Addis Ababa University

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The magnitude of anemia and growth status among selected public school children in a setting of mass deworming in Sululta town, Oromia Region, Ethiopia, 2017

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This is to certify that the thesis prepared by Moges Wordofa, entitled:

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Abbreviations

AOR	Adjusted odd ration
BAZ	Body mass index for age z-score
CBC	Complete blood count
COR	Crude odd ration
CRP	C-reactive protein
EDHS	Ethiopian demographic and health survey
EDTA	Ethylene diamine tetra-acetic acid
EPHI	Ethiopian Public Health Institute
ETB	Ethiopian birr
FGR	Fetal growth restriction
fl	femtoliter
FMOH	Federal ministry of health
HAZ	Height for age Z-score
Hgb	Hemoglobin
ID	iron deficiency
IDA	Iron deficiency anemia
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean Corpuscular volume.
pg	picogram

PLT	platelet
RBC	Red blood cell
SAF	sodium acetate-Acetic Acid-Formalin
SD	Standard deviation
STH	Soil transmitted helminthes
WAZ	Weight for age z-score
WBC	White blood cell
WHO	World health organization

Abstract

Background: Undernutrition and anemia in children continues to be public health problem in developing countries. Large scale implementation of anthelminthics delivered through school system can significantly reduce infection and morbidity among school children with improvement in growth status and reduction in prevalence of anemia.

Objective: To determine the magnitude of anemia and growth status among public school children in setting of mass deworming in Sululta town, Oromia region ,Ethiopia,2017.

Methods: A cross-sectional study was conducted from April to June 2017 in three randomly selected public schools in Sululta town. A total of 510 students aged 5-14 years were included conveniently. Socio-economic and demographic factors as well as food frequency data were collected using structured questionnaire. Anthropometric measurements such as height and weight were carried out and analyzed using WHO Anthroplus v1.0.4. Complete blood count was performed using Sysmex KX-21N automated analyzer. Stool samples were processed using direct wet mount, formol ether concentration and Kato-katz methods. Moreover, H.*Pylori* stool antigen test was also performed. Data was entered and analyzed using SPSS version 21 software. Bivariate and multivariate analyses were computed to assess association between variables .The odds ratio and 95% CI were calculated to assess the strength of the association and P-value< 0.05 was considered as statistically significant

Results: Among 510 study participants, 60.2% were females and 82.7% were dewormed. The magnitude of anemia was 3.7% when adjusted for altitude above sea level. 3.1% and 0.6% of participants had mild and moderate anemia respectively. The magnitude of stunting and thinness were 16.9% and 10.8%, respectively. None of socio-demographic variables of parents and children were significantly associated with anemia. As to growth status, children from small size family (≤5) were more likely to be stunted (P=0.002) compared to large size family (>5). The associations of deworming with anemia and growth status were insignificant.

Conclusion: The magnitude of anemia, stunting and thinness was low. None of the variables including deworming were associated with anemia and similarly with growth status except for family size. Thus further longitudinal study should be conducted.

Key words: Anemia, growth status, stunting, thinness, deworming

1. Introduction

1.1. Background

Anemia is defined as reduced mass of Red blood cells (RBCs) circulating in the blood which are insufficient to meet the body's physiologic needs that vary with a person's age, gender, residential elevation above sea level (altitude), smoking behavior, and different stages of pregnancy [1].

The prevalence of anemia is an important health indicator and globally, it affects 1.62 billion people, which corresponds to 24.8% of the population. Of these, 47.4% and 41.8% are preschool-age children and pregnant women respectively. World Health Organization (WHO) regions of Africa and South-East Asia have the highest risk, where about two thirds of preschool-age children and half of all women are affected [2]. According to Ethiopian national micronutrient survey report conducted by Ethiopian Public Health Institute (EPHI) in 2016, the prevalence of anemia adjusted for altitude among preschool children, school age and non-pregnant women of reproductive age were 34.4%, 25.6% and 17.7 %, respectively [3].

The etiology of anemia involves the interaction between multiple factors. The main causes are: dietary iron deficiency, infectious diseases such as malaria, hookworm infections and schistosomiasis; deficiencies of other key micronutrients including folate, vitamin B and vitamin A; or inherited conditions that affect red blood cells, such as thalassaemia. Globally, the most significant contributor to the onset of anemia is iron deficiency [1] and prevalence of anemia decreased for most causes between 1990 and 2010 [4].

Child growth is internationally recognized as an important indicator of nutritional status and health in a population [5]. Anthropometric nutritional assessment has several public health and development uses, such as overall population assessment, identification of target groups or areas for intervention; continuous nutritional surveillance is a tool for development planning and monitoring nutritional status to determine trends of particular health importance [6].

Anthropometric indices like height for age z-score (HAZ) and weight for age z-score (WAZ) are used to assess nutritional status. HAZ is a measure of height of individual against age and indicate how many standard deviations a data point is from WHO growth standard. WAZ is a measure of weight of individual against age by comparing with WHO growth standard [5].

In developing countries, mean HAZ (height for age Z-score) improved from –1.86 in 1985 to –1.16 in 2011; mean WAZ (weight for age Z-score) improved from –1.31 to –0.84. Over this period, Prevalence of moderate-and-severe stunting declined from 47.2% to 29.9% and underweight from 30.1% to 19.4% .The largest absolute improvements were in Asia and the largest relative reductions in prevalence in southern and tropical Latin America. Anthropometric status worsened in sub-Saharan Africa until the late 1990s and improved thereafter [7].

According to 2016 Ethiopian Demographic and health survey (EDHS) report, 10% of children are wasted and 3% are severely wasted while 38% of children are stunted and 18% are severely stunted. Stunting is slightly higher among male than female children (41% versus 35%). Residency, mother's education and wealth quintiles are the most important predicators of stunting [8].

Fetal growth restriction (FGR) and unimproved sanitation are the leading risk factors for stunting in developing countries. Reducing the burden of stunting requires a paradigm shift from interventions focusing solely on children and infants to those that reach mothers and families and improve their living environment and nutrition [9].

Large scale implementation of anthelmintics delivered through the school system can significantly decrease infection and morbidity among school children. Mass treatment has been shown to decrease in prevalence of *S. mansoni* and *hookworm* infection and significant increase in hemoglobin [10].

The WHO recommends periodic treatment with anthelminthic medicines, without previous individual diagnosis to almost all children living in endemic areas. Treatment is recommended once a year when the prevalence of STH infections in the community is over 20%, and twice a year when the prevalence of the infections in the community exceeds 50%. The strategy is to target drug treatment to at-risk groups: pre-school-age children, school-age children and women of childbearing age [11].

1.2 statement of problem

Anemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It occurs at all stages of the life, but is more prevalent in pregnant women and young children [12]. It is critical health concern since it affects growth and energy level adversely [13]. Anemic women and their infants are at greater risk of dying during perinatal period; children mental and physical development delayed or impaired; physical work capacity and production of manual worker are reduced and there have been many efforts to fight anemia over the past two decades but despite these efforts, the problem is still common **[**1**]**.

Globally, anemia affects 305 million (25.4%) of school age children while in developing countries the prevalence in those age group is 40% and classified as severe public health problem [4]. In Ethiopia, the prevalence of anemia among school age children in some places reaches to 37.6% and the occurrence is directly linked with parent's income, maternal education and food insecurity [14].

Child malnutrition continues to be the leading public health problem in developing countries. Globally, there were 165 million stunted, 99 million underweight, and 51 million wasting children by year 2012. It kills 3.1 million under-five children every year [15]. In Ethiopia under nutrition (stunting and underweight) was associated with 24% of all child mortality with estimated 379,000 deaths within a period 2004 -2009 and the overall result indicates that an estimated of 55.5 billion Ethiopian birr(ETB) was lost in the year 2009 [16].

Intestinal helminthes infections are very common in Ethiopia particularly in school-age children and can reach up to 83.3 % in some regions [17]. As a result, the country launches a mass deworming program in November 2015 to treat at risk children in schools for soil transmitted helminthes (STH) and *Schistosoma* infection [18]. Several studies in Ethiopia confirmed the association of helminthes infection with high prevalence of anemia and undernutrition in children [17, 19] but only a few studies is carried out to evaluate the impact of deworming on anemia [20] as well as on growth status. Thus this study in addition to determining the magnitude of anemia and growth, it also help us to evaluate the impact of mass deworming in improving the health of school children through reducing burden of anemia and undernutrition.

1.3. Significance of the study

- The study finding informs health policy makers and other stakeholders to revise/ strengthen the existing deworming intervention policy.
- The study provides information about the magnitude of anemia and growth status as well as possible associated risk factors in context of mass deworming.
- The study serves as an input for other related studies.

2. Literature review

2.1 Magnitude of anemia

The 2011 WHO estimate showed that anemia affects round 800 million children and women. The highest prevalence was in children (42.6%) and lowest was in non-pregnant women (29%). WHO south East Asia, eastern Mediterranean and Africa region had the highest prevalence of anemia across the population group. Children in Africa region represent highest proportion of individual affected with anemia (62.3%) [21]. concerning the nutritional status, undernutrition is responsible for the death of one-third of children (7.6 million children) in the globe every year [22].

A cross sectional study by Ngui *et al*. involving 550 children in rural areas of west Malaysia showed that the prevalence of anemia, iron deficiency (ID) and iron deficiency anemia (IDA) was 26.2%,54.9% and 16.9%, respectively. *T.trichuria* and *A.lumricoides* were found to be significantly associated with anemia and IDA [23].

Other cross sectional studies among school children by Khatiwad S *et al*. in 2015 in Eastern Nepal and by Turyashemererwa MF *et al.* in 2013 in central Uganda showed similar prevalence of anemia. The Nepalese study revealed that 37.9% of the school children were anemic with the most common cause being lack of sufficient iron in diet [24]. Similarly, 37.7% of school children in central Uganda had anemia of which 36.9% were mild and 0.8% were moderate. None of the children had level of hemoglobin considered as severe anemia. Children who consume only one or two meals per day had 3.5 fold increased odd of being anemic [25]. A community based study in Ethiopia by Assefa S *et al* in 2011 in Jimma town revealed that, 37.6% of children aged 6-14 years had anemia. The prevalence was higher in 6-11 years (40.5%) age group than 12-14 yrs. (30.1%). Regarding severity, 48% of them had mild while 52% had moderate anemia [14].

Another community based cross sectional study in Jimma by Desalegn A *et al*. in 2014 among school children showed the overall prevalence of anemia was 43.7%. Severe, moderate and mild anemia was found in 15.6%, 49.2% and 35.2% of the children. Nutritional iron deficiency anemia was diagnosed in 37.3% of children. Microscopic examination of peripheral blood film showed normocytic normochromic picture in 54.3%, microcytic hypochromic in 36.3% and normocytic hypochromic in 9.4% of school children. The possible explanation for severe anemia

in the study could be due to dietary deficiency and high burden of intestinal parasite [26]. On the other hand, a study that was done by Mesfin *et al*.in 2012 in Kersa district, eastern Ethiopia reported that 27.1% of school children were anemic .13.8%, 10.8% and 2.3% had mild, moderate and severe anemia respectively [27]. However, unlike the above studies in developing countries including Ethiopia, a cross sectional survey among 7572 school children in 142 schools in 11 regions of Ethiopia by Hall *et al*.in 2008 reported relatively low prevalence of anemia (9.8%) when adjusted for altitude [28] .

2.2 Magnitude of nutritional status

Regarding the magnitude of growth status, a study in Sudan by Mohammed S *et al.* on the prevalence on thinness, stunting and anemia among school children in 2015 showed 23.1% and 7.1% of study participants were thinned and stunted respectively [29].

A study in Nigeria by Senbanjo *et al*.in 2011 reported that, of 570 children aged 5-19 years, 17.4% were stunted of which 22.2% were severe [30]. According to study conducted by Mukherje *et al.* in 2008 in Pune among children aged 5-11 years , 13.8%, 6.7% and 9.8% were stunted, wasted and underweight respectively [31].

Herrador Z *et al.* in 2014 studied the malnutrition and associated factor in rural and urban setting of Fogera and Libo Kemkem district, Ethiopia. The data showed that there is high prevalence of stunting (39.8%) and thinness (21.4%) among school aged children. The prevalence of malnutrition was significantly higher in a rural than urban community (53% and 42.1% respectively, P=0.006) [32].

A cross sectional study in Durbete town ,northwest of Ethiopia by Alelign T *et al.* in 2015 indicated that 32.3% of school children were undernourished (27.1% were under weight and 11.2% were stunted). The prevalence of stunting was significantly higher in male and older children (10-15 years) [33]

2.3. Predicators of anemia

A study in Kenya by Ngesa *et al.*in 2010 among children aged 6 months to 14 years revealed age as a predicting factor in which children below 1 year were at highest risk of anemia. The risk of anemia was significantly higher in male than female children. The study also showed that mothers with secondary and above education had a protective effect on the risk of anemia on

their children while children diagnosed with malaria were found to be 4 times more prone to anemia [34]

According to a study by Assefa *et al.*in 2011, mother's education level and average monthly income were found to be important determinants of anemia. Low level of mother's education may affect children nutritional level negatively and low income limit type and amount of food available and about half of children with anemia had lower intake of food from animal source [14].However, according to a study by Alelign T *et al.* in 2015 the odd of being anemic was similar among children with different socio-economic status but the chance was nine times higher in children who were infected with hookworms compared to those who were not infected with any helminthes species (AOR = 8.87, 95 % CI = 2.28, 34.58) [33].

A cross sectional study by Mesfin *et al* in 2012 showed that children with age group of 5-9 yrs., had illiterate father and children who had irregular legume consumption were at high risk of anemia .However maternal education did not show significant association with anemia [27].

2.4. Risk factors associated with nutritional status

Turyashemererwa *et al.* in 2013 also determined risk factors of the anthropometric characteristics of school children aged 5-11 years in central Uganda. The finding indicted that children aged 8-11 years were more wasted (P=0.04) and stunted (p=0.03) and children who come from a small family size (≤3) were significantly (p=0.03) more stunted compared to those with family size >3 [25].

