FEC at baseline (EPG)	8,706	7,935	0.686
(95%CI)	(6,357; 11,375)	(5,583; 10,836)	
Mean FEC at follow-up (EPG)	112	175	0.659
(95%CI)	(0; 332)	(22; 396)	
FECR (%)	98.7	97.8	0.638
(95%CI)	(95.5; 100)	(94.6; 99.7)	
T. trichiura			
N	137	129	
Mean FEC at baseline (EPG)	909	769	0.450
(95%CI)	(672; 1,184)	(543; 1,037)	
Mean FEC at follow-up (EPG)	688	612	0.659
(95%CI)	(461; 967)	(438; 828)	
FECR (%)	24.4	20.4	0.814
(95%CI)	(4.5; 42.2)	(13.3; 40.9)	
Hookworm			
N	56	56	
Mean FEC at baseline (EPG)	355	335	0.789
(95%CI)	(271; 452)	(234; 449)	
Mean FEC at follow-up (EPG)	40	6	0.045
(95%CI)	(9; 88)	(2; 13)	
FECR (%)	88.7	98.1	0.051
(95%CI)	(78.7; 97.2)	(96.2; 99.5)	

CHAPTER FIVE: DISCUSSIONS

Our study assessed the efficacy of the two brands of ALB (Bendex® and Ovis®) against *A. lumbricoides*, *T. trichiura*, and hookworm with recommended dose (400 mg single oral dose). The efficacy was evaluated using FECR as parasitological indicator and the result was interpreted according to the standardized new guideline of WHO-2013 [40].

The present study revealed that both drugs were highly efficacious against *A. lumbricoides*, in accordance with WHO guideline thresholds used for assessing ALB efficacy for *A. lumbricoides* (FECR ≥ 95%), and fall with in the WHO classification of satisfactory [40].

While, in the case of *T. trichiura,* both drugs were unsatisfactory with arithmetic mean FECR below the recommended thresholds (< 40 %). WHO guideline classified such low efficacy as "reduced efficacy" against *T. trichiura* when the arthimetic mean FEC reduction is below 40% [40]. The finding of the present study (low FECR) of both brands of ALB against *T. trichiura* is comparable to the previous report from Ethiopia [49]. In addition a multinational trial of the efficacy of ALB against STH infections in children that was conducted in seven countries including Ethiopia (the present study site Jimma) [10] that confirmed the efficacy of ALB against *T. trichiura* was relatively ineffective. So high prevalence of this helminth and the disappointed result in FECR reported by this study and in some other studies [11, 22, 23] highlights the urgent need for alternative drugs and/or development of novel anthelmintic drugs to tackle the exciting problems.

In the present study the arithmetic mean FECR (98.1%) obtained with Ovis® in the treatment of hookworm was higher than that of Bendex® (FECR 87%); which was almost significant (*P*-value =0.051). This finding which resulted in higher efficacy for the Ovis® and lower efficacy for Bendex® classified the ALB efficacy against hookworm into two different ranges: satisfactory (FECR ≥ 90%) for Ovis® and doubtful (90% > FECR ≥ 80%) for Bendex®according to the newly adopted WHO guideline [40].

Several factors may influence the efficacy of anthelmintic drugs and out of these factors quality of the drug is of great importance [27]. Generic products are now available for all major anthelminthics and monitoring the quality of drugs either imported or manufactured locally is a world-wide challenge, with greater concerns in countries with poor-resource economies.

However result from previous study indicated that concentration of active ingredient in anthelmintic drugs varied for their claimed composition in different brands [28]. Moreover, a study by the same research group also confirmed that *in-vitro* quality of BZ varies with country of origin and/or brands [29].

Thus the amount of active ingredient, the purity, dissolution and bioavailability in general are the different quality attributes responsible for quality variations in different brands of medicines which might have resulted in *in-vivo* efficacy variations. Comparative *in-vitro* quality evaluation (assay and dissolution profile) study of the same products of Ovis® and Bendex® tablets that were used for *in-vivo* efficacy study was conducted in Jimma University Laboratory of Drug Quality (JuLaDQ). The finding reported that assay results for both brands of ALB tablets comply with tolerance limit of 90-110% set in United States Pharmacopoeia (USP). In addition both brands did not reveal any impurity above the reporting threshold of 0.1% set in ICH Q3B. However, there was variation in the *in-vitro* dissolution profile between the two brands which indicated that Ovis® complied with pharmacopoeial specifications, while Bendex® did not; even with the adopted, more "aggressive" method of double revolution per minute (rpm). Maximum percentage release around (50%) was observed for Bendex®, which was by half lower than the corresponding percentage release (100%) observed for Ovis® [50].

Generally speaking, this *in-vitro* drug release variation result could justify the almost significant *in-vivo* efficacy variations (P-value<0.051). The lower *in-vivo* efficacy for Bendex® (88.7%) than that of Ovis® (98.1%) against hookworm might be due to the lower by half dissolution rate of Bendex® than that of Ovis®.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1. CONCLUSION

In conclusion, the results of this study suggest that those two brands of ALB are therapeutically efficacious for ascariasis, but not for trichuriasis. For hookworm infections, while Ovis® is therapeutically efficacious, Bendex® did not meet the WHO criteria. This could possibly be due to poor *in-vivo* release of Bendex® as indicated by dissolution analysis (manuscript under preparation). Literatures have reported reduced efficacy of BZ anthelminthics against *T. trichiura* and our findings also supported the fact. Thus, the reduced efficacy of the two brands studied here and may be elsewhere, is a warning sign especially for our study site.

6.2. RECOMMENDATION

Our study focused only on two different brands of ALB and therefore further studies are highly recommended including detail *in-vivo* efficacy and *in-vitro/in-vivo* correlation studies. And re-consideration of alternative drug regimens for trichuriasis treatment.

REFERENCES

1. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ (2006) Soil-transmitted helminth infections: Ascariasis, Trichuriasis, and hookworm. Lancet 367: 1521–32.
2. Pullan LR, Smith JL, Jasrasaria R, Brooker SJ (2014) Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Parasite and Vectors 7:1-19
3. Hotez PJ, Kamath A (2009) Neglected tropical diseases in Sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Negl Trop Dis* 3(8): e412.
4. Brooker S, Clements CA, Bundy AD (2006) Global epidemiology, ecology and control of soil-transmitted helminth infections. *Adv Parasitol* 62: 221–261.
5. Hotez JP, Brooker S, Bethony MJ, Bottazzi EM, Loukas A, Xiao S (2004) Current concepts hookworm infection. *New England Journal of Medicine* 351(8): 799-807.
6. De Silva N, Brooker S, Hotez PJ, Montresor A, Engels D, Savoli L (2003) Soil transmitted helminth infections: updating the global picture. *Trends in Parasitology* 19(12): 547–551.
7. Tadesse Z, Hailemariam A, Kolaczinski HJ (2008) Potential for integrated control of neglected tropical diseases in Ethiopia.*Trans R Soc Trop Med Hyg*102: 213-214.
8. Abera B, Alem G, Yimer M, Herrador Z (2013) Epidemiology of soil-transmitted helminths, Schistosoma mansoni and haematocrit values among schoolchildren in *Ethiopia.J Infect Dev Ctries* 7(3):253-260.
9. King DJ, Endeshaw T, Escher E, Alemtaye G, Melaku S, Gelaye W, Worku A, Adugna M (2013) Intestinal parasite prevalence in an area of Ethiopia after implementing the SAFE Strategy, enhanced outreach services, and health extension program. *PLoS Negl Trop Dis* 7(6): e2223.
10. Vercruysse J, Behnke JM, Albonico M, Ame MS, Angebault C, Bethony MJ, Engels D, Guillard B, Hoa NH, Kang G, Kattula D, Kotze AC, McCarthy JS, Mekonnen Z, Montreso A, Periago MV, Sumo L (2011) Assessment of the anthelmintic efficacy of albendazole in school children in seven countries where soil-transmitted helminths are endemic. PloS Negl Trop Dis 5(3): e948.
11. Debalke S, Worku A, Jahur N, Mekonnen Z (2013) Soil transmitted helminths and associated factors among school children in government and private primary school in Jimma Town, southwest Ethiopia. *Ethiop J Health Sci* Vol. 23, No. 3.

