ASSESSMENT OF THE MAGNITUDEOF VITAMIN A DEFICIENCY ANDITS PREDICTORS AMONG PRESCHOOL CHILDREN IN ETHIOPIA

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Jimma, Ethiopia

ASSESSMENT OF THE MAGNITUDEOF VITAMIN A DEFICIENCY AND ITS PREDICTORS AMONG PRESCHOOL CHILDREN (6-59 MONTHS) IN ETHIOPIA

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Abstract

Background: Globally, VAD affects an estimated 190 million (33.3%) preschool age children. VAD is responsible for almost 6% of under five deaths in Africa and 8% in South-East Asia. However, currently there is limited information on the magnitude of VADand its associated factors using national level data.

Objective: The study was aimedto assess the national prevalence of vitamin A deficiency and its predictors among preschool children.

Method: A community based cross-sectional study was conducted from March to July 2015. A total of 1216 preschool children were taken. Data on the background characteristics of participants were collected using a structured interviewer administered questionnaire with a Samsung tablet 4. In addition, venous blood was collected in free trace metals tube for determination of serum retinol level. High performance liquid chromatography was used to analyse serum retinol and inflammation status was assessed using C reactive protein and Alpha 1 acid glycoprotein. Data were analysed using SPSS for windows version 20. Bivariate and multivariable logistic regression were used to select independent predictors of VAD. Variables with P-value of <0.05 were declared as statistically significant.

Results: The study found that the national prevalence rate of subclinical vitamin A deficiency (serum retinol < 0.7 µmol/L) was 25% and after adjusting for inflammation the prevalence of vitamin A deficiency became 18%.On multivariable regression analyses, rural residence AOR=[2.24(1.256-3.992)], being anaemicAOR= [1.52(1.089-2.123)], having acute inflammationAOR =[2.78(1.715-4.496)],having chronic inflammationAOR=[1.91(1.431-4.625)], and having diarrhoeal disease AOR=[1.57(1.024-2.396)] were significant predictors of VAD.

Conclusion and recommendation:The national prevalence of vitamin A deficiencyshowed, a moderate public health importance according to WHO criteria. Vitamin A deficiency was high among those preschool children's with anaemia, acute and chronic inflammation, diarrhoea, and living in rural areas.Enhancing the consumption of diversified diet through the health extension workers, health development army and other innovative ways and alsofood fortification is recommended.

Key words: Preschool children; Vitamin A deficiency; Retinol; inflammation.

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Acronyms and abbreviations

AGP	α-1-acid glycoprotein
CI	Confidence Interval
CRP	C–reactive protein
CSA	Central statistical agency
DDS	Dietary diversity score
DHS	Demographic and Health Survey
EA	Enumeration Area
ELISA	Enzyme Linked Immuno Sorbent Assay
ENMS	Ethiopian national micronutrient survey
HDA	Health development army
HEWs	Health extension workers
HPLC	High performance liquid chromatography
MOH	Ministry of Health
PPS	Probability proportional to size
PSC	Preschool-age children
PSU	Primary sampling unit
SPSS	Statistical package for social science
SRS	Simple Random Sampling
SSU	Secondary Sampling Unit
WAZ	Weight for Age Z-score
WHO	World health organization
WHZ	Weight for height Z-score
VAD	Vitamin A deficiency

Chapter One: Introduction

1.1 Background

Protein energy malnutrition and micronutrient deficiencies, continues to be a major health burden in developing countries and accounts for fifty percent deaths in children less than five years of age(1).

Vitamin A is an essential, fat soluble vitamin known needed by the human body for its very diverse physiological importance: proper immune function, promoting visual function, epithelial integrity (normal function of skin and mucous membranes), growth and development of bone tissue and supporting reproduction. Three different forms of vitamin A are active in the body: retinol, retinal, and retinoic acid. Collectively, these compounds are known as retinoids (pre formed vitamin A).Foods derived from animals source provide compounds (retinyl esters) that are readily digested and absorbed as retinol in the intestine(2).

World Health Organization (WHO) defines vitamin A deficiency as tissue concentrations of vitamin A low enough to have adverse health consequences, even if there is no evidence of clinical deficiency(3).Xerophthalmia is a clinical symptom/sign of VAD which with increasing severity, manifests as night blindness, conjunctivalxerosis and Bitot's spot, corneal xerosis and corneal ulceration/ keratomalacia if untreated ends up in partial or total blindness (4).

VAD is caused by insufficient intake of vitamin A food sources, poor absorption and transportation, and increased catabolism(5). In addition it can be due to poor living conditions which are typically expressed as low family income which limits their purchasing power particularly regarding food, or adverse sanitary conditions(6).

Preschool agedchildren, pregnant and lactating mothers, are the groups that are most vulnerable to this nutritional problem and its adverse health consequences. The reasons behind for the pre-schoolers, their nutritional demands are high, consumption of vitamin A rich foods and the dietary fat required for absorption can be limited, and infections can deplete their body reserves of vitamin A(7).

No single indicator can be reliably used to assess vitamin A deficiency. Different aspects of vitamin A status are assessed using clinical indicators, biochemical indicators, functional indicators and histological indicators(8).

Serum retinol is the recommended biomarker by the World Health organization. Circulating serum retinol is reduced in the presence of inflammation or infectious state and as a consequence results in an overestimation of the prevalence of vitamin A(9).

1.2 Statement of the problem

Vitamin A deficiency contributes significantly to morbidity and mortality from common childhood infection, even at sub-clinical levels. Studies suggest that illness and risk of death from some infections are increased even in children who are not clinically deficient but, whose vitamin A body store is depleted(10).Vitamin A deficiency is one of the main nutritional deprivations in children, and is the main cause of avoidable blindness in childhood(4).

Globally, Low serum retinol concentration (<0.70 µmol/l) affects an estimated 190 million (33.3%) preschool age children night blindness affects 5.2 million (0.9%) preschool age children.(3).

Vitamin A deficiency, as indicated by either night blindness or biochemical deficiency, is present in a moderate to severe degree in preschool-age children in 45 and 122 countries, respectively, out of the 193 WHO member states(3).

Approximately one third of the world's preschool-age population is estimated to be vitamin A deficient. Africa and South-East Asia are the region's most affected by VAD, where 44–50% of the affected preschool-age children live in South East Asia(3).

Vitamin A deficiency is endemic throughout Africa and is the leading cause of childhood preventable blindness, and contributory factors to child morbidity and mortality from infectious disease(11).

Vitamin A deficiency is responsible for almost 6% of child deaths under the age of 5 years in Africa and 8% in South-East Asia(12).

WHO regional estimates indicate that the highest proportion of preschool-age children affected by night blindness, 2.0%, is in Africa. This means that Africa has the greatest number of preschool-age children affected with night blindness (2.55 million), and corresponds to almost half of the children affected globally(3).

According to 2009 WHO estimate, the national prevalence of vitamin A deficiency among preschool children in Ethiopia is 46.1% and the prevalence of night blindness is 4.9%(3).

In Ethiopia, studies showed the national prevalence of sub clinical VAD among preschool children were 34.4 % in 2009 (13) and 37.7 % in 2010 (14)but in both studies the prevalence was not adjusted for sub clinical infection. The national prevalence rate of Bitot's spots and night-blindness was 1.7% and 0.8% respectively(14). Another community based cross sectional study conducted in Tigray found prevalence of night blindness was 1.2 % and Bitot's spot was 1.5 %(15).

The risk factors of vitamin A are context-specific (sociodemographic, cultural, environmental, etc.), variations in factors contributing to vitamin A deficiency exist among countries, regions, and localities, underlining the need to assess country/region/area-specific risk factors(13).

To address this devastating problemthe governmentof Ethiopia initiateddifferent measures.However, VAD is still a public health problem in the country and there is no current data on the serum retinol level which provide information on the current adjusted prevalence of VAD. Data are insufficient to show vitamin A status and associated factors among pre-school-age children. Therefore, this study will provide baseline information for policy makers, program planners, researchers and relevant stakeholders for planning, monitoring and evaluation of nutrition programs in Ethiopia.

Chapter Two:Literature review

2.1 Overview

According to World Health Organization (WHO), vitamin A deficiency defined as tissue concentrations of vitamin A low enough to have adverse health consequences, even if there is no evidence of clinical deficiency(3).VAD is defined to be of public health importance if the national prevalence reaches 15% using a serum or plasma retinol concentration < 0.7 μ mol/L.

2.2 Magnitude of Vitamin A deficiency and Associated Factors

Approximately one third of the world's preschool-age population is estimated to be vitamin A deficient; with highest prevalence (44-50%) being reported in regions of Africa and South-East Asia(16).

A cross-sectional population-based study done in the urban zone of 9 cities in the state of Paraiba, North eastern Brazil, on 1,211 children (6 to 59 months) showed that 21.8% of children had vitamin A deficiency, presence of a subclinical infectionwas found to be the significant variable(17). In this study only CRP was used.

A community-based cross-sectional study done in rural areas of West Bengal, India, in preschool children showed that 61% of Vitamin A deficiency and 0.6% prevalence of Bitot's spot,the median blood vitamin A was 16.6 μg/dl, age group of the childrenfound to be the significant variable with the prevalence of Bitot's spots(18). However in this study sub clinical infection was not assessed and vitamin A level was determined from dried blood spot (DBS).

A communitybased cross-sectional study conducted in Aligarh District,India, on 3571 children aged between 0 to 60 months from six villages and four peri urban areas, prevalence of xerophthalmia was 9.1%,vitamin A containing foods,and nutritional status (wasting) were found to be the significant predictors(19).

In another cross-sectional study on a sample of 228 children, 12 - 16 months of age in Primary Health Care units of Goiân, Goiás, Brazil, VAD was14.0%, while median serum retinol concentration was 1.3μmol/L (20).In this study maternal schooling, haemoglobin and

CRP were found to be the significant variables, and sub clinical infection was assessed only by using CRP.

