ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES, SCHOOL OF PUBLIC HEALTH

AFLATOXIN EXPOSURE AND ITS ASSOCIATION WITH STUNTING AMONG YOUNG CHILDREN IN BUTAJIRA DISTRICT, SOUTH-CENTAL ETHIOPIA

MARY AYELE (BSc)

Advisers: Dr. Bilal Shikur (MD, MPH)

Mr. Demewoz Haile (MSc)

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Mary Ayele (Bsc)

Approved by the board of examiners:

Advisor

_____	_____	_____	_____
Full name	Rank	Signature	Date

Advisor

_____	_____	_____	_____
Full name	Rank	Signature	Date

Internal examiner

_____	_____	_____	_____
Full name	Rank	Signature	Date

External examiner

_____	_____	_____	_____
Full name	Rank	Signature	Date

Chair of Dept. Graduate Committee

_____	_____	_____	_____
Full name	Rank	Signature	Date

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ACRONYMS AND ABBREVIATIONS

AF	Aflatoxin
AF-alb adduct	Aflatoxin-albumin adduct
AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
CAST	Council for Agricultural Science and Technology
EDHS	Ethiopian Demographic and Health Survey
ELISA	Enzyme-Linked Immunosorbent Assay
EPI	Expanded Programme for Immunization
EPHI	Ethiopian Public Health Institute
HAZ	Height for Age Z-score
HDSS	Health and Demographic Surveillance Site
HH	House Hold
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ILRI	International Livestock Research Institute
IREC	Institutional Research Ethics Committee
LC/MS/MS	Liquid Chromatography/Mass spectrometry and Liquid chromatography/ Tandem Mass Spectrometry
PCA	Principal Component Analysis
SES	Socio-economic status
SNNPR	Southern Nation Nationalities People's Region
rmp:	Revolutions per minute
TEM:	Technical Error of Measurement
WAZ:	Weight for Age Z-score

ABSTRACT

Background: Aflatoxins are one family of mycotoxins, which are a naturally occurring toxic by-product. Aflatoxin M1 is the major metabolite product of aflatoxin B1, which is excreted in urine of mammals and gives a reliable indication of recent (24-72 hours) exposure to aflatoxin. Recent evidences suggest several mechanisms through which aflatoxin can impair growth & development. Despite the high prevalence of stunting in Ethiopia, there is no well-established evidence showing individual aflatoxin exposure and its association with stunting in young children

Objective: The study assessed aflatoxin exposure and its association with stunting among young children aged 12 to 59 month in Butajira district.

Method: A community based cross-sectional study was conducted in Health and Demographic Surveillance Site, Butajira. We used stratified simple random sampling technique to select the study participants. The study included, 332 children aged 12-59 month. The data were collected in the form of questionnaire, height/length measurement and urine samples collection. Aflatoxin M1 analysis was performed by Enzyme-Linked Immunosorbent assay (ELISA) at Ethiopian Public Health Institute laboratory. The data analysis was carried out using STATA and WHO Antro plus. Multiple logistic regression was used to see the association between stunting and aflatoxin exposure level by adjusting for possible confounders.

Results: The mean age of children participated in the study was 39$\pm$10.9 month. About 62% of the children were exposed to aflatoxin M1 in their urine, at a level ranging from 0.15ng/ml to 0.4ng/ml. Sixty point nine percent (60.9%) of the study participants were stunted. Children with detectable aflatoxin M1 in their urine at a level of 0.4ng/ml were 1.9 times (95% CI: 0.79, 4.46) more likely to develop stunting than those who were not exposed, even though this association was not significant at p-value <0.05 and 95% CI.

Conclusion and Recommendation: The study showed a high prevalence of aflatoxin exposure in the study area. This indicates that strategies and regulations focusing mainly on crop management system and food safety measures need to be revised in order to take in to account the measures for control of aflatoxin exposure.

Keywords: aflatoxin M1, cross-sectional study, ELISA, stunting

1 INTRODUCTION

1.1 Background

Aflatoxins are one family of mycotoxins, which are a naturally occurring toxic by-product, named after a genus of fungus that produces it (Aspergillus flavus and Aspergillus parasiticus) (1). Major types of aflatoxin include B1, B2, G1 & G2, as well as M1 & M2. Aflatoxin B1 is the most toxic of the aflatoxin compounds (2); its primary biotransformation in the liver undergoes detoxification reaction, which generates aflatoxin M1, Q1, B2a, P1 & aflatoxicol (AFL) (3). AFM1 is the major metabolite product of aflatoxin B1, which is usually excreted in milk of dietary cattle & urine of mammals (4).