12. Crompton DWT and Nesheim MC (2002) Nutritional impact of intestinal helminthasis during the human life cycle. *Annu Rev Nutr* 22:35-59.
13. World Health Organization (2005) Deworming for health and development. Report of the third global meeting of the partners for parasitic control. Geneva, WHO
14. Stephenson LS, Latham MC, Adam EJ, Kinoti SN, Pertet A (1993) Physical fitness, growth and appetite of Kenyan school boys with hookworm, *Trichuris trichiura* and *Ascaris lumbricoides* infections are improved four months after a single dose of albendazole. *J Nutr* 123, 1036-1046.
15. Stephenson LS (2001) Optimizing the benefits of anthelmintic treatment in children. *Paediatr Drugs*; 3(7): 495-508.
16. WHO (2012) Soil-transmitted helminthiases: Eliminating soil-transmitted helminthiases as a public health problem in children: progress report 2001-2010 and strategic plan 2011-2020 WHO/HTM/NTD/PCT/2012.4
17. Humphries D, Nguyen S, Boakye D, Wilson M and Cappello M (2012) The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis* 25:584–589.
18. De Clercq D, Sacko M, Behnke J, Gilbert F, Dorny P, Vercruysse J (1997) Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. *Am. J. Trop. Med. Hyg.* 57 (1), 25-30.
19. Albonico M, Bickle Q, Ramsan M, Montresor A, Savioli L, Taylor M (2003) Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. Bulletin of the World Health Organization 81:343-352.
20. Legesse M, Erko B and Medhin G (2004) Comparative efficacy of albendazole and three brands of mebendazole in the treatment of ascariasis and trichuriasis *East African Medical Journal* 81:3.
21. Kumsa B, Tolera A, Nurfeta A (2010) Comparative efficacy of seven brands of Albendazole against naturally acquired gastrointestinal nematodes in sheep in Hawassa, southern Ethiopia. *Turk J. Vet. Anim. Sci* 34(5): 417-425.
22. Keiser J and Utzinger J (2008) Efficacy of current drugs against soil transmitted helminth infections. *JAMA* 299(16):1937-1948.

23. Bennett A and Guyatt H (2000) Reducing intestinal nematode infection efficacy of Albendazole and Mebendazole. *Parasitology today* 16: 2.
24. Prichard RK (2007) Marker for benzimidazole resistance in human parasitic nematodes. *Journals cambridge 134*(8):1087-1092.
25. Geerts S and Gryseels B (2000) Drug resistance in human helminths: Current situation and lessons from livestock. *Clin. Microbiolgy review* 13(2): 207–222.
26. Accelerating work to overcome the global impact of neglected tropical diseases a road map for implementation Roadmap WHO/HTM/NTD/2012.1 Full version.
27. Albonico M (2003) Methods to sustain drug efficacy in helminth control programmes *Acta Tropica* 86: 233-242.
28. Monteiro AM, Wanyangu SW, Kariuki DP, Bain R, Jackson F, McKellar QA (1998) Pharmaceutical quality of anthelmintics sold in Kenya. *Vet Rec* 142 (15): 396-398.
29. Suleman S, Zeleke G, Deti H, Mekonnen Z, Duchateau L, Levecke B, Vercruysse J, D'Hondt M, Wynendaele E, De Spiegeleer B (2014) Quality of medicines commonly used in the treatment of soil transmitted helminthes (STH) and giardia in Ethiopia: a nationwide survey. *PLoS Negl Trop Dis* (in press).
30. Hotez JP, Molyneux HD, Fenwick A, Ottesen E, Sachs ES, Sachs DJ (2006)Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Medicine* 3(5)**:** e102.
31. World Health Organization (2002) Prevention and control of Schistosomiasis and soil-transmitted helminthiasis. WHO Technical Series Report 912. Geneva, WHO.
32. World Health Organization (2011) Helminth control in school aged children: A guide for managers of control programmes, Geneva, WHO second edition.
33. Montresor A, Gyorkos TW, Crompton DWT, Bundy DAP and Savioli L (1999) Guidelines for monitoring the impact of control programmes aimed at reducing morbidity caused by soil-transmitted helminths and schistosomes, with particular reference to school-age children. WHO/CDS/CPC/SIP/99.3.
34. UpToDate.19.3 Albendazole: Drug information Copyright 1978-2011 Lexicomp, Inc. All rights reserved.

35. Albonico M, Engels D, Savioli L (2004) Monitoring drug efficacy and early detection of drug resistance in human soil-transmitted nematodes: a pressing public health agenda for helminth control. *International Journal for Parasitology* 34:1205-1210.
36. Tchuem Tchuenté LA (2011) Control of soil-transmitted helminths in sub-Saharan Africa: Diagnosis, drug efficacy concerns and challenges. *Acta Tropica* 120S S4–S11.
37. Levecke B, Speybroeck N, Dobson RJ, Vercruysse J, Charlier J (2011) Novel insights in the fecal egg count reduction test for monitoring drug efficacy against soil-transmitted helminths in large-scale treatment programs. *PLoS Neglected Tropical Diseases* 5(12): e1427.
38. Levecke B, Behnke JM, Ajjampur SS, Albonico M, Ame SM, Charlier J, Geiger SM, *et al.* (2011) A Comparison of the sensitivity and fecal egg counts of the McMaster egg counting and Kato-Katz thick smear methods for soil-transmitted helminths. *PLoS Negl Trop Dis* 5(6): e1201.
39. World Health Organization (1999) Report of the WHO Informal consultation on monitoring of drug efficacy in the control of schistosomiasis and intestinal nematodes. WHO/CDS/CPC/SIP/99.1, Geneva, World Health Organization.
40. World Health Organization (2013) Assessing the efficacy of anthelminthic drugs against schistosomiasis and soil-transmitted helminthiases. WHO/HTM/NTD/PCT/4.
41. Albonico M, Mathema P, Montresor A, Khakurel B, Reggie V, Pandey S, Savioli L (2007) Comparative study of the quality and efficacy of originator and generic albendazole for mass treatment of soil-transmitted nematode infections in Nepal.*Trans R Soc Trop Med Hyg* 101: 454-460.
42. Ramalingam S, Sinniah B, Krishnan U (1983) Albendazole, an effective single dose, broad spectrum anthelminthic drug. *Am. J. Trop. Med. Hyg*. 32(5): 984-989.
43. Steinmann P, Utzinger J, Du ZW, Jiang JY, Chen JX, Hattendorf J (2011) Efficacy of single-dose and triple-dose albendazole and mebendazole against soil-transmitted helminths and taenia spp: a randomized controlled trial. *PLoS ONE* 6(9): e25003.
44. Jongsuksuntigul P, Jeradit C, pornpattanakul S, Charanasri U (1993) A comparative study on the efficacy of albendazole and mebendazole in the treatment of ascariasis, hookworm infection and trichuriasis. *Southeast Asian J Trop Med Public Health* 24(4):724-729.

