PREVALENCE AND RISK FACTORS OF ANEMIA AMONG SCHOOL AGE CHILDREN IN FILTU TOWN, SOMALI REGION, SOUTH EAST ETHIOPIA



BY:

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A RESEARCH PAPER SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCE SPECIALTY IN HEMATOLOGY AND IMMUNOHEMATOLGY

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## JIMMA UNIVERSITY

# COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES DEPARTMENT OF MEDICAL LABORATORY SCIENCE AND PATHOLOGY

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#### ABSTRACT

**Introduction:** Anemia is one of the major public health problems affecting more than half of school age children in developing countries. Anemia among children has been demonstrated in many studies resulting in impaired cognitive and intellectual performance, motor development, language development, and scholastic achievement.

**Objectives:** To determine the prevalence and risk factors of anemia among school-age children in Filtu town, Somali region, South-East Ethiopia.

**Methodology:** A community based cross-sectional study was conducted between July and August, 2013 in Filtu town. A total of 355 school age children between 5-15 years old were included in the study. Sociodemographic data were obtained from each participant using pretested questionnaire. Hemoglobin was estimated using HemoCue 201+ photometer (HemoCue, Angelholm, Sweden) analyzer and Anthropometric data of the children were taken. Intestinal parasitosis determined by formol-ether concentration technique and both thick and thin blood films for malaria parasites were performed. Descriptive statistical analysis, binary and multiple logistic regression analysis were done using SPSS version16; P<0.05 was considered as statistically significance.

**Results:** The prevalence of anemia was found to be 23.7% among school age children. Mild, moderate and severe anemia was 74.5%, 24.3% and 1.2%, respectively. Morphologically, 58.3% were microcytic hypochromic anemia, 40.5% were normocytic normochromic anemia and 1.2% was macrocytic anemia. Results of a multivariate analysis showed that, being stunted [OR=5.5, 95%CI=: 2.83,10.72, P<0.001], being underweight [OR=2.1, 95%CI: 1.06- 4.05, P=0.034], infection with intestinal parasite [OR=3.0, 95%CI: 1.05-8.46, P= 0.040] and low family income [OR=9.4, 95%CI: 2.88, 30.99, P<0.001] were the independent risk factors of anemia among school age children.

**Conclusion:** The prevalence of anemia among school age children in Filtu town was 23.7%. Stunting, underweight, intestinal parasite (Ascaris lumbricoides and Giardia lamblia) and low family income were the predictors of anemia.

**Recommendations:** Awareness of the communities on role of nutrition on anemia and interventions targeting both nutritional deficiencies and parasitic infections should be employed. *Key words:* Anemia; risk factors; school age children.

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# LISTS OF ABBREVIATIONS

| <b>BMI</b> – Body | mass | index |
|-------------------|------|-------|
|-------------------|------|-------|

- **CBC-** Complete blood count
- **EDHS** Ethiopian demographic and health survey
- HAZ Height- for- age Z-score
- Hgb-Hemoglobin
- **IDA** Iron deficiency anemia
- **IP** Intestinal parasite
- NCHS National center for health statistics
- OR- Odds Ratio
- **PSAC** Pre-school age children
- **RBC-** Red blood cell
- SAC School age children
- SPSS Statistical package for social sciences
- **STH** Soil transmitted Helminthes
- **UNICEF** United Nations Children's Fund
- WAZ Weight for age Z –score
- WHO World health organization

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#### **CHAPTER ONE: INTRODUCTIONS**

#### **1.1 Background**

Anemia is defined as a condition where there is reduction in hemoglobin level or reduction in red blood cell (RBC) count or both in the body which is below normal for the same age group, sex and geographical locations, resulting in lower quantities of oxygen delivered to support the body's activities(1).

Anemia among children results from one or more of the following processes: Defective red cell production due to lack of essential nutrients in the diet (iron, vitamin A, vitamin  $B_{12}$  and folic acid), poor bioavailability of iron, or increased utilization of nutrients during rapid growth of the children and chronic infections like HIV/AIDS and TB etc. And also anemia is caused by Blood loss, resulting from intestinal worm infestation, notably hookworm, or heavy menstrual in girls. The other is, it caused by increased red cell destruction (hemolysis) due to parasitic diseases such as malaria or genetic conditions such as sickle cell anemia or thalassemia (2, 3).

Malaria, especially from the protozoan *Plasmodium falciparum*, causes anemia by rupturing circulated red blood cells and by suppressing the production of new red blood cells. Malaria does not, however, cause iron deficiency, because much of the iron in hemoglobin released from the ruptured cells stays in the body (4).

Helminthes such as hookworm's species can cause blood loss and therefore iron loss. Adult hookworms attach themselves to the gut wall, where the mature larvae and adult worms ingest both the gut wall and blood. Hookworm species change feeding sites every 4–6 hours and during feeding secretes anticoagulant, resulting in secondary blood loss from the damaged gut wall after the worms has stopped feeding. The number of adult hookworms and the fecal egg count, which is an indirect estimate of the number of worms, are strongly correlated with the amount of blood lost, which if chronic can result in iron deficiency anemia (5, 6).

The nematodes *Trichuris trichiura* can cause anemia when the worm burden is heavy. Heavy infections also cause inflammation and dysentery, which in turn can cause further blood loss.

The trematode *Schistosoma mansoni*, can cause significant blood loss in severe infection. Shistosomiasis' emerging eggs rupture the intestinal lining, resulting in the leakage of blood and other fluids and nutrients into the lumen (7, 8).

Although anemia has a variety of causes, it is generally assumed that 50% of cases are caused by iron deficiency. The main risk factors for iron deficiency among young children are low intake (malnutrition) and the high requirement of iron during child growth i.e. accelerated development, hormonal changes, and starting of menstrual periods in girls, high rate of infection and worm infestation are major causes in this period (9 -11).

The etiology of anemia in Ethiopia is not well established and the information available is limited in representativeness of the whole country. Various researchers came up with different conclusions despite the problem being among the ten top morbidities reported by most health institutions in the country. Some studies in the past have documented the problem as being rare in Ethiopia attributed to consumption of *Eragrostis teff* ("teff") a cereal which has high iron content mainly due to contamination with the soil while others have concluded the issue as is a mild to moderate public health problem due to factors such as parasitic infections in addition to nutritional deficiencies (12, 13).

The signs and symptoms of anemia among children are non-specific and difficult to detect. However, findings suggest that chronic anemia includes irritability, pallor (usually not seen until hemoglobin levels are less than 7g/dl), fatigue, glossitis, a systolic murmur, and growth delay. Children with acute anemia often present more dramatically with clinical findings including jaundice (yellow skin), tachypnea, tachycardia, splenomegaly and congestive heart failure (14).

Anemia is diagnosed by different methods; complete blood count (CBC) which includes RBC indices, hemoglobin determinations or hematocrit, reticulocyt count and by peripheral blood film morphology which can show unusual size, shape and color of RBC. WHO recommend that, hemoglobin is well validated, and widely accepted diagnoses of anemia (1, 15).

Anemia is a public health problem only when the prevalence exceeds 5.0% of the population and also the WHO has also laid down the classification of countries with respect to the level of public health significance of anemia; a prevalence of <4.9% no public health problem, 5-19.9% mild public health problem, 20-39.9 moderate public health problem and >40% severe public health problem (1).

Due to the multifactorial conditions, the complexity of the risk factors of anemia, and potential interactions among them, a single strategy to control anemia in developing countries may have little success. In developing countries, an integrated strategy for anemia control and prevention are : dietary improvement through education to encourage selection of iron-rich foods to improve iron content and bioavailability, fortification (adding iron to common foods) and fermentation (reducing inhibitors of iron absorption), iron supplementation and de-worming; distributing pills by means of the health system, malaria control, linking intervention strategies to related health and nutrition programs (16, 17).

#### **1.2 Statements of the problem**

Anemia is a global public health problem affecting both developed and developing countries with major consequences for human health as well as social and economic development. It occurs at all ages, but is more prevalent in pregnant women and young children (1).

According to the 2001 global WHO report, anemia affects 1.62 billion people, which corresponds to 24.8% of the World population. It is a major problem among women and young children, but there is growing evidence, it is also a problem among school age children (1).

According to information from the period 1993-2005 on the worldwide, prevalence of anemia among school age children was 37%. The prevalence was highest among the lowest category of national level of socioeconomic development, and vice versa (18).

The highest prevalence of anemia exists in the developing world where its causes are multifactorial. In the developing world, 42% of children less than five years of age and 53% of school aged children are anemic (19).

Anemia is a public health problem in developing countries, especially among children and pregnant women. In sub-Saharan Africa, it is estimated that between 50% and 70% of all pregnant women are anemic, with 5-15% being severely anemic and prevalence of anemia in pre-school and school-age children is estimated to be 50% and 46%, respectively (1, 20).

Different studies have shown high prevalence of anemia in African populations. As the study conducted in 2000 among school age children in eight African and Asian countries found that 40%-60% of school aged children in Mali, Tanzania, Mozambique, Ghana, Malawi and Kenya and 12% and 30% of those in Vietnam and Indonesia suffered from anemia. The analysis of the hemoglobin concentrations of nearly 14, 000 children enrolled in basic education in these eight countries in Africa and Asia indicated a large burden of anemia, which mostly in Africa (21).

According to the study conducted by the Food Security Nutrition Analysis Unit (FSNAU-Somalia) in conjunction with the UN bodies such as UNICEF, WFP and WHO indicated that nearly half of all women and children in Somalia were anemic and Vitamin A deficient. The report showed that about 50% of all women, 30% of all school aged children and 60% of children less than five years were anemic (22).