Aflatoxins predominately occur in hot and humid regions of the world (1) and are largely associated with commodities produced in the tropics and subtropics, such as maize, rice, sorghum, barley, rye, wheat, groundnut, soya bean and cottonseed (5). Pre- and post-harvest crop management has a significant influence on the accumulation of aflatoxin in dietary staples (6); thus populations highly reliant on these staples and with limited agricultural capacity and storage facilities, are most frequently exposed through diets (7).

Human aflatoxin exposure is primarily through dietary contamination (8). Aflatoxin may enter the food supply by direct contamination resulting from mold growth on food, or by indirect contamination through the use of contaminated ingredients in processed food or through use of animal products such as milk, milk products, eggs, or meat (9).

Human and animal studies indicate that aflatoxin exposure causes immunosuppression (which in turn can lead to repeated infections and consequently, growth retardation in young children), impairs protein synthesis, and causes changes in the hepatic metabolism of micronutrients (10). There are three biologically plausible pathways through which aflatoxin may affect growth: enterocyte damage, zinc deficiency and inhibition of protein synthesis (11).

Aflatoxin is fat soluble and can be measured in blood as an aflatoxin-albumin adduct, in urine (aflatoxin- M1) as an aflatoxin-guanine adduct and in milk as aflatoxin-M1 (2). The use of aflatoxin metabolites as biomarkers reflects not only the dietary exposure of the individual, but also the uptake, toxicokinetics and toxicodynamics (12). Urinary aflatoxin biomarkers of exposure & effect for aflatoxins have been validated in comprehensive

studies in animals (13); where assessing the level of aflatoxin adducts in urine gives a reliable indication of recent (24-72 hours) exposure to aflatoxin. Given that aflatoxins are carcinogens, there is no safe threshold for exposure (13).

1.2 Statement of the problem

In many parts of the developing world, chronic exposure to aflatoxins at high levels remains a significant health burden (13). It is estimated that approximately 4.5 billion people, predominantly those living in developing countries, are at risk of exposure to aflatoxins, with people in some regions experiencing chronic exposure at high levels (7).

According to the International Agency for Research on Cancer (IARC), both AFB1 and AFM1 are classified as class I carcinogens (14). Aflatoxin-B1, which is the most potent aflatoxin, has been associated with child growth impairment (15), suppressed immune function (16), hepatomegaly (17) and death due to acute poisoning (18).

Animal studies provide evidence that chronic aflatoxin exposure retards growth and interferes with micronutrient absorption and utilization (2). Aflatoxins are lipophilic, are able to cross the placental barrier and can be bio activated in utero. This exposure has been shown to continue in infancy and once children are weaned, they have similar high prevalence and level of exposure as observed in adults. The period of breastfeeding is generally associated with lower levels of exposure (19) because the mother's metabolism limits transfer of dietary aflatoxins into the milk (20). Biomarkers in biological fluids, such as blood & urine are quantitative dosimeter of individual exposure, as well as initial cautioning signs of health effects (21).

In Ethiopia, poor child growth is prevalent, where the proportion of stunting is 38% in children under 5 years old (22). There have been interventions to improve child nutritional status in Ethiopia, including vitamin A supplementation and immunization against vaccine preventable diseases, but overall, the prevalence of impaired growth is still unacceptably high.

Ethiopia is most favourable for aflatoxicogenic fungi and aflatoxin contamination (23). High contamination of food and feed products in Ethiopia may be due to, conducive climatic conditions, traditional crop production practices, inadequate harvesting, drying and storage practices, policy and institutional capacity, lack of awareness and high reliance on one or two crops for food. In addition, major staple grain crops in the country are contaminated with aflatoxin (23).

Despite the high prevalence of stunting in Ethiopia, there is no well-established evidence showing individual aflatoxin exposure and its association with stunting in young children.

1.3 Significance of the study

This study will show the aflatoxin exposure level among young children in Ethiopia, which helps to develop strategies and interventions targeting children and the general public from exposure to harmful mycotoxins and prevent the health consequences following it.

The results obtained from this study will help to bring an impact on the policy of our country; in the Agricultural sector, to improve the pre- and post- harvest crop management system and to prevent contamination of animal feed and dairy products with aflatoxin. Through the health sector, it will help to develop strategies to prevent the health consequences following aflatoxin exposure, mainly through food safety measures.

This research will also be as a base for further studies under this area in Ethiopia, so as to ensure food safety and nutrition measures to prevent the short and long term consequences of aflatoxins.

2 LITERATURE REVIEW

2.1 The Metabolites of Aflatoxin B1 in human urine

According to a review by Godfrey, humans and animals get exposed to aflatoxins by two major routes; one is through direct ingestion of aflatoxin-contaminated food or ingestion of aflatoxins carried over from feed in to milk & milk products like cheese & powdered milk as well as other animal tissue mainly as AFM1 and the other is by inhalation of dust particles of aflatoxins especially AFB1 in contaminated foods in industries & factories (24).