45. Soukhathammavong PA, Sayasone S, Phongluxa K, Xayaseng V, Utzinger J, Vounatsou P, (2012) low efficacy of single-dose albendazol and mebendazole against hookworm and effect on concomitant helminth infection in lao pdr. *PLoS Negl Trop Dis* 6(1): e1417.
46. Knopp S, Mohammed KA, Speich B, Hattendorf J, Khamis IS, Khamis AN, Stothard JR, Rollinson D, Marti H, Utzinger J (2010) Efficacy of albendazole and mebendazole alone or in combination with ivermectin against *Trichuris trichiura* and other soil-transmitted helminths in school children in Zanzibar, Tanzania. *Clinical Infectious Diseases* 51(12):1420–1428.
47. Olsen A, Namwanje H, Nejsum P, Roepstorff A, Thamsborg SM (2009) Albendazole and mebendazole has low efficacy against *Trichuris trichiura* in school-age children in kabale district, Uganda. *Trans R Soc Trop Med Hyg*103: 443-446.
48. Adugna S, Kebede Y, Moges F, Tiruneh M (2007) Efficacy of mebendazole and albendazole for *Ascaris lumbricoides* and hookworm infections in an area with long time exposure for anthelminthics, Northwest Ethiopia. *Ethiop Med J* 45(3):301-306.
49. Mekonnen Z, Levecke B, Boulet G, Bogers J-P, Vercruysse J (2013) Efficacy of different albendazole and mebendazole regimens against heavy intensity *Trichuris trichiura* infections in school children, Jimma Town, Ethiopia. *Pathogens and global health* 107(4).
50. Belew S, Getachew M, Mekonnen Z, Suleman S, Spiegeleer BD, *et al* (2014) Comparative assay and dissolution profile study for two brands albendazole tablets: Bendex® and Ovis® (Manuscript under preparation).

ANNEXES

1. Informed Consent (English) Form- for parents/guardians

For parents/guardians of children participating in a study in Hamle-19 and Jiren n° 1 elementary schools in Jimma Town

Investigators: Mestawet Getachew (PI)

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Organization: [1]Department of Pharmacy, College of Public Health and Medical Sciences, Jimma University, Ethiopia

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Project title: Assessment of efficacy of two brands of albendazole from local market against soil transmitted helminths (STH) among school children in Jimma Town, southwest Ethiopa

This informed consent has two parts:

Part I- Information sheet, and

Part II- Certificate of Consent

Read and give a copy of the full informed consent form to the participants.

Part I- Informed sheet

Introduction:

My name is Mestawet Getachew (pharmacist & principal investigator) from Jimma University. I conducting a study to assess the efficacy of albendazole bought on local market against soil transmitted helminths among school children in Jimma Town, southwest Ethiopia

Purpose of the research

Studies have shown that intestinal worm infections are so common among school-aged children in Jimma and as well in Ethiopia. These worms live within the intestine and feed on blood or nutrients, which make children, feel ill, often resulting in low red blood cell counts (anemia), absences from school and reduction in growth. Thus, researchers from Jimma University, needs to carry out a study to assess the efficacy of albendazole against those intestinal worms among

students of elementary schools in Jimma. We are asking children aged between 5-18 years from the schools to participate. The school your child is going to has been one of the schools chosen for this study. Therefore, we invite you to help us by taking part in this study.

Procedures

After agreeing that your child can take part, one of the research staff will visit the school on a certain day and give your child a small plastic container and ask him/her to produce a sample of his/her faeces. The plastic container with the stool will be collected the next morning in the school. We will check the stool to see if your child has worms and we will provide him/her with the appropriate treatment albendazole. This will make your child healthier. albendazole is a very safe drug; sometimes a child may experience a minor side effect after taking the tablet. These effects, if they occur, include headache, feeling dizzy, itching and nausea. They last for a short time and disappear by themselves. In case of any persistent problem you are advised to inform the research team nurse or the principal investigator using the phone number indicated here.

Confidentiality

The information obtained during the conduct of this study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the Informed Consent form will not be permitted. The results of the research study may be published, but subjects' names or identities will not be revealed. Records will remain confidential. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office in Jimma University and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will permitted only for authorized personnel directly involved with the study and will be password protected. Subject-specific information may be provided to responsible local medical personnel only with the subject's permission. Stool samples collected will not be used for research purposes other than those outlined in the protocol, and will be safely disposed of after the completion of the study.

Feed-back after the survey has been completed

At the end of the survey, the results of the study will be communicated to the Jimma Town health bureau. In turn, the head of the health bureau will inform the community of the results through public meeting in your child's school. The Department of pharmacy, Jimma University and the department Virology, Parasitology and Immunology, Faculty of Veterinary Medicine,

Ghent University, will coordinate the international dissemination of the study results, including publication.

Safety

For this survey, you child is inquired to provide their feaces only, which is a non invasive procedure. Therefore, we do not expect any harm to occur on your child. If your child is infected with any of the soil transmitted helminthes he/she will be treated with albendazole. The drugs will only be administered by qualified health professional.

Benefits

Children participating in the survey will directly benefit by being investigated for STH infections and receiving appropriate treatment. The study results will help to guide administrators of health bureau to take measure to prevent and control the infection and also to monitor the efficacy of the anthelmintic drugs.

Right to refuse or withdraw

We assure you that our best care will be taken of your child if you agree to let him/her take part in the study. You should also know that you are free to withdraw your child from the study at any time and that he/she will not be discriminated in any form for education or health services

Who to contact

If you have any questions about the study at any time, you can contact:

Mrs. Mestawet Getachew (Investigator of the project) Tel: +251 911 076564 (Mobile)

Part II – Certificate of consent for parents/guardians of children participating in the research study

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study. I agree to enroll my child in this study:

Print name of subject, date and signature or thumb impression of subject

_____,____/____/____ (dd/mm/yy)

If illiterate

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____, ____/____/____ (dd/mm/yy)

Print name of researcher, date and signature of researcher_____,____/____/____ (dd/mm/yy)

2. Informed Consent (Amharic) Form for parents/guardians

ቅ□ 7፡2 በጂማ ከተማ በሀምሌ 19 ና በጅሪን ቁዓር አንድ አንደኛ ደረጃትምህርት ቤት ተማሪዎች ላይ ከጅማ ከተማ ፋርማሲዎች ከሚሸጠው አልቤንዳዞል መድሀኒት ለሆ□ ትላትል ህክምና ያለውን የማከም ብቃቱን ለማወቅ የሚደረግ ጥናት ለሚሳተፉ ወላጆች/ህጋዊ አሳዳጊዎች ማብራሪያና የተሳተፊነት ፊርማ ቅጽ

የዋናው ተመራማሪ ስም፡

መስታወት ጌታቸው

ሱልጣን ሱሌማን

ዘለቀ መኮንን

ቡሩኖ ሌቪኬ

ድርጅት፡ ጅማ ዩኒቨርሲቲ የማሕበረሰብ ጤናና ህክምና ሳይንስ ኮሌጅ □ፋርማሲ ት/ክፍል

የጥናቱ ርዕስ፡ በጂማ ከተማ በሀምሌ 19 ና በጅሪን ቁዓር አንድ አንደኛ ደረጃ ትምህርት ቤት ተማሪዎች ላይ ከጅማ ከተማ ፋርማሲዎች ከሚሸጠው አልቤንዳዞል መድሀኒት ለሆ□ ትላትል ህክምና ያለውን የማከም ብቃትለማወቅ የሚደረግ ጥናት