In a cross-sectional study carried out on 101 infants, aged 18-24 months in the urban area of Viçosa city, Southeastern Brazil, prevalence of vitamin A deficiencywas39.6%, and median serum retinol was 21.8µg/dl, this study reported thatfather's educational level, number of residents in homeand child's age were significant predictors associated with VAD(21).

In a survey conducted in the Republic of the Marshall Islands involving 281 children, 141 boys and 140 girls, aged 1–5 years, 8.5% had night blindness and 52% had serum retinol <0.70 µmol/L. Of 248 children who had both AGP and CRP measured, 49.6% had inflammation (22).

A cross-sectional study carried out among 303 children aged 1-3 years in Bungoma district - western Kenya showed that 76.6% had vitamin A deficiency (serum retinol <0.7µmol/l), conjunctival xerosis was detected in 168(59%), 5(2%) had Bitot's spots(23), in this study VAD wasn't adjusted for sub-clinical infection.

A community-based cross sectional survey among 1,630 children (aged 6-23 months) was undertaken in 2015 in western Kenya, 20.1% of the children were found to have vitamin A deficiency,age of children, and vitamin A capsule intake were found to be significantly associated(24). In this study retinol level was assessed by using RBP from dried blood spots and only CRP was assessed.

A population based cross-sectional study was done on national sample of 1,350 children between the age group of 0-6 years in 2009, 34.4 % were noticed to have vitamin A deficiency from the nine regions in Ethiopia, vitamin A supplementation, family size, illness over two weeks prior to the study, vaccination status, and religion weresignificantly associated (13).In this study sub-clinical infection (CRP and AGP) were not measured.

A population based cross sectional study on national sample of 23,148 children between 6 and 71 months of age for clinical examination and 1,200 children for blood sample.The national prevalence rate of Bitot's spots and night-blindness was 1.7% and 0.8% respectively.The national prevalence rate for sub clinical vitamin A deficiency was 37.7%,

prevalenceof Bitot's spots among children wassignificantly associated with sex, residence, and age(14).In this study sub clinical infection (CRP and AGP) were not determined.

A community based cross sectional study was conducted in northern Tigray from January 27 to March 7, 2014, in a total of 1230 preschool childrenaged from 24 to 59 months. The prevalence of night blindness was1.2 % and Bitot's spot was 1.5 %, mother's literacy status, family size and sex of the child were found to be significantly associated with Bitot's spot(15).in this study sub clinical VAD was not assessed.

2.3 Significance of the study

Vitamin A deficiency, which receives little attention in the face of overt hunger, is a major factor in the morbidity,mortality, and physical deformities among pre-school children. Vitamin A deficiency in Ethiopia is a cause for economically and socially significant problems that could potentially cost the country enormous human capacity and economic loss unless a major action is taken.Preschool children are one of the most vulnerable group of population to VAD.

Cognisant of the aforementioned facts, the government of Ethiopia has developed a nutritional guideline for prevention and control of micronutrient deficiencies since 2005 and interventions including universal biannual supplementation and diseases targeted supplementation of preschool children 6-59 months and behaviour change communications on the importance of consuming vitamin A friendly foods has been going on through the health extension workers and recently through the health development army.

However, there is no current data on the serum retinol level which provide information on the current adjusted prevalence of VAD at the national levels in Ethiopia. Data are insufficient to show vitamin A status and associated factorsamong pre-school-age children. Therefore, this study will providebaseline information for policy makers, program planners, researchers and relevant stakeholders for planning, monitoring and evaluation of nutrition programs in Ethiopia.

2.4 Conceptual framework

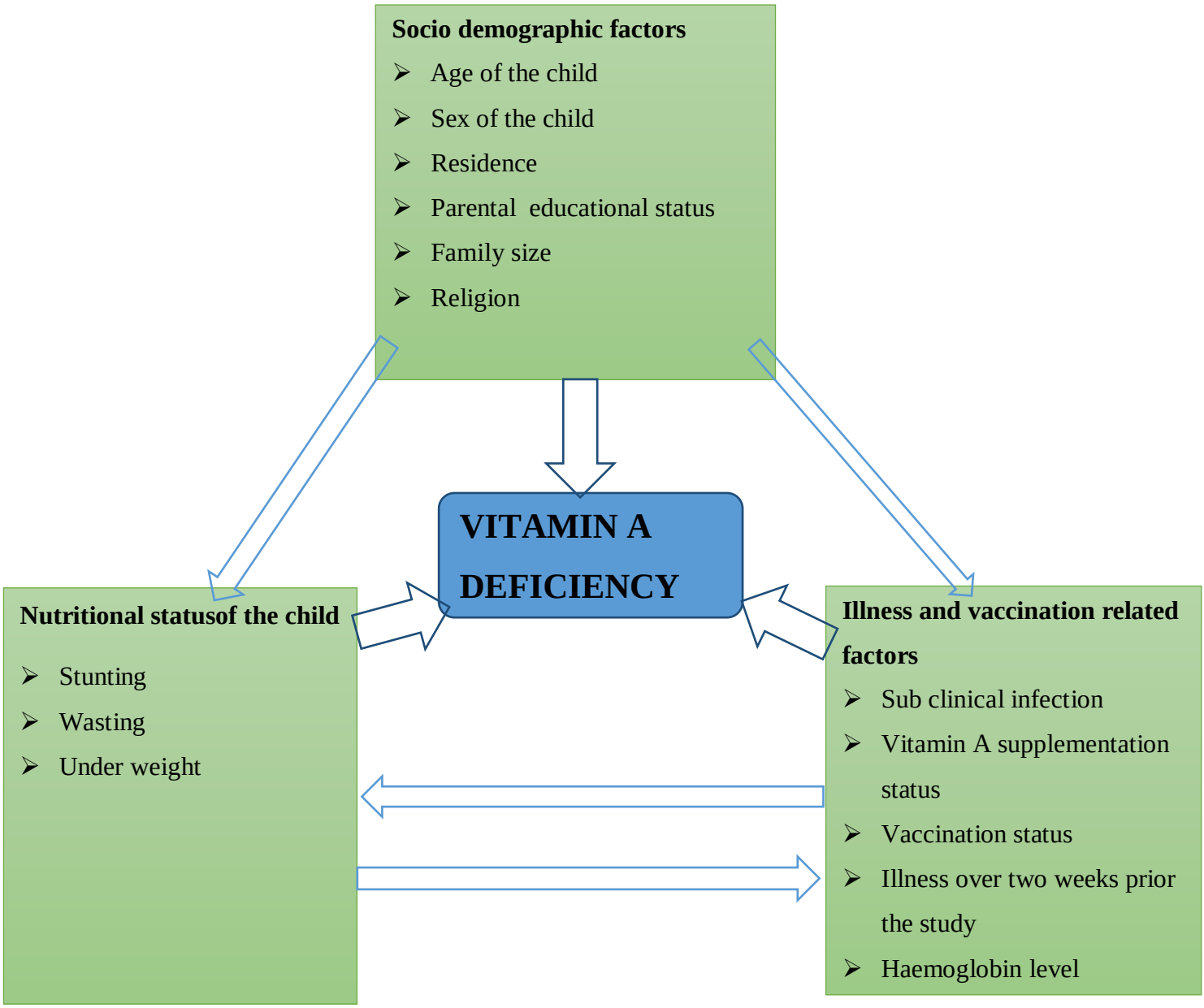

Figure 1**:** Conceptual frame work developed by the investigator after review of different literatures

Chapter Three: Objective

3.1 General objective

- To assess the national prevalence of vitamin A deficiency and its predictors among preschool children in Ethiopia.

3.2 Specific objectives

- Todeterminethe prevalenceof vitamin A deficiency among preschool children in Ethiopia.
- To identifypredictors ofvitamin A deficiency among preschool children in Ethiopia.

ChapterFour: Method and material

4.1 Study area and period

Data for this study comes from the national micronutrient survey commissioned by the Federal Ministry of Health to determine the national prevalence of micronutrient deficiencies in Ethiopia. The study was coordinated by the Ethiopian Public Health Institute with the involvement partners from various universities abroad and it is part of a PhD work. The Federal Democratic Republic of Ethiopia is a landlocked, mountainous and developing country located between 33 °and 48 °East longitudes and 3° and 15° North latitudes. Ethiopia is characterized by a rugged and mountainous topography with altitudes ranging from 4,620m above sea level at Mount Ras Dejen in North Gondar to110m below sea level at the Dallol depression in Afar.Ethiopiawith a total area of over 1.1 million kilo meter square, is the second most populated country in Sub-Saharan Africa.The country is divided into 9 regions and two city administrations. The population is very young and is one of the least urbanized in the world(25,26). This study is part of the national micronutrient survey and was carried out from March to July 2015.

Source:Wikipedia, Administrative regions and zones of Ethiopia Available at: https://en.wikipedia.org/wiki/Ethiopia

Figure 2: Map of Ethiopia

According to the projection by CSA, Ethiopian population for the year 2015 was 90,078,005 of which 45,250,993 are males and 44,827,012 are females. And concerning pre-school children, 6,656,641 are males and 6,447,968 are females making 13,104,609 the total preschool children in Ethiopia(27).

4.2. Study design

A population based cross-sectional study design was used.

4.3. Population

4.3.1 Source population

All mothers or care givers with preschool childrenfound in Ethiopia.

4.3.2 Study population

All mothers or care givers with preschool children found in randomly selected households in Ethiopia.

4.3.3 Study unit

Selected pre-school child.

4.4 Inclusion and Exclusion criteria

Inclusion criteria

All mothers or care givers with preschool children's aged between 6 – 59 months.

Exclusion criteria

Critically ill mothers/Care givers.

Critically ill children and children's with gross physical deformities.