A study conducted by Senbanjo OI *et al.*in 2011 showed that only low level of mother's education was significantly associated with stunting [30]. Whereas a study by Mukherje *et al.*in 2008 on determinants of nutritional status implied that children from mothers of low education level and large family size had increased risk of being malnourished [31].

According to cross sectional study in Bangladesh among preschool by Jesmin A *et al*. in 2007, height of mothers, birth weight of children ,education of fathers ,knowledge of mothers on nutrition and frequency of feeding were the most significant factors that had independent and direct influence on the stunting of children [35].

Another cross sectional study in Addis Ababa, Ethiopia by Degarege D *et al*., in 2014 showed that 32.9% of school children were undernourished (19.6% stunted and 15.9% underweight). Having a birth order greater than two, meal frequency at most three times a day, large family size (6-8), being born to a mother less than 20 years old, living in mud/soil floored house and being male were significantly associated with increased odds of undernutrition in the multivariate regression model. Children having employed mothers were at greater risk of stunting than those having housewife mother [36].

2.5. Anemia and growth status in the context of mass deworming

School deworming programs has been implemented in several countries as prevention and control strategy of intestinal helminthes and hence reduces the associated morbidities and mortality. Accordingly several investigations have been carried out to support and generate evidence for such programs. For example, Watkin EW *et al* in 1993 showed that for Guatemala school children with a heavy burden of *Ascaris* and light burden of *Trichuris*, successful removal of *Ascaris* and slight reduction of *Trichuris* after two round of treatment with albendazole led to modest gain in weight (0.18kg, p=0.057) at six months after the first treatment compared to control group. However, the result suggested that even inexpensive, effective deworming drug such as albendazole is not a quick fix when administered only one or two times and a long period of treatment is necessary to effect change of greater biological parameter [37].

On the other hand, a randomized controlled trial in Peru by Joseph SA *et al* in 2015 among preschool children showed that soil transmitted helminthes (STH) infection was of low prevalence and predominantly light intensity. There was no statistical difference in weight gain in any of deworming intervention group compared to control group. Low prevalence and intensity of infection in particular may have limited the impact of deworming which reduces morbidity primarily through reduction in moderate and heavy intensity infection. In deworming intervention, nutrition improvement are not direct consequence of drug administration but as result of elimination of parasite that compete for nutrition and when intervention is administered to population with low prevalence and/or intensity the short term benefit could be difficult to measure [38].

A twelve months longitudinal intervention study in 123 Bangladesh children aged 2-5 years by Northropclewes *et al.*in 2001 revealed that anthelminthic administration successful reduced the

prevalence of *A.lumbricoides, T.trichuria and Hookworm* infection. Despite this, treated children did not show any significant improvements in growth. Low intensity helminthes infection predominantly *A.lumbricoides* and *T.trichuria do* not contribute significantly to poor growth and biochemical status [39].

However in contrast to the above studies, Sur D *et al.* in 2002 showed that the mean weight increased significantly in albendazole group compared to control group at three, six and nine months after treatment. Albendazole group also experienced few episodes of diarrhea than their counterparts with 28% reduction. Thus, periodic mass deworming with albendazole would seem to be safe and effective method. The study noted that relatively high prevalence of *Ascaris* was observed in control and albendazole group at base line [40].

A longitudinal study in Uganda by Kabatereine BN *et al*. in 2003 in 1871 randomly selected school children showed that treatment with praziquantel and albendazole led to significant decrease in the intensity of *Schistosoma mansoni* by 70% after one and 82% after two years of treatment. The intensity of hookworm infection also decreased. There was significant increase in hemoglobin level after one year (0.135g/dl) and two years (0.303g/dl) of treatment and a significant decrease in anemia prevalence and signs of clinical morbidity. Improvements in hemoglobin level were greatest among children who were anemic or harboring heavy *S.mansoni* infection at base line [10].

A study in Zanzibar in 1994 by Stoltzfus JR *et al*. indicated that deworming program significantly reduce the burden of iron deficiency anemia and moderate to severe anemia in school children in the first year of implementation. The most striking benefit was prevention of moderate to severe anemia in children with heavier hookworm infection at baseline which is due to cross sectional relation between hookworm infection and anemia in these children at base line. However, the program had no detectable effect on average hemoglobin concentration or prevalence of mild anemia [41].

In Ethiopia, Yimam Y *et al.* studied the impact of anthelminthic treatment on helminthes infection and related anemia among school children in 2011. The finding indicated that anthelminthic treatment resulted significant reduction in prevalence of helminthes infection and significant increase in hemoglobin level one month after treatment. Increase in the age of

children, low hemoglobin level and low nutritional status before anthelminthic treatment were significant predicators of better hemoglobin gain after treatment. The finding could be due to increased intensity and prevalence of multiple infections in those with low hemoglobin level at base line [20].

According to FMoH and EPHI, both schistosomiasis and STH are endemic in Ethiopia and represent significant health burden [42]. The number of school age children requiring treatment for schistosomiasis is estimated to be 13.1 million while it is 23.3 million for STH [43]. Thus, the country launched mass deworming program in November 2015 targeting to treat 6.4(48.8%) million and 16.4(70.4%) million against schistosomiasis and STH respectively [18]. There are no published studies assessing the impact of such deworming programs yet. Earlier studies as reviewed above have documented a high rate of anemia and undernutrition in school children. The current study, tried to investigate magnitude of anemia and growth status in the context of mass deworming.

3. Conceptual frame work

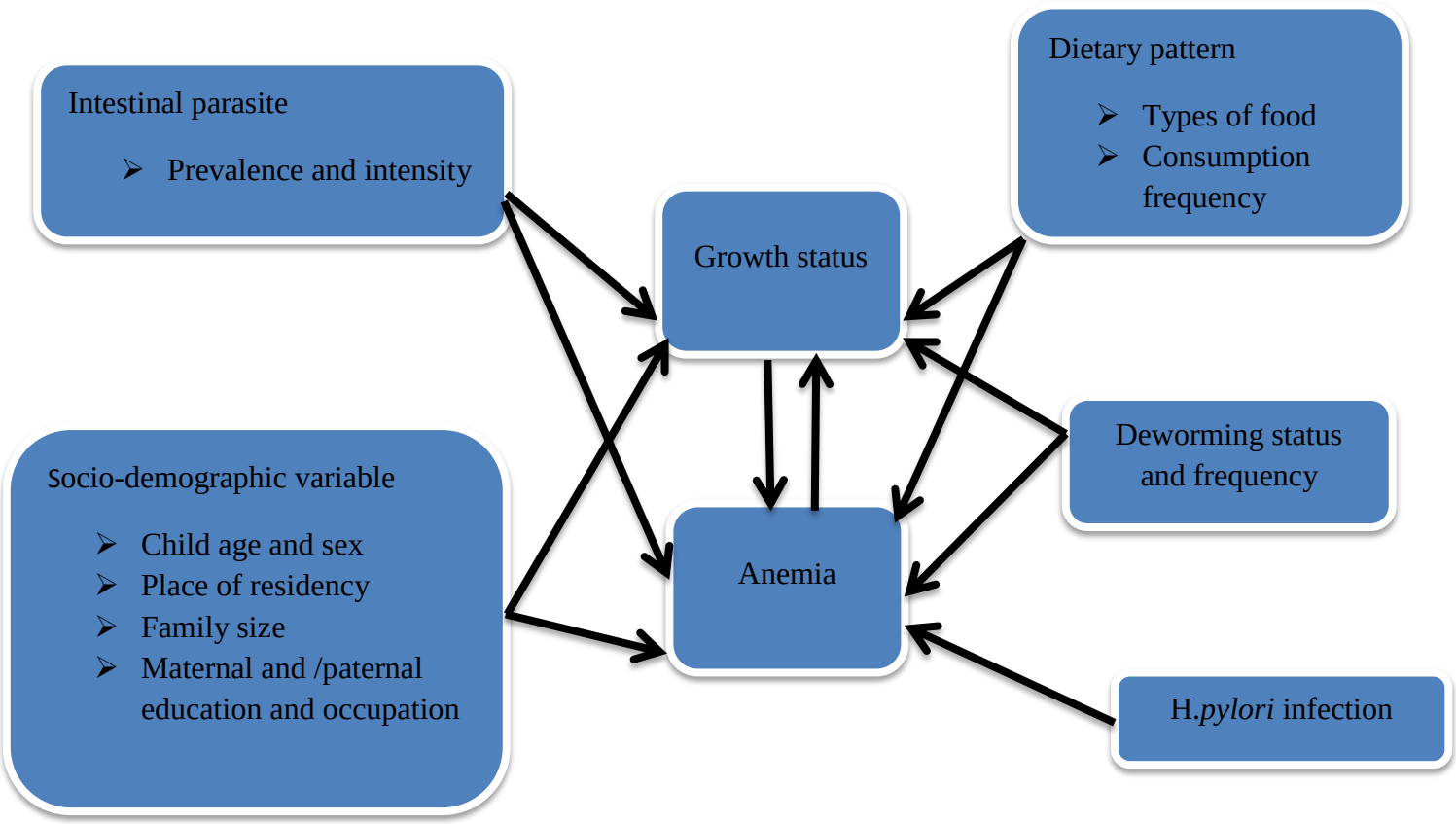

Figure 1.Conceptual frame work of risk factors associated with anemia and growth status

4. Objectives

4.1. General objective

- To determine the magnitude of anemia and growth status among public school children in setting of mass deworming in Sululta town, Oromia region, Ethiopia.

4.2. Specific objectives

- To determine the magnitude of anemia among study participants.
- To determine the magnitude of growth status among school children.
- To assess association of anemia with deworming status among study participants.
- To assess the association of growth status with deworming among school children
- To assess various associated risk factors of anemia
- To assess different associated risk factors of undernutrition.

5. Hypothesis

The magnitude of anemia, under nutrition and associated factors are not different from what has been reported in Ethiopia and is higher among non-dewormed than dewormed study participants.

6. Materials and Methods

6.1. Study Area

The study was conducted in Sululta town located in north Shoa zone of Oromia region 21 km to the northwest of Addis Ababa. The town is geographically located at 9^{o}26'N and 38^{0}39'E.The area has an altitude of 2450 m above sea level. It has temperature that ranges from 15-18^{o}c. The total population of the town is 49,000. Sululta has nine governmental and twenty eight private primary schools.

Sululta town was selected for this study for two main reasons. First, preliminary visits to health centers in Addis Ababa and surrounding showed that the magnitude of intestinal helminthes was very low in their records, Sululta was better. Second, since fresh whole blood and stool samples have to be transported to Addis Ababa for hematological and parasitological analysis, the site is better in this regard as well.

6.2. Study design and period

A cross-sectional study was conducted from April - June 2017.

6.3. Population

6.3.1. Source population

The source population was students in all public primary schools in Sululta town during the study period.

6.3.2 Study population

School children with age of 5-14 years in the selected primary public schools were the study population.

6.4. Eligibility criteria

6.4.1. Inclusion criteria

- ✓ School children under the age of fifteen.
- ✓ Children who were voluntary and whose parents/guardians gave consent to participate.

6.4.2. Exclusion criteria

- Under five years children
- Children currently taking iron supplements drugs (ferrous sulphate, ferrous gluconate and Heam up syrup)
- Children currently taking drugs for *H .Pylori* infection (triple therapy: Amoxicillin ,Omeprazole and metronidazole) as well as parasitic infection (albendazole, mebendazole)

6.5. Study Variables

6.5.1. Dependent variables

Anemia and severity, growth status (stunting (HAZ< -2), thinness (BAZ < -2)) and severity

6.5.2 Independent variables

- ➢ age, sex and grade of the children

- maternal variable(educational status, employment)
- paternal variable (educational status, employment)
- Family size
- Dietary information
- *H. pylori* infection
- Intestinal parasites infection
- Deworming status

6.6. Data collection and measurement

6.6.1. Sample size determination

The minimum number of sample required for this study was determined by using single population proportion formula considering the following assumptions:

Where:-n, = minimum sample size required for the study

Z, =standard normal distribution (Zα/2=1.96) with confidence interval of 95%

P= since similar study was not done in the context of mass deworming in Ethiopia, thus, p value of 0.5 was used

d= absolute precision or tolerable margin of error (d) =5 %(0.05)

q= (1-p)

$$n = \frac{(z_{\alpha/2})^2 \cdot pq}{d^2}$$

$$n = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2}$$

n= 384

The total sample size of the study was 384.

To compensate for the likelihood of non-compliance, 10% of the samples were added to the initially estimated sample size (384). As a result, the minimum sample size required was 422. However, we recruited additional 88 samples and our total sample size was 510.

6.6.2 Sampling Technique

Three public schools were selected randomly using lottery method. These were: - Laga dima, Wasarbi and Abdi Boru. There were total of 1183, 941 and 1069 students in all age group in

Laga dima, Wasarbi and Abdi Boru schools respectively. Convenient sampling technique was used to select participants from each schools and grades since only those who were volunteer as well as whose parent signed informed consent could participates.

6.6.3. Data collection procedures

After obtaining signed consent and/assent, an interviewer-administrated questionnaire was used to gather relevant information from participants on status of deworming, socio-demographic characteristic of children and parents (age and sex of a child, family size, paternal and maternal occupation and education). Frequency of food intake was assessed using a food frequency questionnaire that was developed for the study. The questionnaire was first designed in English and then translated and pretested in Amharic and Oromiffa languages.

Anthropometric measurement such as height and weight were obtained and properly recorded. Stool sample was collected and analyzed to determine prevalence and intensity of intestinal parasites and H.*Pylori* stool antigen test was performed to determine the correlation between *H.Pylori* infection and anemia. Complete blood count (CBC) was performed using Sysmex Kx-21 to determine the magnitude of anemia. Thin smear was prepared and stained using wright stain to evaluate RBC morphology and C-reactive protein was determined to check inflammatory condition in study participants.