ይህ ሰነድ ሁለት ክፍሎች አሉት፡፡

1. **ስለ ጉዳዩ መረጃ የሚሰጥ ክፍልና**
2. **□ተሳታፊዎች የስምምነት ሰነድ**

የስምምነቱ ሰነድ አንድ ኮፒ ይሰጥዎታል፡፡

□□ል ሀ፡ ማብራሪ□

ጤና ይስጥልኝ፡፡ መስታወት ጌታቸው እባላለሁ፡፡ እኔ በጅማ ዩኒቨርሲቲ □□ሊኒካል ፋርማሲ ማስተር ተማሪ ነኝ፡፡እንደሚታወቀዉ የሆድ ትላትል በሽ□ የሀገራችን ዋነኛ የጤና ችግር ነዉ፡ በጅማ ከተማ በተለይ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛል፡፡ ስለ□ህ በሀምሌ 19 አንደኛ □ረ□ ና በጅሪን ቁዓር አን□ ትምህርት ቤት ተማሪዎች ላይ ይህንን የጤና ችግር የሆነውን የሆድ ትላትል ለማከም የምንጠቀመው የአልቤንዳዞል መድሀኒት ለሆ□ ውስጥ ትላትል ህክምና ያለውን የማከም ብቃቱን ለማወቅ በጅማ ከተማ ፋርማሲዎች ከሚሸጠው አልቤንዳዞል መድሀኒት ላይ ጥናት ማድረግ ይፈልጋል፡

አላማ

በሀገራችን ኢትዮጵያ የሆድ ትላትል በሽታ በተለ□ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛ ል። በዚህ በከተማችንም በብዛት ተስፋፍቶ ይገኛል። በልጆች ላይ የደም ማነሰ እና የእድገት መቀጨጭን ያስከትላል። ስለዚህ እኔ ከጅማ ዩኒቨርሲቲ ስለ አልቤንዳዞል ለሆድ ውስጥ ትላትል ያለውን የማከም ብቃት ለማዓናት አቅጃለሁ፡፡ በመሆኑም ልጆዎ ከሚማርበት ት/ቤት እድሜያቸው ከ5-18 አመት የሆኑትን በዘህ ጥናት እንድሳተፉ እ□ል□ለሁ። ስለ□ህ ል□- በ□ህ ዓናት ውስጥ እንዲሳተፍ ፍቃ□ኛ እንዲሆኑ በትህትና እ□□ቆታለሁ።

የአካሄድ ቅድመ ተከተል

በጥናቱ ላይ ለመሳተፍ ከተስማሙ ከተመራማሪዋ አንድ ወይ ከዚያ በላይ የሚሆኑ ሰዎች ወደ ት/ቤት ይመጡና በጥናቱ ቀን ለልጆዎ የሠገራ ናሙና በሚቀጥለው ቀን ጠዋት ይዞ እንዲመጣ □ስ□ራ ማም□ እቃ እንሰጠዋለን። የሰገራው ናሙናዉ ትላትል እንዳለዉ ለማረጋገጥ ላቦራቶሪ ተወስ□ ምርመራ □□ረፅበታል። ትላትል ከተገኘበት ለል□- □አልቤንዳዞል መድሐኒት እንሰጠዋለን መድሐኒቱን ከወሰደ በኌላ አንዳንድ ጊዜ ትንሽ ራስምታትና ማቅለሽለሽ ሊሰማው ይችላል። ይህም ምልክት አጭርና ቶሎ የሚጠፋ ነው። አጋጣሚ ሆኖ የሚፀናበት ከሆነም ክትትል በሚያደርጉለት ሐኪሞች በአቅራቢያ በሚገኙ የጤና ጣብያ እንድታከሙ ይደረጋል።

ሚስጥራዊነት

ከምርምሩ የምንሰበስበው መረጃ በሚስጥር ይያዛል። የምርመራ ውጤት ከራሶ ፈቃድ ውጪ ለማንም አይሰጥም።ከልጆዎ የሚገኘው መረጃ የሚከማችበት መዝገብ ውስጥ በልጆዎ ስም □ተ□□ □□ል እንዲኖር አይደረፅም።በቁዓር ማለትም በኮ□ መል□ □መ□□ላል።□ቱ ኮ□ ወ□ም ቁዓር የየትኛዉ ተሳተ□ ፅለሰብ እንደሆነ □ማተወቅበት ሰነድ በተቆለ□ ቦታ በጅማ ዩኒቨርስቲ ዉስዓ □ቀመ□ል።□ህ ሚስዓር ምርምሩን ለሚያቀናጁ □ስፖንሰሮች ለዋና ተመራማሪና ከሃኪሞ በስተቀር ለሌላ ለማንም ሰዉ አይሰዓም። □ተሰበሰቡት የሰገራ ናሙናዎች ከታሰበ□ ዓላማ ዉጪ ለሌላ ዓላማ አይዉሉም። በመጨረሻ በጥንቃቀ እንድወገዱ ይደረጋል።

ጥናቱ ከተጠናቀቀ በኃላ የጥናቱን ዉጤት ስለማሳዋቅ

በጥናቱ መጨረሻ የተገኘዉ ዉጠት ለጅማ ከተማ የጤና ቢሮ ይላካል፡ በተማሪዎች የወላጆች ቀን ላይ የተገኘዉን ዉጤት በጂማ ከተማ የጤና ቢሮ ኀላፊ ለህብረተሰቡ ይገለጻል። በ□ለም አቀፍ ደረጃ በዓለም አቀፍ የጤና ድርጅት አማከይነት ማሳተምን ጨምሮ በዓለም ይሰራጫል፡

ጥንቃቄ ስጋትና ጉዳት

ለዚህ ጥናት ልጆዎ የሰገራ ናሙና ብቻ እንዲሰጥ ይጠየቃል። ይህም በልጆዎ ላይ ምንም አይነት ገዳት አያስከትልበትም። የልጆዎ ሰገራ ተመርምሮ የሆድ ትላትል ቢገኝበት በሀኪም አማካኝነት አልቤንዳዞል የተባለ ለበሽታዉ ተገቢ የሆነ መድሃኒት እንዲዉጥ የደረጋል።

ጥቅሞች

በዚህ ጥናት ቢሳተፉ ልጆዎ ከሆድ ትላትል ነፃ እንዲሆን ይረዳል። በተፊ ማሪ የኢልቤንዳዞል የማከም ብቃቱን ለማወቅ ይረዳል። የመከላከያ ዘዴዎችን በማመላከት በሃገራችን በሽታዉን ለመከላከል በሚደረገው ጥረት ከፍተኛ አስተዋጽኦ ያደርጋሉ።

ያለመሳተፍና ከተሳታፊነትየማቋረዓ መብቶች

በዚህ ጥናት ልጆዎ ላይ ምንም አይነት ጉዳት እንደማይደርስ ለማረጋገጥ እንወዳለን። በጥናቱ ልጆዎን ለመሳተፍ ፈቃደኛ ባይሆኑ የሚደርስብዎት ምንም አይነት ተጽእኖ □ለም። ል□-መሳተፍ ከጀመረም በኋላ በማናቸውም ሰዓት ከጥናቱ ሊያስወጡ ይችላሉ።ለልጆዎ የሚገባዉ የህክምና አገልግሎት ጥናቱን ስላቋረጠ አይነፈገዉም።

ማንን ማነጋገር እንዳለብዎ

ማንኛዉም አይነት ጥያቄ ካለዎት አሁን ወይም ሌላ ጊዜ ሊጠይቁ ይችላሉ።ሌላ ጊዜ ለመጠየቅ ቢፈልጉ ከዚህ በታች ያሉትን ግለሰቦ ማነጋገር ይችላሉ።

መስታወት ጌታቸው **ስል□ ቁዓ ር** ***0911076564***

□□ል ለ፡ □ተሳታፊዎች የስምምነት ሰነድ

በጥናት ምርምሩ ላ□ ል□ ተሳታ□ □እንዲሆን ጥሪ ቀርቦልኛል። ከላይ የተገለፀትን ነገሮች በሙሉ አንብቤያለው ወይም ተነቦልኛል። ግልፅ ያልሆነልኝ ነገር ካለ ጥያቄዎች እንድጠይቅ እድል ተሰቶኛል። አጥጋቢ መልስም አግኝቻለሁ።በሙሉ ፍቃደኝነት ልጄ በዚህ ጥናት እንዲሳተፍ ፈቅጄ ተስማምቻለዉ።