4.5 Sample size determination and sampling procedure

4.5.1 Sample size determination

To determine the minimum sample size required for prevalence studies single population proportion formula was used:

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

Where;

N = Sample size

$Z_{\alpha/2}$ = Standard normal variable corresponding to the 95% confidence level = 1.96

P = Prevalence = 37.7(14)

D = Margin error (precision) = 0.04

DEFF = Design effect = 2

NRR = Non response rate (%)= 10%

N = $\frac{1.96*1.96*0.377(1-0.377)}{0.04*0.04}$ *2= 1128 then by adding 10% NRR (113) = 1241

So the final sample size was 1241.Since this study is part of the mega project on ENMS in which a total of 2200 preschool children's were approached and after final data collection because of non-response and other technical issues a total of 1216 preschool children became ready for analyses and to meet this specific study objective all data were analysed.

4.5.2 Sampling procedure

The sampling frame used for the 2015Ethiopian national micronutrient survey wasthe list of Central Statistics Agency (CSA) enumeration areas (EAs) from the 2007 Ethiopia Population and Housing Census. The EAs were developed through a cartographic mapping conducted between 2005 and 2007. Each EA contains 150 to 200 households in rural and urban areas as a measure of size.

The nine regional states and two city administrations in Ethiopia were treated as separate strata, allowing for national parameter estimates, as well as for estimates by region as there may be regional differences in micronutrient deficiencies.

For the first stage of sampling in each region or city administration, EAs (PSU) was randomly selected using fourth root probability proportional to size (PPS) method. At the second stage of sampling, one segment (division) of EA(SSU) was taken by simple random selection. At the third stage of sampling, eleven Householdstertiary sampling units were selected from all eligible households listed in each segments of EA using simple random selection and finally all preschool children were studied in the selected households. Prior to the actual survey, each selected EA was visited by a team to conduct a listing of all the HHs within the boundary of each selected EA. EAs was further segmented in to locally known smaller geographic units of 40-60households or less and one segment was randomly selected for the survey. In each household, a census of the people living in the household was conducted. The data from this exercise was used for random selection of households.

Table 1: The total number of EAs and HHs selected for each region for the research on the assessment of vitamin A deficiency among preschool children in Ethiopia, 2015

Region/City Administration	Number of EAs per regions	Number of selected HH per regions
Tigray	36	396
Afar	29	319
Amhara	44	484
Oromiya	46	506
Somali	26	286
BenishangulGumuz	28	308
SNNPR	42	462
Gambela	27	297
Hareri	27	297
Addis Ababa	34	374
DereDawa	27	297
Total	366	4026

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Sampling procedure

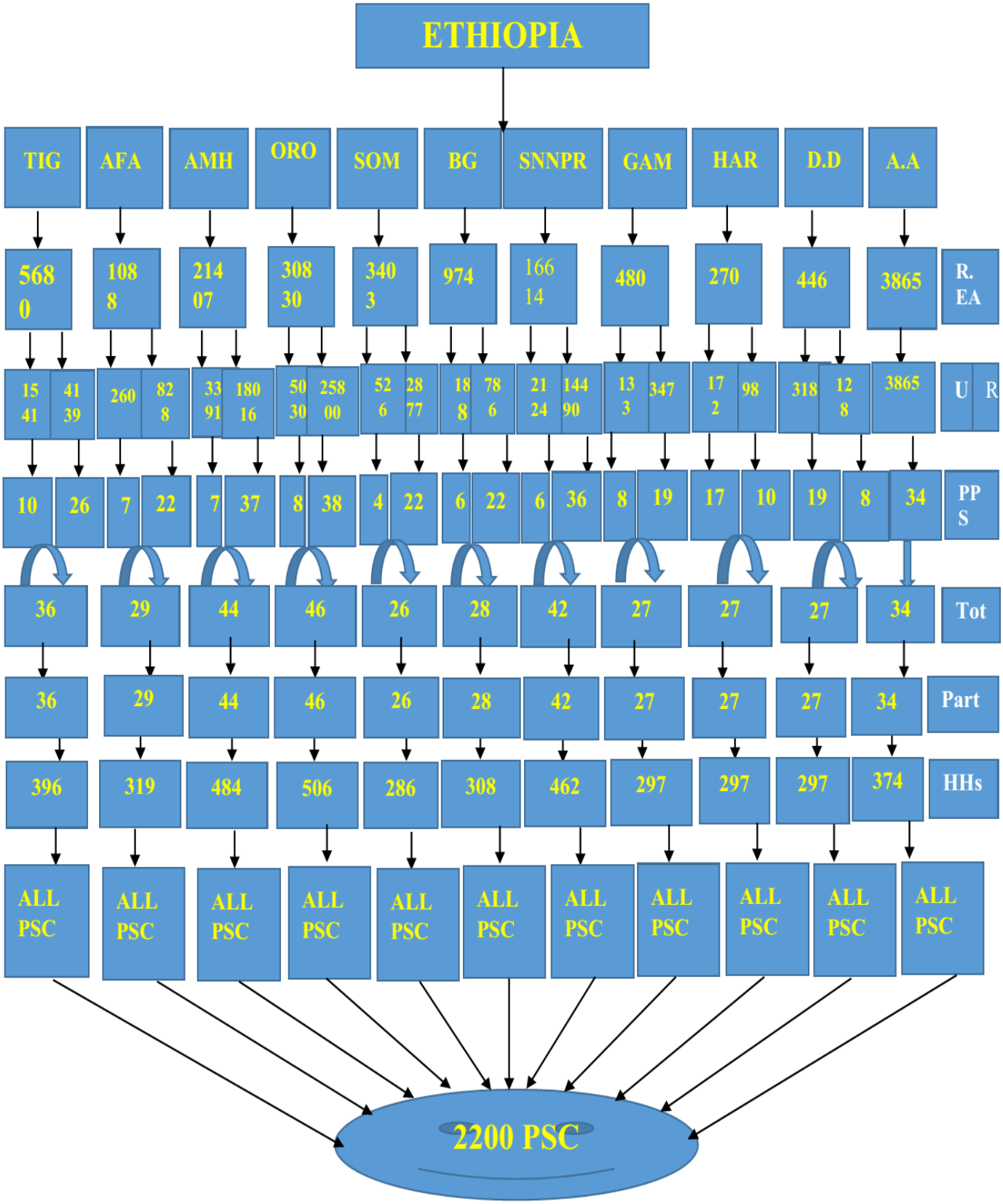

Figure 3**:** Schematic presentation showing the sampling procedure

4.6 Study variables

Dependant variable

- Sub clinical vitamin A deficiency (Serum retinol level <0.7µmol/L)

Independent variables

Socio demographic factors

- Age of the child
- Sex of the child
- Residence
- Parental educational status
- Family size
- Religion

Nutritional statusof the child

- Stunting
- Wasting
- Underweight

Illness and vaccination related factors

- Vitamin A supplementation status
- Vaccination status of the child
- Sub clinical inflammation
- Illness over two weeks prior the study
- Haemoglobin level

4.7 Data collection tool and technique

4.7.1 Data collection tools

Data on the background characteristics of the study participants were collected using a structured interviewer administered questionnaire with a Samsung tablet 4(Open data kit program).All the mothers or care givers were interviewed for their socio demographic information, vaccination/supplementation related factors and illness related factors. Anthropometric measurements and biochemical specimens were taken at end of the interview from the preschool children.

Vitamin A supplementation status, vaccination status (measles and polio) were assessed for the last two weeks. Illness were assessed with fever, diarrhoea and cough for the last two weeks.

Anthropometric,clinical and biological specimen measurements

Height and weight of each child was taken using standardized and calibrated equipment. Weight was measured with light clothing, no shoes and recorded to nearest 0.1 kg using Seca 874U electronic scale with dual display (Germany). Length/Height was measured with no shoes, pins and braids from the hair that could affect the measurement and recorded to the nearest 0.1cm.Height was measured with the head of participants at the Frankfurt Plane, knees straight and the heels, buttocks and the shoulders blades touching the vertical stand of the stadiometer and length was measured in a recumbent position on a portable measuring board with a fixed head piece and an adjustable foot piece. Anthropometric indices of length/height-for-age, weight-for-age and weight-for-length/height wasdeveloped for the children aged 6 to 59 months using current WHO growth standards(28).Then all indices were converted to indicator using the WHO cut-off values of < -2 SD for malnutrition.

Biochemical assessment

A venous blood sample of six ml was collected using a vacutainer from the cubital area. At the time of the sample collection, the unique individual ID number with each respondent's ID was affixed to the questionnaire and vacutainer. Universal precaution was undertaken during biological sample collection. Serum retinol was analysed using the high-performance liquid chromatography (HPLC) technique. The internal standard retinyl acetate is added to serum sample, which is then rapidly deproteinised and precipitated using 100% methanol. The extracting solvent (HPLC grade Hexane) is added and the mixture is then subjected to vortex for one minute and centrifuged at 3000rpm for 2 minutes. The supernatant upper layer was removed and kept in a second tube. The extraction was repeated and the two extracts were

evaporated at 37°C water bath under a gentle stream of white spot nitrogen gas. The residue was reconstituted with 100µL of the mobilized phase and 30µL is injected into HPLC column. Serum retinol was calculated from a standard curve by linear regression.

Roche Immunoassay kits for analysis of Acute Phase Proteins (CRP, AGP)

C-reactive protein (CRP) and α-1 Acid glycoprotein (AGP) are acute phase proteins which are markers of inflammation, there level were assayed and measured with the immune-turbidimetry method using Roche Immunoassay kits and the P-module of the Roche eCobas Integra 400 clinical analyser. Both were used to adjust or eliminate the results of some nutritional tests (retinol, retinol binding protein (RBP) and ferritin).

Vitamin A deficiency: children with serum retinol level below 0.70 µmol/L (29). And in whose children with inflammation retinol value was adjusted to either AGP or CRP. Those inflamed children retinol values were adjusted with this equation, retinol adjusted = original retinol value – (beta coefficient of AGP or CRP * AGP or CRP value).