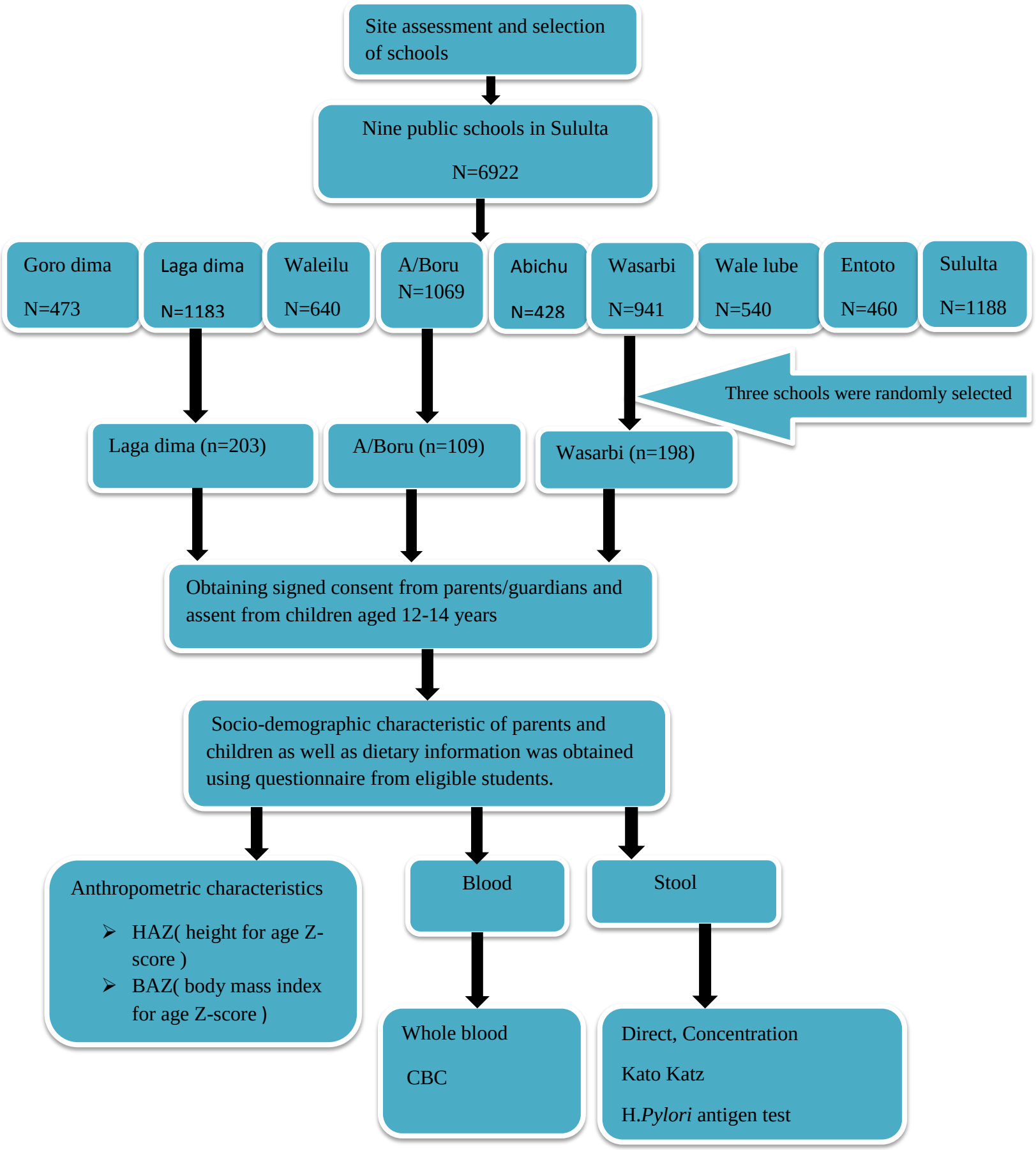

Fig 2:-Data collection procedures

6.6.4. Anthropometry measurement

Anthropometric parameters measured were height and weight. Body weight was measured to the nearest 0.1 kg on a battery powered digital scale and height was measured to the nearest 0.1 cm using a fixed base portable wooden length measuring board with a sliding head bar and the measurements were done while children were in bare foot and lightly-worn clothes. Anthropometric indices were calculated using WHO Anthroplusv1.0.4 software. The indices: - height for the age (HAZ) and body mass index for age (BAZ) were expressed as Z-score and children were categorized as stunted, thinned or normal based on WHO guidelines [5].

6.7. Laboratory analysis

6.7.1. Hematological Analysis

After having the ethical clearance and obtaining consent from parents/guardians and assent from children, 5 milliliter (ml) of whole blood was collected using vacutainer test tube containing EDTA for CBC. Additionally, about 4ml blood was drawn using serum separator test tube for C-reactive protein qualitative and semi-quantitative test analysis from study participants by trained laboratory personnel and principal investigator following the standard venous sample collection procedure . The standard sample collection procedure is shown in Annex VIII.

CBC (Complete blood count) determination

Laboratory testing Principles and procedure

CBC was performed by Sysmex KX-21N Hematology analyzer using whole blood.

Principle: The Sysmex KX-21N Hematology analyzer performs analysis of 18 parameters including a 3-part WBC differential, plus histograms for RBC, PLT and WBC in blood. It employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block using the DC detection method. The hemoglobin detector block measures the hemoglobin concentration using the noncyanide hemoglobin method.

Sheath flow DC detection is the basic method for measuring the number of blood cells and distinguishing their types by size.as electric current passed through a solution. This method measures change in electrical resistance that occur when blood cells pass through a detection aperture. Therefore, blood cell can be counted by measuring the number of times an electric current resistance is generated. Larger blood cell produces greater resistance allowing distinguishing cell by type [45]. See the detail in the Annex VIII.

6.7.2. Stool sample collection and analysis

The school children were provided with small plastic cup and clean wooden applicator stick and informed to bring sufficient stool sample. The samples were then immediately processed for direct wet amount and Kato-Katz microscopic examination at the study site. Egg counts for helminthes were made on the spot within 30 min of slide preparation whereas the remaining portion of stool sample were transported by using SAF(sodium acetate-Acetic Acid-Formalin) to Addis Ababa University, Medical Laboratory Science department parasitology laboratory for formol ether concentration technique [46] . See detail in Annex VIII.

6.7.3. H. *Pylori* Stool antigen test

H.Pylori Ag Rapid test uses a colloid gold conjugated monoclonal anti-*H.Pylori* antibody and another monoclonal anti- *H.Pylori* antibody to specifically detect *H.Pylori* antigen present in the faecal specimen of an infected patient. The test is user friendly, accurate, and the result is available within in 15 minutes [47] .The detailed procedure is presented in Annex VIII.

6.7.4. CRP (C-reactive protein)

C-reactive protein (CRP) is the most widely used indicator of an acute-phase response for the early indication of infections, inflammation or other disease associated with tissue injury. Normally, the serum concentration of CRP is 0.1 mg/dl or less. It is synthesized only in the liver, and synthesis is stimulated by IL-6 and IL-1.

Test principle: Rapid latex agglutination test

The agglutination test is based on the reaction between patient serum containing CRP as the antigen and the corresponding antihuman (CRP) antibody coated to the treated surface participles. The coated particles enhance the detection of an agglutination reaction when antigen is present in the serum [48]. Detail test procedures are found in the Annex VIII

Deworming program

As part of the national mass deworming program, 500 mg of mebendazole was given orally to school children of age 5-19 years to treat soil-transmitted helminthes (Ascariasis, trichuriasis and hookworm disease) by trained health extension workers at schools. Deworming program was the first round at the study site. Samples were collected after 2-3 months of deworming. After the program, the school record showed that some students were found not taking the treatment during the deworming period. The reasoning for non-deworming was lack of awareness and absence from school during the program implementation period.

6.8. Operational definition

Deworming is anthelmintic treatment given to children free of charge without stool examination periodically.

Public school: school supported by governmental funds.

Since the study site is at altitude of 2450 m above the sea level, hemoglobin adjustment was mandatory.

Hemoglobin adjustment = -0.032 (altitude x 0.0032808) +0.022 (altitude x 0.0032808)^2 [49].

Thus based above formula, 1.16g/dl hemoglobin value was added to the WHO cut off value to define anemia and severity [50].

Anemia :-A reduction of hemoglobin (Hgb) value below 12.66 g/dl for 5-11yrs age ; below 13.16g/dl for the age 12-14yrs adjusted for altitude above sea level

Normocytic: refers to red cells with normal size i.e., MCV (mean corpuscular volume) value between 80 and 100 fL and implies a red cell that has a size of 6 to 8 μm in diameter.

Microcytic: when MCV is below 80 fL

Macrocytic: when MCV is above 100 fL

Normochromic: red cells with normal hemoglobinization, which implies that the amount of Hgb per packed red cell is in the appropriate concentration. MCHC (Mean corpuscular hemoglobin concentration = 32% to 36%))

Mild anemia: Hemoglobin 12.16 -12.56 g/dl for children aged 5-11years; 12.16 -13.06 g/dl for children aged 12-14 years.

Moderate anemia: Hemoglobin 9.16 -12.06 g/dl for children aged 5-14 years.

Severe anemia: Hemoglobin lower than 9.16 g/dl for children aged 5-14 years.

Growth status: was assessed by using anthropometric indices namely, height-for-age Z-score (HAZ) and body mass index for age Z-score (BAZ)

Stunted: - HAZ < -2SD of WHO growth standard media for 61months to 19 years.

Severely stunted: - HAZ < -3SD of WHO growth standard media for 61 months to 19 years.

Thinned: - BAZ<-2SD of WHO growth standard media for 61months to 19 years.

Severely thinned:-BAZ < -3SD of WHO growth standard media for 61months to 19 years [5]

Stunting: - signifies slowing in skeletal growth. Growth rate may be reduced from birth but significant degree of stunting representing accumulated consequences of retarded growth and may not be evident for some years. Stunting is frequently found to be associated with poor overall economic conditions, especially mild to moderate, chronic or repeated infections, as well as inadequate nutrient intake.

Thinness:-indicates a deficiency in a tissue and fat mass compared with the amount expected in child of the same height or length and may result either from failure to gain weight or from actual weight loss. It may be precipitated by infection or some other household crisis and usually occurs in situations where the family food supply is limited and the food intake of children is low [6].

6.9. Data management and analysis

The filled questionnaire and checklist were be checked for completeness, consistency and be coded by the principal investigator. Data cleanup was performed to check for accuracy and consistencies. Any error identified was corrected immediately.

Bivariate and multivariate statistical analysis was performed to determine the association of anemia and growth status with independent variables. P-value less than 0.05 were considered as statistically significant. The strength and the direction of the association were determined by the odds ratio and 95% confidence interval. Statistical analyses were performed using SPSS statistics version 21.

6.10. Data quality assurance

The quality of data was maintained in pre-analytical, analytical and post analytical phases of the research process.

Pre analytic:-with regard to the quality of the questionnaires, it was pretested to check for any typing errors and inconsistency before proceeding to data collection.

- accuracy and precision of weight balance and height measuring device was checked
- Blood and stool sample was collected using the right container, labeled correctly and transported at the appropriate temperature.
- During data collection, filled questionnaire was randomly selected to check all the necessary information were included.

Analytic:-Internal quality control was performed continuously to ensure reliable operation of Sysmex kx-21 and the reagents

- The reagents were stored at appropriate temperatures
- All the tests were performed using standard operational procedures
- The stability of controls and reagents was checked

Post-analytical

- All results were recorded and kept confidential in the appropriate prepared log sheet and some critical sample was stored for further examinations if needed and the remaining was discarded by disinfecting.

6.11. Ethical considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, School of Allied Health Sciences, and Department of Medical Laboratory Sciences. Written informed consent and assent for participation in the study was obtained from the children themselves and/or their parents/guardian prior to being enrolled in the study. Confidentiality of data was maintained throughout the study.

6.12. Dissemination of results

This study on completion could serve as a reference material to physicians or any health professionals, researchers, experts and policy makers for intervention. To reach these bodies the finalized paper will be submitted to Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Laboratory Sciences so it can serve as a reference in the library. Additional effort will also be made to present on conferences to reach the medical/scientific community and publish the article on reputable Journals after the final reports.

7. Results

7.1. Demographic characteristic of children and their parents/guardians

A total of 510 school children aged 5-14 were involved in this study. Their mean age was 10.86 (±2.51 SD) and 53.9% were in the age group between 5-11 years. Of the total 510 study participants, 60.2% were females; while 82.7% participants were dewormed. Most of the study participants were urban dwellers (76.7%) and 65.9% of the participants were from small family size (≤5).

As to the maternal occupation and education, 59.6% were house wives and half of them were illiterate. On the other hand, 25.7 % of fathers were daily laborer and over one- third (35.3%) of them can only read and write (Table 1).

Table 1:- Socio-demographics characteristic of school children aged 5-14 years and their parents/guardians in three selected public schools in Sululta town, Oromia region, Ethiopia, 2017. (n=510)

Socio-demographic variables	Frequency	percentage
Sex		
Male	203	39.8
Female	307	60.2
Residency		
Urban	391	76.7
Rural	119	23.3
Age group(year)		
5-11	275	53.9
12-14	235	46.1
Mean age(± SD)	10.86	2.51
Family size		
≤5	336	65.9
>5	174	34.1
Deworming status		
Yes	422	82.7
No	88	17.3
Parents/guardians characteristics		
Maternal occupation		

Civil servant	32	6.3
House wife	304	59.6
Private organization	30	5.9
Farmer	9	1.8
Daily laborer	73	14.3
Merchant	62	12.2
Maternal education		
Illiterate	270	52.9
Read and write only	90	17.6
Primary	94	18.4
High school	36	7.1
Higher education	20	3.9
Paternal education		
Illiterate	98	19.2
Read and write only	180	35.3
Primary	147	28.8
High school	58	11.4
Higher education	27	5.3
Paternal occupation		
Civil servant	95	18.6
Private organization	84	16.5
Merchant	127	24.9
Daily laborer	131	25.7
Farmer	48	9.4
No work	25	4.9

7.2. Anthropometrics characteristics of study participants

The mean height for age Z-score (HAZ) and body mass index for the age Z-score (BAZ) were -1.11(±0.98 SD) and -0.88(±1.02 SD) respectively. According to WHO growth reference standards, taking -2SD as a cut-off point [5], the overall magnitude of stunting (HAZ<-2) and thinness (BAZ <-2) in our study participants were 16.9% and 10.8%, respectively. Most of the cases were moderate while only 1.6% of thinness and 3.1% stunting were severe (Table 2).