□ተሳታፊ ስም፡ ቀን፡ ፊርማ/የጣት አሻራ

ቀን----------ወር----------□ም ----------

ማንብብና መጻፍ የማይችሉ ከሆነ

□□ለተኛ ምስክር ስም፡ ቀንና ፊርማ(በተቻለ ምስክሩ ከጥናትና ምርምር አድራጊዉ ቡድን ጋር ምንም ግንኙነት የሌለዉና በራሱ በተሳታፊዉ ቢወከል ይመረጣል

----------ቀን----------ወር---------- □ም

የጥናትና ምርምር አድራጊዉ ወይም አስተባባሪዉ ስም፡ ቀንና ፊርማ

----------ቀን----------ወር---------- □ም

3. Informed Consent (Afan Oromo) Form for parents/guardians

Guca haayyama Hirmaannaa maatiifi ykn Kunuunsitootaafi ijoollee mana barnoota sadarkaa 1ffaa Hamile - 19 magaala Jimmaaffi Jireenlakoofsa 1

Qorattoonni Mestawet Getachew (Qorataa duraa)

Sultan Suleman

Zeleke Mekonnen

Bruno Leveke

Dhaabbata: Muumme Qorichaa, kolleejii fayyaa hawaasaa fi saayinsii Medisinii Yuunivarsiiitii Jimma :

Mata dureen qorannichaa: yaalii hammam fayyisuu albendazolii mana dawaa adda addaa irraa bitameef kan raammolee karaa biyoo dadarban,irratti baratoota sadarkaa 1ffaa magaala Jimma kibalixa itiyoopiyaa. Irratti hojjetamu

- Gucinni hayyammi hiirmaanna tun kutaa lama qaba

  Kutaa 1. Fuula odeefannoo fi

  Kutaa 2. Warqa ragaa Eeyyamaa

  Dubbisiitii koopii guutuu quca eeyaama kana hirmaatotaaf kenni

  Kutaa 1- Odeefannoo/ibsa

  Seensa:

Maqaan koo Mestawet Getachew (Ogeessa Qorichaaa fi qorataa isa duraati). Ani fi qorattoonni kannen yuunivarsiitii Jimmaa fi Yuuniersity Giyeenti irraa dhufne. Qoratoota kun fayyisuu Albendazoolii mana dawaa biyya keenya kessaa bitame irratti baratoota sadarkaa 1ffaa magaala Jimmaa kibalixa itiyoopiyaa keessatti qowannaa gageesina

Kaayyoo qorannichaa

Itiyoopiyaa keesatti Ijooleen uumuriin isaanii mana barumsaaf gahan raamolee karaa biyyoo darbaniin ni faalamu. Kunis as magaala Jimma keessatisi baayyee kan mul'tuu fi ijoolleen hanqina dhiiigaa diimaatiin rakkisuu fi guddina isaan ni xiqqesessa. Qorattootni Yuunivarsiitii Jimma fi walda fayya addunyaa qorannoo ammam walaansi qorichaa raamolee kana akka ijoolee raamoleetiin faalaman fayyisuu ilaaluuf gaggessuu barabbadan. Qorichiichi albendazole kan jedhamu bifa tabileetiitiin ykn liqinfamuun kename ijooleen alanfachuun bishaaniin kana liqimsanidha. Albendazoliin qoricha addunyaa irratti raamolee walaanuuf balinnani fayyadudha akkasumas Vermox jedhamee kan beekamu dha.

Kanaafuu, ijoolleen umuriin isaanii waggaa 5-18 taanii manneen barnoota keessatti argaman akka qorannoo kana irratti hirmaataa gaafaanna. Mebedaazooliin dubartii ulfaa fi hin kennamu. Manni barumsa ijooleen keessan itti baratu mana barnoota filataman keessaa isa tokko ta'a. Kanaaffu akka ijoolleen keessaan qorannoo kana irratti hirmaatan eeyyam akka laattaniif sin affeerra.

Adeemsa

Akka ijoolleen keessan irratti hirmaantan erga eeyyamtanii booda, hojjetoota qorannoo keenya mana barumsa mucaan keessan itti argamu/ttu dhaquun mucaa kessaniif qabdu iddattoo pilaastikii irraa tolfamte itti kennuun akka inni/isheen iddattoo bobbaa ho'aatilmaamaan 6 gm ta'u fidu/fiddu gaafatu.

Iddattoo bobbaa, akka ijoolleen keessaan raammolee marri'immaniin qabamanii fi hin qabamne ilaalele firii irratti hunda'uun walaansa barbaachisaa keenninaaf, kun mucaan keessan fayyaa akka ta'u/taatu ni taasisa. albendazoli qoricha baa'ee miidhaa hin qabnedha. Haa ta'u malee yeroo tokko tokko, ijoolleen yemmuu qoricha albendazole fudhatan dhiibbaa/dukkabbii bicuu/xiqqoo isaanirraan gahuu danda'a. DHukkubbiwwan tunis, yoo jiraatan matabowwoo, muka'uun namatti dhagahamuu, dhaqina hooqsisuu fi garaa hammeessuu dha. Haa ta'u malee kun kan turu yeroo baayyee bicuu taatee fi kan ofi badudha. Akka carra yoo kan turuu fi cimaa ta'e mucaa keessan gara buufata fayyaa bakka ogeeyyonni leejjii fudhataniif geessuun walansisuun akka qabdan isiniinjenna.

Guyyaa 14 booda hagam qorichi kun akka hojjete ilaaluuf mucaa keessan iddattoo bobba biraa akka nuukennu/tuu ni gaafana. Iddatto bobba raammoon marri'imanii keessatti argame kutaa laboraatoorii keessa kuusudhan qorannoon biraa yoo barbaachise ni hojjena. Dhuma qorannich irratti of eeganoodhan iddattoowwan kun ni gatamu.

Icitii

Odeeffannoon yommuu qorannoo kanaa argamu icitiidhaan eegama. Odeeffannoon argame kun qaama biraaf dabarfame hin kennamuu. Argannoo qo'annoo kanaa maxxansumuu ni danda'a haata'u malee maqaaf eenyummaan namootaa hin ibsamu. Galmeewwn icitiin eegamu. Iciitii kana eeguudhaaf, qorataan galmeewwan kana sanduqa furtuu qabu keessa Biiroo Yunivarsitti Jimmaatti argamu keessa kaa'a. Friin qorannoo taasifamuu kun koodiin itti kennamee akka eenyummaan namoota fedhiitiin hirmatamanii akka hin beekamnne ta'a. Namoota firii laaboraatorii ilaalani allatti akka ragaan kampiitera keessa taa'u ni taassifama. Kompuuterichis

koodiitiin cufaa ta'a. odeeffannoon namoota dhuunfaa yoo fedhii saanii ta'e qofa ogeessota fayyaaf kennamuu danda'a. Bobbaa funaanamu haala protokolii keessaatti ibsameen alatti qo'annoo biraatiif osoo hin ooliin haala miidhaa hin finneen qo'annoon kun erga dhumee booda ni gatama.

Of-eegannoo

Kan isaan irra dheegamu bobba furdaa qofaa kennuu dha. Yaaliin tun albendaazolii yeroo ammaa kana gabaa irratti argaman waan ilaaluuf bu'aa malee miidhaa hin qabu.. qorichis kan kennamu ogeessa fayyaati qofaani dha.

Faayidaawwan

Ijoleen kana keessatti hirmaatan raamolee marri'imaanii irraa bilsa akka ta'anii fi hin taane qoratamuufi wala'ansa barbaachisaa argachuun ni fayyadamu. Ijoolleen dhuma irrattis raamootiin qabamanis haala isaanii irratti hunda'uun yaalii atattamaa ni argatu.