Values of C-reactive protein higher than 5.0 mg/L and α-1 acid glycoprotein higher than 1.0 g/L was taken as acute and chronic inflammation(30).

Haemoglobin level was measured by using a Hemo-Cue (Hb-201) instrument.

Anaemia is defined as when a child's haemoglobin value of less than 11g/dl and haemoglobinvalue was adjusted for those children living in altitudes 1000 meters and above(31).

$$\text{Hb adjustment} = -0.032 \times (\text{altitude} \times 0.0032808) + 0.022 \times (\text{altitude} \times 0.0032808)^2$$

4.7.2 Data collection technique

Eighteen Teams or a total of around 191 different health professionals conducted the survey in the field.Out of which 11 were regional coordinators, 18 overall quality control supervisors, 36 Enumerators, 18 Enumerators supervisors, 36 Laboratory technician, 18 Laboratory supervisors, 54 were drivers and there were also local guides. Each team were consist of one Enumeration Sub-Team, one Laboratory (biological sample collection) Sub-Team, one overall Quality Control Supervisor, and a driver. Each team were responsible for completing 18 - 22 clusters.

- The Enumeration sub-team was consist of one enumeration supervisor and two enumerators (interviewers). This team conducted interviews.
- The Laboratory sub-team was consist of one lab supervisor and two lab technicians. This team collected all the biological samples collection and perform anthropometry measurements. The laboratory sub-team was responsible for maintaining the cold chain and processing biological specimens in the field as necessary.
- The Quality control supervisor conducted community sensitization and oversee the quality of the data, specimen collection and cold chain in each cluster. This person went to the next cluster first (one sub-team may remain in a cluster to complete a final call-back interview or specimen collection while the other team moves to the next cluster).

A unique household barcode and individual ID number were assigned to each respondent consenting to sample collection.

The child's mother or caretakerrespond to the questionnaires on behalf of the children. All children aged 6-59 months in all eleven selected households in each cluster were eligible to participate in the survey.When eligible occupants of a house were not present, two return visits to the household were made. If no eligible respondents were available, selected households was not be replaced.

4.8 Standard and Operational definition

Acute inflammation: C-reactive protein greater than 5.0 mg/L(32).

Anaemia: A child with haemoglobin value less than 11g/dL and whose haemoglobin level is adjusted for altitude if he lives in higher altitude than 1000 meters(31).

Chronic inflammation: α-1-acidglycoprotein greater than 1.0 g/L(32).

Enumeration Area (EA): is a unit of land delineated for the purpose of enumeration housing units and population without omission and duplication. An EA usually consists of 150 to 200 households in rural and urban areas.

Household (HH): is defined as a group of people who share a common cooking pot.

Parental Educational status: Educational status of the mother or father (mother's or father's).

Preschool children: A child whose age is 6 – 59 months.

Stunted: A child with height for age z-score < –2 SD.

Sub clinical infection: when there is either acute or chronic inflammation.

Underweight: A child with weight for age z-score < –2 SD.

Vitamin A deficiency (VAD): Children with serum retinol level of less than 0.70 µmol/L and whose retinol value is adjusted to either AGP or CRP(8).

Wasting (Thinness): A child with weight for height z-score <–2 SD.

4.9 Data Processing and Analyses

Data collection was conducted using Samsung tablet 4 and sent to the EPHI server every night up on completion of every household and reviewed by supervisors. All the data (questionnaires, anthropometry and laboratory) were taken from the server with CSV data extension andwasexported to SPSS for windows version 20.Then data were cleaned and analysed using SPSS for windows version 20.Anthro plus was used to analysed anthropometric data. Data backup was done regularly to avoid any loss.

Data analyses wasconducted in two steps. First, descriptive statistics was used to examine the frequency and distribution of preschool children's sociodemographic, illness, vaccination and nutritional status related characteristics.

Secondly,chi square test was used to check for assumptions, then both binary and multivariable logistic regression analyses were conducted. Binary logistic regressionwas done to determine the association between the dependent& independent variables. Variables with p-values are < 0.25onbivariate logistic regression analyses were entered to the multivariable logistic regression analyses to identify an independent effect of the variables that showed significant association with vitamin A deficiency. The Hosmer and Lemeshow's goodness-of fit test wasinsignificant. Multicollinearity was also checked. To evaluate the association between VAD and each independent variables, both crude odds ratio (COR) and adjusted odds ratio (AOR) with 95% confidence intervals were reported. The result was presented by frequency tables, graphs and discussed with previous study findings. P-values < 0.05 were declared as statistically significant.

4.10 Data Quality Control

The questionnaire was written in English first and translated into three different languages (Amharigna, Oromiffa, and Tigrigna) and back translated into English.

The anthropometric measurements (height, weight) were taken using standardized and calibrated equipment in duplicate for each participant. Weighing scales (Seca 874U Electronic Scale with dual Display) were checked against zero reading after weighing every child &were calibrated with known weight object regularly.Children's age were based on birth, health records available at the household or self-report of the mother/caretaker using an event calendar.

The tools were pretested prior to the survey training. A three-week training were given to the data collectors, supervisors and overall quality control.

The Ethiopian Public Health Institute (EPHI) and the Federal Ministry of Health (FMOH) conducted regional level administrative sensitization meetings with key political and health leaders prior to the survey implementation. Emails, phone calls and text messages were also made to appropriate political and community leaders to provide information about the survey prior to implementation. They also provided national public service announcements through print and electronic media to publicize the survey well in advance of survey implementation. Sensitization and an explanation of the survey were also provided during the household listing exercise.

Each team vehicle maintained a self-contained field lab that includes a portable centrifuge to allow for immediate centrifugation and aliquoting serum in cryovials. These vehicle were also include a -20^{o}C freezer that can be powered by battery or electricity for fast freezing of serum samples in the field. This freezer was also used to maintain frozen gel packs for distributing in each cool box that goes to the field during sample collection. In each EA (cluster) a temporary field laboratory were set up in a central site such as a school, pharmacy, health centre or other location for the technologist to immediately centrifuge the samples brought in from the field and aliquot the serum into appropriate cryovials. When there were no electricity in the EA, the field labs were set up in the vehicle. All samples were processed within <2 hours of collection.

Experienced phlebotomists collected blood from an arm by venepuncture using trace metal free evacuated tube collection system, it was necessary to collect whole blood into different Vacutainers. The first Vacutainer contain a separator gel (blue top), was free from trace metals with a non-rubber stopper and utilized specifically for zinc, retinol, and folate.We collected six ml of blood, stored it immediately in a cold box and centrifuged within2 hours to separate the serum, at the field lab serum was transferred into four labelled cryovial (1.8 mL), store the cryovials at -20^{o}C and finally transport all cryovials to EHNRI Lab for analysis. A second vacutainer (red top) was used specifically for ferritin, vitamin B12, AGP,CRP and other mineral analysis.In which five ml of blood was collected, stored the it immediately in a cool box, centrifuge to separate the serum, At the field lab theserum was transferred into two labelled cryovials (1.8 mL each) which we stored cryovials at -20^{o}C.

The samples were transported as soon as possible after collection at the household in cold boxes containing frozen gel packs (<8 ^{o}C) by local guides hired specifically to assist each lab technician in rapidly carrying the samples to the centralised temporary field lab site.
Finally the packed blood cells were stored at -20^{o}C and all cryovials transported to EPHI Lab for analysis. EPHI Lab undertookthe laboratory analysis for serum retinol, CRP and AGP.

Laboratory Analyses

The appropriate amount of serum sample were dispensed into cryovials, 250µl for serum retinol, 250µlfor CRP and AGP, and stored at <-20^{o}C. All samples were processed within 2 hours of collection.

Micronutrient analyses

Serum retinol was assessed in all samples and alsoanalysed using the high-performance liquid chromatography (HPLC) technique.

Sub clinical Infection

Acute phase proteins: C-reactive protein (CRP) and Alpha-1-acid-glycoprotein (AGP) were measured to adjust for inflammation when interpreting retinol value. These measures were assessed using the immune-turbidimetry assay.

The quality control supervisor insures the biological samples were collected and properly labeled, stored for spot testing and the specimens were shipped to EPHI.

4.11 Ethical consideration

Written informed consent were obtained from all households before data collection begins. The study was conducted after getting written approval from EPHI, the national scientific and ethical review committee. Each respondent were informed about the benefits and objective of the study; Confidentiality was kept at each step of data collection and processing. The participants were assured that they have full right to participate or withdraw any time during the study.When we found children withxerophthalmia, low MUAC or bilateral oedema theywere referred to the nearest health institution.

4.12Dissemination plan

Findings of the study will be submitted to Jimma University, College of Health Sciences, Department of Population and Family Health. The report will be distributed to the Federal Ministryof Health, Ethiopian Public Health Institute, different professional associations', regional health offices, and other stakeholders.There will be a workshop to discuss final results and develop programmatic recommendations. Survey findings will also be disseminated and presented at regional conferences, meetings and workshops.Finally effort will be made to publish in peer-reviewed journals so that the findings are of benefit throughout the country.

Chapter Five:Result

5.1 Socio-demographic characteristics of the preschool children

A total of 1216 preschool childrenwho had adequate biological sample were taken. Six hundred twenty nine (51.7%) were males. The proportions of preschool children representing the urban and rural areas were 17% and 83%,respectively. Muslims accounted for 512 (42.1%) the religion categories followed by Orthodox Christians 444 (36.5%). The average family size was 5.8 andover half of the parents 674 (55.4%)were not able to read and write.

Table 2: Demographic characteristics of preschool children and households in Ethiopia, 2015

Variables		Frequency (n)	Percent (%)
Sex of child	Male	629	51.7
	Female	587	48.3
Age of child	6-11	80	6.6
	12-23	201	16.5
	24-35	269	22.1
	36-47	311	25.6
	48-59	355	29.2
Residence	Rural	1009	83.0
	Urban	207	17.0
Religion	Muslim	512	42.1
	Orthodox	444	36.5
	Protestant	225	18.5
	Others	35	2.9
Parental educational status	Not attended	674	55.4
	Primary	378	31.1
	Secondary	108	8.9
	Vocational and above	56	4.6
Total		1216	100

5.2 Nutritional status related characteristics of the Preschool children

Thisstudy found that prevalence of stunting in the preschool children was 424(34.9%) while those wasted and underweight were140 (11.5%) and 298 (24.5%) respectively.