Animal studies have shown that under normal conditions, 50% of the orally administered dose of AFB1 is quickly absorbed from the duodenal region of the small intestine and enters the liver through the hepatic portal blood supply, where it is metabolised in to different derivatives (25). The metabolites of AFB1 detected in human urine include aflatoxin P1 (AFP1), aflatoxin Q1 (AFQ1), aflatoxin M1 (AFM1) and DNA-adduct (AFB1-N7Guanine). The excretion rate of the different aflatoxin metabolites in human urine is not clearly defined (26). AFM1 has been well established as a biomarker of exposure for the recent ingestion of AFB1 and the most frequently detected urinary aflatoxin (27).

A study done in Tanzania in 2017, compared urinary aflatoxin M1 (AFM1) and aflatoxin-albumin adducts as a biomarker for assessing aflatoxin exposure in under-five children. The study found a highly significant correlation ($r=0.468$, $p<0.001$) between the two biomarkers; concluding that urinary AFM1 is a good biomarker of recent exposure to aflatoxin in children, and this biomarker correlates well with AF-alb biomarker in children (28).

2.2 Aflatoxin M1 (AFM1) Exposure Level and the Method of Analysis

According to Partnership for Aflatoxin Control in Africa (PACA) report in 2014, children under 5 remain particularly vulnerable to aflatoxin exposure significantly hindering children's growth and development while damaging their immunity (29). A review done by Cardwell and Henry in 2004; provides evidences that in developing countries, many children are frequently exposed to high levels of toxic fungal metabolites (aflatoxins) in their diet (30).

A cross sectional study done in Ethiopia in 2017; Aflatoxins were detected in 17% of urine samples using LC/MS/MS, were AFM1 (7%) was the most detected urinary Aflatoxin metabolite with a mean concentration of 0.064ng/ml (31). Similarly, in study done in Cameroon using urinary biomarkers to assess mycotoxin exposure in young children, AFM1(14%) was detected with a mean concentration of 0.33pg/mg (32). In another study done in Ghana to detect urinary AFM1 exposure in children aged 6month to 2 years, all urine samples were positive for AFM1 at a range 24.7-8368.9 pg/mg creatinine using HPLC fluorescent detector (4).

In a cross-sectional study done in Sao Paul, Brazil to determine urinary biomarkers for assessing human exposure to aflatoxin using LC/MS/MS, AFM1 was detected in 61% of urine samples at levels ranging from 0.19 -12.7 pg.mg/creatinine. Residues of aflatoxin B1, B2, G1, G2 & aflatoxicol (AFL) were not detected in any urine samples (3)**.** Another cross-sectional study done in Malaysia, to examine the association between aflatoxin M1 excreted in human urine samples with the consumption of milk & dairy products. The study found that respondents with intake of milk & dairy products above median (67.79 g/day) had significantly high level of AFM1 in urine compared to those with low intakes and AFM1 was detected in 61.3% of samples with level ranging from 0- 0.0747ng/ml (33)**.**

Generally, the choice of which analytical process is appropriate will take into account the target molecule, chemical features, complex matrix, timing of testing, and required limits of detection/quantification (34).

2.3 The Association between Aflatoxin Exposure and Child Stunting

Although there are few human data, animal studies provide evidence that chronic aflatoxin exposure retards growth and interferes with micronutrient absorption and utilization (2). There are three biologically plausible pathways through which aflatoxin can affect growth: 1) Enterocyte damage or environmental enteropathy, which is caused as a result of ingestion of toxins, leading to structural abnormalities of the small intestine, altered barrier integrity and mucosal inflammation which ultimately leads to chronic systemic immune activation; repeated infections and consequently growth retardation. 2) Zinc deficiency: altered intestinal architecture can result in loss of enzymes, leading to malabsorption of variety of nutrients; most notably zinc deficiency. 3) Inhibition of protein synthesis: absorption of non-essential amino acids occurs in the small intestine, damage to the small intestine due to the toxins leads to malabsorption of amino acids; inhibition of protein synthesis leading to impaired metabolism (11).