Mirga diduu fi addan kutuu

Yoo akka ijoolleen keessan qorannoo kana keesatti hirmaatan eeyyamtan, of eegannoo guddoo akka isaaii goonu isin mirkaneesina. Akkasumas yeroo barbaadanii irratti mucaa keessaan hirmanaa qoranichaa akka addan kutu/kuttuu gochuuf mirga guutuu qabdu. Kanaaf immoo miidhaan/loogiin mucaa keessaniira karan barnootaa fi waalansaa irra ga'u hin jiru. Yoo gaaffi qabaattan battaluma aadde Mestawet Getachew (tel. 0911076564) gaafachuu dandeessan

Kutaa II – Guca raga Heeyyamaa

Odeefannoo armaan olii dubbiseera ykn naa dubbisameera carraan gaafii gaafachuu naa kennameera gaaffii kootifisi deebii gahaan argadheera. Fedhii kootiin mucaan koo akka qorannoo kana keessatti hirmaatu/hirmaattu heeyyamee:

Maqaa maatii hirmaata _____Mallato _____

guyyaa _____ g/j/w

Kan hin baranneef

Maqaa nama barate adda kan raga ta'uu, guyyaa fi mallattoo (yoo danda'ame namni kun hirmaatotaan osoo filatamee fi garee qoranicha waliin hariiroo osoo qabachuu baate gaarii ta'a).

_____ Guuyyaa (Jia/waggaa

Maqaa qorataa guyyaa fi mallattoo qorataa _____guu/j/w_____ g/j/w

4. Consent (English) Form for children older than 12 years

Project title: Assessment of efficacy of two brands of albendazole from local market against soil transmitted helminths (STH) among school children in Jimma Town, southwest Ethiopa

Principal investigators Mestawet Getachew (PI)

Sultan suleman

Zeleke Mekonnen

Bruno Levecke

Part I- Informed sheet (statement)

Introduction:

My name is Mestawet Getachew (pharmacist & principal investigator) and I and my colleagues are from Jimma University. We, together with researchers from Ghent University are conducting a study to assess the efficacy of albendazole bought on local market against soil transmitted helminths among school children in Jimma Town, southwest Ethiopia

Purpose of the research:

Studies have shown that intestinal worm infections are so common among school-aged children in Jimma and as well in Ethiopia. These worms live within the intestine and feed on blood or nutrients, which make children, feel ill, often resulting in low red blood cell counts (anemia), absences from school and reduction in growth. Thus, researchers from Jimma University, needs to carry out a study to assess the efficacy of albendazole against those intestinal worms among students of elementary schools in Jimma. The school you are going has been one of the schools chosen for this study. Therefore, we invite you to help us by taking part in this study.

Procedures:

If you agree to participate, we will visit you at the school and give you a small plastic container and ask you to collect your fresh stool sample. Then we will check the stool to see if you have infection of intestinal worms. If you do, we will provide you appropriate treatment with a single dose of albendazole in tablet form. Sometimes a child may experience one or more side effects after taking a tablet of anthelmintics. These effects, if they occur, include headache, feeling dizzy, itching and nausea. However, these side effects usually last only a short time and disappear without the need for treatment. In case of any persistent problem you are advised to

inform the research team nurse or the principal investigator using the phone number indicated here

Confidentiality:

The information obtained during the conduct of this study will remain confidential. The results of the research study may be published, but subjects' names or identities will not be revealed. Records will remain confidential. Only the researchers doing the study and principal investigator will use these forms. All will have a duty of confidentiality to you as a research participant.

Safety:

For this survey, you are inquired to provide your feaces only, which is a non invasive procedure. Therefore, we do not expect any harm to occur on you. If you are infected with any of the soil transmitted helminthes you will be treated with albendazole. The drugs will only be administered by qualified health professional.

Benefits:

By participating in the survey you will directly benefit by being investigated for intestinal worm infections and receiving appropriate treatment free of charge.

Right to refuse or withdraw:

We assure you that our best care will be taken for you if you agree to take part in the study.You are free to withdraw from the study at any time and no one will force you to participate and you will not be discriminated in any form for education or health services. If you have questions, feel free to ask Mrs. Mestawet Getachew (pharmacist & principal investigator, Tel. 0911076564)

Part II: Certificate of consent

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I will participate in this study by giving my stool for intestinal worm diagnosis and management and I understand that I have the right to withdraw from the study at any time.

Print name of subject, date and signature

_____ ____/____/____ (dd/mm/yy)

5. **Consent (Amharic) Form for children older than 12 years**

ቅ□ 7፡5 □□ሜያቸው ከ12 □መት በላ□ □ሆኑትና በጥናቱ ላ□ በሚሳተፉት ተማሪዎች □ሚሞላ ተፊ ማሪ □ስምምነት ቅ□

የጥናቱ ርዕስ፡ በጂማ ከተማ በሀምሌ 19 ና በጅሪን ቁዓር አንድ አንደኛ □ረ□ ትምህርት ቤት ተማሪዎች ላይ ከጅማ ከተማ ፋርማሲዎች ከሚሸጠው አልቤንዳዞል መድሀኒት ለሆ□ ትላትል ህክምና ያለውን የማከም ብቃቱን ለማወቅ የሚደረግ ጥናት ፡

የዋናው ተመራማሪ ስም

መስታወት ጌታቸው

ሱልጣን ሱሌማን

ዘለቀ መኮንን

ቡሩኖ ሌቪኬ

□□ል ሀ፡ ማብራሪ□

ጤና ይስጥልኝ፡፡ መስታወት ጌታቸው እባላለሁ፡፡ እኔ በጅማ ዩኒቨርሲቲ □□ሊኒካልፋርማሲ ማስተርተማሪ ነኝ፡፡እንደሚታወቀዉ የሆድ ትላትል በሽ□ የሀገራችን ዋነኛ የጤና ችግር ነዉ፡ በጅማ ከተማ በተለይ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛል፡፡ ስለ□ህ በሀምሌ 19 ና በጅሪን ቁዓር አን□ አንደኛ ደረጃ ትምህርት ቤት ተማሪዎች ላይ ይህንን የጤና ችግር የሆነውን የሆድ ትላትል ለማከም የምንጠቀመው የአልቤንዳዞል መድሀኒት ለሆ□ ውስጥ ትላትል ህክምና ያለውን የማከም ብቃት ለማወቅ ከጅማ ከተማ ፋርማሲዎች ከሚሸጠው አልቤንዳዞል መድሀኒት ላይ ጥናት ማድረግ ይፈልጋል፡፡

አላማ

በሀገራችን ኢትዮጵያ የሆድ ትላትል በሽታ በተለ□ በታዳጊ ልጆች ላይ በብዛት ተስፋፍቶ ይገኛ ል፡፡ በዚህ በከተማችንም በብዛት ተስፋፍቶ ይገኛል፡፡ በልጆች ላይ የደም ማነሰ እና የእድገት መቀጨጭን ያስከትላል፡፡ ስለዚህ እኔ ከጅማ ዩኒቨርሲቲ ስለ አልቤንዳዞል ለሆድ ውስጥ ትላትል ያለውን የማከም ብቃት ለማዓናት አቅጃለሁ፡፡ በመሆኑም በሀምሌ 19 ና በጅሪን ቁዓር አንድ አንደኛ □ረ□ት/ቤት እድሜያቸው ከ5-18 አመት የሆኑትን በዘህ ጥናት እንድሳተፉ እ□ል□ለሁ፡፡ ስለ□ህ አንተ ወይም አንቺ በ□ህ ዓናት ውስጥ እንድትሳተፍ/ፊ □ቃ□ኛ እንድትሆን/ኝ በትህትና እ□□ቃለሁ፡፡