Table 3: Nutritional status related characteristics among preschool children in Ethiopia, 2015

Variables		Frequency (n)	Percent (%)
Stunting	Normal	792	65.1
	Stunted	424	34.9
Wasting	Normal	1076	88.5
	Wasted	140	11.5
Underweight	Normal	918	75.5
	Underweight	298	24.5
Total		1216	100

5.3 Illness and vaccination related characteristics of the preschool children

The prevalence rate of subclinical infection was 44.4%. Regarding anaemia413 (34%) of the preschool children were anaemic. Four hundred sixty six (38.3%)of the preschool children had received Vitamin A supplementation in the last six monthswhile 860 (70.7%) and 291 (23.9%) had received measles and polio vaccination in the last six months, respectively.

Table 4: Illness and vaccination related characteristics of preschool children in Ethiopia, 2015

Variables		Frequency (n)	Percent (%)
Diarrhoea	Yes	280	15.8
	No	1495	84.2
Cough	Yes	371	20.9
	No	1402	79.1
Fever	Yes	264	14.9
	No	1508	85.1
Sub clinical infection	Yes	540	44.4
	No	676	55.6
Anaemia	Yes	413	34.0
	No	803	66.0
Vaccination status of polio	Yes	291	23.9
	No	925	76.1
Vitamin A supplementation	Yes	466	38.3
	No	705	58.0
	Don’t know	45	3.7
Vaccination status of measles	Yes	860	70.7
	No	191	15.7
	Not applicable	49	4.1
	Don’t know	116	9.5

5.4 Prevalence of vitamin A deficiency

The study found that prevalence of subclinical vitamin A deficiency was 25% and after adjusting for inflammation, the prevalence of VAD decreases to 18%, thus representing a 7% overestimation in the unadjusted prevalence of VAD.

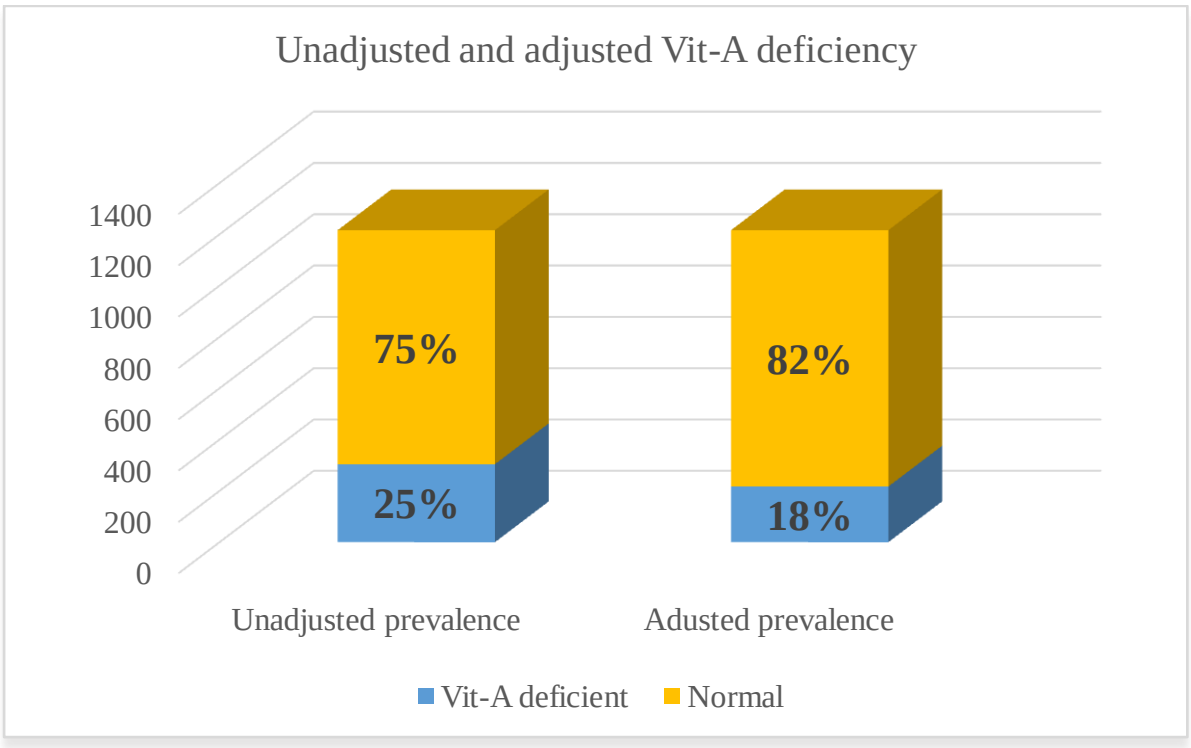

Figure 4: Unadjusted and adjusted prevalence of vitamin A deficiency among preschool children in Ethiopia, 2015

5.5 Factors associated with vitamin A deficiency

Both bivariate and multivariable logistic regression analyses were done using Entermethod to isolated factors associated with vitamin A deficiency. On the bivariate analyses age, residence, religion, family size, stunting, wasting, acute inflammation, chronic inflammation, diarrhoeal disease, cough and anaemia became candidate variables for multivariable analyses(Table 5).

Table 5: Bivariate logistic regression model predicting the likelihood of sub clinical vitamin A deficiency among children 6-59 months in Ethiopia, 2015

Variables		Sub clinical (VAD3)			
		Deficient	Normal	COR	P
Age	6-11	23	57	1	
	12-23	43	158	0.67	0.335
	24-35	45	224	0.49	0.079
	36-47	56	255	0.54	0.112
	48-59	66	289	0.57	0.128
Residence	Urban	20	187	1	
	Rural	195	814	2.24	0.002
Religion	Orthodox	62	382	1	
	Protestant	42	183	1.41	0.104
	Muslim	106	406	1.61	0.004
	Others	10	25	2.46	0.177
Stunting	Normal	273	519	1	
	Stunted	169	255	1.26	0.144
Wasting	Normal	108	968	1	
	Wasted	20	120	1.49	0.059
Chronic inflammation	Yes	93	416	1.41	0.091
	No	97	610	1	
Acute inflammation	Yes	46	145	1.81	0.002
	No	153	872	1	
Diarrhoea	Yes	51	138	1.92	0.050
	No	166	861	1	
Cough	Yes	57	174	1.63	0.052
	No	165	820	1	
Anaemia	Yes	102	311	1.81	0.051
	No	123	680	1	
Family size	< 5	76	300	1	
	5 to 7	104	516	0.79	0.172
	>7	37	183	0.79	0.308

On multivariable logistic regression analyses, after adjusting for other variables, living in rural areas, being anaemic, having both acute and chronic inflammation, and having diarrhoea were positively associated with vitamin A deficiency(P<0.05). It was observed that children who live in rural areaswere 2.24 times more likely to be vitamin A deficient as compared to preschool childrenliving in urban areasAOR=[2.24(1.256-3.992)]. Similarly, the odds of VAD was 1.52 times higher amonganaemic preschool children, AOR= [1.52(1.089-2.123)].Preschool children with acute inflammation were almost three times more likely to be Vitamin A deficient as compared their counterparts without acute inflammation, AOR =[2.78(1.715-4.496)]. Likewise, the odds of VAD was 1.91 times higher among those children with chronic inflammation than their counter parts, AOR = [1.91(1.431-4.625)].Preschool children with diarrhoea in the last two weeks were 1.57times as likely to be vitamin A deficient compared to preschool childrenwithout diarrhoea, AOR=[1.57(1.024-2.396)] (Table 6).

Table 6: Multivariable logistic regression model predicting the likelihood of vitamin A deficiency among children 6-59 months in Ethiopia, 2015

Variables		Sub clinical VAD		COR	P	AOR (95% CI)
		Deficient	Normal			
Acute inflammation	Yes	46	145	1.81	0.000	2.78(1.715-4.496)*
	No	153	872	1		1
Chronic inflammation	Yes	83	426	1.09	0.000	1.91(1.431-4.625)*
	No	107	600	1		1
Anaemia	Yes	102	311	1.81	0.014	1.52(1.089-2.123)*
	No	123	680	1		1
Residence	Rural	195	814	2.24	0.006	2.24(1.256-3.992)*
	Urban	20	187	1		1
Diarrhoea	Yes	51	138	1.92	0.038	1.57(1.024-2.396)*
	No	166	861	1		1

* Variables having stastically significant association (P < 0.05)

Chapter Six: Discussion

It is crucial to realize that many children who are vitamin A deficient will not have the eye signs. This means that children with the eye signs are only the “tip of the iceberg(33).

The prevalence of vitamin A deficiency in this study is 25%, but because of the effect of inflammation on serum retinol value, the prevalence need to be adjusted.The adjusted prevalence was 18%, this prevalence was overestimated by 7% which can be considered as a moderate public health problem, according to WHO criteria.

This adjusted prevalence (18%) was almost similar to that of studies conducted in other countries among children less than five years, which showed that prevalence was 20.1% in western Kenyan children aged 6 to 23 months(34) and 21.8% in north eastern Brazilian 6 to 59 months(17). However, the prevalence of VAD was lower than that of national studies conducted among children less than five years, in Ethiopia. A national survey conducted in Ethiopiaamong preschool school children 0-48 months in 2009, found prevalence of 34.4%(13)and another national study in preschool children aged 6-71 months in 2010, found prevalence of 37.7%(14).This differences can be attributed to the fact that both studies did not adjust or eliminate their retinol values with sub clinical infection and there is considerable time gap between this study and those two studies conducted. Similarly this study found alower prevalence compared to others cross sectional studies, 76.6% in Bungoma district western Kenya(23), 47% in Marshal islands(22). This discrepancy might be due to the differences in socio economic status, lower level of illness and anaemia,and the Kenyan studydid not adjust or eliminateretinol value for inflammation status.