The study shows in Ethiopia, the 2011 EDHS tested over 9,000 children age 6 to 59 months and over 15,000 women for anemia. More than 4 in 10 children are classified as having any anemia, most of whom have mild or moderate anemia. Even if anemia has decreased from 54% of children in the 2005 to 44% of children in 2011, it is the major public health problem in the country (23).

Although prevalence of anemia in Ethiopia at the national level is considered to be mild, some regions in the lowlands exhibited extremely high prevalence of anemia. For instance, Central Statistical Agency, "Ethiopia Demographic and Health Survey 2005 report showed that 85.6% of children in Somali Region suffer from anemia and nearly 50% of the women were anemic. This is further compounded by the fact that unlike most regions in the country the Somali regions did not see a significant decline in the prevalence of anemia in the previous five years (the 2011 EDHS report). Therefore, in this lowland region anemia can be considered a significant public health problem affecting the lives of women, and children which contributing significant to maternal and child deaths (23, 24).

The consequences of anemia among school age children are, lowered resistance to disease, increased susceptibility to infection, poor cognitive development, impaired physical development and poor school performance, and reduced work capacity with impaired social and economic development of the country (25, 26).

In Ethiopia, most of the studies on prevalence of anemia were conducted among preschool age children and pregnant women but few studies on School aged children, so there is a need for more studies related to anemia and its risk factors among school age children. Hence this research was conducted to determine the magnitude of anemia and its possible risk factors among school age children in Filtu town, Somali Region of South-East Ethiopia.

# **1.3 Significance of the study**

Anemia is one of the most widespread public health problems, especially in developing countries with greater risk in school age children. There is also evidence that anemia result in reduced growth, impaired cognitive development, reduced physical work capacity and increased morbidity among school age children.

So, this study contributes some information regarding to the prevalence of anemia and its risk factors which can help health administrators and programme managers to develop and implement suitable strategies for preventing and controlling.

Furthermore, this study will be used as base line data for further studies.

#### **CHAPTER TWO**

#### Literature review

There is little recent evidence to suggest the prevalence of anemia among school age children in world wide. Indeed, based on 2000 ACC/SCN data base it is estimated that 7.8% of school-age children in developed countries and 53% of developing countries suffer from anemia. The highest prevalence is reported in Asia 58.4% followed by Africa 49.8% (19, 27).

In the survey on anemia and risk factors in rural Qinghai and Ningxia elementary schools of China in 2010; out of 4, 000 students involved in the study, the overall anemia rate was 24.9%. According to the study female school age children and those whose parents had lower level of educations were more likely to be anemic than those females whose parents had higher level of educations (p<0.05) (28).

The study conducted in 2012, on prevalence of anemia among school aged children at Governmental school of Rishikesh, Uttrakhand, India showed that, out of 200 school children enrolled in the study, 56.5% (113/200) were found to be anemic. A significantly higher number of girls were anemic at all age 66.6%. At almost all ages significantly more (65.2%) vegetarian children were anemic. The most common blood picture among the study groups was Microcytic hypochromic 62 (54.9%), normocytic normochromic accounts 48 (42.5%) and 03 (02.5%) were dimorphic. According to the report hemoglobin showed a rising trend with improved socio-economic status and (90.9%) of the children belonging to lower socio-economic groups were anemic (29).

In the study conducted in rural Punjab, India in 2011 on anemia and risk factors among young children (n=4320); the prevalence of anemia in young females was 89.5%, of which 49.8% had mild, 38.2% moderate and 1.5% severe anemia respectively. On the other hand the prevalence of anemia in young males was 89.9%, from which 51.2% had mild, 38% moderate and 0.7% severe anemia, respectively. According to the report both males and females who were in the younger age group, underweight and who belonged to a lower socio-economic status had a higher prevalence of anemia (30).

Anemia in adolescence school aged girls in India at different periods, 2009, 2008-2011, 2012 were reported as 78.5%, 72.9% and 78.75%, respectively which indicates a major health problem in the study groups. In the study conducted in 2012 among adolescent school girls in Chennai, Tamil Nadu there was varying degrees of anemia ranging from mild (37.5%) to severe (6%). The researcher reported that anemia was significant association with family size (P=0.019, weight (P= 0.010), and mother's educational status (P= 0.031) (31-33).

In the study conducted on the frequency and etiology of anemia among children between 6 and 16 years of age in Sanliurfa, in the Southeast region of Turkey: anemia was found to be 5.4% of the children which was 7.8% in children 6-11 years of age group, and 1.5% in the 12-16 years of age group. In the report causes of anemia were, iron deficiency accounted 58.9% in children, beta-thalassemia heterozygosity in 6.3% of children, chronic disease that causes anemia of inflammation in 19.0% of children, intestinal parasitic infections in 10.8% children and in 5.1% of children, the cause of anemia could not be determined. The study's results showed that iron deficiency anemia and chronic and parasitic disease are important problems in schoolchildren of Sanliurfa, while beta-thalassemia and hemoglobinopathies have less importance (34).

A cross sectional study conducted in 2007, on anemia and intestinal parasite infection among school children of 400 students in Vietnam indicated , anemia prevalence of 25%. The youngest group(5-6 years old) showed the highest anemia prevalence (38%). According to the result of the study compared to girls, boys had a higher prevalence of anemia (28.8% compared to 21.1%). The author reported that Children infected with *Trichuris trichuira* showed a lower hemoglobin concentration and a higher prevalence of anemia (AOR= 1.96, 95% CI: 1.07-3.59) (35).

According to the study conducted in 2001 in Deshna and Armant Districts of Qena Governorate, Upper Egypt, the overall anemia prevalence among school children was 12%. According to the report no case of severe anemia (< 7.0 g/dl) was detected and the prevalence of anemia was 12.8% among boys and 11.2% among girls, but this difference was not statistically significant. The authors interpreted that the lower prevalence of anemia among school-age children was the dietary intake in the area is currently just sufficient to satisfy the iron needs of the majority of children (36). As the study conducted among school age children in Kenitra Morocco 2008, the overall prevalence of anemia in the studied population was 12.2 %. The result of the study showed that there was a significant relationship between socio-demographic characteristics of the families like educational level of the mother and anemia in children (p=0.010) (37).

From the result of the study conducted in Abidjan, Côte D'ivoire among school age children: the prevalence of anemia (hemoglobin< 11.5 g/dl) was 30.3 % with 33.3 % of males and 29.1 % for females. The researcher reported that among the anemic children; 18.1 % were microcytic hypochromic anemia, 39.4 % were nornocytic normochromic anemia and 4.3 % macrocytic normochromic anemia this could be explained by a deficiency of micronutrients and in addition, haemoglobinopathies was found in these children (38).

According to the study conducted in rural communities of Abia state, Nigeria among school age children; the prevalence of anemia was 82.6% with rates of mild, moderate and severe anemia being 9.6%, 71.6% and 1.4%, respectively. According to report anemia was significantly associated with helminthes infestation and malaria parasite infection (p<0.05). From result of the study majority of the children had a mean weight and height below the recommended standards. Out of the 249 children in the study, 77% were both stunted and underweight while 56% were wasted (39).

In a survey attempted to estimate the prevalence and risk factors of anemia among school age children of 316 students in three rural communities of the Ovia North east local government area of Edo State, Nigeria in 2010; the overall prevalence of anemia was 38.6%. But for each community the prevalence of anemia was varies: 75.9% of children in Evbuomore, 42.3% in Isiohor and 26.8% in Ekosodin were anemic. And also the result of the study showed malnutrition was high which indicated that, 37.0% of the children were stunted, 19.3% wasted, and 44.0% underweight. There was a statistically significant association between hookworm and *Ascaris lumbricoides* infection with anemia (P < 0.05) (40).

The study conducted in united republic of Tanzania 2001, to evaluate the efficacy of schoolbased anti-helmintic treatments against anemia at the base line indicated that the overall prevalence of anemia was 56%. A total of 54% of the children had hemoglobin values < 11 g/dl, and 10% had moderate-to-severe anemia (<9 g/dl) and Severe anemia (<7 g/dl) was identified in fewer than 2% of the children. Logistic regression analysis revealed that age group AOR= 0.59, 95%CI (0.44–0.79), stunting AOR= 1.57, 95CI% (1.14–2.15) and the intensity of infection with hookworm AOR= 1.17, 95% CI (1.05–1.32) and *S. haematobium* AOR= 1.39, 95% CI (1.19–1.62) were the important predictive variables for anemia (Hgb < 110 g/l) (41).

As cross-sectional survey conducted to determine Risk factors of anemia in schoolchildren in 20 randomly selected schools in Tanga Region of Tanzania in 2008; out of 845 schoolchildren age 7-14 years randomly selected, the prevalence of anemia was 79.6%. According to the report infections with hookworm, shistosomiasis, underweight and deficiency in Vitamin A were the most significant factors predicting of anemia among the children (p<0.05) (42).

The study conducted in Kenya in 2004 among adolescence schoolgirls indicated that the mean  $(\pm 95\%$  confidence intervals (CI)) of Hgb and prevalence of anemia (<120 g/l) was 129 g/l (127–132g/l) and 21.1%, respectively. Only one girl had Hgb less than 70g/l. The prevalence of anemia using the 112 g/l altitude adjusted race-specific WHO cutoff was 10.8%. Red cell morphology indicated that 27.2% of the anemic girls were Microcytic-hypochromic anemia. Macrocytosis was rare (0.6%). According to the report none of the geohelminths tested were associated with anemia in the girls (43).

Another cross-sectional survey conducted from period 2008-2010 in Kenya among school-aged children for 16, 941students (aged 5–16), to estimate the contribution of parasitic infections and nutrition for anemia; the mean altitude-adjusted Hgb concentration was 122.1 g/l; with 35.3% prevalence of anemia was reported. The result of the study shows, severe malnutrition (p<0.001) and infections with *P. falciparum* (p<0.001) and hookworm infections (p<0.001) were significantly associated with lower Hgb, with greater impacts seen for co infected children (44).