የአካዜድ ቅድመ ተከተል

በጥናቱ ላይ ለመሳተፍ ከተስማማህ/ሽ/ በጥናቱ ቀን የሠገራ ናሙና በምቀጥለው ቀን ጥዋት ይዞ እንድመጣ የሰገራ ማምጫ እቃ እንሰጥሃለን፡፡ የሰገራው ናሙናዉ ትላትል እን□ለ□ ለማረጋገጥ ላቦራቶሪ ተወስዶ ምርመራ ይደረግበታል፡፡ ትላትል ከተገኘበት አልቤንዳዞል መድሐኒት እንሰጥሃለን/ሻለን መድሐኒቱን ከወሰድክ/ሽ/ በኋላ አንዳንድ ጊዜ ትንሽ ራስምታትና ማቅለሽለሽ ሊሰማህ/ሽ/ ይችላል፡፡ ይህም ምልክት አጭርና ቶሎ የሚጠፋ ነው፡፡ አጋጣሚ ሆኖ የሚፀናብህ/ሽ/ ከሆነም ክትትል በምያደርጉልህ/ልሽ/ ሐኪሞች በአቅራብያ በምገኙ የጤና ጣብያ እንድትታከም/ሚ/ ያደርጋሉ፡፡

ሚስጥራዊነት

ከምርምሩ የምንሰበስበው መረጃ በሚስጥር ይያዛል፡፡ ከጥናቱ በሚገኘው ውጤት የግለሰብ ማንነት በማይ□ወቅ መልኩ ሊታተም ይችላል መዝገቦች በሚስጥርነት ይያዛሉ ጥናቱ □ያሚ□ክሄ□ው፡ ተመራማሪ የጥናቱ ድጋፍ ሰጭ /ጌንት ዩኒቨርስቲ እና የተለየ የጥናቱን ስነምግባር የሚከታተል ኮሚቴ ስለ አንተ መረጃ ሊሰጣቸው ይችላል፡፡ ይህ ደግሞ ጥናቱ ደረጃወን የጠበቀ እንዲሆን ለማድረግ ነው፡፡ እነዚህ አካላትም ምስጢርነቱን የመጠበቅ ኃላፊነት አለባቸው ::

ጥንቃቄ ስጋትና ጉዳት

ጉዳት፡ የሰገራ ናሙና መስጠት ምንም አይነት ጉዳት አይኖረውም

ጥቅሞች

በዚህ ጥናት የማሳተፉ ልጆች ከሆድ ትላትል ነፃ እንዲሆኑ ይረዳል፡፡ በተፊ ማሪ የአልቤንዳዞል የማከም ብቃቱን ለማወቅ ይረዳል፡፡ የመከላከያ ዘዴዎችን በማመላከት በሃገራችን በሽታዉን ለመከላከል በሚደረገው ጥረት ከፍተኛ አስተዋጽኦ ያደርጋሉ፡፡

ያለመሳተፍና ከተሳታፊነት የማቋረጥ መብቶች

በዚህ ጥናት ላይ ብትሳተፍ /ብትሳተፊ/ ምንም አይነት ጉዳት እንደማይደርስ ለማረጋገጥ እንወዳለን፡፡በ□ሮ□□ቱ ላለመሳተ□ □ቃ□ኛ ባትሆን/ኚ የሚደርስብህ/ሽ ምንም አይነት ተጽንኦ □ለም፡፡ በጥናቱ መሳተፍ ከጀመርክ/ሽ/ በኋላ በማናቸውም ሰዓት ከጥናቱ መወጣት ወይም ማቋረጥ ይቻላል፡፡ ከጥናቱ በመውጣትህ/ሽ/ በማቋረጥህ/ሽ/ የሚገባህን/ሽ/ የህክምና አገልግሎት አይነፈግህም/ሽም/ :: ጥያቄ ካለህ/ሽ መስታወት ጌታቸው /ዋና አስተባባሪ 0911076564/ መጠየቅ ይቻላል፡፡

□□ል ለ፡ □ተሳታፊዎች የስምምነት ሰነድ

ከላ□ የተገለፁትን ነገሮች በሙሉ አንብቤያለው ወይም ተነቦልኛል። ግልፅ ያልሆነልኝ ነገር ካለ ጥያቄዎች እንድጠይቅ እድል ተሰቶኛል ለጥያቄም በቂ ምላሽ አግኝቻለሁ። በሙሉ ፍቃደኝነት በዚህ ጥናት ላይ የሰገራ ናሙና በመስጠት በመሳተፍ በፍቃደኝነት ተስማምቼአለሁ።

□ተሳታፊ ስም፡ ቀን፡ ፊርማ/የጣት አሻራ

ቀን----------ወር----------□ም

6. Consent (Afan Oromo) Form for children older than 12 years

Guca haayyama Hirmaannaa ijoollee waggaa 12 fi isaa oli mana barnoota sadarkaa 1^{ffaa} Hamile - 19 magaala Jimmaaffi Jireenlakoofsa 1

Qorattoonni **Mestawet Getachew (Qorataa duraa)**
Sultan Suleman
Zeleke Mekonnen
Bruno Leveke

Dhaabbata: Muumme Qorichaa, kolleejii fayyaa hawaasaa fi saayinsii Medisinii Yuunivarsiiitii Jimma:

Mata dureen qorannichaa: yaalii hammam fayyisuu albendazolii mana dawaa adda addaa irraa bitameef kan raammolee karaa biyoo dadarban,irratti baratoota sadarkaa 1^{ffaa} magaala Jimma kibalixa itiyoopiyaa. Irratti hojjetamu

- Gucinni hayyammi hiirmaanna tun kutaa lama qaba

  Kutaa 1. Fuula odeefannoo fi

  Kutaa 2. Warqa ragaa Eeyyamaa

Kutaa 1- Odeefannoo/ibsa

Seensa:

Maqaan koo Mestawet Getachew (Ogeessa Qorichaaa fi qorataa isa duraati). Ani fi qorattoonni kannen yuunivarsiitii Jimmaa fi Yuuniersity Giyeenti irraa dhufne. Qoratoota kun fayyisuu albendazoolii mana dawwaa biyya keenya kessaa bitame irratti baratoota sadarkaa 1ffaa magaala Jimmaa mana barumsaa hamle 19Jimmaaf fiJireenlakoofsa 1 itiyoopiyaa keessatti qowannaa gaggeessuufi.

Kaayyoo qorannichaa

Itiyoopiyaa keesatti Ijooleen uumuriin isaanii mana barumsaaf gahan raamolee karaa biyyoo darbaniin ni faalamu. Kunis as magaala Jimma keessatisi baayyee kan mul'tuu fi ijoolleen hanqina dhiiigaa diimaatiin rakkisuu fi guddina isaan ni xiqqesessa. Qorattootni Yuunivarsiitii Jimma, Yuuniersiitii Giyent fi Walda Fayya Addunyaa qorannoo hammam walaansi qorichaa raamolee kana akka ijoolee raamoleetiin faalaman fayyisuu ilaaluuf gaggessuu barabbadan. Qorichiichi Albendazole kan jedhamu bifa tabileetiitiin ykn liqinfamuun kename ijooleen

alanfachuun bishaaniin kana liqimsanidha. albendazoliin qoricha addunyaa irratti raamolee walaanuuf balinnani fayyadudha akkasumas Vermox jedhamee kan beekamu dha. Kanaafuu, ijoolleen umuriin isaanii waggaa 12 fi isaaa ol taaatan qorannoo kana keessatti akka hirmaattan afferamtaniittu.