The result of this study showed that preschool children living in rural areas were2.24 times more likely to be vitamin A deficient compared to preschool children living in urban area settingsAOR=[2.24(1.256-3.992)]. This finding is consistent with the study done in Ethiopia in 2010(14).The reason for this might a higher prevalence of illness, lower consumption of vitamin A–rich foods, poorer access of information and health servicein rural areas.

The odds VAD was 1.57 times higher among preschool children with diarrhoea in the last two weeks before the survey compared to preschool childrenwithout diarrhoea AOR=[1.57(1.024-2.396)]. The difference in vitamin A deficiency between preschool children with and without illness has been reported by different studies.A study conducted in Ethiopia in 2009 showed a similar finding(13). The reason for strong association of vitamin A deficiency with illness is well-described and can be due to the fact that illnesses can reduce dietary intake, increase the catabolism and urinary excretion, Specificallydiarrhoeal diseases can affect vitamin A status by increasing loss of nutrients, whileintestinal parasites, such as GiardiaandAscaris,could reduce nutrients absorption(13,35). Conversely, VAD is a risk factor for the development of infectious diseases, since it decreases the immune resistance to these conditions, thus creating a vicious cycle between VAD and illness among children(35).

In this study, statistically significant association were seen between VAD and inflammation, showing higher vitamin A deficiency prevalence amongchildren's with both acuteAOR =[2.78(1.715-4.496)] and chronic inflammation AOR=[1.91(1.431-4.625)]. This relationship between VAD and inflammation is clear in literatures, in cross sectional studies done in state of Paraiba 2013(17), and Goias 2015(35), Brazil.The possible reasons are: Serum retinol concentration can be reduced in inflammation processes, even if the vitamin stores in the liver are at normal levels (30), and inflammationdecreases the absorption and increases catabolism of the vitamin(17,35).

Regarding anaemia,the odds of VAD was 1.52 times higher amonganaemic preschool children AOR=[1.52(1.089-2.123)]. This finding is supported by a study conducted in Goiás(20) Brazil 2015,haemoglobin concentration was positively correlated with the serum retinol concentration. It can be explained by inadequate consumption of diversified diet especially foods of animal source and decreased resistance to infection in anaemic children's.

Although several interventions has been going on to prevent and control vitamin A deficiency in preschool children the findings of this study imply that Vitamin A deficiency is still at the level where WHO declares it to be problem of public health importance in Ethiopia. VAD in this segment of the population has a strong negative repercussion on their health, survival, growth and wellbeing. This poses arguments on the need for the programs aiming at achieving the sustainable development goals and the heath transformation plan of Ethiopia should look into innovative strategies of addressing this problem.

Strength of the study

Since this study is part the national micronutrient survey it assessed sub clinical vitamin A deficiency in the country. During data collection, ODK (open data kit program) was used and temporary field labs were maintained which have refrigerator that can be powered with either electricity or battery. High performance liquid chromatography which is highly sensitive and specific, was used to assess serum retinol level and retinol values were adjusted to inflammation.

Limitation of the study

Even though this study is expected to addresses very important issues, there are some variables that were not addressed in this study. There might be social desirability bias from respondents.

Chapter Seven: Conclusion and recommendation

Conclusion

It is concluded that the national prevalence of subclinical vitamin A deficiency among preschool children shows a moderate public health problem based on WHO biochemical criteria. Vitamin A deficiency was associated with residence, anaemia, illness (diarrhoea), acute and chronic inflammation.

Recommendation

For the government organizations

- FMOH need to strengthen the measures that may have a medium to long-term effects include efforts to improve the health status of preschool children especially control of diarrhoea and anaemia.
- FMOH should strengthen and intensify on-going VAD control program in preschool age children.
- FMOH need to strengthening attempts to enhance the consumption of diversified diet with focus of rural areasthrough the health extension workers, health development armies and other innovative ways.
- FMOH and other concerned bodies need to consider food fortification.
- FMOH and other concerned bodies should give due attention for rural areas in the prevention and control of VAD.
- Ministry of agriculture needs to strengthen researches related to nutrient reach products.
- Ministry of education in collaboration with other responsible stakeholders should strengthen adult learning program.

For the researchers

- There is a need for longitudinal studies that will enable an assessment of vitamin A containing foodswith VAD.

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Annex

Annex one:Informed Consent for Preschool Child Interview(0-59) months

Ethiopian national micronutrient survey 2015

Ethiopian Federal Ministry of Health Enrolment Informed Consent for Preschool Child Interview

As I mentioned earlier, we are trying to learn more about the health of children also. Among all the preschool children 0-59 months old in Ethiopia your children have been chosen to participate in this survey. We would like to continue asking you questions about your preschool children.

This information will help the government to plan health and nutrition services. The survey usually takes between 30 and 45 minutes to complete.

Among infants less than 6 months of age, we would like to just ask some questions about what they eat.Among children 6-59 months, we would like to find out more about how well they are by collecting a sample of yourchild's blood and stool. We will also measure your child's height and weight and ask questions related to what they are eating and their health habits.

If your child is 6 month old or older, the benefit to you for taking part in this survey is that you will get results for your child's weight, height, malaria, and anaemia. The other information you give us will not benefit you in a direct way. However, we will add the information you give us to that of other houses in Ethiopia, and will create a report. The report will contribute to the good of your community. What you say is important and valuable, and will help the Ministry of Health to improve their health and nutrition programs.

If you are not interested you do not have to take part in this survey. If I ask you any question youdon't want to answer, just let me know and I will go on to the next question. You may choose to stop the interview at any time. Refusing to answer will not affect your family's access to health services.

All of the answers you give will be confidential and will not be shared with anyone other than members of our survey team. This form with your answers will be kept under lock and key. You don't have to be in the survey, but we hope you will agree to answer the questions since your views are important.

If you have any question about this survey please call our manager (Dilnesaw Zerfu) at the mobile (0911421720).

Do you have any questions for me? …….

May I begin the interview now? .....

Participant's name (print) …….....

Survey staff conductingSurvey staff signature and date ……..

Respondent agrees to be interviewed……..……1

Respondent does not agree to be interviewed……....2

Annex Two:Informed Consent for Preschool childanthropometry and biochemical sample collection

CONSENT STATEMENT FOR ANTHROPOMETRY AND BIOCHEMICAL SAMPLE COLLECTION

For Caretaker of Preschool Child 6-59 months

As part of this survey, we are asking people all over the country to take an anaemia and malaria test. We would also like to assess the vitamins and minerals in your 6-59 month old child's body. We are not collecting samples or measuring children under 6 months of age. Anaemia is a serious health problem that usually results from poor nutrition, infection, or chronic disease. This survey will assist the government to develop programs to prevent and treat anaemia.

We wouldlike to measure your child's height, weight, and check him/her for oedema. We would also like to take a sample of his/her blood and stool. The tests are safe. Some tests may cause your child slight discomfort, such as taking a blood sample. For the blood sample, your child will have blood drawn from a vein in the arm with a needle. The equipment used in taking the blood is clean and completely safe. It has never been used before and will be thrown away after each test. We would also like you to collect a sample of stool from the same child in a cup.By giving us his/her stool to test, you will help the Ministry of Health learn more about parasites that make people sick in Ethiopia.

Your child's blood will be tested for anaemia and malaria immediately, and the result told to you right away. We will also provide information on your child's weight and height. The result will be kept strictly confidential and will not be shared with anyone other than members of our survey team.

We will refer your child to the clinic if s/he has severe anaemia, malaria or oedema.

You can say yes to any of these tests, or you can say no. It is up to you to decide.

Do you have any questions?

May we take your child's weight and height (anthropometry)?

Will you provide a small amount of your child's blood and stool?

Annex Three: Tool

Household id ☐☐☐☐☐
Cluster (3 digit) HH (2 digit)
Ethiopian national micronutrient survey 2015
Ethiopian Federal Ministry of Health, Ethiopian Public Health Institute

Preschool age child bar code label

Questionaire

IDENTIFICATION	
PG01. CLUSTER NUMBER: ☐☐☐	
PG02. HH NUMBER: ☐☐	
PG03. RESPONDENTLINE NUMBER: (SHOULD BE MOTHER/CAREGIVER) ☐☐	
PG04 CHILD LINE NUMBER ☐☐	

ASK FOR ALL PRESCHOOL CHILDREN 0-59 MONTHS

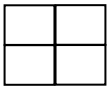
No.	QUESTION	CODING CATEGORIES	SKIP
P1	**What is the birth date of the child? In day/month// year (How many months old is this child?)** NOTE FOR INTERVIEWERS *(Screening question to verify that the date of birth of the child)*	Age in years ☐☐	**If <6mos →P13**
P2	Do you know when the last vaccination campaign here?	No……... 00 Yes ……..……. 01	**00→**P3
P2a	**When was the last vaccination campaign here?** (Write month and Year)	_____/_____ mo / yr	
P3	**Do you have a child clinic/ Vaccination card/ book with (**child's name**) vaccinations?** *(If yes ask:* may I see it please?)	No……... 00 Yes, not seen…… 01 Yes, seen…….. 02	
P4	**Has your child received a vitamin A capsule in the last 6 month?** *(show vitamin A capsules)*	No……... 00 Yes ……..……. 01 Don't know …….. 88	**00→**P5 88→P5
P4a	**Does Vitamin A supplementation date is recorded?**	Date is not recorded……. 00 Date is recorded (specify)… 01 Don't know ……..….. 88	**00→**P5 88→P5
P4b	**Write the most recent date of vitamin A Capsule given**	_____/_____/____ day / mo / yr	
P4c	Source of the date (Information)	From clinic card/Book……. 01 Mothers/family Recall …… 02	