As study conducted on the prevalence of anemia among school age children in different parts of Somalia, in 2009 (North West, North East and South Central) showed that, the overall prevalence of anemia among school age children was 29.8% with high proportion of mild and moderate anemia. There was only one case of severe anemia in North West and six in South Central of Somalia (45).

According to the study conducted on risk factors for intestinal parasitosis, anemia, and malnutrition among school children in Northern Ethiopia; the overall prevalence of anemia was 11% (95% CI: 8-13%). Prevalence of Stunting and thinness were 35% (95% CI: 31-38%), and 34% (95% CI: 30-38%), respectively. According the report poor personal hygiene habits and hookworm infection were associated with anemia and nutritional deficiency (low body mass index) (46).

School aged children are more vulnerable to anemia due to their rapid growth need of high iron and intestinal parasite infections. Therefore, it is a critical health concern among this age group, because it affects physical and mental development, tiredness and decreased physical and intellectual abilities. Despite the multiple consequences of this disease, few investigations are conducted on school aged children in Ethiopia and no study was conducted in the study area (Filtu town) previously. So this study was undertaken to determine the prevalence of anemia and its possible risk factors which contribute information regarding magnitude and determinants of anemia among school age children in Filtu town.

# **CHAPTER THREE: OBJECTIVES**

# 3.1 General objective

• To determine the prevalence and risk factors of anemia among school age children in Filtu town, Ethio-Somali region, South- East Ethiopia

# **3.2 Specific objectives**

- To determine the overall prevalence of anemia among school age children in Filtu town
- To determine the morphological types of anemia among school age children
- To determine the risk factors of anemia among school age children

# **CHAPTER FOUR: MATERIALS AND METHODS**

# 4.1 Study area and periods

The study was conducted from July16/2013 to August 14/2013 in Filtu town, Ethio-Somali region, South East Ethiopia.

Filtu is a zonal town for Liban zone of Somali region which located at the South-East part of the Ethiopia. It is approximately 725 Km away from the capital city of the country (Addis Ababa) and it is around 1, 190 Km away from the capital city of the Somali region (Jigjiga). Liben zone is bordered by Oromia from the west, by Afder zone of Somali from the East by Kenya from the South west, and by Republic of Somalia from the South east.

The climate in Filtu [Liben zone] is arid and it has a bimodal rainfall pattern with low annual rainfall and annual average temperature of 27°C to 29°C and an altitude of around 1300meters from sea level (47).

According to the recent census, the total population of Filtu woreda was 539,048 and population of the urban area comprised 46,634. Most of the communities are pastoralist and dependent on animal products for their consumption. The predominantly livestock-based economy has, for centuries, relied up on herding a primary stock of camels, flocks of sheep and goats, as well as the raising of cattle in settled agricultural areas where conditions are favorable (48, 49).

A small percentage of the communities are merchants, selling materials from the border of the country. And as a whole, most of the communities have ration distribution from the government through the means of work for food within their kebeles.

There are one district government hospital, two governmental elementary schools and one secondary and preparatory school in the town.

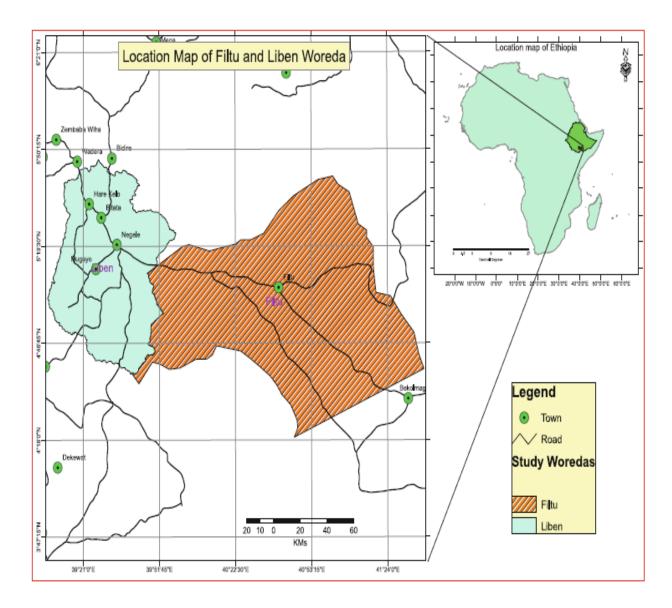


Fig -1: Location map of study areas (Liben Zone, Filtu Woreda, South-East Ethiopia) (50).

# 4.2 Study design

A community based cross sectional study was conducted to determine anemia and its risk factors among school age children in Filtu town.

# **4.3 Source Population**

All school aged children of Filtu town

# 4.4 Study population

All randomly selected school aged children that fulfilled the inclusion criteria

## **Inclusion criteria**

• All of the school aged children between 5 -15 years old.

## **Exclusion criteria**

- Those children on treatment of anemia during data collection
- Children with chronic disease
- Children whose care taker refused informed consent were excluded from the study.

# 4.5 Sample size determination

The sample size for study was determined using single population formula by using the following assumptions:

Level of confidence taken was 95%, margin of error 5%, and P is 30% which taken from the study done in Somalia (22). Based on the above assumptions the actual sample size for the study was computed employing the formula:

$$n = \frac{(Z\alpha/2)^2 P (1-p)}{d^2}$$

Where:

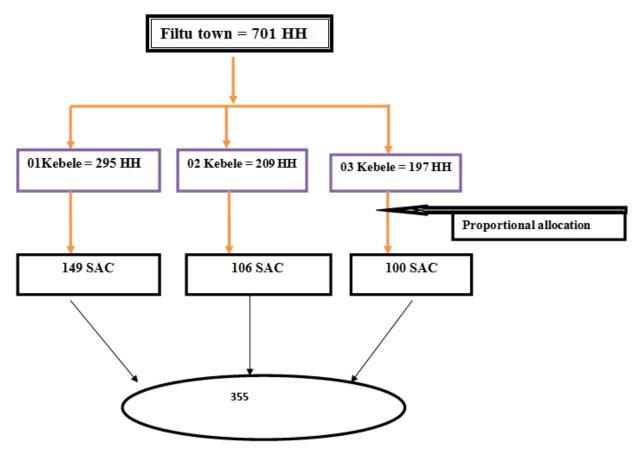
n= sample required Z= 95% confidence interval (1.96) D= margin of error (5%) P= prevalence rate (0.3)

Then  $n = (1.96)^2 (0.3*0.7) = 323$ (0.05)<sup>2</sup>

By considering 10% of non response rate which is calculated to be 32 So, the total sample size was <u>355</u>

#### 4.6 Sampling technique

Systematic sampling technique was used by taking house number as sample frame which obtained from Filtu town health extension workers. The town has three kebeles with the total household contents of the three kebeles, 01, 02 and 03 kebele were 295, 209 and 197, respectively. First proportional allocation was done for the three kebeles'. After that systematic sampling method was used from house to house. This means 'K' was calculated which is the interval size between the houses; K=N/n, after this, random selection of an integer between 1 to k was done which is the starting point. Finally, from systematically selected house hold, one school age children was selected. But, for those children more than one per house hold one individual was included by lottery methods.



HH= House hold

Fig 2: Flow of sampling techniques of school age children in Filtu town, Somali region, South East Ethiopia, 2013.

## 4.7 Data collection

Socio-demographic and socioeconomic data of the children and their parents were collected via pre-tested questionnaire.

## **Hemoglobin Determination**

Hemoglobin (Hgb) concentration was determined by Hemocue HB 201+ analyzer (HemoCue, Angelholm, Sweden). The blood sample was collected by finger pricking after rubbing the finger tip with sterile cotton (immersed in 70% alcohol), and pricking it with a sterile disposable lancet. A drop of blood was allowed to enter the optical window of the microcuvette through capillary action. The microcuvette was placed into the cuvette holder for photometric determination of hemoglobin level. Then, the concentration of hemoglobin level was quantitatively determined and read in g/dl (**Annex-I**).

School age children with hemoglobin levels lower than 11.5g/dl and 12g/dl was considered as anemic for age ranges of 5-11 and 12-15 years respectively. Mild anemia was defined as an Hgb of 10-11.9 g/dl in males 12-15 years and an Hgb of 10-11.4 g/dl in males 5-11 years and females. Moderate anemia was defined as an Hgb of 7-9.9 g/dl and severe anemia as an Hgb of < 7 g/dl in both males and females (1).

#### **Stool collection and examination**

The children were provided with clean, leak-proof stool cup and clean wooden applicator stick for stool specimen collection. The children were informed to bring about 2mg stool sample of their own. The stool samples were processed for microscopic examination using both direct saline method and formol-ether concentration techniques (**Annex-II**).

## Blood film for Red blood cell morphology assessment

Two (2) thin blood films were prepared, stained with Wright stain and examined for RBC morphology. The anemia was typed by looking the red cell size, and hemoglobin content (color) (Annex-III).

#### Blood film examination for diagnosis of Hemoparasites

Thick and thin blood films were prepared by collecting blood from finger prick and stained with Giemsa stains. The thick blood film provides enhanced sensitivity of the blood film technique and used for detection of low levels of parasitemia. The thin blood film was fixed with methanol and stained with diluted Giemsa-stain using buffered water at pH 7.2 to emphasize the parasite inclusions in the RBC which used for morphological identification of the parasite to the species level and it provides greater specificity than the thick-film examination (**annex-IV**).