Adeemsa

Yoo fedha qabaattan hojjetoota qorannoo keenya mana barumsa keessan dhaquun qabdu iddattoo pilaastikii irraa tolfamte sinii kennuun akka iddattoo bobbaa ho'aatilmaamaan gm 6 fiddan isin gaafatu. Firii qorannoo keessan irratti hundaa'uudhaan qorichi albendaazoli isin tola kenname ni fayyitu. Haa ta'u malee yeroo tokko tokko, ijoolleen yemmuu qoricha albendazole fudhatan dhiibbaa/dukkabbii bicuu/xiqqoo isinirra gahuu danda'a. Dhukkubbiwwan tunis, yoo jiraatan matabowwoo, muka'uun namatti dhagahamuu, dhaqina hooqsisuu fi garaa hammeessuu dha. Haa ta'u malee kun kan turu yeroo baayyee bicuu taatee fi kan ofi badudha. Akka carra yoo kan turuu fi cimaa ta'e gara buufata fayyaa bakka ogeeyyonni leejjii fudhataniif geessuun wal'aanmu dandeessu.

Guyyaa 14 booda hagam qorichi kun akka hojjete ilaaluuf iddatto bobba biraa akka nuukennitu ni gaafana. Iddatto bobba raammoon marri'imanii keessatti argame kutaa laboraatoorii keessa kuusudhan qorannoon biraa yoo barbaachise ni hojjena. Dhuma qorannich irratti of eeganoodhan iddattoowwan kun ni gatamu.

Icitii

Odeeffannoon yommuu qorannoo kanaa argamu icitiidhaan eegama. Odeeffannoon argame kun qaama biraaf dabarfame hin kennamuu. Argannoo qo'annoo kanaa maxxansumuu ni danda'a haata'u malee maqaaf eenyummaan namootaa hin ibsamu. Galmeewwn icitiin eegamu. Iciitii kana eeguudhaaf, qorataan galmeewwan kana sanduqa furtuu qabu keessa Biiroo Yunivarsitti Jimmaatti argamu keessa kaa'a. Friin qorannoo taasifamuu kun koodiin itti kennamee akka eenyummaan namoota fedhiitiin hirmatamanii akka hin beekamnne ta'a. Namoota firii laaboraatorii ilaalani allatti akka ragaan kampiitera keessa taa'u ni taassifama. Kompuuterichis koodiitiin cufaa ta'a. odeeffannoon namoota dhuunfaa yoo fedhii saanii ta'e qofa ogeessota fayyaaf kennamuu danda'a. Bobbaa funaanamu haala protokolii keessaatti ibsameen alatti qo'annoo biraatiif osoo hin ooliin haala miidhaa hin finneen qo'annoon kun erga dhumee booda ni gatama.

Of-eegannoo

Kan isin irra dheegamu bobba furdaa qofaa kennuu dha. Yaaliin kun albendaazolii yeroo ammaa kana gabaa irratti argaman waan laatuuf bu'aa malee miidhaa hin qabu.Qorichis kan kennamu ogeessa fayyaati qofaani dha.

Faayidaawwan

Isin ijoleen kana keessatti hirmaattan raamolee marri'imaaniif faalamuu fi hin faalamne qorannoon adda baasa fi wala'ansa barbaachisaa argachuun ni fayyadamtu. Kan dhuma irrattis raamootiin qabamtanis haala tokko tokko irratti hunda'uun yaalii atattamaa ni argattu.

Mirga diduu fi addan kutuu

Yoo qorannoo kana keesatti hirmaattan of eegannoo guddoo akka goonu isinii mirkaneesina. Akkasumas yeroo barbaadanii hirmanaa qoranichaa addan kutuuf mirga guutuu qabda. Kanaaf immoo miidhaan/loogiin karaa barnootaa fi waalansaa isin irraan ga'u hin jiru. Yoo gaaffi qabaatte battaluma aadde Mestawet Getachew (tel. 0911076564) gaafachuu dandeessa.

Kutaa II – Guca raga Heeyyamaa

Odeefannoo armaan olii dubbiseera ykn naa dubbisameera carraan gaafii gaafachuu naa kennameera gaaffii kootifisi deebii gahaan argadheera. Fedhii kootiin qorannoo keessan keesatti hirmaachuu akkan barbaade isin mirkaneessa:

Maqaa hirmaata _____Mallato _____

guyyaa _____ g/j/w

Maqaa qorataa guyyaa fi mallattoo qorataa _____guu/j/w_____ g/j/w

7. Data collection format for baseline parasitological exam

Laboratory method ……

Name of the school…… Date of data collection……..

Serial no	Name of the students	Grade	Age	Sex	Ref no	PreEPG A.lumbricoides	PreEPG T. trichiura	PreEPG Hook worm	Previous history of drug allergy	Pregnancy for female	Diarrhea & severe medical condition	Name & signature of lab technicians
1												
2												
3												
4												
5												

8. Data collection format for post treatment parasitological exam

Laboratory method ……

Name of the school…… Date of data collection……..

Serial no	Name of the students	Grade	Age	Sex	Ref no	Post EPG *A.lumbricoides*	Post EPG *T. trichiura*	Post EPG Hook worm	Adverse drug events	Name & signtureof lab technician
1										
2										
3										
4										

9. Materials and reagents

- ✓ 60-ml containers;
- ✓ digital scales (precise to 0.01 g);
- ✓ stirring device (fork, spatula, tongue depressor, spoon);
- ✓ measuring cylinder;
- ✓ Pasteur pipettes and rubber teats;
- ✓ strainer;
- ✓ saturated NaCl solution to be prepared at least 1 day before use and kept at room temperature (specific density = 1.2, verified with a densitometer);
- ✓ McMaster slides;
- ✓ compound microscope;
- ✓ 5 l distilled water;
- ✓ 3 kg NaCl.
- ✓ Gloves
- ✓ Wire mesh screens
- ✓ McMaster counting chambers

10. SOP for McMaster technique

Flotation solution (to be prepared 24 h before processing samples):

- heat 5 l water to 50 °C;
- gently add NaCl while stirring the suspension;
- stop adding NaCl when a sediment appears;
- Keep the solution at room temperature.

McMaster egg counting technique

- place a 60-ml container on the electric scale;
- tare the scale (the display should show 0.00 g);
- homogenize the stool with a wooden spatula;
- weigh exactly 2 g of stool on the scale;
- add 30 ml of saturated NaCl;
- Homogenize and pour the faecal suspension three times through a tea strainer to withhold large debris. During the last sieving step, the filtrate must be squeezed dry;
- rinse the McMaster slide and tap it on a hard surface;
- 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;
- Allow the McMaster slide to stand for 2 min, place under a light microscope and examine 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;
- Calculate the number of eggs per gram of faeces by multiplying the total number of eggs under the two grids by 50. This is done for each parasite species.

DECLARATION

AS AN EXAMINER I CERTIFY THAT I HAVE READ AND EVALUATED THE PAPER PREPARED BY MESTAWET GETACHEW AND EXAMINED THE CANDIDATE. WE RECOMMENDED THAT THE THESIS BE ACCEPTED AS FULFILLING THE THESIS REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL PHARMACY.

_____ _____ _____

EXAMINER'S NAME SIGNATURE DATE

I, THE UNDERSIGNED, SENIOR CLINICAL PHARMACY STUDENT, DECLARE THAT THIS THESIS IS MY ORIGINAL WORK IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS FOR THE DEGREE OF MASTER IN CLINICAL PHARMACY. ALL THE SOURCES OF THE MATERIALS USED FOR THIS THESIS AND ALL PEOPLE AND INSTITUTIONS WHO GAVE SUPPORT FOR THIS WORK ARE FULLY ACKNOWLEDGED.

NAME: MESTAWET GETACHEW

SIGNATURE_____

DATE OF SUBMISSION_____

PLACE OF SUBMISSION: DEPARTMENT OF PHARMACY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES

JIMMA UNIVERSITY