P5	**Has your child received Measles vaccine in the last 6 month?** **(Ask this question only for Child more than 9 months)**	No…… 00 Yes ……..…… 01 Not applicable …… 02 Don't know …….. 88	**00→**P6 88→P6
P5a	**Does Measles supplementation date is recorded?**	Date is not recorded……. 00 Date is recorded (specify)… 01 Don't know 88	**00→**P6 88→P6
P5b	Write the most recent date of measles vaccination	_____/_____/____ day / mo / yr	
P5c	Source of the date (Information)	From clinic card/Book…… **01** Mothers/family Recall **02** ……	
P6	**Has your child received polio vaccine in the last 6 month?**	No……... 00 Yes ……..…… 01 Don't know …….. 88	**00→**P7 88→P7
P6a	**Does Polio supplementation date is recorded?**	Date is not recorded……. 00 Date is recorded (specify)… 01 Don't know ……..…… 88	**00→**P7 88→P7
P6b	WRITE THE MOST RECENT DATE OF POLIO VACCINATION	Polio _____/_____/____ day / mo / yr	
P6c	Source of the date (Information)	From clinic card/Book…. 01 Mothers/family Recall 02 ……	
P7	**During the last six months, did** (child's name) **take any multivitamin tablets, multivitamins or syrups?** (SHOW TABLETS AND SYRUP) ASK *TO SEE THE TABLETS* AND SYRUPS	No…… 00 Yes…… 01 Don't know…….. 88	**00→P9** **88→P9**
P8	**How many days did** (child's name) **take any of these products in the last week (7 days)**	Number of days…… ☐☐ (If none, enter 00) (If don't know, enter 88)	
P9	**During the last six months, did (**child's name**) take any iron tablets/syrups?** (SHOW TABLETS AND SYRUP) ASK *TO SEE THE TABLETS* AND SYRUPS	No……... 00 Yes ……..…… 01 Don't know …….. 88	**00→P11** **88→P11**
P10	**How many days did (**child's name**) take iron tablets/syrups in the last week (7 days)?**	Number of days…… ☐☐ (If none, enter 00) (If don't know, enter 88)	
P11	**Does (**child's name**) eat soil or earth from any source (for example, walls of mud houses, the market or the yard)?**	No……... 00 Yes ……..…… 01 Don't know …….. 88	**00→P13** **88→P13**
P12	**Over the last week (last 7 days), how many days did (**child's name**) eat soil or earth from any source (for example, walls of mud houses, the market or the yard)?**	Number of days…… ☐☐ (If none, enter 00) (If don't know, enter 88)	
(ASK FOR ALL PRESCHOOL CHILDREN 0-59 MONTHS) CHILD HEALTH QUESTIONS: Now I would like to ask you some questions about **(child'sname)** health.			

P13	**Has (**child's name**) been diagnosed with anaemia in the past 6 months?**	No…….. Yes ……..…… Don't know……..	00 01 88	**00→**14 88→14
P13a	If yes ask **did (**child's name**) take any iron tablet or syrup?**	No…….. Yes ……..…… Don't know……..	00 01 88	
P14	**Did (**child's name**) take any drugs for intestinal worms in the past 6 months?**	No…….. Yes ……..…… Don't know……..	00 01 88	
P15	**Has (**child's name**) been ill with diarrhoea in the past 2 weeks?** (DEFINED AS 3 OR MORE LOOSE OR WATERY STOOLS IN A 24-HOUR PERIOD)	No…….. Yes ……..…… Don't know……..	00 01 88	**00→P17** **88→**P17
P16	**Was he/she given any of the following to drink at any time since he/she started having the diarrhea:** A) fluid made from a special ORS packet like LEMLEM? (SHOW EXAMPLE) B) homemade fluid of salt, sugar, and water?	No…….. Yes ……..…… Don't know……..	00 01 88	
P17	**Has (**child's name**) been ill with a cough or breathing problems (in the past 2 weeks)**	No…….. Yes ……..…… Don't know……..	00 01 88	**0→P20** **88→**P20
P18	**When (**child's name**)had an illness with a cough, did he/she breathe faster than usual with short, rapid breaths or have difficulty breathing**?	No…….. Yes ……..…… Don't know……..	00 01 88	**0→P20** **88→**P20
P19	**Was the fast or difficult breathing due to a problem in the chest or a blocked or runny nose?**	Chest only …….. Nose only . …….. Both ……. Other Specify_____ Don't know ……..	01 02 03 77 88	**88→P20**
P20	**Has** (child's name)**been ill with a fever in the past 2 weeks?**	No…….. Yes ……..…… Don't know……..	00 01 88	
P21	**Has** (child's name)**been ill with malaria in the past 2 weeks?**	No…….. Yes ……..…… Don't know……..	00 01 88	
P22	**Has (**child's name**) had any hospitalization and /or clinic visits due to illness in the last 2 weeks?**	No……. Yes ……..…… Don't know……..	00 01 88	**0→P24** **88→**P24
P23	**Where did you seek health care assistance when (**child's name**) was sick for the last 2 weeks** *Anywhere else?* *PROBE FOR ALL SOURCES* *MULTIPLE RESPONSES ALLOWED*	No assistance sought Hospital/Clinic…. Health center…… Health post….. Mobile clinic…. Pharmacy….. Pvt doctor…… Market/Shop……. Traditional healers …… Other Specify_____	00 01 02 03 04 05 06 07 08 77	
P24	**At any time during the illness, did (child's name) take any drugs for the illness in the last 2 weeks?**	No…….. Yes ……..…… Don't know ……..	00 01 88	

Child feeding (breast feeding and complementary feeding) (0 to <24 months)

Next we would like to ask you questions about what your child eats.

P25	**Has (**child's name**) ever been breastfed?**	No……..... 00 Yes ……..……. 01 Don't know ……... 88		**0→P34** **delete** **1→P27** **Add** **88→P34**
P26	IF NO,WHYWASN"T(NAME)BREASTFED?	Motherill/weak…… …1 Child ill/weak……...2 Childdied…… ….3 Nipple/breastproblem…… …4 Insufficientmilk……5 Motherworking……6 Child refused…… 7 Other(specify)……..……77		**All→P34**
P27	**How long after birth did you first put (**child's name**) to the breast?** IF RESPONDENT REPORTS SHE PUT THE INFANT TO THE BREAST IMMEDIATELY AFTER BIRTH, CIRCLE '00' FOR 'IMMEDIATELY'. IF LESS THAN 1 HOUR, CIRCLE '1' FOR HOURS ANDRECORD'00' HOURS. IF LESS THAN 24 HOURS, CIRCLE '1' AND RECORD NUMBER OF COMPLETED HOURS, FROM 01 TO 23. OTHERWISE, CIRCLE '2' AND RECORD NUMBER OF COMPLETED DAYS.	IMMEDIATELY..... 00 HOURS…..... 01 DAYS..... 02 		
P28	Whatdidyoudowiththe firstmilk (colostrum)? ***Colostrums****is thefirst yellowmilk"inger"*	Givetochild……..…… …1 Throwaway…… ….2 Other (specify)_____ 77 Don't know ……..88		
P29	Inthefirstthreedays after delivery, was (name)givenanythingto drink other thanbreastmilk?	No……..... 00 Yes ……..……. 01 Don't know ……... 88		**0→P31** **88→P31**
P30	What was (name)giventodrink? (morethanone answer is possible)	Milk(otherthanbreastmilk)… 01 Holy water……..…… 02 Sugar with water orglucose… 03 Fruitjuice…… 04 Infantformula…… 05 Tea/Infusion…… 06 … 07 Honey…… 08 Rawbutter…….. 09 Ersho…… 10 Abishwater…….. 77 Other,specify_____ _		

P31	**Is the child still breast feeding?**	No…….. Yes ……..…….	00 01	**0→P34**
P32	**Was (**child's name**) breastfed yesterday during the day or at night? That is since this time yesterday until now? (to emphasize 24 hours)**	No…….. Yes ……..……. Don't know ……...	00 01 88	**0→P34**
P33	**How many times did (**child's name**) drink breast milk yesterday during the day or at night?That is since this time yesterday until now? (**to emphasize 24 hours**)**	Number of times ☐☐ Don't know……..….88		
Next I would like to ask you about some liquids that ***(NAME)*** may have had yesterday during the day or at night Did ***(NAME)*** have any ***(***ITE***M*** FRO***M L***IST***)***? Note to interviewer: R*ead the list of liquids one by one starting from water and mark yes or no* accordingly.				
P34	Plain water?	No…….. Yes ……..……. Don't know ……...	00 01 88	
P35	Infant formula (for example S26, Bay luck, Nestle,……..)	No…….. Yes ……..……. Don't know ……...	00 01 88	**00→P36** **88→P36**
P35a	IF YES: How many times since yesterday, during the day or at night, did (NAME) drink infant formula? NUMBER OF TIMES DAY OR NIGHT IF 7 OR MORE TIMES, RECORD '07'. DRANK FORMULA	Number of times ☐☐ Don't know……..88		
P36	Milk such as tinned, powdered, or fresh animal milk?	No…….. Yes ……..……. Don't know ……...	00 01 88	**00→P37** **88→P37**
P36a	IF YES: How many times since yesterday, during the day or at night, did (NAME) drink milk? NUMBER OF TIMES DAY OR NIGHT IF 7 OR MORE TIMES, RECORD '07'. DRANK MILK	Number of times ☐☐ Don't know…….88		
P37	Juice or juice drinks?	No…….. Yes ……..……. Don't know ……...	00 01 88	
P38	Clear broth? (Such as meat broth or vegetable broth)	No…….. Yes ……..……. Don't know ……...	00 01 88	
P39	Yogurt?	No…….. Yes ……..……. Don't know ……...	00 01 88	**00→P40** **88→P40**
P39a	IF YES: How many times since yesterday, during the day or at night, did (NAME) eat yogurt? NUMBER OF TIMES DAY OR NIGHT IF 7 OR MORE TIMES, RECORD '07'. ATE YOGURT	Number of times ☐☐ Don't know…….88		
P40	Thin porridge/Gruel?	No…….. Yes ……..……. Don't know ……...	00 01 88	
P41	Any other liquids such as [**list other water-based liquids available in the local setting**]? For Example Abishe (Fenugreek)	No…….. Yes ……..……. Don't know ……...	00 01 88	
P42	Any other liquids?	No…….. Yes ……..……. Don't know ……...	00 01 88	