#### Anthropometric measurements

Anthropometric measurements of height and weight were measured with children in light clothing. Weight measurement was taken using a portable digital scale to the nearest 0.1 kg. A fixed base portable wooden constructed scale carefully calibrated was used for height measurement to the nearest 0.1 cm. Every measurement was performed two times, and the mean values were used for analysis.

All the data were transformed and expressed in Z-scores and calculated using WHO anthroPlus1.0.4 statistical packages program for ages 5–19 years (WHO, Geneva, Switzerland with reference growth standards from the year 2007). The mean Z scores were calculated and under nutrition was defined for a child, who has less than-2 z-scores (-2SD) from the NCHS median reference population values. This was used as cut-off points for determination of malnourishment.

# 4.8 Study variables

## 4.8.1 Independent variables

- Socio-demographic and socioeconomics variables
  - Age of the children
  - Sex of the children
  - Family income
  - Family size
  - Educational status of the family

- Family occupation
- Nutritional status of the children
- Intestinal parasitosis.
- Malaria parasite infection

# 4.8.2 Dependent variable

Prevalence of anemia

# 4.9 Quality control measures

Training was given for data collectors before the start of data collection and daily supervision was made for the data collectors (laboratory technicians and nurses) to discuss the need for quality data. All the activities of the work was carefully monitored and supervised by principal investigator.

HemoCue photometer (HemoCue201+) was checked for calibration by using low level and high level Quality control solution each day prior to use according to manufacturer's quality control guideline. Precision of HemoCue was checked by repeated measure of 20 samples, it gave CV less than 5%.

For anthropometric testing, trained nurses were collecting the data. Trainees Performed replicate measurements of height (until agreement within 0.5 cm) on 10 healthy volunteer children on the same day. The portable digital weight scales was checked at the beginning of each working day against a standard of known kilo-gram stone which is kept for this purpose until the end of the study.

For evaluations of stained blood smear, both macroscopically and microscopically evaluation of Stained Blood films was performed. Macroscopically, blood films appear purplish when the stains are at neutral PH. If blue, the buffered water was too alkaline; if pink to red, the buffered water was too acid. Microscopically, low-power (10 X) scan was used to determine the overall quality of the blood smear and to scan the edge and the center the slide to be sure there are no clumps.

The formol-ether concentrated stool slides were examined microscopically by (10X and 40X) by the technician and, all negative and positive slides were immediately (within one hour) observed by another senior technician and principal investigator in Filtu district Hospital laboratory department.

Standard operating procedures (SOPs) were followed for all laboratory analysis.

#### 4.10 Data processing and analysis

Data were coded, cleaned prior to analysis, and then entered and analyzed using SPSS V16.0 statistical software. Cross tabulation was done for describing the socio economic and sociodemographic data and to determine the prevalence of anemia. Bivariate and multivariate analyses were done to look for association between the prevalence of anemia and independent variables. Ninety five percent (95%) confidence intervals were used and p-*value* less than 0.05 were considered as statistically significant.

#### 4.11 Operational definitions

School aged children – Is defined as, children whose age is between 5–15 years old (21).

**Anemia**- Is defined as a low level of Hgb in the blood which is < 11.5 g/dl for children aged 5 to 11 years and <12 g/dl for children aged 12-15 years, resulting in lower quantities of oxygen available to support the body's activities (1).

**Normocytic Norm chromic anemia**: The presence of normal size red cells (equal to the nucleus of small lymphocyte) with normal color (central pallor), in which Hgb filling cytoplasm when appeared in blood film

**Microcytic Hypochromic anemia**: The presence of small red cells (less than the nucleus of small lymphocyte) and with pale color with only rim of hemoglobin appeared in stained blood film.

**Macrocytic normochromic anemia**: The presences of large red cells (greater than the nucleus of small lymphocyte) and mostly normal hemoglobin filling cytoplasm when appeared in stained blood film.

## 4.12 Ethical considerations

Ethical clearance was obtained from Jimma University ethical review board and permission was obtained from Filtu woreda health bureau to conduct the study. After informing parents/caregivers about the objective of the study, written consent was obtained from the parents and oral assent was obtained from the children those greater than 7years old. Anyone who was not willing to participate excluded from the study. The results of the samples were kept confidential. Confidentiality was maintained by numeric coding of samples and questionnaires. At the end of the study, all the subjects found positive for intestinal and Hemoparasites were treated free of charge at Filtu district hospital.

# 4.13 Data dissemination plan

The result of the study will be disseminated to Jimma University College of public health and medical sciences, Filtu woreda health bureau and other concerned and interested organizations. Finally, publication of the research in local or international journals will be made.

# **CHAPTER FIVE: RESULTS**

#### 5.1 Descriptions of Sociodemographic characteristics

A total of 355 school age children were participated on the study. Out of 355 study participants, 187(52.7%) were males and 168(47.3%) were females. Majority of children 289(81.4%) belong to age group 5 to 11 years. The mean age of the children was  $8.5 \pm 2.8$ SDyears.

Majority of the children's mothers and fathers were illiterate which accounted 79.2% and 67.3%, respectively. Regarding family occupation, most of the mothers were house wife 217(61.1%), 69(19.9%) were merchant, 29(8.2%) were governmental employee. Fathers occupations status shows that majority 83(23.4% were daily laborer. With Regard to the families' monthly income which was classified based on from other study conducted in Ethiopia [56], indicated that 13(3.7%) earned <500 Ethiopian birr, 206(58.0%) earned 500-1999ETB and 136(38.3%) were earned >2000 ETB (**Table 1**).

# 5.2 Descriptions of nutritional status, intestinal parasites and malaria parasite infections

Anthropometrics measurement of the school age children showed that 117(33.0%) of the children were stunting for their age, 104(29.3%) were underweight, and BMI for age revealed that, 114(32.1%) of the children were low body mass index for their age (BMI below 5<sup>th</sup> percentile for their age) (**Table2**).

Stool examination showed that 29(8.2%) school age children were positive for intestinal parasites from which 23(6.5%) were *Ascaris lumbricoides* and 6(1.7%) were *Giardia lamblia*. Malaria parasite was detected and 13(3.7%) of the study children were positive for *plasmodium Vivax* but no *plasmodium falciparum* parasites were detected in the study area (**Table 2**).

| Variables             | Number (%  |  |  |
|-----------------------|------------|--|--|
| Sex                   |            |  |  |
| Male                  | 187 (52.7) |  |  |
| Female                | 168 (47.3) |  |  |
| Age                   |            |  |  |
| 5-11                  | 289 (81.4) |  |  |
| 12-15                 | 66 (18.6)  |  |  |
| Mother's occupation   |            |  |  |
| House wife            | 217(61.1)  |  |  |
| Merchant              | 69 (19.4)  |  |  |
| Governmental employee | 29 (8.2)   |  |  |
| Private employee      | 12 (3.4)   |  |  |
| Daily laborer         | 19 (5.4)   |  |  |
| Others                | 9 (2.5)    |  |  |
| Father's occupation   |            |  |  |
| Farmer/pastoralist    | 77 (21.7)  |  |  |
| Merchant              | 62 (17.5)  |  |  |
| Governmental employee | 66 (18.6)  |  |  |
| Private employee      | 40 (11.3)  |  |  |
| Daily laborers        | 83 (23.4)  |  |  |
| Others                | 27 (7.6)   |  |  |
| Mother's educations   |            |  |  |
| Illiterate            | 281 (79.2) |  |  |
| Primary school        | 36 (10.1)  |  |  |
| High school           | 17 (4.8)   |  |  |
| Collage and above     | 21 (5.9)   |  |  |
| Father's educations   |            |  |  |
| Illiterate            | 239 (67.3) |  |  |
| Primary school        | 41(11.5)   |  |  |
| High school           | 25 (7.1)   |  |  |
| Collage and above     | 50 (14.1). |  |  |
| Family size           |            |  |  |
| <5                    | 87 (24.5)  |  |  |
| >5                    | 268 (75.5) |  |  |
| Family income ETB*    | · · · ·    |  |  |
| <500                  | 22 (6.2)   |  |  |
| 500-1999              | 198 (55.8) |  |  |
| >2000                 | 135 (38.0) |  |  |

Table- 1: Sociodemographic characteristics' of school age children in Filtu town, Somali region, South- East Ethiopia, 20013 (n=355).

*ETB*\*= *Ethiopian birr* 

| Variables                 | Number (%) |
|---------------------------|------------|
| Stunting (Z score<-2SD)   |            |
| Yes                       | 117 (33.0) |
| No                        | 238 (67.0) |
| Underweight(Z score<-2SD) |            |
| Yes                       | 104 (29.3) |
| No                        | 251 (70.7) |
| Intestinal parasites      |            |
| Positive                  | 29 (8.2)   |
| Negative                  | 326 (91.8) |
| Malaria parasites         |            |
| Positive                  | 13 (3.7)   |
| Negative                  | 342 (96.3) |

Table 2: Descriptions of nutritional status, malaria and intestinal parasites of school age children of Filtu town, Somali region, South East- Ethiopia, 2013 (n=355).

## 5.3 Prevalence, severity and types of anemia

#### 5.3.1 Prevalence of anemia

The overall prevalence of anemia among the school aged children was 23.7%. The prevalence was high in males (27.8%) compared to female school aged children (19.0%). The Hgb ranged from 6.7 to 15.3g/dl with mean Hgb levels of  $12.4 \pm 1.4g/dl$ . The anemia prevalence was 26.0% among the age group 5-11 years old and 13.6% among 12-15 years age groups (**Table 3**).