Table 2:-Magnitude and severity of growth status among school children by age group in three selected public schools in Sululta town, Oromia region, Ethiopia, 2017

Nutritional status*	Age groups(yrs.)	Mean(SD)	N (%) <-z score	Nutritional status	
				Moderate	severe
HAZ	5-11	-1.0841(1.049)	50(18.2)	42(15.3)	8(2.9)
	12-14	-1.1297(0.9012)	36(15.3)	28(11.9)	8(3.4)
	Total	-1.1051(0.983)	86(16.9) stunted	70(13.7)	16(3.1)
BAZ	5-11	-0.8467(1.029)	27(9.8)	23(8.4)	4(1.5)
	12-14	-0.911(1.013)	28(11.9)	24(10.2)	4(1.7)
	Total	-0.8765(1.021)	55(10.8) thinned	47(9.2)	8(1.6)

*HAZ: -Z-score for height for age calculated for ages 5-14yrs.

*BAZ: - Z-score for body mass index for age calculated for ages 5-14yrs.

Regarding the dietary pattern, 51.4 % and 75.5% of the participants eat legumes and cereals respectively once a day while 39.8%, 60.2% ,82.9% ,72.1% and 75% of them occasionally eat root and tubers, vegetables , meat , milk and milk products and eggs respectively (Figure 3).

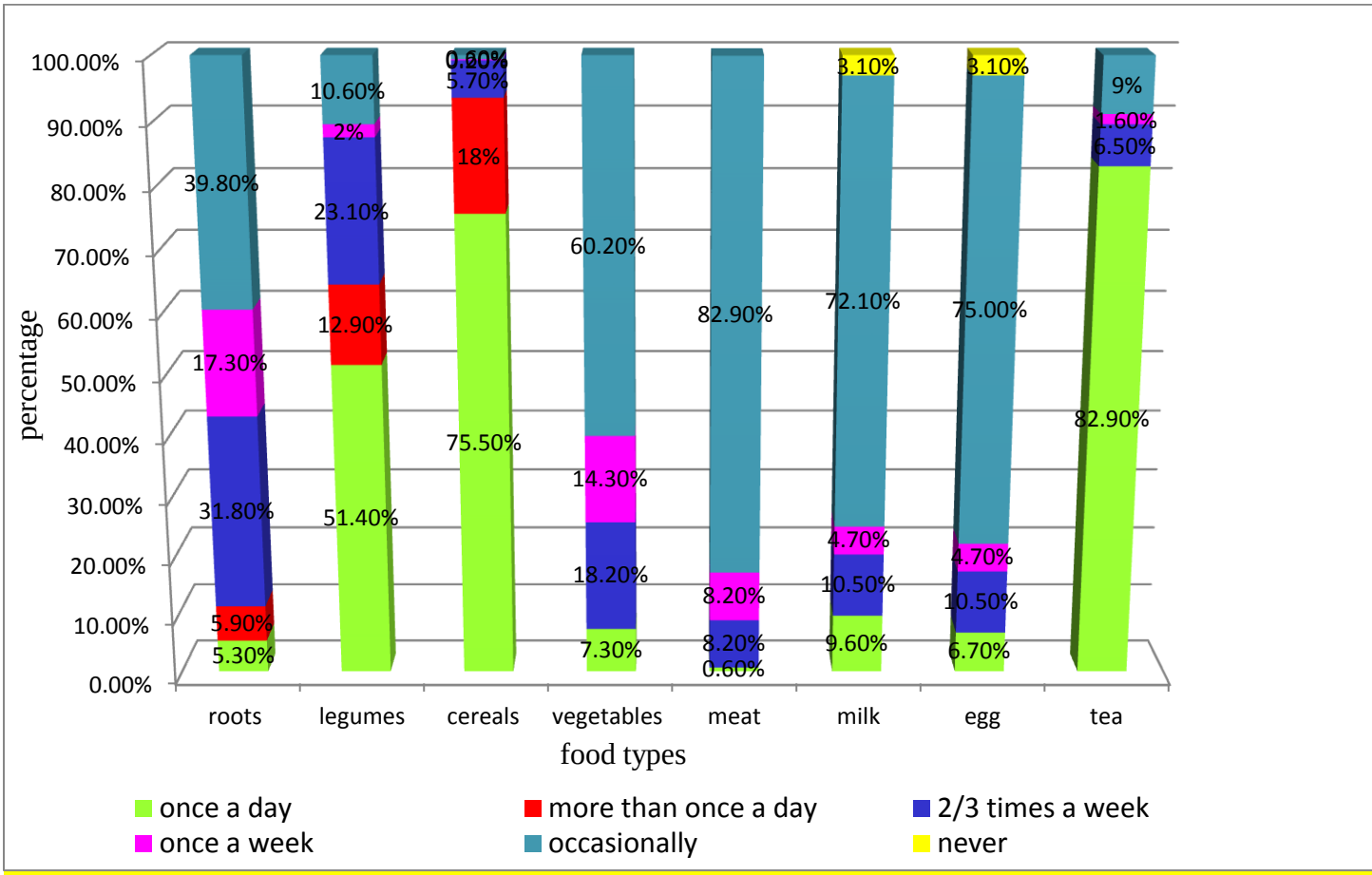

Figure 3: Food type and consumption frequency among school children in three selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

7.3 .Magnitude and severity of anemia

The overall prevalence of anemia as defined by Hgb <12.66 g/dl for 5-11yrs age and Hgb <13.16g/dl for the age 12-14yrs adjusted for altitude above sea level was 19(3.7%). The magnitude was similar among the age groups (3.6% for 5-11yrs age and 3.8% for the 12-14yrs age), between males (3.4%) and females (3.9%) and slightly higher in non-dewormed (4.5%) than dewormed (3.6%) but not statistically significant (P=0.66). Regarding the severity, 3.1% and 0.6% of participants had mild and moderate anemia respectively. There was no severe anemia among study participants during the study period (Table 3).

Table 3:- Magnitude and severity of anemia among school children by age groups, sex and. deworming status in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

	Anemia			Severity	
Age category	No	Yes	Total	Mild	Moderate
5-11 age	265(96.4)	10(3.6)	275	8(80)	2(20)
12-14 age	226(96.2)	9(3.8)	235	8(88.9)	1(11.1)
Sex					
Female	295(96.1)	12(3.9)	307	9(75)	3(25)
Male	196(96.6)	7(3.4)	203	7(100)	0
Deworming status					
Yes	407(96.4)	15(3.6)	422	13(3.1)	2(0.5)
No	84(95.5)	4(4.5)	88	3(3.4)	1(1.1)
Total	491(96.3)	19(3.7)	510	16(3.1)	3(0.6)

7.4. Association of socio-economic and demographic factors of school children and parents/guardians with anemia

Bivariate statistical analysis showed that none of the socio-demographic characteristic of children and parents (age and sex of children, parent occupation and educational status, family size and address) was associated with anemia (Table 4).

Table 4:- Socio-demographic characteristics of school children and their parents/guardians as predicators of anemia in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

Characteristics	Total	Anemia		Unadjusted odd ration (95% CI)	P-value
		Yes n (%) n=491	No n (%) n=19		
Sex					
Female	307	12(3.9)	295(96.1)	1.14 (0.44 ,2.94)	0.79
Male	203	7(3.4)	196(96.6)	1	
Age category(yrs.)					
5-11	275	10(3.6)	265(96.4)	0.95(0.38,2.37)	0.91
12-14	235	9(3.8)	226(96.2)	1	
Address					
Rural	119	4(3.4)	115(96.6)	0.87(0.28 ,2.68)	0.81
Urban	391	15(3.8)	376(96.2)	1	
Maternal occupation					
House wife	304	14(4.6)	290(95.4)	0.73(0.16 ,3.39)	0.69
Private organization	30	0	30(100)		
Farmer	9	0	9(100)		
Daily laborer	73	2(2.7)	71(97.3)	0.42(0.06, 3.14)	0.41
Merchant	62	1(1.6)	61(98.4)	0.25(0.02 ,2.82)	0.26
Civil servant	32	2(6.3)	30(93.8)	1	
Paternal occupation					
Private organization	84	3(3.6)	81(96.4)	0.79(0.17 ,3.67)	0.77
Merchant	127	4(3.1)	123(96.9)	0.71(0.17, 2.89)	0.63
Daily laborer	131	6(4.6)	125(95.4)	1.04(0.29 ,3.79)	0.95
Farmer	48	1(2.1)	47(97.9)	0.48(0.05, 4.4)	0.51
No work	25	0	25(100)		
Civil servant	95	5(5.3)	90(94.7)	1	
Maternal education					
Illiterate	270	12(4.4)	258(95.6)	1.2(0.29 ,4.8)	0.8
Read and write only	90	3(3.3)	87(96.7)	1.1(0.26 ,5.0)	0.86
Primary	94	2(2.1)	92(97.9)	1.1(0.25 ,4.8)	0.91
High school	36	2(5.6)	34(94.4)	1.3(0.24 ,6.4)	0.79
Higher education	20	0	2(100)	1	

Paternal education					
Illiterate	98	5(5.1)	93(94.9)	0.49(0.05 ,4.71)	0.54
Read and write only	180	6(3.3)	174(96.7)	0.32(0.03,2.91)	0.31
Primary	147	4(2.7)	143(97.3)	0.25(0.03 ,2.51)	0.24
High school	58	4(6.9)	54(93.1)	0.53(0.05 ,5.67)	0.60
Higher education	27	0	27(100)	1	
Family size					
>5	174	6(3.4)	168(96.6)	0.89(0.33 ,2.38)	0.81
≤5	336	13(3.9)	323(96.1)	1	

7.5. Association of anthropometrics parameters, intestinal parasite infection H.*Pylori* infection and dietary pattern with anemia

Only 4.7 % of stunted (HAZ <-2) and 5.5% of thinned (BAZ <-2) study participants had anemia. There was no statistically significant association of stunting (P= 0.62) and thinness (P=0.48) with anemia (Table 5).

The overall prevalence of intestinal parasite and helminthes in particular was 19.6% and 15.7%, respectively. H.*nana* accounts for relatively highest proportion (31%) while *Ascaris lumbricoides*, *Hookworm*, *Trichuris trichuri*a and *teania* spp. accounts 16%, 11%, 9% and 11% respectively. The magnitude of intestinal parasite was similar in dewormed (19.7%) and non-dewormed (19.3%) study participants. The occurrence of anemia was 4% among intestinal parasite infected and 3.7% among non-infected children. The intensity of helminthes infection was determined by counting the number of helminthes egg excreted in a faeces (expressed egg per gram, epg) using kato-katz technique. Using WHO threshold value for light, moderate and heavy infection for each parasite [51], there were 1.8%, 2.7% and 1.2% of light infection of *Hookworm*, *Ascaris lumbricoides* and *Trichuris trichuria*, respectively. There was no moderate and heavy helminthes infection in this study. Individuals with light *hookworm* infection had 3.4-fold increased odd of being anemic. However, the association was not statistically significant (P=0.27) (Table 5).

The magnitude of anemia was similar between H.*Pylori* infected and non-infected participants with no significant association. None of dietary pattern was significantly associated with anemia (Table 5).

Table 5:- Bivariate analysis of anthropometric parameters, intestinal parasite, H.*Pylori* infection and dietary pattern as predictors of anemia among school children in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

Characteristics	Total	Anemia		Crude odd ratio COR (95% CI)	P-value
		Yes n (%) N=19	No n (%) N=491		
HAZ<-2					
Yes	86	4(4.7)	82(95.3)	1.33(0.43,4.1)	0.62
No	424	15(3.5)	409(96.5)	1	
BAZ<-2					
Yes	55	3(5.5)	52(94.5)	1.58(0.45 ,5.62)	0.48
No	455	16(3.5)	439(96.5)	1	
Intestinal parasite					
Yes	100	4(4)	96(96)	1.09(0.36 ,3.38)	0.87
No	410	15(3.7)	395(96.3)	1	
Intestinal helminthes					
Yes	80	4(5.0)	76(95)	1.46(0.47 ,4.51)	0.51
No	430	15(3.5)	415(96.5)	1	
***Hookworm* infection**					
Light (1-1999 epg)	9	1(11.1)	8(88.9)	3.35(0.39 ,28.3)	0.27
Negative	501	18(3.6)	483(96.4)	1	
***Trichuris* infection**					
Light (1-999 epg)	6	0	6(100)		
Negative	504	19(3.8)	485(96.2)		
***Ascaris* infection**					
Light (1-4999 epg)	14	0	14(100)		
Negative	496	19(3.8)	477(96.2)		
H.*Pylori* infection					
Positive	56	2(3.6)	54(96.4)	0.95(0.21, 4.23)	0.95
Negative	454	17(3.7)	437(96.3)	1	
CRP					
Positive	51	1(2.0)	50(98.0)	0.5(0.06, 3.74)	0.49
Negative	459	18(3.9)	441(96.1)	1	
Roots and tubers					
>1 a day	30	0	30(100)		
2/3 times a week	162	6(3.7)	156(96.3)	1.16(0.33, 4.03)	0.82
Once a week	88	2(2.3)	86(97.7)	1.1(0.29 ,4.1)	0.89
Occasionally	203	11(5.4)	192(94.6)	1.24(0.36,4.24)	0.73
Once a day	27	0	27(100)	1	
Legumes					
>1 a day	66	2(3.0)	64(97)	0.79(0.17 ,3.68)	0.76
2/3 times a week	118	3(2.5)	115(97.5)	0.66(0.18, 2.4)	0.53
Once a week	10	1(10)	9(90)	2.8(0.32, 24.3)	0.35
Occasionally	54	3(5.6)	51(94.4)	1.5(0.39 ,5.58)	0.56