Two key studies done in west Africa, focusing on the relationship between AF exposure & growth retardation in children, both undertaken by the same research group; found that serum aflatoxin-albumin adducts were associated with stunting in children aged <5 years, and those children with stunting or who were underweight had 30-40% higher mean aflatoxin-albumin concentrations (With $p = 0.001$ for height for age, $p = 0.005$ for weight for height) (35). The same group subsequently conducted a longitudinal study and found that the highest quartile of AF-albumin adduct was associated with a 1.7cm mean height reduction compared with the lowest quartile (36). In contrast with this, a longitudinal study done in Tanzania among children aged 6-14 months; found no significant association between mean aflatoxin albumin levels from all sampling times and length-for-age Z-scores (95% CI: -0.07- 0.05; $p= 0.084$). However, the result of the study revealed high prevalence of aflatoxin and fumonisin exposure, as well as high prevalence of growth impairment (37). The difference among the studies can be attributable to various factors, such as difference in age, food contamination levels or individual variation in the toxicokinetics of mycotoxin.

In a study done in Ethiopia in 2017, no association was found between the presence of urinary aflatoxin biomarkers and being stunted (r:-0.038 & p=0.598) (31). Consistent to this finding a study done in Cameroon in 2013 found no significant association between

urinary aflatoxin biomarkers and stunting (32). This may be due to the small sample size or factors causing stunting (confounders) were not well assessed in the studies.

2.4 Contamination of Food staples with Aflatoxin

The growth of aflatoxicogenic fungi is directly related with the production of aflatoxin, so conditions suitable for these fungal growth is favorable of aflatoxin production. The primary factors influencing fungal growth in stored food products are the moisture content (more precisely, the water activity) and the temperature of the commodity. Thus delay in drying to safe moisture levels increases risks of mould growth and mycotoxin production (23).

There are different surveys conducted in Ethiopia showing the aflatoxin level in food. In a survey done by Ayalew A in 2010, aflatoxins were detected in 88% of maize samples with a concentration of 4.1µg/kg (38). Another survey done by Dereje A et al in Ethiopia; found that 100% of groundnut samples were positive for Aspergillus species, were A.flavus had the highest incidence (77.2%) and Aflatoxin B1 was detected in all the samples with concentration range of 0.1-397.8ppb. In addition 83.9% of the total samples were unsafe for direct consumption (39). Similarly, a study done by Alemayehu et al in 2014, found that all the samples of sorghum and finger millet from Ethiopia were contaminated with Aspergillus species (40). According to a report by USAID in 2011, aflatoxin B1 was detected in four major crops of Ethiopia: barley, sorghum, teff and wheat (41). A survey from Addis Ababa and its surrounding cities indicated that all milk samples were contaminated with AFM1 in a range of 0.028µg/L-4.98µg/L(42).

Different studies tried to see the association between aflatoxin exposure and the diet consumed by the study participants. A cross sectional study from Benin, found that the mean aflatoxin-albumin level was higher in the group consuming maize more frequently ($p<0.01$) than those who consumed less frequently (35). A study done in Iran in 2015; found a significant association between aflatoxin M1 (AFM1) excretion and consumption of nuts by assessing intake of dietary foods suspected to be contaminated with aflatoxins in the recent 72 hours (43). Another cross-sectional study done in Malaysia in 2012; found a significant association between the consumption of milk and dairy products with the level of AFM1 detected in urine samples ($p<0.01$) (33). A study done in Brazil in 2009 was inconsistent with the above finding. The study assessed the dietary intake of suspected foods to be contaminated with aflatoxin using 24-hour recall and feeding

frequency inquiry (FFI) on the monthly consumption, but no significant association was found between food consumption and AFM1 concentration in urine. Which can be attributed to the small sample size used by the study (44).

2.5 Factors associated with stunting

According to a cross-sectional study in 2017 done in Butajira town and surrounding districts, Ethiopia; the prevalence of stunting was 52.5% in children age between 24-59 months. The results of the study showed that child age, number of under-five children in the household, marital status of the mother, repeated acute respiratory tract infection attack and duration of breast feeding were significantly associated with stunting (45). In another cross-sectional study done in Lalibela town, Ethiopia; the prevalence of stunting was 47.3% and the study found that age and sex of the child, deworming service, overall breastfeeding status and household socioeconomic status were significantly associated with stunting (46). A study in Bule Hora district, South Ethiopia found a prevalence of stunting 47.6%. Sex of the child, diarrhoea disease in the past two weeks and pre-lacteal feeding at the time of birth were significantly associated with stunting (47). Outside Ethiopia, a cross-sectional study done in Vietnam in 2008 found a prevalence of stunting 44.3%. Region of residence, maternal educational status, household size, number of under-five children in the household, duration of exclusive breastfeeding were significantly associated with stunting (48).

A case-control study done in Meskan district, Gurage zone in 2014; found that number of family member in the household, mother’s occupation, overall duration of breast feeding as well as duration of exclusive breastfeeding were significantly associated with stunting (49). Another case-control study done in Mozambique in 2017 found that birth weight, sex of the child, residence and number of under-five children in the household were significantly associated with stunting (50).