The prevalence of anemia in children whose parents illiterate were 21.4% and 17.7% for mothers and fathers, respectively and anemia prevalence among children who had five or less family members was 9.2%, while for those with more than five family members was 28.4% (**Table 3**).

| Variables                     | Anemic N (%) | Non-anemic N (% |
|-------------------------------|--------------|-----------------|
| Sex                           |              |                 |
| Male                          | 52 (27.8)    | 135(72.2)       |
| Female                        | 32 (19.0)    | 136 (81.0)      |
| Total                         | 84 (23.7)    | 271(76.3)       |
| Age                           |              |                 |
| 5-11                          | 75 (26.0)    | 214 (74.0)      |
| 12-15                         | 9 (13.6)     | 57 (86.4)       |
| Total                         | 84(23.7)     | 271(76.3)       |
| Mother's occupations          |              |                 |
| House wife                    | 59 (27.2)    | 158 (72.8)      |
| Merchant                      | 10 (14.5)    | 59 (85.5)       |
| Governmental employee         | 3 (10.3)     | 26 (89.7)       |
| Private employee              | 3 (25.0)     | 9 (75.0)        |
| Daily laborer                 | 6 (31.6)     | 13 (68.4)       |
| Others                        | 3 (33.3)     | 6 (66.7)        |
| Father's occupation           |              |                 |
| Farmer /pastoralist           | 28 (36.4)    | 49 (63.6)       |
| Merchant                      | 11 (17.7)    | 51 (82.3)       |
| Governmental employee         | 9 (13.6)     | 57 (86.4)       |
| Private employee              | 7 (17.5)     | 33 (82.5)       |
| Daily laborer                 | 20 (24.1)    | 63 (75.9)       |
| Others                        | 9 (33.3)     | 18 (66.7)       |
| Mother's education            |              |                 |
| Illiterate                    | 76 (27.0)    | 205 (73.0)      |
| Primary school                | 5 (13.9)     | 31 (86.1)       |
| High school                   | 2 (11.8)     | 15 (88.2)       |
| Collage or above              | 1 (4.8)      | 20 (95.2)       |
| Father's educations           |              |                 |
| Illiterate                    | 64 (26.8)    | 175 (73.2)      |
| Primary school                | 10 (24.4)    | 31 (75.6)       |
| High school                   | 2 (8.0)      | 23 (92.0)       |
| Collage or above              | 8 (16.0)     | 42 (84.0)       |
| Family monthly income in ETB* |              |                 |
| <500                          | 11(50)       | 11 (50)         |
| 500-1999                      | 53 (39.3)    | 82 (60.7)       |
| >2000                         | 20 (10.1)    | 178 (89.9)      |
| Family size                   |              |                 |
| <5                            | 8 (9.2)      | 79 (90.8)       |
| >5                            | 76 (28.4)    | 192 (71.6)      |

Table -3: Distribution of anemia prevalence among school age children by socio-demographic factors in Filtu town, Somali regions, South- East Ethiopia, 2013(n=355).

ETB\*= Ethiopian birr

The prevalence of anemia was found to be 47.0% among stunted children and 41.3% among underweight children. Anemia prevalence among school age children positive of malaria parasites were 53.8% and 22.5% among children negative for malaria parasites. Concerning intestinal parasitosis, the prevalence of anemia among children infected with intestinal parasitosis (*Ascaris lumbricoides* and *Giardia lamblia*) were 41.4% (**Table 4**).

| Variables                 | Anemic     | Non anemic |  |  |
|---------------------------|------------|------------|--|--|
|                           | N (%)      | N (%)      |  |  |
| Stunting (Z score<-2SD)   |            |            |  |  |
| Yes                       | 55 (47.0)  | 62 (53.0)  |  |  |
| No                        | 29(12.2)   | 209 (87.8) |  |  |
| Underweight(Z score<-2SD) |            |            |  |  |
| Yes                       | 43 (41.3.) | 61 (58.7)  |  |  |
| No                        | 41 (16.3)  | 210 (83.7) |  |  |
| Intestinal parasite       |            |            |  |  |
| Positive                  | 12 (41.4)  | 17 (58.6)  |  |  |
| Negative                  | 72 (22.1)  | 254 (77.9) |  |  |
| Malaria parasite          |            |            |  |  |
| Positive                  | 7 (53.8)   | 6 (46.2)   |  |  |
| Negative                  | 77 (22.5)  | 265 (77.5) |  |  |

Table -4: Anemia prevalence by nutritional status, intestinal parasite and malaria infections among school age children in Filtu town, Somali region, South-East Ethiopia, 2013 (n=355).

## 5.3.2 Severity of anemia among school age children

The anemia was graded according to WHO standards which indicated that: the rates of mild, moderate, and severe anemia among the children were 62/84(73.8%), 21/84(25.0%) and 1/84(1.2%), respectively (**Figure 3**).

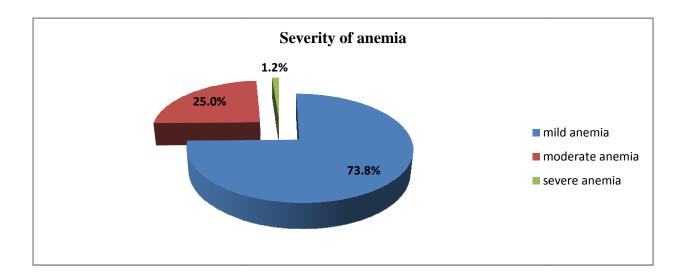


Figure-3: prevalence of severity of anemia among school age children in Filtu town, Somali region, South-East Ethiopia, 2013 (n=84).

# 5.3.3 Morphological types of anemia among school age children

The commonest blood picture was microcytic hypochromic anemia 49(58.3%) with few percent of target cell followed by normocytic normochromic anemia 34(40.5%) and only 1(1.2%) Macrocytic normochromic anemia was seen (**Figure 4**).

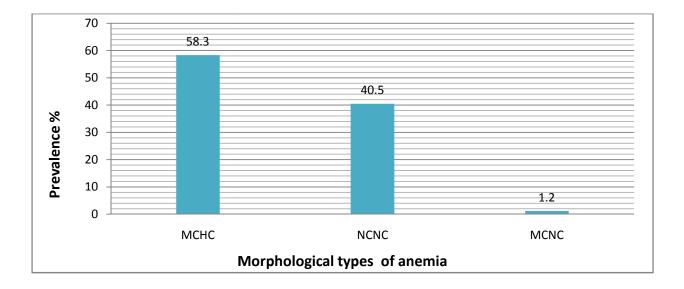


Fig 4: Morphological types of anemia among school age children in Filtu town Somali region south east Ethiopia, 2013.

## 5.4 Factors associated with prevalence of anemia

Prevalence of anemia among 5 - 11 age groups was 75(26.0%) and 9(13.6%) among 12 - 15 age groups which was statistically significant (p=0.037). Compared to boys, girls had a higher prevalence of anemia (27.8% compared to 19.0%) (**Table5**).

Anemia was also found to be more prevalent, which was 33.3% among children whose mothers have no occupation due to old age and body disability than those children whose mothers were governmental employee. Bivariate analysis showed that, no association of maternal occupation and prevalence of anemia among school age children which was not statistically significant (P>0.005). Regarding father occupations; being the children's fathers were governmental employee or private employee, the more children were protective (p<0.05). Concerning mothers educational level, even if high prevalence of anemia among children whose mothers illiterate (27.0%), mother's educational level was not significantly associated with anemia (P>0.005) and also, no significant association between father's educational status and prevalence of anemia (p>0.05) among the children (**Table 5**).

In regard to family size, the prevalence of anemia among children who had five or less family members was 8(9.2%), while for those with more than five family members was 76(28.4%). This difference was statistically significant (P<0.001) (**Table 5**).

Regarding family income, those children who came from low monthly family income (<500ETB) had high prevalence of anemia (50%) compared to those from high monthly family income (>2000 ETB) which 10.1%. The difference was statistically significant (P<0.001) (**Table 5**).