Once a day	262	10(3.8)	252(96.2)	1	
Cereals					
>1 a day	92	4(4.3)	88(95.7)	1.3(0.41 ,4.1)	0.65
2/3 times a week	29	2(6.9)	27(93.1)	2.1(0.46 ,9.9)	0.34
Once a week	1	0	1(100)		
Occasionally	3	0	3(100)		
Once a day	385	13(3.4)	372(96.6)	1	
Vegetables					
2/3 times a week	93	3(3.2)	90(96.8)	1.2(0.12 ,11.9)	0.88
Once a week	73	1(1.4)	72(98.6)	0.5(0.03 ,8.2)	0.63
Occasionally	307	14(4.6)	293(95.4)	1.7(0.2 ,13.5)	0.61
Once a day	37	1(2.7)	36(97.3)	1	
Fruits					
2/3 times a week	53	1(1.9)	52(98.1)	1.08(0.12 ,9.46)	0.95
Once a week	63	1(1.6)	62(98.4)	1.07(0.12 ,9.14)	0.95
Occasionally	385	17(4.4)	368(95.6)	1.2(0.16 ,9.14)	0.87
Once a day	9	0	9(100)	1	
Meat					
2/3 times a week	42	3(7.1)	39(92.9)	1.33(0.04 ,48.3)	0.88
Once a week	42	0	42(100)		
Occasionally	420	15(3.6)	405(96.4)	1.15(0.04, 38.3)	0.94
Never	3	1(33.3)	2(66.6)		
Once a day	3	0	3(100)	1	
Milk and milk products					
2/3 times a week	54	1(1.9)	53(98.1)	1.08(0.33, 3.49)	0.90
Once a week	24	0	24(100)		
Occasionally	367	18(4.9)	349(95.1)	1.22(0.49, 3.0)	0.67
Never	16	0	16(100)		
Once a day	49	0	49(100)	1	
Egg					
2/3 times a week	67	1(1.5)	66(98.5)	1.06(0.25 ,4.54)	0.94
Once a week	45	2(4.4)	43(95.6)	1.20(0.26,5.45)	0.82
Occasionally	329	12(3.6)	317(96.4)	1.16(0.32 ,4.25)	0.83
Never	46	4(8.7)	42(91.3)	1.42(0.32 ,6.26)	0.65
Once a day	23	0	23(100)	1	
Tea/coffee					
2/3 times a week	33	1(3.0)	32(97)	0.98(0.35 ,2.78)	0.97
Once a week	8	0	8(100)		
Occasionally	46	3(6.5)	43(93.5)	1.13(0.48,2.66)	0.79
Once a day	423	15(3.5)	408(96.5)	1	

7.6. Association of deworming status with anemia and growth status

While assessing the study sites, some students were found not being part of the deworming program for various reasons. The magnitude of anemia and thinness was compared between dewormed and non-dewormed study participants in a bivariate analysis. The result indicated that deworming is not associated with anemia (P=0.66) and thinness (P=0.85). However, as shown in Table 6, at unadjusted odd ration, the magnitude of stunting is found to be higher in dewormed than non-dewormed with significant association (P=0.036).

Table 6:- The association of deworming status with anemia and growth status among school children in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510).

Deworming status	HAZ < -2 (stunting)			BAZ <-2 (thinness)			Anemia		
	N (%)	COR(95% CI)	P	N (%)	COR(95% CI)	P	N (%)	COR(95% CI)	P
No	8(9.1)	0.44(0.21 ,0.95)	0.036	10(11.4)	1.1(0.52 ,2.2)	0.85	4(4.5)	1.3(0.42, 4.0)	0.66
Yes	78(18.5)	1		45(10.7)	1		15(3.6)	1	

7.7. Factors associated with nutritional status

At unadjusted odd ratio, children whose fathers were merchant (OR= 2.3, 95% CI=1.05-4.99, P=0.038) and illiterate (OR=8.4, 95% CI=1.09 -65.5, P=0.04) had 2.3-fold and 8.4 fold increased odd of being stunted, respectively. Family size was also significantly (P=0.001) associated with stunting. Children from a small family size were more likely to be stunted than those having large family. On the other hand, intestinal parasite (P=0.028) and helminthes (P=0.014) were significantly associated with thinness (Table 7).

Table 7: Bivariate analysis of associated risk factors for stunting and thinness among school children in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

Characteristics	Stunting			Thinness		
	N (%)	COR(95% CI)	P-value	N (%)	COR(95% CI)	P-value
Sex						
Female	44(14.3)	0.6(0.40, 1.02)	0.062	30(9.8)	0.77(0.44,1.35)	0.37
Male	42(20.7)	1		25(12.3)	1	
Age category(yrs.)						
5-11	50(18.2)	1.23(0.77 ,1.96)	0.39	27(9.8)	0.81(0.46 ,1.41)	0.45
12-14	36(15.3)	1		28(11.9)	1	
Address						
Rural	63(16.1)	1.25(0.74, 2.12)	0.41	42(10.7)	1.02(0.53 ,1.97)	0.96

Urban	23(19.3)	1		13(10.9)	1	
Family size						
>5	16(9.2)	0.39(0.22 ,0.69)	0.001	22(12.6)	1.33(0.75 ,2.36)	0.33
≤5	70(20.8)	1		33(9.8)	1	
Maternal occupation						0.07
House wife	45(14.8)	0.8 (0.29 ,1.93)	0.56	34(11.2)	1.89(0.43, 8.26)	0.4
Private organization	3(10)	0.5 (0.11, 2.13)	0.34	7(23.3)	4.57(0.87, 24.1)	0.07
Farmer	3(33.3)	2.2 (0.42 ,11.2)	0.36	2(22.2)	4.28(0.51 ,35.9)	0.18
Daily laborer	15(20.5)	1.1 (0.39, 3.21)	0.83	2(2.7)	0.42(0.06,3.14)	0.4
Merchant	14(22.6)	1.3 (0.43,3.68)	0.67	8(12.9)	2.2(0.44, 11.15)	0.33
Civil servant	6(18.8)	1		2(6.3)	1	
Paternal occupation						0.74
Private organization	10(11.9)	1.1(0.43, 2.75)	0.87	14(16.7)	2.05(0.81, 5.17)	0.13
Merchant	28(22.2)	2.3(1.05, 5.0)	0.038	12(9.5)	1.08(0.42,2.76)	0.87
Daily laborer	24(18.5)	1.8(0.82, 4.0)	0.14	14(10.8)	1.24(0.49,3.08)	0.65
Farmer	7(15.2)	1.4(0.51, 4.06)	0.49	5(10.9)	1.25(0.39, 4.06)	0.71
No work	6(25.0)	2.7(0.86, 8.29)	0.09	2(8.3)	0.93(0.19 ,4.71)	0.93
Civil servant	10(11.1)	1		8(8.9)	1	
Maternal education						0.08
Illiterate	52(19.3)	1.35(0.38, 4.79)	0.64	24(8.9)	1.85(0.24,14.46)	0.56
Read and write	10(11.1)	0.71(0.18,2.85)	0.63	13(14.4)	3.21(0.39,26.1)	0.28
Primary	16(17.0)	1.16(0.3, 4.4)	0.83	16(17.0)	3.89(0.49, 31.3)	0.2
High school	5(13.9)	0.91(0.19 ,4.3)	0.91	1(2.8)	0.54(0.03, 9.18)	0.67
Higher education	3(15)	1		1(5.0)	1	
Paternal education						0.65
Illiterate	24(24.5)	8.4(1.09 , 65.48)	0.04	11(11.2)	1.01(0.26, 3.92)	0.99
Read and write only	26(14.4)	4.4(0.57, 33.76)	0.16	19(10.6)	0.94(0.26, 3.43)	0.93
Primary	28(19)	6.1(0.79, 47.02)	0.08	19(12.9)	1.12(0.33 ,4.32)	0.79
High school	7(12.1)	3.6(0.42 ,30.57)	0.25	3(5.2)	0.44(0.08 ,2.32)	0.33
Higher education	1(3.7)	1		3(11.1)	1	
Intestinal parasite						
Yes	22(22)	1.5(0.89 ,2.62)	0.13	17(17)	2.0(1.08 ,3.72)	0.028
No	64(15.6)	1		38(9.3)	1	
Intestinal helminthes						
Yes	18(22.5)	1.6(0.86 ,2.78)	0.15	15(18.8)	2.25(1.18 ,4.3)	0.014
No	68(15.8)	1		40(9.3)	1	
***Hookworm* infection**						
Light (1-1999 epg)	1(11.1)	0.6 (0.08 ,4.96)	0.65	0		
Negative	85(17)	1		55(11.1)		
***Trichuris* infection**						
Light (1-999 epg)	2(33.3)	2.5(0.45 ,13.87)	0.3	1(16.7)	1.67(0.2 ,14.5)	0.64
Negative	84(16.7)	1		54(10.7)	1	
***Ascaris* infection**						
Light (1-4999 epg)	2(14.3)	0.8(0.18 ,3.72)	0.79	2(14.3)	1.39(0.3 ,6.39)	0.67
Negative	84(16.9)	1		53(10.7)	1	

In the multivariate analysis, at adjusted odd ratio, children whose fathers were illiterate had 7.2 fold of being stunted but the association was marginally significant (P=0.07). However, family size was the only independent predicators of stunting with a statistically significant level (P=0.002) at multivariate analysis when adjusted for all the variables stated in Table 8. As to the thinness, children whom their mothers work in private organization and had primary education level had 2.8-fold and 3.6-fold increased odd of being thinned. In addition, children infected with helminthes had 2 fold increased odd of being thinned. However none of these predicators were statistically significantly associated with thinness (Table 9).

Table 8:- Multivariate analysis of associated risk factor for stunting among school children in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

Characteristics	Total	Stunting			P-value
		Yes n(%) n=86	No n(%) n=424	Adjusted odd ration AOR (95% CI)	
Sex					
Female	307	44(14.3)	263(85.7)	0.75 (0.46 ,1.23)	0.25
Male	203	42(20.7)	161(79.3)	1	
Family size category					
>5	174	16(9.2)	158(90.8)	0.38 (0.21 ,0.69)	0.002
≤5	336	70(20.8)	266(79.2)	1	
Paternal occupation					
Private organization	84	10(11.9)	74(88.1)	0.7 (0.28 ,1.86)	0.5
Merchant	127	28(22)	99(78)	1.4 (0.64 ,3.24)	0.37
Daily laborer	131	24(18.3)	107(81.7)	0.9 (0.43 ,2.3)	0.98
Farmer	48	7(14.6)	41(85.4)	0.9 (0.29 ,2.58)	0.8
No work	25	6(24)	19(76)	1.8 (0.56 ,5.82)	0.33
Civil servant	95	11(11.6)	84(88.4)	1	
Paternal education					
Illiterate	98	24(24.5)	74(75.5)	7.2 (0.84 ,60.6)	0.07
Read and write only	180	26(14.4)	154(85.6)	3.6 (0.44 ,29.7)	0.23
Primary	147	28(19)	119(81)	5.3 (0.65 ,43.2)	0.12
High school	58	7(12.1)	51(87.9)	2.9 (0.3 ,26.3)	0.34
Higher education	27	1(3.7)	26(96.3)	1	
Intestinal parasite					
Positive	100	22(22)	78(78)	1.2 (0.36 ,3.85)	0.78
Negative	410	64(15.6)	346(84.4)	1	
Intestinal helminthes					
Positive	80	18(22.5)	62(77.5)	1.2 (0.35 ,4.4)	0.74
Negative	430	68(15.8)	362(84.2)	1	
Deworming status					
No	88	8(9.1)	80(90.9)	0.5(0.23,1.1)	0.09
Yes	422	78(18.5)	344(81.5)	1	

Table 9:- Multivariate analysis of associated risk factor for thinness among school children in selected public schools in Sululta town, Oromia region, Ethiopia, 2017 (n=510)

Characteristics	Total	Thinness			
		Yes n(%) n=55	No n(%) n=455	Adjusted odd ration AOR (95% CI)	P-value
Maternal occupation					0.08
House wife	304	34(11.2)	270(88.8)	1.1 (0.19 ,6.35)	0.9
Private organization	30	7(23.3)	23(76.7)	2.8 (0.41 ,18.9)	0.3
Farmer	9	2(22.2)	7(77.8)	2.3 (0.23,24.5)	0.48
Daily laborer	73	2(2.7)	71(97.3)	0.2 (0.02 ,1.96)	0.17
Merchant	62	8(12.9)	54(87.1)	1.2 (0.19 ,8.1)	0.8
Civil servant	32	2(6.3)	30(93.8)	1	
Maternal education					0.15
Illiterate	270	24(8.9)	246(91.1)	1.9 (0.17 ,20.3)	0.61
Read and write only	90	13(14.4)	77(85.6)	3 (0.26 ,34.1)	0.38
Primary	94	16(17)	78(83)	3.6 (0.32 ,39.8)	0.3
High school	36	1(2.8)	35(97.2)	0.5 (0.02 ,9.4)	0.61
Higher education	20	1(5.0)	19(95)	1	
Intestinal parasite					
Positive	100	17(17)	83(83)	1.3 (0.29 ,6.2)	0.71
Negative	410	38(9.3)	372(90.7)	1	
Intestinal helminthes					
Positive	80	15(18.8)	65(81.3)	2.1 (0.42 ,10.2)	0.38
Negative	430	40(9.3)	390(90.7)		

8. Discussion

This cross-sectional study aimed at determining the magnitude of anemia and growth status among selected public school children in a setting of mass deworming in Sululta town, northwest of Addis Ababa. It also investigated predictors of anemia and growth status. The study revealed that the overall magnitude of anemia among school children was 3.7%. The magnitude observed in a current study is considered as insignificant (not in the public health problem category) based on WHO category of public health problem [13]. According to WHO, the prevalence of anemia as a public health problem is categorized as follows: <5%, no public health problem; 5-19.9%, mild public health problem; 20-39.9%, moderate public health problem; ≥40%, severe public health problem [2]. The magnitude of stunting (16.9%) and thinness (10.8%) was also low.

The result is lower compared to a study by Hall *et al.* who in their nutrition and health survey in Ethiopian school children reported an anemia prevalence of 9.8%, adjusted for altitude [28]. The anemia in their case is categorized as a mild public health problem. However, relatively high prevalence of anemia in the moderate and severe public health significance range (27.1% to 43.7%) was observed in other related studies in Ethiopia by Mefin *et al.,* 2012 [27]; Assefa *et al*., 2011 [14]; Desalegn *et al*., 2013 [26] and neighboring African countries like Kenya 28.8% [34], and Uganda 37.7% [25]. It is also higher than reports from Nepal 37.9% [24] and Malaysia 26.2% [23].