Now I would like to ask you about (other) liquids or foods that ***(NAME)*** ate yesterday during the day or at night. I am interested in whether your child had the item even if it was combined with other foods. For example, if ***(NAME)*** ate a millet porridge made with a mixed vegetable sauce, you should reply yes to any food I ask about that was an ingredient in the porridge or sauce. Please do not include any food used in a small amount for seasoning or condiments (like chilies, spices, herbs, or fish powder), I will ask you about those foods separately. **Yesterday during the day or at night, did*(Child's name)*drink/eat:**				
P43	Did your child eat foods made out of any of the following cereals, such as bread, pasta, thick-grained porridge, injera or kita? **(Read each food type from the list)** Multiple response is allowed	No …….. Teff……. Maize…… Wheat …….. Barley ……. Sorghum ……..…… Millet …… Oat …… Other (specify) _____	00 01 02 03 04 05 06 07 77	
P44	Pumpkin, carrots, squash or orange flash sweet potatoes that are yellow or orange inside?	No……... Yes ……..…… Don't know …….....	00 01 88	
P45	White potatoes, white yams, bulla, kocho, manioc, cassava, white sweet potato, or any other foods made from roots?	No……... Yes ……..…… Don't know …….....	00 01 88	
P46	Any dark green, leafy vegetables like kale, spinach, or amaranth leaves, pumpkin leafy?	No……... Yes ……..…… Don't know …….....	00 01 88	
P47	Ripe mangoes or papayas?	No……... Yes ……..…… Don't know …….....	00 01 88	
P48	Any other fruits, avocado, banana, guava, lemon,….?	No……... Yes ……..…… Don't know …….....	00 01 88	
P48a	Any other fruits or vegetables, bamboo shoot, bean, cabbage, tomato …….?	No……... Yes ……..…… Don't know …….....	00 01 88	
P49	Liver, kidney, heart or other organ meats?	No……... Yes ……..…… Don't know …….....	00 01 88	
P50	Any meat, such as beef, pork, lamb, goat, chicken, or duck?	No……... Yes ……..…… Don't know …….....	00 01 88	
P51	Egg?	No……... Yes ……..…… Don't know …….....	00 01 88	
P52	Fresh or dried fish or shellfish?	No……... Yes ……..…… Don't know …….....	00 01 88	
P53	Any foods made from beans, peas or lentils?	No……... Yes ……..…… Don't know …….....	00 01 88	
P53a	Any foods made from nut?	No……... Yes ……..…… Don't know …….....	00 01 88	

P54	Cheese or other food made from milk?	No…….. Yes ……..…… Don't know …….....	00 01 88	
P55	Any oils, fats, or butter, or foods made with any of these?	No…….. Yes ……..…… Don't know …….....	00 01 88	
P56	Any sugary foods such as chocolates, sweets, candi pastries, cakes, or biscuits	No…….. Yes ……..…… Don't know …….....	00 01 88	
P57	Condiments for flavor, such as berbere, chilies, spices, herbs, or flavoring powders?	No…….. Yes ……..…… Don't know …….....	00 01 88	
P58	Foods made with red palm oil, red palm nut, or red palm nut pulp sauce?	No…….. Yes ……..…… Don't know …….....	00 01 88	
P59	Any commercially fortified baby food, like Fafa, Cerilak, Cerifam, Mother's Choice?	No…….. Yes ……..…… Don't know …….....	00 01 88	
P60	**Did (**child's name**) eat any solid, semi-solid, or soft foods yesterday during the day or at night?**	No…….. Yes ……..…… Don't know …….....	00 01 88	**00→P62** **88→P62**
P61	**How many times did (**child's name**) eat solid, semi-solid, or soft foods other than liquids yesterday during the day or at night?** (ASK THE RESPSONDENT THIS QUESTION AND RECORD THE ANSWER.)	Number of times ☐☐ Don't know……...88		
P62	**Did (**child's name**) drink anything from a bottle with a nipple yesterday during the day or night?**	No…….. Yes ……..…… Don't know …….....	00 01 88	
P63	**How old was** (child's name)**when he/she was introduced to solid, semi- solid or soft solid food (complementary feeding) for the first time?** Example of solid foods include: meat, fish; Semi solid foods include: porridge, rice, lentils; Soft solid foods include: bananas (VERIFY THE AGE IN MONTHS COMPLETE)	Not yet introduced….. 00 Months (complete) ☐☐ Don't know…..**.....**88		
P64	**Is the mother/caretaker of this child fasting?**	No…….. Yes ……..…… Don't know …….....	00 01 88	
P65	Record time: End of Interview (Ethiopian time)	___ ___ : ___ ___		

P66: FINAL INTERVIEW RESULT:	1 COMPLETED 2 NOT AT HOME 3 REFUSED 4PARTLY COMPLETED 5INCAPACITATED 6 OTHER (SPECIFY) _____-

IF CHILD IS GREATER THAN 6 MONTHS OF AGE ASK TO OBTAIN CONCENT AND CONTINUE WITH SAMPLE COLLECTION)

IF CHILD IS LESS THAN 6 MONTH OF AGE THANK THE RESPONDENT AND MOVE TO NEXT QUESTIONNAIRE.

Consent given for: **PL01** Blood ☐ **PL02** Stool ☐ **PL03** Anthropometry ☐ (Y OR N)	
PL04 Anthropometrist Code: ☐☐	Anthropometrist Name:_____
PL05 Code for Laboratory Technician: ☐☐	Lab Tech Name_____
PL06 WEIGHT IN KILOGRAMS Refused = 777.7 Not measured = 000.0	KG. ☐☐☐.☐
PL07 LENGTH (for children 6 to <24 month) / HEIGHT (≥ 24 month) IN CENTIMETERS Refused = 777.7 Not measured = 000.0	CM. ☐☐☐.☐
PL08 MUAC (Mid upper arm circumference) In centimeter **Refused = 77.7** **Not measured = 00.0**	☐☐.☐
PL09 Edema (Present on both legs?)	No…….. 00 Yes…….. 01
PL10 Does your child have difficulty with his/her vision during the day? **ONLY ASK CHILDREN 24 MONTHS OR OLDER**	No…….....00 Yes …… 01 Don't know…… 88
PL11 Does your child have difficulty with his/her vision at night ("Dafent" night blindness in local language)?	No…….....00 Yes ……01 Don't know……88
PL12 Bitot Spot	No……00 Yes……01
PL13BLUE TOP TUBE (METAL FREE) Not collected =00.0 Refused = 77.7	ML. ☐☐.☐
PL14PURPLE TOP TUBE (EDTA) Not collected =00.0 Refused = 77.7	ML. ☐☐.☐
PL15RED TOP TUBE (EDTA) Not collected =00.0 Refused = 77.7	ML. ☐☐.☐

PL16 Date blood sample taken (Ethiopian Day/Month/Year)	Date: _____/_____/_____ Day/Month/Year
PL17TIMEBLOOD DRAW (Ethiopian time)	Blood draw ___ ___ : ___ ___ Hour Minute
PL18When did you eat your most recent meal (food)?(Ethiopian time)	___ ___ : ___ ___ Hour Minute
PL19MALARIA RESULTS (RDT)	NEGATIVE…….....0 POSITIVE P FALCIPARUM ……..…….....1 Positive P VIVAX……..….2 INVALID…….....3
PL20 FEVER in last 24 HR?	NO……..…….. 0 YES …….....……....1
PL21HEMOGLOBIN RESULTS	g/dL ☐☐.☐
In order to determine if you have blood in the urine or worms we would like to collect a stool sample from your child. If you can provide this now, we appreciate it. If not now, we can come back to pick up the sample at a later time. *INSTRUCTIONS IF UNABLE TO PRODUCE AT WILL*: **For stool**:We will return tomorrow to pick up your stool. We would like the freshest stool you can give us. Please use one cup to collect the first stool you pass.	
PL22STOOL COLLECTED?	NO…….....0 YES ……...……....1
PL23 Date stool sample taken (Ethiopian Day/Month/Year)	Date: _____/_____/_____ Day / Month / Year
PL24TIME: STOOL PASSED (Ethiopian time)	___ ___ : ___ ___ Hour Minute
PL25TIME: STOOL COLLECTED Ethiopian time (as recorded on cup)	___ ___ : ___ ___ Hour Minute
PL26 Time Blood centrifuged (Ethiopian time)	___ ___ : ___ ___ Hour Minute
PL27 Referral given	No……... 00 Yes ……...…… 01
P28: FINAL INTERVIEW RESULT:	RESULT CODES: 1 COMPLETED 2 NOT AT HOME 3 PARENT REFUSED 4 CHILD REFUSED 5PARTLY COMPLETED 6INCAPACITATED 7OTHER (SPECIFY) _____

Thank you for` completing this interview.

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Declaration

Assurance of Principal Investigator:

I, the undersigned, agree to accept responsibility for the scientific Ethical and technical conduct the research project and for provision of required progress reports as per terms and

conditions the health sciences in effect at the time of grant are forwarded the result of this application.

Name of the student: __Tadesse Mekonen____

Date_____ Signature _____

Approval of the advisors:

This thesis has been submitted with my approval as University advisor.

Name of the first advisor: ___Prof. Dr. Tefera Belachew_____

Signature _____ Date _____

Name of the second advisor: _____

Signature _____Date _____

Date of submission: _____