| Varia   | bles                  | Anemic    | Non anemic | COR*(95%CI)                      | P-value |
|---------|-----------------------|-----------|------------|----------------------------------|---------|
| Sex     |                       |           |            |                                  |         |
|         | Male                  | 52 (27.8) | 135 (72.2) | Ref(1)                           |         |
|         | Female                | 32 (19.0) | 136 (81.0) | 0.6 (0.30, 1.008                 | ) 0.054 |
| Age     |                       |           |            |                                  |         |
|         | 5-11                  | 75 (26.0) | 214 (74.0) | 2.2 (1.05, 4.70)                 | 0.037   |
|         | 12-15                 | 9 (13.6)  | 57 (86.4)  | $\operatorname{Ref}(1)$          |         |
| Mothe   | er's occupations      |           |            |                                  |         |
|         | House wife            | 59 (27.2) | 158 (72.8) | $\operatorname{Ref}(1)$          |         |
|         | Merchant              | 10 (14.5) | 59 (85.5)  | 0.7(0.28, 1.771)                 | 0.461   |
|         | Governmental employee | 3 (10.3)  | 26 (89.7)  | 0.5(0.10, 2.61)                  | 0.413   |
|         | Private employee      | 3 (25.0)  | 9 (75.0)   | 0.9(0.13, 5.22)                  | 0.849   |
|         | Daily laborer         | 6 (31.6)  | 13 (68.4)  | 1.5(0.44, 5.15)                  | 0.508   |
|         | Others **             | 3 (33.3)  | 6 (66.7)   | 0.9(0.13, 6.87)                  | 0.941   |
|         |                       |           |            |                                  | 0.741   |
| Fatha   | 's occupation         |           |            |                                  |         |
| Fattiel | Farmer /pastoralist   | 28 (36.4) | 49 (63.6)  | $\mathbf{D} \circ \mathbf{f}(1)$ |         |
|         | Merchant              | 11 (17.7) | 51 (82.3)  | Ref(1)                           | 0.017   |
|         | Governmental employee | 9 (13.6)  | 57 (86.4)  | 0.4(0.17, 0.84)                  | 0.017   |
|         | Private employee      | 7 (17.5)  | 33 (82.5)  | 0.3(0.12, 0.64)                  | 0.003   |
|         | Daily laborer         | 20 (24.1) | 63 (75.9)  | 0.4(0.15, 0.95)                  | 0.038   |
|         | Others**              | 9 (33.3)  | 18 (66.7)  | 0.6(0.28, 1.10)                  | 0.092   |
|         | oulors                | ) (55.5)  | 10 (00.7)  | 0.9(0.35, 2.21)                  | 0.777   |
| Mothe   | r's education         |           |            |                                  |         |
|         | Illiterate            | 76 (27.0) | 205 (73.0) | 7.4 (0.98, 56.20)                |         |
|         | Primary school        | 5 (13.9)  | 31 (86.1)  | 3.2 (0.35, 29.68)                |         |
|         | High school           | 2 (11.8)  | 15 (88.2)  | 2.7 (0.22, 32.23)                | 0.440   |
|         | Collage or above      | 1 (4.8)   | 20 (95.2)  | Ref (1)                          |         |
| Father  | 's educations         |           |            |                                  |         |
|         | Illiterate            | 64 (26.8) | 175 (73.2) | 1.9 (0.86, 4.31)                 | 0.114   |
|         | Primary school        | 10 (24.4) | 31 (75.6)  | 1.7 (0.56, 4.79)                 | 0.320   |
|         | High school           | 2 (8.0)   | 23 (92.0)  | 0.5 (0.09, 2.33)                 | 0.346   |
|         | Collage or above      | 8 (16.0)  | 42 (84.0)  | Ref (1)                          |         |
| Family  | y monthly income ETB  |           |            |                                  | 0.001   |
|         | <500                  | 11 (50)   | 11 (50)    | 8.9 (3.43, 23.13)                |         |
|         | 500-1999              | 53 (39.3) | 82 (60.7)  | 5.8 (3.23, 10.30)                | < 0.001 |
|         | >2000                 | 20 (10.1) | 178 (89.9) | Ref (1)                          |         |
| Family  | y size                |           |            | 0.2(0.12, 0.5c)                  | 0.001   |
|         | <5                    | 8(9.2)    | 79(90.8)   | 0.3(0.12, 0.56)                  | 0.001   |
|         | >5                    | 76(28.4)  | 192(71.6)  | Ref (1)                          |         |
|         | /5                    | . ,       |            |                                  |         |

Table -5: Sociodemographic factors associated with anemia among school age children in Filtu town, Somali region, South- East Ethiopia, 2013 (n=355).

COR\*= Crude odds ratio, ETB=Ethiopian birr; others\*\*=no job due to body disability and old age

The prevalence of anemia was high in the stunted children which accounted 55(47.0%) and in normal children 29(12.2%). It showed that school age children who were being stunted were 6.4 times more likely to be anemic (COR =6.4, 95%CI: 3.76, 10.88) than normal children and also anemia prevalence among underweight was high 43(41.3%) compared to normal children 41(11.5%) , the difference was statistically significant (COR=3.16, 95%CI: 2.16, 6.01, P<0.001)(**Table 6**).

Anemia prevalence among school age children positive of malaria parasite were 53.8%. Bivariate analysis showed that the association between malaria parasite and anemia was statistically significant (P=0.015).Concerning intestinal parasite, those children infected with *Ascaris lumbricoides* and *Giardia lamblia* were higher anemic 41.4% than those children not infected with intestinal parasitosis(22.1%), which was statistically significant (p=0.023) (**Table 6**).

| Variables                 | Anemic (%) | Non-anemic (%) | COR*(95%CI)      | P-value |
|---------------------------|------------|----------------|------------------|---------|
| Stunting (Z score<-2SD)   |            |                |                  |         |
| Yes                       | 55 (47.0)  | 62 (53.0)      | 6.4(3.76, 10.88) | 0.000   |
| No                        | 29 (12.2)  | 209 (87.8)     | Ref (1)          |         |
| Underweight(Z score<-2SD) |            |                |                  |         |
| Yes                       | 43 (41.3)  | 61 (58.7)      | 3.2(2.16, 6.01)  | 0.000   |
| No                        | 41 (16.3)  | 210 (83.7)     | Ref (1)          |         |
| Intestinal parasite       |            |                |                  |         |
| Positive                  | 12 (41.4)  | 17 (58.6)      | 2.5(1.14, 5.45)  | 0.023   |
| Negative                  | 72 (22.1)  | 254 (77.9)     | Ref (1)          |         |
| Malaria parasite          |            |                |                  |         |
| Positive                  | 7 (53.8)   | 6 (46.2)       | 4.0(1.31, 12.30) | 0.015   |
| Negative                  | 77 (22.5)  | 265 (77.5)     | Ref (1)          |         |

Table -6: Nutritional status, malaria and intestinal parasites associations with anemia among school age children in Filtu town Somali region, South- East Ethiopia, 2013 (n=355)

COR\*=Crude odds ratio, SD=standard deviation

### 5.4 Multiple logistic regressions of factors associated with anemia

Since there might be confounding variables, all the factors that had a p value of  $\leq 0.25$  on bivariate analysis were entered into the multiple logistic regression models to establish those that were independently associated with anemia. The variables included in the final model of logistic regression analysis were, sex of the children, age of the children, family size, family income, fathers' occupational status, nutritional status of the children, intestinal parasite infections and malaria parasite (**Table 7**).

In multivariate analysis by using multiple logistic regressions model, sex of the children, age of the children, fathers' occupational status, family size and malaria parasite were not retained in the final model. Factors like malnutrition (stunting and underweight), family income and intestinal parasites (*Ascaris lumbricoides* and *Giardia lamblia*) were remained independently risk factors of anemia.

Regarding family income, after controlling confounding effect the likely hood of being anemic among children whose families income <500ETB were 9.4 times higher than among children from their families monthly income >2000 ETB (AOR= (9.4, 95% CI: 2.88- 30.99, P<0.001). Similarly the odds of being anemic was 4.7 times higher among children from family income 500-1999 than family monthly income >2000 ETB (AOR = (4.7, 95% CI: 2.31, 2.31, P<0.001) (**Table-7**).

The odds of being anemic were 5.5 times higher among stunted children than among normal (AOR= 5.5, 95%CI: 2.83, 10.72, P< 0.001). Adjusted odds ratio revealed that School aged children who underweight were 2.1 times more likelihood of anemic than normal (AOR=2.1 95%CI: 1.06, 4.05, P= 0.034). Being infected with intestinal parasite increase the likelihood of anemia by 3 times than the non- infected children (AOR= 3.0, 95%CI: 1.05, 849, P= 0.040) (**Table 7**).

| Varia   | bles                  | COR* (95%CI*)     | <b>P-value</b> | AOR* (95%CI)      | P-value |
|---------|-----------------------|-------------------|----------------|-------------------|---------|
| Sex     |                       |                   |                |                   |         |
|         | Male                  | Ref (1)           |                |                   |         |
|         | Female                | 0.6 (0.30,1.008)  | 0.054          | 0.5 (0.1, 2.01)   | 0.100   |
| Age     |                       |                   |                |                   |         |
|         | 5-11                  | 2.2 (1.05,4.70)   | 0.037          | 2.2 (0.88,5.71)   | 0.091   |
|         | 12-15                 | Ref (1)           |                | Ref (1)           |         |
| Mothe   | er's occupations      |                   |                |                   |         |
|         | House wife            | Ref (1)           |                | Ref(1)            |         |
|         | Merchant              | 0.45(0.22, 0.95)  | 0.035          | 0.71(0.28, 1.771) | 0.461   |
|         | Governmental employee | 0.31(0.09, 1.06)  | 0.062          | 0.50(0.10, 2.61)  | 0.413   |
|         | Private employee      | 0.89(0.23, 3.41)  | 0.868          | 0.84(0.13, 5.22)  | 0.849   |
|         | Daily laborer         | 1.24(0.45, 3.40)  | 0.082          | 1.51(0.44, 5.15)  | 0.508   |
|         | Others **             | 1.34(0.32, 5.53)  | 0.687          | 0.93(0.13, 6.87)  | 0.941   |
| Father  | r's occupation        |                   |                |                   |         |
|         | Farmer /pastoralist   | Ref(1)            |                | Ref(1)            |         |
|         | Merchant              | 0.38(0.17, 0.84)  | 0.017          | 0.75(0.27, 2.05)  | 0.571   |
|         | Governmental employee | 0.28(0.12, 0.64)  | 0.003          | 0.45(0.15, 1.35)  | 0.156   |
|         | Private employee      | 0.37(0.15, 0.95)  | 0.003          | 0.39(0.12, 1.21)  | 0.100   |
|         | Daily laborer         | 0.56(0.28, 1.10)  | 0.038          | 0.85(0.36, 2.01)  | 0.704   |
|         | Others**              | 0.88(0.35, 2.21)  | 0.092          | 0.96(0.30, 3.13)  | 0.704   |
| Family  | y size                |                   |                |                   |         |
|         | <5                    | Ref (1)           |                | Ref (1)           |         |
|         | >5                    | 3.9 (1.80, 8.48)  | 0.001          | 2.2 (0.85, 5.74)  | 0.103   |
| Family  | y income              |                   |                |                   |         |
|         | <500                  | 8.9 (3.43, 23.13) | 0.000          | 9.4 (2.88, 30.99) | <0.001  |
|         | 500-1999              | 5.6 (3.23, 10.30) | 0.000          | 4.7 (2.31, 2.31)  | <0.001  |
|         | >2000                 | Ref (1)           |                | Ref (1)           |         |
| Stunti  | ng                    | 6.4 (3.76, 10.88) | 0.000          | 5.5 (2.83, 10.72) | <0.001  |
| Under   | weight                | 3.6 (2.10, 6.04)  | 0.000          | 2.1 (1.06, 4.05)  | 0.034   |
| Malar   | ia                    | 4.0 (1.31, 12.30) | 0.015          | 2.3 (0.58, 8.75)  | 0.242   |
|         |                       | 7.0 (1.31, 12.30) | 0.013          | 2.3 (0.30, 0.73)  | 0.242   |
| IP infe | ections               | 2.3(1.14, 5.45)   | 0.023          | 3.0 (1.05, 8.49)  | 0.040   |

Table -7: Multivariate logistic regressions of selected factors associated with anemia among school age children in Filtu town, Somali region, South- East Ethiopia, 2013.