When anemia was categorized by severity based on hemoglobin levels, mild and moderate anemia was recorded in 3.1% and 0.6% of the participants, respectively. No severe anemia (Hgb<9.6g/dl) was detected in this study. However, unlike the current finding, Desalegn *et al* [26] indicated high level of moderate (49.2%) and mild (35.2%) anemia as well as 15.6% of severe anemia which was due to high burden of parasite and dietary deficiency. Similarly Assefa *et al* [14] also reported high level of mild (48%) and moderate (52%) anemia.

The possible explanation for very low prevalence of anemia among school children in this study could be due to low prevalence and light intensity of parasite infection and regular consumption of legumes (51.4% and 23.1% participants eat once a day and two or three times a week respectively) which contain phytoferritin that serve a good source of iron absorption compared to other non-heme iron source [44].

Of the factors associated with anemia, in contrast to other studies which showed significant association of anemia among school children with age groups, fathers education level (Mesfin *et al*., 2012), mother education level and average monthly income (Assefa *et al*., 2011), the current study indicated that none of socio-demographic characteristics of children and parents was associated with anemia [27, 14] Turyashemererwa *et al* have reported less than twice consumption of meals per day as a predictor of anemia [25]. Absence of association is also observed in a study by Alelign *et al*., 2010 [33]. This could be due to homogeneity of respondent's socio-economic and demographic characteristic in this study and difference in life style, culture and environmental condition which contributes to the difference in risk factors in different communities.

In alignment with study by Assefa *et al.* [14] the magnitude of anemia in this study among stunted participants was low (4.7%) with no significant association. Similarly only 5.5% of thinned participants had anemia which is contrary to Assefa *et al*. report of 59.1% of thinness among anemic participants with a significant association. The current study also showed similar prevalence of anemia between helminthes infected and non-infected participants. However, Ngui *et al*. indicated a significant association of anemia with *Trichuris trichuria* and *Ascaris lumricoides* [23]. Alelign *et al* also illustrated that the risk of anemia was approximately nine times higher in children who were infected with *hookworm* compared to children who were not infected with any helminthes species [33]. Very low intensity of infection might partly explain the absence of association between anemia and intestinal parasites. Though difficult to measure in this cross sectional study, the ongoing mass deworming program might have contributed for the reduction of overall load of intestinal parasitic infection.

Nutritional assessment in a community is essential for accurate planning and implementation of intervention program to reduce morbidity and mortality associated with undernutrition. In the population investigated, the overall prevalence of stunting (16.9%) and thinness (10.8%) were low. The finding was comparable with other studies by Senbanjo *et al*., 2011 [30] and Mukharje *et al*., 2008 [31]. However, Mohammed *et al* [29] reported relatively high prevalence of thinness (23.1%) and low prevalence of stunting (7.1%) which is contradictory to the current study. In Ethiopia, the majority of malnourished children live in a rural area [32]. Twelve months longitudinal study in rural Bangladesh demonstrated the effect of low intensity of helminthes as insignificant factor to poor growth status [39]. Low prevalence of stunting and thinness observed

in the current study could be attributed to urban (76.7% of participants) nature of study area as well as low burden of parasitic infection.

Of factors associated with nutritional status, children from small size family (≤5) were more stunted compared to the large family. The current finding is consistent with result obtained by Turyashemereraw *et al.* [25]. It is, however, expected that children from smaller families have better nutrition and low prevalence of undernutrition [31, 36].The discrepancy cannot be explained and further study may be required to explore this.

In a present study socio-demographic characteristics of parents (level of education and occupation) were not associated with stunting and thinness. The finding is congruent with study by Turyashemereraw *et al* [25] and Alelign *et al* [33]. However, low educational level of mothers was important determinants of children growth status in many studies [30, 31]. As the level of education of mothers' increase so does their finance and their contribution to the total family income. The economic empowerment enables them to make a decision to improve nutrition and health of their children. In the current finding ,even though over half of mothers were illiterate, they could have good nutritional knowledge and besides it could be argued that these mothers are most likely to be house wives (considering that 59.6% were house wives) having a chance to stay home and care for their children thus reducing the risk of stunting [36].

As it is observed in a present study, helminthes infection in general was not associated with stunting and thinness. The intensity of helminthes infection was light. As the result the infection may not have significant impact on nutritional status of children. The finding is consistent with Alelign *et al* [33].

The magnitude of thinness and anemia was similar between dewormed and non-dewormed study groups. However, relatively high magnitude of stunting (18.5%) was observed in dewormed than non-dewormed (9.5%) study groups. In this study, no significant association of deworming with anemia and growth status observed after two months of school deworming. The finding is inconsistent with Yimam *et al* [20]. In contrary to the current study, high prevalence and intensity of helminthes infection could contribute to pronounced effect of anthelmintic treatment after one month in those with low hemoglobin value at base line [20]. On the other hand, selective treatment of only infected individuals in Yimam *et al.* could explain for the discrepant finding.

Current finding also contradict with a study in Uganda among school children of age 6-14 years by kebaterein e*t al.,* 2003 [10]. As opposed to the present study, longitudinal nature of the study and two years follow up as well as combined treatment of albendazole and praziquantel which are effective against STH and *Shistosoma* could be responsible for the discrepancy. However, unlike the above two studies Stolzus *et al* reported no detectable effect of deworming on average hemoglobin value or prevalence of mild anemia [41].

Regarding the effect of deworming on growth status, our result is consistent with Watkins *et al* [37]. Even though the burden of *Ascaris* was high in their study, short follow up period (six months) and less deworming frequency (twice within six months) contributed to less impact of albendazole on growth. On the other hand, only one round of deworming and two months of follow in the current study could possibly elucidate the lack of benefit from intervention. Our result is also in line with randomized control trial in Peru by Joseph *et al*., 2015 [38]. Low prevalence and intensity of helminthes infection in both studies could explain for the consistency of the results and would make the effect of deworming less evident. On the other hand, the current finding contradict with double blind randomized control trial in urban slum of India by Sur *et al.*2002, which indicated the significant impact of albendazole on growth and diarrhea after three, six and nine months of treatment [40]..

WHO recommends periodic deworming as a means of controlling morbidity from helminthes infection which indirectly improves nutritional status through maintaining low level of these infections in childhood. As discussed above, both supportive and contradicting evidences are available from different countries employing different study designs.

9. Strengths and limitation of the study

9.1Strength of the study

- Use of kato-katz technique to determine the intensity of helminthes infection.
- Several parameters (growth status, intestinal parasites, *H pylori*, *CRP*) measured to determine their association**.**
- Fairly good number of students studied by collecting both maternal and paternal information.

9.2. Limitation of the study

- Cross sectional nature of study
- Anemia due to micronutrient deficiency such as iron, folate and vitamin B_{12} were not assessed
- Short follow up period after the school based deworming

10. Conclusion and Recommendation

10.1. Conclusion

In general the magnitude of anemia, stunting and thinness was low in this study. None of socio-economic and demographic factors of participants were associated with anemia and similarly, except for family size, association of parents and children study variables with undernutrition was trivial.

Regarding the effect of deworming, no definite association with anemia and growth was observed. This could be due to:

- Cross sectional nature of the study design makes it difficult to establish cause and effect relation.
- Low prevalence and intensity of helminthes infection as deworming effect has been evident in heavy infection settings
- Short follow up period (two months) and only one round of deworming

10.2. Recommendation

Longitudinal study design should be used to determine the effectives of deworming program as well as the association of different socio-demographic factors and intestinal parasite with growth status and anemia among school children.

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12. Annexes

Annex I. Participant information sheet

Investigator: Moges Wordofa

Advisors:

Aster Tsegaye (MSc, PhD)

Kassu Desta (MSc, PhD Candidate)

Binyam Taye (MPH, PhD)

Name of organization: Addis Ababa University, College of Health Science, School of Allied Health Science, Department of Medical Laboratory Science

Title of the study

The magnitude of anemia and growth status among school children in setting of mass deworming in selected public schools in Sululta town, 2017.

Purpose of the study

The objective of this study is to determine the magnitude of anemia and growth status among school children in setting of mass deworming in three selected public schools in Sululta town.

Procedures to be carried on the study participants

1**.** Height and weight of school children will be measured in barefoot**.**

2 .About 5 ml venous bloods will be collected in EDTA and about 4ml in serum separator tube

3. Each individual will be provided with labeled clean plastic container, toilet tissue paper and pieces of applicator sticks to collect stool sample

Risks associated with the study

There is no risk on the study participants other than little discomfort related to blood collection

Benefits of the study

You will be diagnosed for anemia using hemoglobin as well as H.*Pylori* antigen without any cost and if there are positive results, we will facilitate for the available treatment option. We will also evaluate the effectiveness of school based deworming in terms of improving health of school children through reducing the burden of intestinal parasite and anemia.

Confidentiality of your information

All information obtained will be kept private

Voluntary participation

Participation is only by willingness. You have the right to withdraw your child from the research at any time without in any way affecting his/her further medical care. I would also like to inform you that this study was approved by Research and Ethics Review Committee (DRERC) of Addis Ababa University, College of Health Sciences, School of Allied Health Sciences, and Department of Medical Laboratory Sciences .If you have any question you can contact:

The address of investigator: Moges Wordofa

Email: - heranmakmow@gmail.com

Mobile: - 0984742173

Department of Medical Laboratory Science research ethics office: +251 11 275 5170

Annex- II. Information sheet for study participants (Amharic version)

የተሳታፊዎች መረጃ ቅጽ

ጥናቱን የሚያጠናው ፤ ሞገስ ዎርዶፉ

የጥናቱ አማካሪወች፣ ዶክተር አስቴር ፀጋየ፣ዶክተር ቢኒያም ታየ፣አቶ ካሱ ደስታ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት

የጥናቱ አላማ

የጥናቱ አላማ ከተማሪዎች የሰገራ ናሙና እና የደም ናሙና በመውሰድ የአንጀት ጥገኛ ትላትል ምርመራ ማካሄድ እንዲሁም የትላትሉን መጠንና አይነት መለየት እንዲሁም የደም ማነስ እና የእድገት ሁኔታን ለማወቅ ነው፡: ጥናቱም ኦሮሚያ ክልላዊ መንግስት በሱሉለታ ወረዳ መጀመሪያ ደረጃ ት/ቤቶች ይካሄዳል.

በጥናቱ ወቅት ከልጅዎ የሚጠበቀው በጥናቱ ለመሳተፍ ፈቃደኛ ከሆነ ክብደት እና ቁመት ይለካል ከዚያ ቀጥሎ የሰገራ ናሙና እና የደም ናሙና መስጠትነው፡፡

ለጥናቱ ተሳታፊዎችያለው ልዩ ጥቅም

በጥናቱ ለሚሳተፉ ፍቃደኛ ተሳታፊዎች ምንም አይነት የገንዘብ ክፍያ የለውም ነገር ግን ከጥናቱ የሚገኘው ውጤት ለርስዎ ህክምና ተጨማሪ መረጃ ለማግኘትና ተላላፊ የሆኑ በሽታዎች ለመቆጣጠር ይጠቅማል እንዲሁም ውጤቱ ያለ ክፍያ ይሰጠወት እና በአካባቢው ባለ ጤና ጣቢያ ህክምና እንዲያገኙ ይደረጋል::

በጥናቱ ተሳታፊዎች ላይያ ለውጉዳት

በጥናቱ መጀመሪያም ይሁን መጨረሻ በዚህ ጥናት ላይ በመሳተፍዎ ደም ሲወሰድ ከሚሰማ ትንሽ ህመም በስጠቀር ሊደርስብዎ የሚችል አንድም ጉዳት አይኖርም፡፡ ለጥናቱ ከተመደበው 30 ደቂቃ ውጭ በጥናቱ ምክንያት የሚያባክኑት ተጨማሪ ጊዜ አይኖርም፡፡

የመረጃ ሚስጥራዊ አጠባበቅ

የሚሰጡት መረጃ በጥናቱ ወቅትም ሆነ ከዚያ በኋላ ባሉት ጊዜያት ሙሉ በሙሉ ሚስጥራዊነቱ የሚጠበቅና መረጃውም የሚያዘዉ በስም ሳይሆን በመለያ ቁጥር ይሆናል፡፡

በጥናቱ ላይ ያለ መሳተፍ መብት አለዎት፡፡

ይህ መረጃ በጥንቃቄ የሚያዝ ይሆናል። በመጨረሻም የጥናቱ ውጤት ለሚመለከተዉ አካል ለጥናቱ አላማና ለህክምና ባለሙያዎች ብቻ የሚገለፅ ይሆናል። ያስታውሱ፤ ስለዚህ ጥናት ማንኛውም ጥያቄ ካለዎት በማንኛውም ጊዜ ከዚህ በታች በተጠቀሱት አድራሻዎች መጠየቅ ይችላሉ። እኔም የጥናቱ ተሳታፊ ይህንን በመገንዘብ ጥናቱ ላይ ለመሳተፍ ተስማምቼ ያለሁ።

ፊርማ ----------

መረጃውን የሰበሰበው ግለሰብ ስም----------

ፊርማ ----------

የዋናተመራማሪው አድራሻ፤ ሞገስ ዎርዶፉ ኢ-ሜይል፣ heranmakmow@gmail ስልክ ፣ +2510984742173

የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤናሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

Annex III: English version of the Assent form

Code of study participant: _____

I have been requested to participate on this study, which plans to evaluate the magnitude of anemia and growth status among public school children in setting of mass deworming .I have been informed that the study involves collecting of Stool and blood specimen as well as anthropometrics measurements such as height and weight. During collection of the specimen I have been told that there will be no problem other than a little pain during blood sample collection procedure and I have also read the information sheet or it has been read to me. I have been also informed that all information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and drop out of the study if I want and that none of my actions will have any bearing at all on my overall health care access.

It is therefore with full understanding of the situations that I agreed to give consent voluntarily Thus I have given my consent to participate in the study, and I_____ hereby to approve my agreement with my signature provided my parent/guardian gives consent.

Participants' signature: _____Date_____

Witness for those you could not read the assent form:

1.

2.