3 OBJECTIVES

3.1 General Objective:

To assess aflatoxin exposure and its association with stunting in young children (12-59 month), in Butajira District, South-Central Ethiopia.

3.2 Specific Objectives:

1. To determine aflatoxin exposure level in young children (12-59 month) by measuring aflatoxin M1 (AFM1) level in urine in Butajira District, South-Central Ethiopia.
2. To examine the association between urinary aflatoxin M1 (AFM1) level and stunting in young children (12-59 month) in Butajira District, South-Central Ethiopia.

4 METHODS AND MATERIALS

4.1 Study setting and period

The study was conducted in the Health and Demographic Surveillance (HDSS) site for School of Public Health, Addis Ababa University located in Butajira. Butajira is a town and separate Woreda in south central Ethiopia. HDSS site is in Meskane and Mareko district, which is located 130km south of Addis Ababa and 50km west of Zeway town in the rift valley, 8.20 north latitude and 38.50 east longitudes with an estimated size of 797 km2. The HDSS area covers 10 kebeles of which 9 are rural and 1 is urban with an estimated total population of 80,369, from which children 12-59 month of age account for 5067. It has three agro ecology zones (Kola, Dega and Weynadega). Gurage is the main ethnic group; Islam is the main religion, followed by Orthodox Christianity. Guragigna is the major language but, Amharic; the national language, is also widely spoken in the area, and is a written language. Maize, sorghum and false banana are the staple foods in the area. There are two hospitals of which one is governmental and the other is non-governmental hospital. In addition, there are health centers, several private clinics and dispensaries which gave health care to the population. The study was conducted from July 2018 to August 2018.

4.2 Study design

Community based cross-sectional study design was used.

4.3 Population

4.3.1 Source population

All young children who reside in Butajira district

4.3.2 Study population

All randomly selected young children aged 12-59 month, who resided in the ten kebeles of Butajira HDSS during the study period.

4.4 Inclusion criteria

All young children aged 12-59 month in the study area

4.5 Exclusion criteria

Critically ill children were excluded, because it was difficult to take measurements.

4.6 Sample size calculation

The sample size for the first objective is calculated using single population proportion formula based on the following assumptions: P= prevalence of Aflatoxin M1 in urine as 7%, from a study done in Ethiopia in 2017 (31).

D (margin of error) = 3%

95% of confidence interval

N= number of sample

Sample size determination will be as follows

$N= Z^2\alpha/2\ p\ (1-p)/d^2$

N= 278

Sample size for the second objective is calculated using two population proportion formula in open epi software, based on the following assumption:

P_1= prevalence of Stunting as 52.5%, from a study done in Butajira in 2017 (45)

Zα/2= standard score corresponding 95% confidence interval (1.96)

Zβ= standard score corresponding 80% power (0.84)

Odds ratio= 2

r= ratio between group one & group two as 1

By using the following assumption, the sample size will be 151 in each group, making a total sample size of 302

Since, sample size for the second objective is greater than the first, 302 is taken.

Adding a contingency of 10% for non-respondent

- **Final sample size is 332**

4.7 Sampling Procedure

The Butajira HDSS contains 10 kebeles divided by three agro ecology zones; Highland, Midland and Lowland. To identify the study population stratification was first done by agro ecology zone.

After stratification, the final sample size was allocated proportionally to each stratum. The list of households with children aged 12-59 month years in each agro-ecology zone was obtained from HDSS database. This list was used as a sampling frame. Assuming there is a homogenous distribution in each stratum, the study population (household) was identified by simple random sampling technique. Only one child was recruited from each selected household. If more than one child was present in the selected household, lottery method was applied to select one child.