CI\*= Confidence interval, COR\* = Crud odds ratio, AOR\*= Adjusted odds ratio, IP=intestinal parasites

## **CHAPTER SIX: DISCUSSIONS**

The result of the study showed that the overall prevalence of anemia among school aged children was 23.7%, suggesting that anemia is a public health problem among the school aged children in this area. No previous similar study was done in the study area; however, 11% prevalence was reported from Northern part of Ethiopia by Mahmud MA *et al* (46). The larger regional variation might be due to differences in geographical variation and difference in life style.

The prevalence of anemia in our study site is higher than those similar studies reported from different areas like, Egyptian children 12% by Barduagni P *et al* (36), among school age children in Kenitra Morocco 12.2% by EL Hioui M *et a l* (37) and among Sanliurfa, South-east Turkish children 5.4% by Koc A *et al* (34). The higher the prevalence might be due to low socioeconomic status and lower nutritional status of school aged children in the study area than those reported from elsewhere.

However, in our study the prevalence of anemia was lower than most similar studies conducted in developing countries in Africa and Asia. For example, anemia in Tanzania was estimated to affect 79.6% children by Tatala SR *et al* (42), 35.3% prevalence among children in Kenya by LeenstraT *et al* (44), 82.6% in Abia State, Nigeria by Onimawo IA *et al* (39). The lower prevalence of anemia in our study area compared to the other developing countries can be explained by the fact that malaria which one of the major causes of anemia were less prevalent (3.7%) in this study compared to the previous study. Onimawo IA *et al* reported that Malaria parasite, which, may also contribute to the etiology, and severity of anemia through several mechanisms including destruction of red blood cells, was confirmed in majority of the children (93.2%) with *Plasmodium falciparum* as the primary cause of severe malaria (39.8%) and also hook worm and shistosomiasis which is the major causes of anemia were not detected in our study area but they were the major cause for the study reported from Tanzania (42), and Kenya (43, 44).

Jain N et al (29) assessed the prevalence of anemia among 200 school children aged between 5 and 16 years from Rishikesh, Uttrakhand, India. They found that the prevalence of anemia was

56.5% and it was significantly higher in girls. In comparison with our results no significant difference between girls and boys in the rate of anemia is found.

Mild anemia prevalence was the highest (74.5%) in our study which might be due to data was collected from healthy school aged children.

More than half of the children presented with a blood picture of Microcytic hypochromic anemic (58.3%) and the other types included Normocytic normochromic 40.5% and 1.2% were Macrocytic. The higher Microcytic hypochromic anemia in our study group might be explained by high malnutrition rate among the children that may cause iron deficiency anemia. Our result was consistence with the study conducted in India among school children (31), which reported that the commonest blood picture was Microcytic hypochromic seen in 54.9%. The authors conclude that the most common cause could be nutritional defections 48.7%, followed by different worm infestation in 17.7%.

The prevalence of anemia changed according to socio-demographic characteristics of children and their families' which showed statistically significant differences for some sort of variables and not for others. For example low families' income was statistically significant AOR=9.44 95%CI :( 2.9, 31.0) because families living at or below the poverty level may not be get enough iron-rich foods and diets of children living in poor families is usually monotonous. This is similar with the study conducted among Students of Ningxia and Qinghai's Poor Counties of Rural China's by Luo R, et al (28).

Multiple logistic regression analysis showed that malnutrition was independently associated with anemia (stunting with AOR=5.50, 95% CI: 2.83, 10.72, underweight with AOR=2.07, 95% CI: 1.06, 4.05) this might be due to long term effect of low iron intake and micronutrient deficiencies. Our study in agreement with the study conducted in different areas like: Tanzania by Tatala SR et al (42), they reported that under weight significantly associated with anemia (p<0.05) by suggesting that the anemia found could be diet related which is long term effect of low iron intake and Vitamin A deficiency among the children.

Children with *Ascaris lumbricoides* were also shown in the present study to have raised likely hoods of having anemia this might be due to Ascariasis decreases the appetite, as a result decrees in nutritional intake among the children which leads to anemia. This is similar with the studies reported in elsewhere in Tanzania by Tatala SR et al (42) and the study in Edo state, Nigeria by Osazuwa et al (40). The explanation to their effect has been suggested to be *A. lumbricoides* do not suck blood but their association with anemia is nonspecific lowering of Hgb during infection-the so called anemia of chronic disease and Osazuwa et al reported that Ascariasis influences nutritional status but its impact on anemia is less clear. *Giardia lamblia* also significant association with anemia in the present study it might be due to heavy infections that causes dysentery and inflammation.

Concerning malaria, although literature indicates there is indeed a strong association of malaria with increased prevalence of anemia through several mechanisms including destruction of Red Blood Cells (40), our study did not find this association probably due to low prevalence of malaria in our study area, because the present study did not consider seasonal variation of malaria and *P. falciparum* which major cause of anemia reported in different literature were not detected in our study.

## Strength and limitations of the Study

Strength of the study:

- The results of this study are generalizable because it is community based study.
- The study tried to identify independent risk factors for the problem

Weakness of the study:

• Food habits of the children were not assessed which limit to interpret anemia in children is associated with insufficient intake of the micro nutrient or consumption of foods that inhibit iron absorption.

# CHAPTER SEVEN: CONCLUSIONS AND RECOMMENDATIONS

# 7.1 Conclusions

It is conclude that anemia is a moderate significant health problem among school aged children with the present prevalence of 23.7%. Majority of school age children were mildly anemic. More than half of the children presented with a blood picture of Microcytic hypochromic anemic (58.3%) and the other types included Normocytic normochromic anemic 40.5% and 1.2% were Macrocytic normochromic.

The results of this study showed that the factors such as: lower nutritional status (being stunted and being underweight), having intestinal parasite infections, socio economic status (low family income) were the factors contributing to the prevalence of anemia among school age children.

# 7.2 Recommendations

Based on the result obtained from this study the following recommendations were forwarded:

- Health education is required to motivate families and the children to prevent and control intestinal parasite infections through health extinction workers.
- Creating awareness of the communities on nutrition, role of healthy diet and consequences of anemia by woreda health bureau.
- Designing for prevention of anemia in school health programs, interventions for both nutritional deficiencies and parasitic infections should be considered by coordination of regional health bureau, woreda health bureau and local NGOs.
- Further studies are needed to consider evaluation of iron indicators like serum ferritin, serum transferrin and also evaluation of serum folate and serum Vit-B<sub>12</sub> etc.

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# ANNEXES

### Annex-I: HemoCue 201+ Hemoglobin Measurement

#### Principle

The HemoCue hemoglobin measures the concentration in whole blood as azidemethemoglobin, utilizing a microcuvette containing a dry reagent system and a dual wavelength photometer. Sodium desoxycholate lyses the erythrocytes, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to the ferric state to form methemoglobin. The methemoglobin then combines with azide to form azidemethemoglobin and is measured photometrically at two wavelengths, 570nm and 880nm (51).

### **Procedure one**

- Turn the photometer on using the switch in the back. The display screen should now read "Hgb."
- 2. Pull the black cuvette holder out to the insertion position which will be noted by a distinct stop. After about two seconds the screen should read "READY" with three flashing dashes.
- 3. Remove a cuvette from the vial and immediately replace cap tightly to avoid humidity damage to the remaining cuvette.
- 4. Hold the cuvette by the winged end and introduce the cuvette tip into the middle of the drop of blood. Avoid getting blood on the outside of the cuvette.
- 5. Analyze the filled cuvette(s) immediately and at the latest ten minutes after it has been filled.
- 6. Put the filled cuvette in the holder and push it in to the stop point.
- 7. After 15 45 seconds, the hemoglobin value is displayed in the window.
- 8. Discard the cuvette in an appropriate bio-hazard container.
- When the measurement series has been completed, push the photometer power switch to "Power Off" position.

## Annex II: Formol-ether concentration technique

## Principle

Faeces are emulsified in formol water, the suspension is strained to remove large faecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formol water. Faecal fat is dissolved in the ether (53).

## **Materials required**

Formol water, 10% v/v Diethyl ether or ethyl acetate Sieve (strainer) with small holes, preferably  $400-450\mu m$  in size

### Procedure

- Using a rod or stick, emulsify an estimated 1 g (pea-size) of faeces in about 4 ml of 10% formol water contained in a screw-cap bottle or tube.
- 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.
- 5. Stopper the tube and mix for 1 minute. If using a Vortex mixer leave the tube unstoppered and mix for about 15 seconds.
- 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.
- 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 re-suspend 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 40X objective to examine small cysts and eggs.
- 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.