If you have any question or comments, you can contact the principal investigator:

Moges Wordofa E-mail:heranmakmow@gmail.com Phone:-0984742173.

Guardian/parent consent form

Name of the parents/guardians_____

My child has been requested to participate in this study which plans to determine the magnitude of anemia and growth status in context of mass deworming. I have been informed that the procedures will not pose any health threat to my child and rather the research finding will be an input for improvement of health service. I am also informed that the result will be kept confidential .Thus having understood this ,I have allowed my child to participate on the research and I have also agreed give correct and sufficient information to the researcher and I _____Here by approve my agreement with my signature

Guardian/parent signature: _____Date_____

Principal Investigator's signature: _____Date _____

Annex IV. Assent form (for participants, Amharic Version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ የሰገራ ናሙና እና የደም ናሙና በመውሰድ የአንጀት ጥገኛ ትላትል ፤ የእድገት ሁኔታ እና የደም ማነስ ምርመራ በማካሄድ እንዲሁም የትላትሉን መጠንና አይነት በመለየት በልጅነት የዕድሜ ክልል በሚገኙ ህጻናት ስለሚደረገው ጥናት በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ እጅ ላይ የሚወሰድ የደም ናሙና እና የሰገራ ናሙና እንደሚያስፈል ተገልጾልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የተሳታፊዉ ፊርማ ----------

የምስክር ሙሉ ስም ፊርማ

1. ---------- ----------
2. ---------- ----------
3. ---------- ----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃ ሰብሳቢው ስም ---------- ፊርማ ----------ቀን----------

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለው አድራሻ ይጠቁሙ፡፡

ሞገስ ዎርዶፉ ኢ-ሜይል፣ heranmakmow@gmail ስልክ ፣ +2510984742173

የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤናሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

Annex V. Afaan Oromo version of consent and Assent form

Afaan Oromo version of Assent form

Lakk.Addaa hirmataa _____

Ani qorannoo faca'iinsa hir'ina dhiigaa fi haala guddina ijoollee umrii waggaa kudha shanii hanga kudha afurii mana barnoota keenya keessatti taasifamu irratti akkan hirmaadhu gaafatamee jira. Qorannoon kun dhiigaa fi bobba'a irratti kan taasifamu yeroo ta'u ,adeemsa kana keessa miidhaa ana irra gahu akka hin jirre naaf ibsamee jira.Kanaafuu qorannoo kana irratti kanan hirmaadhu fedhii kootiin fi barbaachisummaa qorannoo sirritti ergan hubadhee ta'uu naaf ibsameera.bu'aa qooranno dhiiga fi boba'a akka naaf ibasamuu fi yaala barbaachisu akkaan argadhu haalli akka mijaa'u naaf ibsameera. Kanaafuu qooran kan irratti akkan hirmaadhu yoo fedhii maatii kiyyaas ta'e , qooranno kana irratti hirmaachuu fedhii koo akka ta'e ibsaa, kanaafis mallatoo kiyyaan mirkaneessa.

Mallattoo barataa /Hirmaaataa _____. Guyyaa_____

Mallattoo ragaa _____ Guyyaa_____

Afaan Oromo version of consent form

Lakk. Addaa maatii_____

Mucaan koo qoranno haala faca'iinsa hir'ina dhiiga fi guddina ijoollee magaalaa Sulultaa mana barumsa mootummaa filatame irratti akka hirmaatu fedhii koo gafatameen jira.Qorannoon kun miidhaa fayyaa daa'ima koo irratti geessisu akka hin jirree fi bu'aan qorannoo fayyaa ijoollee foyyessuu keessatti ga'ee guddaa akka qabu naaf ibsameera.Akkasumas bu'aan qorannoo iccitiin isaa akka egamu naaf ibsameera .Haala kana hubachuun ,daa'ima koo qorannoo kana irratti akka hirmaatu fedhii koo akka ta'ee fi anis odeeffannoo barbaachisu kennuuf qophii akkan ta'ee fi kanaafis mallattoo kiyyaan mirkaneessa.

Mallattoo Maatii _____ Guyyaa_____

Mallattoo qoorata _____ Guyyaa_____

Qorannoo kana ilaalchise yoo gaafii ykn yaada qabatan

Qorataa: Moges Wordofa

E-mail: heranmakmow@gmail.com Lakk. Bilbila: 0984742173

Annex VI. English version of the questionnaire

The study title is to determine the magnitude of anemia and growth status in setting of mass deworming among public school children in Sululta town, Oromia region.

Interview

We thank gratefully for your agreement to participate in this study. Now we are going to undertake interview with you and the interview is about general socio-economic and demographic characteristics, dietary information and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore, we politely ask you to give us the right response.

1. Back ground information of students

1. Name of school:-_____

2. Address: - 1) rural 2) Urban

3. Sex. M ☐ F ☐

4. Age of child _____

5. Grade _____

6. Weight (kg) of child_____

8. Height (cm) of child _____

2. Maternal and paternal characteristics

2.1. Maternal occupation 1) Civil servants 3) Private organization 5) Daily laborer

2) House wife 4) Farmer 6) Merchant

2.2. Maternal Education status

0) illiterate 1) read and write only 2) primary school 3) high school 5) higher education

2.3. Paternal Occupation 1) Civil servant 3) Merchant 5) farmer
2) Private organization 4) Daily laborer 6) no work

2.4. Paternal educational status

0) illiterate 1) read and write 2) primary 3) high school 5) higher education

2.5. Family size _____

3. **Child dietary information**

3.1. How often do you eat roots and tubers (potato, sweet potato, Ensat and cassava?**)**

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.2. How often do you eat legumes (Beans, peas, chicken pea?)

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.3. How often do you eat cereals (corn, teff, wheat, sorghum, etc.) ?

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.4. How often do you eat vegetables (Tomato, cabbage)?

1) Once a day 2) more than once day 3) 2/3 times a week 4) occasionally 5) never

3.5. How often do you eat fruit (Orange, banana, etc.)?

1) Once a day 2) more than once day 3) 2/3 times a week 4) occasionally 5) never

3.6. How often do you eat meat (including poultry, fish)?

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.7. How often do you eat milk and milk product (butter, cheese, yoghurt etc.)?

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.8. How often do you eat Egg?

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

3.9. How often do you drink tea/ coffee?

1) Once a day 2) more than once a day 3) 2/3 times a week 4) occasionally 5) never

4. Clinical data

4.1 did your child taken any anthelminthic drugs in the last 6 months in the school or at the health center? 1) Yes 2) no

4.2. Have the child been recently (two weeks) treated for infection with *H.Pylori* 1) Yes 2) no

Thank you very much for your participation in this study!
Name of principal investigator _____

Annex VII. Amharic version of questioner

ጠቅላላ መረጃ

1.የት/ቤት ስም፡----------

2.አድራሻ፡ 1)ገጠር 2) ከተማ

3. ጾታ ፡ ወ ☐ ሴ ☐

4.የልጁ እድሜ ----------

5. የክፍል ደረጃ----------

6. የልጁ ክብደት (ኪ.ግ) ----------

7. የልጁ ቁመት (ሴ.ሜ) ----------

2.የቤተሰብ ሁኔታ

2.1. የእናት ስራ ሁኔታ 1) የመንግስት ሰራተኛ

2) የቤት እመቤት

3) የግል ተቀጣሪ

4) ገበሬ

5) የቀን ሰራተኛ

6) ነጋዴ

2.2. የእናት የት/ት ደረጃ

0) ያልተማረች 1) መጻፍና ማንበብ ብቻ 2) የመጀመሪያ ደረጃ ት/ት 3)ሁለተኛ ደረጃ ት/ት

5) ከፍተኛ ት/ት

2.3.የአባት የስራ ሁኔታ 1) የመንግስት ሰራተኛ

2) ነጋዴ

3) የግል ተቀጣሪ

4) የቀን ሰራተኛ

5) ገበሬ

6) ስራ የለውም

2.4. የአባት የት/ት ደረጃ

0) ያልተማረች 1) መጻፍና ማንበብ ብቻ 2) የመጀመሪያ ደረጃ ት/ት 3)ሁለተኛ ደረጃ ት/ት

5) ከፍተኛ ት/ት

2.5. የቤተሰብ መጠን----------

3. ስለልጁ አመጋገብ መረጃ

3.1. ልጁ ስራሰር በሳምንት ምንያክል ጊዜ ይመገባል

1) በቀን አንዴ 2)በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.2. ጥራጥሬ በሳምንት ምንያክል ጊዜ ይመገባል

1) በቀን አንዴ 2)በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.3. ልጁ እህል በሳምንት ምንያክል ጊዜ ይመገባል (በቆሎ፣ጤፍ፤ገብስ፤ ማሽላ፣ወዘተ)

1)በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.4. ልጁ አትክልት በሳምንት ምንያክል ጊዜ ይጠቀማል (ቲማቲም፣ጎመን፣ወዘተ)

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.5. ልጁ ፍራፍሬ በሳምንት ምንያክል ጊዜ ይጠቀማል (ብርቱካን፣ ሙዝ፣ወዘተ)

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.6. ልጁ ስጋ በሳምንት ምንያክል ጊዜ ይመገባል

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.7. ልጁ ወተት እና የወተት ውጤቶችን በሳምንት ምንያክል ጊዜ ይጠቀማል

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.8. ልጁ እንቁላል በሳምነት ምንያክል ጊዜ ይመገባል

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

3.9. ልጁ ሻይ/ቡና በሳምንት ምንያክል ጊዜ ይጠቀማል

1) በቀን አንዴ 2) በቀን ከአነድ ጊዜ በላይ 3) በሳምንት ከ2-3 ጊዜ 4) አልፎአልፎ 5) በጭራሽ

4. **የክሊኒካል ዳታ መረጃ**

4.1.**ልጁ የትላተል መዳህኒት በዚህ ስድስት ወር ውስጥ በት/ቤት ወይም በጤናተቀም ወስጥወስዶል**

1) **ወስዶል** 2) **አልወሰደም**

4.2. **ልጁ በዚህ ሁለት ሳምንት ውሰጥ የጨጋራ መዳህኒት ወስዶል**

1) **ወስዶል** 2) **አልወሰደም**

በጥናቱ ስለተሳተፉ በጣም እናመሰግናለን

ጥናቱን የሚያካሂደው ስም ----------

Annex VIII. Afaan Oromo version of the questioner

Mata-duren qoranno, faca'insa hirina dhigaa fi haala guddina ijollee umurii wagga shanii hanga wagga kudha afurii mana barumsa mootumma magalaa sululta, naanno Oromiya. Qooranno kan hiramachuu keessaniif galatooma jeecha oddefannno sirrii ta'ee akka nuuf kennitan kabajan siin kafanna

1 .Odefanno barataa

1.1.Maqaa mana barumsa _____

1.2.Iddoo jireenya:- 1) Magaala 2) Baadiya

1.3.Saala :- 1) Dhiira 2) Duubartii

1.4.Umurii_____

1.5.Kutaa_____

1.6.Ulfinaa(Kg)_____

1.7.Dheerina(cm) _____

1.8.Daa'immini keessan ji'oota jahaan darbaan kessaa qooricha raamo mana barumsattii ykn buufata faayyatti fuudhatee beeka? 1) Eeyyen 2) hin fudhanne

2. Odefannoo haala maatii

2.1. Hojii haadha mana

1) Hojjata mootumma 3) Hojjata dhaabata dhuunfa keessa 5) Hojii guyya

2) Hojii mana keessa 4) Qote bula 6) Daldalaa

2.2. Haala barumsa haadha mana

0) Kan hin baranne 1) duubisu fi barressu qofa 2) sadarkaa 1ffaa xumuree 3) sadarkaa 2ffaa xumuree 4) barumsa sadarkaa ola'anaa xumuree

2.3. Hojii Abba warraa

1) Hojjata mootumma 3) Daldalaa 5) Hojii guyya

2) Hojjata dhaabata dhuunfa keessa 4) Qote bula 6) Kan hojii hin qabanne

2.4. Haala barumsa Abba warraa

0) Kan hin baranne 1) duubisu fi barressu qofa 2) sadarkaa 1ffaa xumuree 3) sadarkaa 2ffaa xumuree 4) barumsa sadarkaa ola'anaa xumuree

2.5. Lakkofsa maatii _____

3. Odefannoo haala nyaata barataa/hirmaata

3.1. Daa'immini kessaan gosa nyaata hiidda Kan akka dinichaa, sukkar diinichaa fi k.k.f ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.2. Daa'immini keessan nyaata kana akka baaqela, ataraa, shumburaa fi k.k.f. ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.3. Daa'immini keessan nyaata Kan akka xaafii, booqolo, garbuu fi k.k.f ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.4. Daa'immini kessaan nyaata fuduraa fi kuduraa Kan akka salaaxa, timaatimi, muuzii, burtukaan fi k.k.f ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.5. Daa'immini keessan nyaata foonii ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.6. Daa'immini keessan aanani fi bu'aa aananii Kan akka dhadhaa, aayibii fi k.k.f ala meeqa soorata?

1)Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.7. Daa'immini keessan bupha'a ala meeqa soorata?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) siruma

3.8. Daa'immini keessan buna ykn shaayii ala meeqa dhugaa?

1) Guyyaatti ala tokko 2) Guyyaatti ala lama 3) torbanitti ala lama ykn sadii 4) darbee darbe 5) sirum

4. Odefanno haala fayyaa ijoolle

4.1. Daa'iminni keessan qooricha raamoo ji'oota jahaan darbee kessaa fudhatee beeka ?
1) Eyyeen 2) hin-fudhanne

4.2. Daa'iminni keessan qooricha baakteria H.pylori torban lamaan darbee keessa fudhatee beekaa? 1) Eyyeen 2) hin-fudhanne

Annex IX. Standard operating procedures (SOP).

1. SOPs for Blood Sample Collection

- ✓ Introduce yourself to the study participants , and ask the him/her to state their full name and demographic information
- ✓ Ask whether he/she has phobias or ever fainted during previous injections or blood draws.
- ✓ If he/she is anxious or afraid, reassure the person and ask what would make them more comfortable.
- ✓ Make the child to be comfortable
- ✓ Discuss the test to be performed and obtain verbal consent.