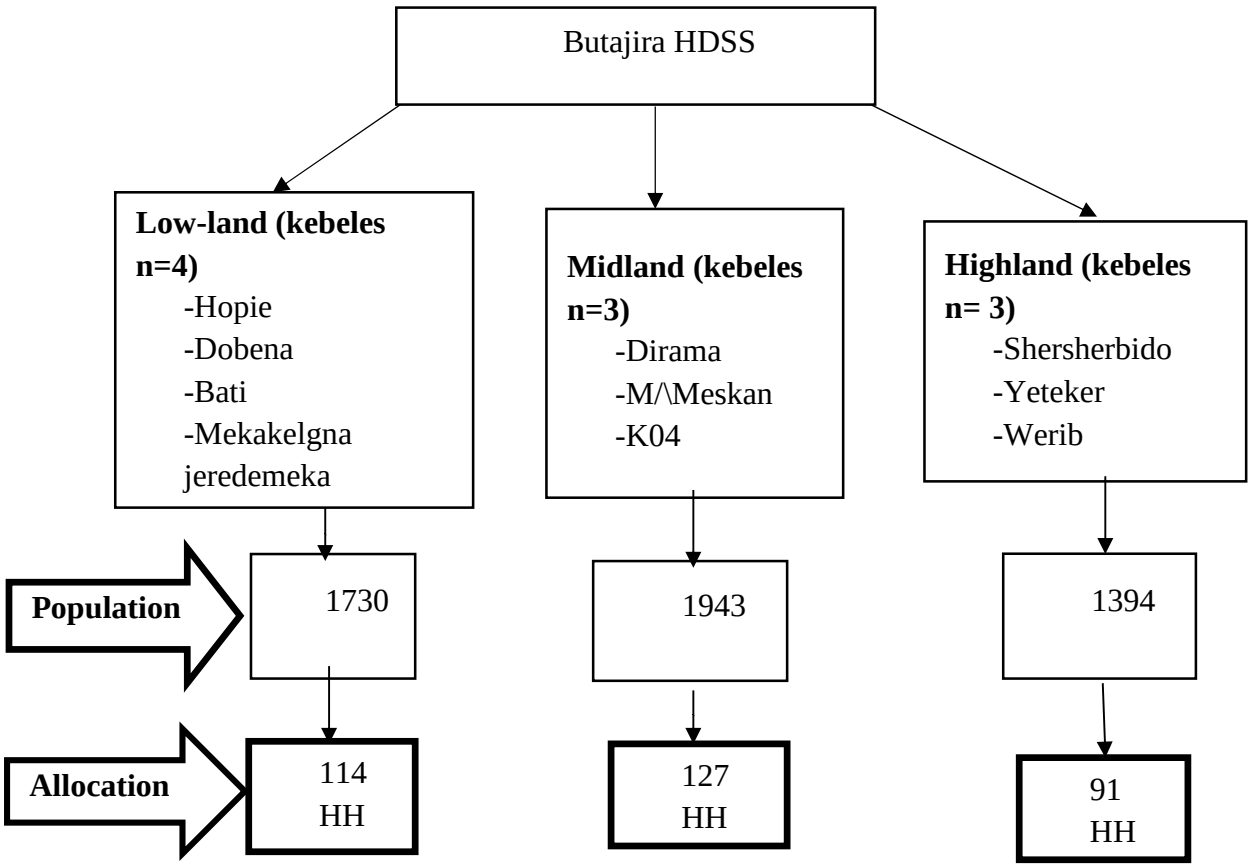

Figure 1: Sampling procedure for children in Butajira HDSS, 2018

4.8 Variables

4.8.1 Dependent variable

- ❖ Stunting

4.8.2 Independent variable

- Aflatoxin exposure
- General child health status: type of illness in the past two weeks and seek treatment/advice for the specified illness
- Number of under five children at the household
- Dietary diversity
- Type of food consumed, frequency of consumption and the usual portion size in the preceding three days (72 hours) before data collection
- Water source for drinking
- Type of toilet used by the household
- Place of storage for crops
- Duration for storage of crops in the household
- Maternal occupation
- Maternal age
- Household wealth index
- Maternal educational status
- Age of the child
- Sex of the child
- Marital status
- Agro-ecology zone

4.9 Data collection methods

The data was collected using; structured and pretested questionnaire, height measurement using calibrated measuring scale and urine sample collection. The data was collected by five data collectors with health background, two supervisors and one coordinator (researcher). To maintain the quality of the data collected, interviewers and supervisors were trained on the significance of the research, sampling procedure, arts of interview, height/length measurement procedures, urine sample collection techniques, appropriate labelling and storage of samples and other relevant information on the questionnaire for

three (03) days. Pilot study was conducted on 5% of the sample size and the participants were excluded from the main study. After the pilot study, corrections were made for clarity of contents.

4.9.1 Questionnaire

Structured questionnaire was designed by reviewing EDHS 2016 and previous similar literatures. The questionnaire was first developed in English, and then translated in to Amharic, and then back to English to check for consistency. The questioners were employed to obtain information on the immediate, underlying and basic predictor of stunting. The data on the questionnaire was collected using face-face interview technique, after all the information on the subject information sheet was explained to the mother/care giver and informed consent was obtained.

4.9.2 Food Frequency Questionnaire

The food frequency questionnaire included the type of food item consumed by the child in the previous three days (72 hrs) before the day of the data collection, the frequency of consumption within the three days and the usual portion size consumed in grams. The food items listed were included after reviewing different literatures, especially those done in Ethiopia; which assessed the level and presence of aflatoxin in common staple foods in the country. Photograph of weighted food items and utensil (cup, bowl, and spoon) were used to determine the portion size consumed by the child.