# Annex III: Blood smear preparation and staining

## Principle of Wright's stain

The Wright's stain is a Romanowsky stain. A Romanowsky stain is any stain combination consisting of eosin Y or eosin B with methylene blue and/or any of its oxidations products. A cationic or basic dye (methylene blue or its oxidation products such as azure B), which binds to anionic sites and gives a blue-grey color to nucleic acids (DNA or RNA), nucleoproteins, granules of basophils and weakly to granules of neutrophils. An anionic or acidic dye such as eosin Y or eosin B, which binds to cationic sites on proteins and gives an orange-red color to hemoglobin and eosinophil granules (54)

### The wedge smears preparation procedure

- 1. Place a drop of blood, about 2 mm in diameter approximately inch from the frosted area of the slide.
- 2. Place the slide on a flat surface, and hold the narrow side of the non-frosted edge between your left thumb and forefinger.
- 3. With your right hand, place the smooth clean edge of a second (spreader) slide on the specimen slide, just in front of the blood drop.
- 4. Hold the spreader slide at a 30 angle, and draw it back against the drop of blood.
- 5. Allow the blood to spread almost to the edges of the slide.
- 6. Push the spread forward with one light, smooth, and fluid motion. A thin film of blood in the shape of a bullet with a feathered edge will remain on the slide.
- 7. Label the frosted edge with patient name, ID# and date.
- 8. Allow the blood film to air-dry completely before staining. (Do not blow to dry. The moisture from your breath will cause RBC artifact.)

## **Procedure of Wright stain's**

- 1. Place the air-dried and fixed smear film side up on a staining rack (two parallel glass rods kept 5cm apart).
- 2. Cover the smear with Wright stain and leave for 2-3 minute
- 3. Add equal the volume of pH 6.8-buffered water (i.e., the same number of drops as the stain)
- 4. Mix by blowing until a metallic sheen appears.
- 5. Allow the diluted stain to act for 3-5 minutes
- 6. Wash off the stain with running tap water/wash bottle
- 7. Don't tip off the stain, because this will leave a fine deposit covering the film.
- 8. Wipe the back of the slide clean and stand it in a draining rack for the smear to dry (head part down).
- 9. The blood film should appear neither too pink nor too blue (check the results microscopically)

# Annex IV: Thick and thin blood films on the same slide stains for malaria parasite Giemsa stains procedure (55)

- 1. Allow the thick film to air dry thoroughly
- 2. Fix air-dried thin film in absolute methanol by dipping the film briefly (two dips) in a Coplin jar containing absolute methanol. Be sure not to get the alcohol or its fumes on the thick film by slightly tilting the slide.
- 3. Remove and let air dry with the *thick film up*. Be sure slide is thoroughly dry before staining. Introducing even a minute amount of methyl alcohol into the stain dilution will interfere with the lysing of the RBCs in the thick films.
- 4. Stain the entire slide with diluted Giemsa stain (1:20, vol/vol) for 30 min. Place the slide in the stain, *thick film down* to prevent the debris caused by dehemoglobinization from falling onto the thin film or keeping the slides in a horizontal position.
- 5. Rinse the thin film by briefly dipping the film in and out of a Coplin jar of buffered water (one or two dips).
- 6. Let air dry in a vertical position with the *thick film down*.

# **Annex – V Questionnaires**

| Date of | sample | collection |
|---------|--------|------------|
|---------|--------|------------|

Age\_\_\_\_

| House | no |
|-------|----|
|-------|----|

| Sex: male $\Box$ female $\Box$ | temale $\square$ |
|--------------------------------|------------------|
|--------------------------------|------------------|

## Families Socio demographic factors

- 1. What is Mother's occupation?
  - (a) House wife
  - (b) Merchant
  - (c) Government employee
  - (**d**) Private employee
  - (e) daily laborer
  - (f) Others
- 2. What is Father's occupation?
  - (a) Farmer
  - (b) Merchant
  - (c) Government employee
  - (d) daily laborer e (private employee)
  - (e) Others
- 3. What is mother education level?
  - (a) Unable to read/write (Illiterate)
  - (**b**) Primary school
  - (c) High school
  - (d) College or above
- 4. What Father's Educational level?
  - (a) Unable to read/write (Illiterate)
  - (b) Primary school
  - (c) High school
  - (**d**) College or above

- 5. Number of family size\_\_\_\_\_
- 6. Average monthly family income \_\_\_\_\_in Birr

# Part II

# Laboratory investigation and anthropometry measurement

- 7. Hemoglobin value\_\_\_\_g/dl
- 8. Blood film for Malaria parasites
  - (a) Yes
  - (b) No
  - (c) If yes what species \_\_\_\_\_
- 9. What is the morphology of red blood cell?

## 10. Stool examinations for intestinal parasitosis

 $\Box$  Type of ova/cyst (specify) \_\_\_\_?

## 11. Anthropometry measurement

- □ Height\_\_\_\_ cm
- $\Box$  Weight \_\_\_\_\_ kg

# Questionnaire by Somali version

- Maalinkalaqaadeysheybarka\_\_\_\_\_
- Kodkacunuga\_\_\_\_\_
- Waxyabahakusaabsanreerkaaga\_\_\_\_\_

# 1. Shaqadahoyadaguriga?

- A) Gurijog
- **B**) Nagaadi
- **C**) Shaqaaledawladeed

## 2. Shaqadaaabbo

- A) Beerqode
- B) Bagaadi
- **D**) Xoogsade(shaqa u gaara)

# 3. Heerkaaqoontahooyada

| A) WaxbaakhriyiKarin,waxnaqoriKa | C) Dugsigasare                    |
|----------------------------------|-----------------------------------|
| in(waxbaanbaranin)               | <b>D</b> )Koleejiyowixiikasareeya |
| <b>B</b> ) Dugsigahoose          |                                   |

## 4. Heerkaaqoontaaabbo

| A)WaxbaakhriyiKarin,waxnaqori | C) Dugsigasare                   |
|-------------------------------|----------------------------------|
| Karin                         | <b>D</b> )Koleejiyowixiikasareey |
| <b>B</b> ) Dugsigahoose       |                                  |

- Lambarkaguriga\_\_\_\_\_
- Guga\_\_\_\_\_
- Jinsiga Rag □ Dumar □ •
  - **D**) Shaqo ii gaara
  - E) Xoogsato
  - **F**)Kuwo
  - **C**) Shaqaaladowladeed
  - **E**)Kuwakale

## а

5. Waa imisa tirade dadkaqoyskiinutiroahaan

6. Waaimisadhaqaalahabishiisoo gala qoysku? \_\_\_\_\_

# **Annex VI- Informed consent**

I am a post graduate student from the department of medical laboratory science and pathology, Jimma University. I am here to study anemia and related risk factor among school age children in your town (Filtu). I am requesting your children to participate in this study.

## **Study procedure**

During the study, the following will be done

- 1) You will be asked questions about your sociodemographic and socioeconomic factors.
- We shall collect samples of blood and stool from your child. During sample collection, strict aseptic measures will be used to make sure that your child does not get exposed to infection.

When your children are found positive for intestinal parasites, malaria and anemia, they will receive standard drugs free of charge. The information in your records is strictly confidential. Your participation in this study is completely voluntary and you can refuse to participate.

Do you understand what has been said to you? If not, you have the right to get proper explanation.

I, the, undersigned have been informed about the study objectives. I have also been informed that all the information is to be kept confidential and that I have the right to decline from or to cooperate in the study. Therefore, with full understanding of the study objective I agree to give the informed consent voluntarily to the researcher to identify the parasites and measure hemoglobin concentration and anthropometry of my children.

Study area\_\_\_\_\_

| Name         | Signature | _Date |      |
|--------------|-----------|-------|------|
| Investigator |           |       |      |
| Name         | Signature |       | Date |

## Annex VII: Consent form [Somali version]

Kusodacida kluseeyso anigoo magaceygaah\_\_\_\_\_\_ waxanke shaqeeyhaya sida amigo xog haruurinaya ka barteymac hadka quudinta iyo tig u oolijiyada ilmaga cuntada ;qaybtiisa quudinta laqaateyee Jimma University. Waxan ka warey saneyney hooyoyin ka sidaay uquudiyan uurku jirta iyo ilmaha yarku wasoo ay kujiraan qodobada aasaaskaah ,si'aan isugu biirin xogta lagameermanka ahee qorsheynta, diyaargarowoga kaleeboon si'aan u hormariwo waxqabdaka quudinta uurka jirta.Si aan uheluo ujeedadaan daacaduimaada iyo kaqayb qaadashada dhabta ah ka jowaabista ku aadan suaalhan kuwoo muhimah iyo aadna loo guriyaqay.

Awoodsiin: waxu doonayaa iwa weydiyoo suaalo ah kuwaasoo aad umaleneyso iney adag yihiin jawaabahooda. Magacaga laguma qori doona form kan ,lomaha istimaahlidoono khaadka qorshee kastaoo inaad shee gtey .Qorshee kasta oo aad nasiisey halkan aya lagu ilaalin.

Waxan akhiriyay foomkan arma waa la iigu kahariyay luuqad aan fahmey gabahaan xaalada kor lagu xusey. Sida darted waxa rajeyuyayaa ihaan kaga qeybqaato dhigashadan

| Saxiixa                |          |           |  |
|------------------------|----------|-----------|--|
| Magac malgaliyaha guud | Saxiixa_ |           |  |
| Magac wareystaha:      | Saxiixa: |           |  |
| Magac agaasinka:       | Saxiixa: |           |  |
| Wakhiiga la dhameeyay  |          |           |  |
| Mamulaha magacisa      | saxiixa  | Tarikhada |  |
|                        |          |           |  |

Hadi maya ugudub takale kaqeybqajayasha ayagoo qorayo sababta isaga/iyada udideen.

# **Annex VIII: Declaration**

I, the undersigned, declare that this thesis is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. Where other peoples work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

| Name of the Principal investigator | Signature | Date |
|------------------------------------|-----------|------|
| Bekele Gutema (BSc)                |           |      |
| Approval by Internal examiner      | Signature | Date |
| Head of the Department             | Signature | Date |