Reassurance of person/study participant

- Describe procedure to the child
- Get oral consent from the child
- Gain child's confidence
- Describe the use of the study to the child.

Assemble supplies and position the person

- Inspect all supplies for possible defect and applicable expiration dates.
- For participant's safety, draw all specimens with the participants/child seated comfortably in an appropriate chair.

Each specimen must be clearly labeled with the following:

- Participants/child name and code as well as date and time of collection
- Type of specimen

Apply Tourniquet

- Tourniquet is used to increase intravascular pressure, which facilitates vein palpation and filling of the tube (s) or syringe.
- Tourniquet application should not exceed one minute as localized stasis with hemo-concentration and infiltration of blood into tissue can occur.
- If the patient has a skin lesion at the intended tourniquet location, consider an alternative draw site, or apply the tourniquet over the patient's gown (cloth)
- Wrap the tourniquet around the arm 3-4 inches (7.5-10 cm) above the puncture site.
- Ask the child to form a fist, but avoid vigorous hand exercise.
- Collect blood form median capital (H pattern) and median (M pattern) veins because these veins are typically closer to the surface of the skin, more stationary, less painful upon needle insertion, and less likely to injure nerves if needle placement is not accurate.

Put On Gloves

- The phlebotomist must put gloves on before the vein puncture is performed.

Cleanse vein-puncture Site

- Use a gauze pad with 70% isopropyl alcohol solution, or
- Cleanse the site with a circular motion from the center to the periphery.
- Allow the area to air dry.
- If the vein-puncture proves difficult and the vein must be touched again to draw blood, the site must be cleansed again.

Vein-puncture procedure using needle and syringe

- Break the seal and look for any defects, check the plunger.
- Prepare the child by informing him/her that the vein puncture is about to occur.

- With the bevel up, puncture the vein with the needle at angle of insertion of 30 degrees or less.
- Keeping the needle as stable as possible in the vein, slowly withdraw the desired amount of blood and ask the patient to open his arm.
- Release the tourniquet as soon as possible, after the blood begins to flow.
- Transfer the blood from the syringe to a venous blood collection tube. Allow the tube to fill without applying pressure to the plunger until flow ceases. This will helps to maintain the correct ratio of blood to additive if an additive tube is being used.
- Mix the additive tubes by inversion. Do not shake tubes. Rubber stoppers should not be removed from venous blood collection tubes to transfer blood to multiple tubes.

Additional Considerations

Hemolysis

To prevent hemolysis; allow the vein-puncture site to air dry after cleansing, never draw blood through a hematoma & make sure the needle is fitted securely on a syringe to avoid frothing.

Hematoma

To prevent hematoma, the phlebotomist should make sure the needle fully penetrates the uppermost wall of the vein, remove the tourniquet before removing the needle, use the major superficial veins, apply small amount of pressure to puncture site.

2. SOPs for complete blood count (including RBC parameters)

Principle:-Sysmex KX-21 Hematology analyzer uses Sheath flow DC detection for determining WBC, RBC, and PLT count. As electric current passed through a solution, this method measure change in electrical resistance that occur when blood cell pass through a detection aperture. Therefore blood cell can be counted by measuring the number of times an electric current resistance is generated. Larger blood cell produces greater resistance allowing distinguishing cell type.

Sysmex KX-21 Hematology Analyzer

Analysis Mode

- **Whole blood mode:** This is the mode of analyzing collected blood sample in the whole blood status. The tube cap is opened and the sample is aspirated through the sample probe one after another.

Procedures: Samples are processed in the following steps:

- Collecting and preparing samples
- Selecting whole blood mode
- Inputting sample no.
- Analyzing samples

Collecting and preparing samples

A specified amount of sample, corresponding to the amount of EDTA anticoagulant, is collected from the vein. Tubes up to 80 mm in height should be used. The volume of sample that can be aspirated is 50µl.

Analyzing samples

- Mix the sample sufficiently
- Remove the plug while taking care not to allow blood scatter
- Set the tube to the sample probe, and in that condition, press the start switch

- The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing," remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.
- When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures.

Reagents of Sysmex KX-21

- **Cellpack:** is ready to use for impedance and photoelectrical analysis of whole blood, its ingredients are: sodium chloride, boric acid, sodium tetra borate, EDTA-2K
- **Stromatolyzer WH**: is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC Free and easy to count; whole blood sample by resistance measurement and photometric measurement, and its ingredients are: non ionic surfactant, organic quaternary ammonium salt
- **Cell clean:** is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of sysmex analyzer.
- It is a detergent to clean the instrument, to remove residual and blood proteins from the hydraulic systems, transducer, sample rotor valve, whole blood aspiration tube and Hgb flow cell. Ingredients: sodium hypochlorite

3. C-reactive protein/ CRP-Latex Determination

C- reactive protein slide test.

Principle

CRP-Latex Test is a rapid slide agglutination procedure based on a modification of the latex fixation method developed for the direct detection and semi-quantitation of C-reactive protein (CRP) in serum. The assay is performed by testing a suspension of latex particles coated with anti-human CRP antibodies against unknown serum. The presence of a visible agglutination indicates an increase of the CRP level above the upper limit of the reference interval in the samples tested.

Reagent composition

R-CRP-Latex Reagent - Suspension of polystyrene latex particles coated with specific antihuman C-reactive protein antibodies in a buffered saline solution. Contains 0.95 g/L of sodium azide.

Control (positive) - Human serum with a CRP concentration >6 mg/L. Contains 0.95 g/L of sodium azide.

Control (negative)-Animal serum with a maximum concentration of human CRP of 1 mg/L. Contains 0.95 g/L of sodium azide.

Precautions: Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

Warning: The reagents in this kit contain sodium azide. Do not allow contact with skin or mucous membranes.

Packaging content

REF 2410005, kit 50 tests. 1 vial CRP-Latex Reagent, 1x1 ml Positive control, 1x1 ml Negative control, 3 Test cards and 1x50 disposable stirrers.

REF 2410010, kit 100 tests. 2 vials CRP-Latex Reagent, 1x1 ml Positive control, 1x1 ml Negative control, 3 Test cards and 2x50 disposable stirrers.

Storage and stability -Store at 2-8ćC. Do not freeze. Frozen reagents could change the functionality of the test. Reagent and Controls are stable until the expiry date stated on the label.

Reagent preparation -Reagent and Controls are ready to use.

Samples -Use fresh, clear serum collected by centrifuging clotted blood.

After the clear serum has been separated it may be stored at 2-8^{o}C for up to one week or longer periods at –20^{o}C or the sample must be frozen.

If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 72 hours after collection.

As in all serological tests, hemolytic or contaminated serum must not be used. Do not use plasma!

Materials requaired - Automatic pipettes.

- Saline solution (0.9% NaCl, only for semi-quantitation procedure).
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

Procedure

A. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 1).
2. Suspend the Reagent vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control serum into two additional circles.
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2 minutes (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control

Reactive: Any degree of agglutination visible macroscopically

B.Semi-quantitative test evaluation

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction.

1. For each specimen to be tested place with an automatic pipette 50 åL of 0.9% saline solution into each of the circles of a card. Do not spread diluents.
2. To circle one add 50 µL of specimen to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid and transfer 50 µL of the mixture to the saline solution in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the sixth circle, and discard 50 µL from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64.(Test each dilution as described in steps 4-7 for the Qualitative)
4. Add 1 drop of CRP-Latex Reagent to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide means of a mechanical rotator (100 r.p.m.) for a period of 2minutes
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative. If the highest dilution tested is reactive repeat the test starting with a preliminary 1:16 dilution. Use a 1:50 dilution of negative control serum in 0.9% saline solution to the replace the 0.9% saline solution in the new 2-fold dilution series. The approximate CRP level (mg/L) present in the sample may be obtained multiplying the titer of the last positive dilution by the minimum detectable unit

4. Stool analysis

4.1. Direct wet mount

Principle: Intestinal parasites can be identified by examination of fresh stool samples. In stool samples we can find worms (e.g. *Ascaris lumbricoides*) and segments of worms (e.g. *Taenia* species) visible to the eye. By microscopic examination of fresh stool samples, we can find eggs (e.g. *Hookworm*) and larvae of worms (e.g. *Strongyloides stercoralis).*We also find protozoa trophozoites (e.g. Amoeba) .In heavy and moderate infection, a direct smear examination with normal saline and/or iodine to stain cysts, is usually sufficient. For light infections, a concentration of the stool sample might be required to find helminthes eggs and protozoa by microscopic examination.

Procedure

1. Place a drop of normal saline on a clean slide
2. Using a piece of stick, place a small amount of specimen, including blood and mucus in one end of the slide and cover it with a cover slide
3. First examine microscopically using 10 x objectives to give good contrast and use the 40x objective to identify trophozoites of protozoa.

Reporting: Report the name of the parasite found.

4.2. Formol ether concentration technique

1. Using a rod or stick, emulsify an estimated 1gm (pea-size) of faeces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.

   Note: Include in the sample, faeces from the surface and several places in the specimen.
2. Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
3. Sieve the emulsified faeces, collecting the sieved suspension in a beaker.
4. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4 ml of diethyl ether or ethyl acetate.

Caution: Ether is highly flammable and ethyl acetate is flammable, therefore use well away from an open flame, e.g. flame from the burner of a gas refrigerator, Bunsen burner, or spirit lamp. Ether vapour is anaesthetic; therefore make sure the laboratory is well-ventilated.

5. Stopper* the tube and mix for 1 minute. If using a Vortex mixer leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube).

   Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.
6. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
7. Centrifuge immediately at 750–1 000 g (approx. 3000 rpm) for 1 minute. After centrifuging, the parasites will have sediment to the bottom of the tube and the faecal debris will have collected in a layer between the ether and formol water
8. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
9. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
10. Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the 40xobjective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass. Although the motility of *Strongyloides* larvae will not be seen, the non-motile larvae can be easily recognized.
11. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces

4.3 .Kato-Katz technique

Principle

People infected with STH or intestinal schistosomes pass the eggs of the worms through their faeces. In the Kato-Katz technique faeces are pressed through a mesh screen to remove large particles. A portion of sieved sample is then transferred to the hole of a template on a slide. After

filling the hole, the template is removed and the remaining sample is covered with a peice of cellophane soaked in glycerol. The glycerol clears the faecal material from around the eggs. The eggs are then counted and the number calculated per gram of faeces .Finally, multiply the number of eggs by 24 to give the number of eggs per to give the number of eggs per gram (epg) the standard measurement to assess the intensity of gram (epg) the standard measurement to assess the intensity of infection.

Specimen
Any fresh stool sample that has not been refrigerated

Reagent/materials

- Kato-set (Template with hole, screen, nylon or plastic, plastic spatula)
- Newspaper or glazed tile
- Microscope slides
- Cellophane as cover slip, soaked in Glycerol-malachite green
- Fresh stool ,Glove

Procedures

- Label a glass slide with the sample number and then place a plastic **t**emplate on top of it.
- Place a small amount of the faecal sample on a newspaper and press a piece of nylon screen on top. Using a spatula, scrape the sieved faecal material through the screen so that only the debris remains.
- Scrape up some of the sieved faeces to fill the hole in the template, avoiding air bubbles and leveling the faeces off to remove any excess.
- Carefully lift off the template and place it in a bucket of water mixed with concentrated detergent so that it can be reused.
- Place one piece of the cellophane, which has been soaked overnight in glycerol malachite green solution, over the faecal sample.
- Place a clean slide over the top and press it evenly downwards to spread the faeces in a circle. Carefully remove the slide by gently sliding it sideways to avoid separating the cellophane strip. If done well, it should be possible to read newspaper print through the stool smear. Place the slide with the cellophane upwards.

Bench Aids for the Diagnosis of Intestinal Parasites. WHO, 1994

5. H. *Pylori* Stool Antigen

Principle:- The H .pylori Ag Rapid test is a lateral flow chromatographic immunoassay for the qualitative detection of *H. pylori* antigen in human faecal specimen. Any reactive specimen with the *H.Pylori* Ag Rapid test must be confirmed with alternative testing method(s) and clinical finding.

H.Pylori Ag Rapid test uses a colloid gold conjugated monoclonal anti-*H.Pylori* antibody and another monoclonal anti- H.*Pylori* antibody to specifically detect *H.Pylori* antigen present in the faecal specimen of an infected patient. The test is user friendly, accurate, and the result is available within in 15 minutes.

Reagents and Materials Provided

- Individually sealed foil pouches containing: Cassette test device ,desiccant
- Sample extraction tubes, containing extraction buffer.
- Plastic droppers for transferring watery stool.
- One package inserts (instruction for use)

Procedure A: Solid stool samples

1. Collect a stool sample in a clean, dry and leak proof container.

2. Open the sample extraction tube by unscrewing the top and use the collection stick to randomly pierce the stool sample in at least five different sites.

3. Ensure stool sample is only in the grooves of the collection stick. Excess stool sample may lead to an invalid test result.

4. Replace the collection stick and tighten securely to close the sample extraction tube.

5. Shake the sample extraction tube vigorously.

Procedure B: Watery stool samples

1. Fill the plastic dropper with the sample; dispense 2 drops (70-85 μL) into the sample extraction tube.

2. Replace the collection stick and tighten securely to close the sample extraction tube.

3. Shake the sample extraction tube vigorously.

Test Procedure

1. Bring the specimen and test components to room temperature if refrigerated or frozen.

2. When ready to test, open the pouch at the notch and remove the test device. Place the test device on a clean, flat surface.

3. Shake the sample collection tube vigorously to ensure a homogenous liquid suspension.

4. Position the sample extraction tube upright and twist off the dispenser cap. Holding the sample extraction tube vertically, dispense 2 drops of the solution into the sample well of the test device. Do not overload sample

5. Set up the timer.

6. Results can be read 15 minutes after adding the specimen. Positive results can be visible in a time period as short as 1 minute. Do not read results after 20 minutes. To avoid confusion, discard the test device after interpreting the result.

13. Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in Addis Ababa University or any other universities. I also declare that all sources of materials used for the thesis have been duly acknowledged.

Name of the MSC candidate: Moges Wordofa

Signature_____

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Date of submission_____/_____/_____

This proposal has been submitted with my approval as university advisor.

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