Dietary diversity had been assessed for all children participated in the study, considering minimum dietary diversity as food intake from at least four food groups. Seven food groups were used; starchy staple (cereals, roots & tubers); legumes and nuts; dairy products (milk, yogurt, cheese); flesh (meat, organ meat, poultry and fish); egg; vitamin A rich fruits and vegetables; and other fruits and vegetables.

4.9.3 Height/Length Measurement

The height/length of all young children participated in the study was measured using accurately calibrated measuring scale. Recumbent length was measured for children under the age of 2; standing height was measured for all other children. During training sessions, Intra and Inter-observer TEM (Technical Error of Measurement) was calculated and repeated measurements were performed by the trainees until they were on the maximum acceptable range. Measurement protocol was prepared and handed for the

trainees, which guided them during the field work. Using the collected data, height-for-age Z-score (HAZ) was calculated using the growth standards published by the WHO in 2006. A Z-score of less than -2 standard deviation (-2SD) is classified as stunting.

4.9.4 Urine Sample Collection

Random urine samples were collected using a 10ml urine cups by the help of the mother/care giver of the child studied. The cups were labelled with unique questionnaire code, household number and date of sample collection and were placed in portable freezers during the time of data collection. At the end of each day, the collected urine samples were checked by the coordinator and placed in Butajira Health center using 10ml polystyrene tubes in -20^0c freezers. After the end of the data collection period, all the collected urine samples were transported to Ethiopian Public Health institute (Paster), for analysis.

All the collected urine samples were detected for the presence and level of Aflatoxin M1 (AFM1) by using ELISA (Bio Rad micro plate reader). The ELISA kits for quantitative assay for Aflatoxin M1 in urine were purchased from Helica Bio system, USA; by the financial support of International Livestock Research Institute (ILRI). The analysis was undertaken by collaboration with Malaria and Neglected Tropical disease's research team at EPHI, based on the protocol obtained from the kit manufactures.

4.10 Laboratory analysis

4.10.1 Assay procedure for determination of aflatoxin M1 in urine using Enzyme Linked Immunosorbent Assay

Five millilitres of urine were liquated into centrifuge tubes and centrifuged at 3000rmp for 10 minutes. Nine hundred and fifty microliters of distilled water were pippeted in to 1.5ml micro tubes. Then 50µl of standards and the supernatant-urine were added into the 950µl of distilled water in the tubes to make-up a total of 1000µl. They were mixed by vortex mixer for 10 second. Two hundred microliters of the assay-buffer were added into the mixing well. Then a 100µl of the diluted standards containing aflatoxin M1 ranging from 0 to 4000ppt (0.0-4.0ng/ml) and the urine samples were added into the mixing well, each sample per well to make-up a total of 300µl. These were mixed by priming pipette at least 5 times. By using new pipette, a 100µl of the mixture were transferred into the Antibody coated micro-wells in duplicate. Then it was incubated for 1hr at room temperature.

Phosphate Buffer Saline-Tween packet (PBS with 0.05% Tween20) was reconstituted by washing out the contents with distilled water into 1 litter glass. This was stored refrigerated when not in use. The contents of the antibody coated micro-wells was decanted in to discard basin, then each micro-wells were filled with PBS-Tween packet and the wash was decanted into a discard basin for 3 times. A hundred microliters of conjugate were then added into each micro-well and incubated at ambient temperature for 15 minutes. The plate was then washed. A hundred microliters of substrate reagent (Tetramethylbenzidine) was then added into each well and incubated at room temperature for 15 minutes in the dark. A hundred microliters of stop solution were added into each of the wells using a multichannel pipette. The intensity of the solution colour in the micro-plate was measured optically using an ELISA reader (Bio-Rad) with an absorbance filter of 450nm as soon as stop solution was added. The optical densities (50) of the samples were compared to the OD's of the standards and an interpretative result is determined. High optical density means aflatoxin concentration is low while a low optical density means aflatoxin levels are high.

4.11 Data Management and Analysis Procedure

The data was checked for completeness and inconsistencies. After appropriate coding was made, the data was entered in Epi-data version 4.0 for windows. Then it was exported to STATA version 14 and WHO Antro Plus for analysis.

Descriptive statistics (mean, median, standard deviation, and range) was calculated for continuous variables.

Principal Component Analysis (PCA) was used to classify the households into low, medium and high socio-economic status.

Height-for-age Z-scores were calculated and compared with reference data from WHO growth chart 2006. Children below -2 standard deviation (-2SD) of WHO median for height-for-age were considered to be stunted.

Binary logistic regression was done to investigate the association between explanatory variables and outcome variable (stunting). Variables that were statistically significant at $p<0.25$ on bivariate analysis, scientifically sound and clinically important variables were fitted in the multiple logistic regression.

Multiple logistic regression was used to examine the association between stunting and aflatoxin exposure by statistically adjusting each variable fitted in the model. Odds ratio (15) with 95% confidence interval (CIs) at p-value <0.05 were used to determine statistically significant association.

4.12 Quality Assurance Procedure

The quality of the data was determined before, during and after the data was collected.

Before data collection: The questionnaire was prepared by reviewing previous similar literatures and EDHS 2016. The consistency of the questionnaire was maintained during translation. Appropriate training was given for data collectors.

During data collection: There had been a close day to day supervision during the data and sample collection process. The completeness of the questionnaire and the appropriate storage of urine samples during the data collection was daily checked.

After data collection: The completeness and consistency of the data was rechecked. Appropriate coding and editing was performed before entering the data into computer software. The collected urine samples were transported under freezer to EPHI laboratory for analysis.

4.13 Ethical Consideration

Ethical approval was obtained from Addis Ababa university, School of Public Health Research Ethical Committee and from ILRI Institutional Research Ethics Committee (IREC). Informed written consent was obtained from the mothers/caregivers of participant's child; after the necessary explanation about the purpose, procedures, benefits, risks and privacy issues of the study was explained.

4.14 Data Dissemination Plan

Full results of the research will be reported to Addis Ababa University, College of Health Sciences, School of Public Health, International Livestock Research Institute (ILRI) and Ethiopian Public Health Institute (EPHI). Summary of main findings of the research will be disseminated to Ministry of Agriculture, Ministry of Health, SNNPR Regional & Gurage Zone Health Office, Butajira Town Health Office and to Butajira HDSS. The participants of the study will not be directly informed about the result of the study, but they will be reached through the HDSS in Butajira. Efforts will also be made to disseminate the result through publication and presentation in scientific conference.

5 RESULTS

5.1 Socio-demographic and economic characteristics of the study subjects and their household

A total of 332 children aged 12 to 59 month with a response rate of 100%, 98.5% and 100% for interview administration, urine sample collection and height/length measurement participated in the study respectively. The mean age of the children participated in the study was 39±10.9 month. Based on gender, 210(63%) of the children were boys and 122(36.75%) of them were girls. Distribution of the study subjects by socio demographic and economic characteristics are presented in Table 1.

At the time of data collection, 106(32%) of the mothers were greater than 35 years with mean age of 30.9 ± 5.9 years and with a range of 18-48 years. Only 6% of the mothers were pregnant with recruited child at age below 19 years. The data on maternal characteristics shows that 262(78.9%) were Muslims, 311(93.7%) were married, 259(78%) were housewife's and 170(51%) of them didn't attend any formal education.

Table 1: Socio-demographic and economic characteristics of children 12-59 months in Butajira District, South-central Ethiopia, 2018

Characteristics	Frequency	Percent (%)
Total children	332	100%
Agro-ecology zone:		
Highland	93	28%
Midland	127	38.3%
Lowland	112	33.7%
Child age in month:		
12-23 month	25	7.5%
24-35 month	81	24.4%
36-47 month	140	42.2%
48-59 month	86	25.9%
Gender:		
Boys	210	63.25%
Girls	122	36.75%
Illness in the past two weeks before data collection:		
Had not been ill	199	59.9%
Malaria/fever	20	8.7%
Flu	27	8.1%
Cough	56	16.9%
Other	8	2.4%
Received treatment/advice after illness:	65	48.9%
Yes	68	51.1%
No		
Maternal current age in years:		
19-24	31	9.6 %
25-29	101	30.4%
30-34	93	28%
≥35	106	32%
Maternal Religion:		
Muslims	262	78.9%
Orthodox	44	13.3%
Protestant	21	6.3%
Catholic	5	1.5%
Marital status:		
Single	12	3.6%
Married	311	93.7%
Divorced	4	1.2%
Widowed	5	1.5%

Maternal occupation:		
Government/private employee	12	3.6%
Merchant	51	15.4%
Housewife	259	78%
Other	10	3%
Maternal Educational Status:		
No formal education	170	51%
Primary education	137	41%
Secondary education	20	6%
Higher education	5	2%
Household SES categories:		
Low SES	111	33.43%
Medium SES	117	35.24%
High SES	104	31.33%
Number of under-five children in the household:		
1	169	50.9%
2	152	45.8%
3	11	3.31%

5.2 Food intake by the young children

Each mother/caregiver was asked to indicate the types of food they fed to their child in the three days prior to the data collection, the frequency and the usual portion size of consumption through an FFQ. Based on this three days' recall, majority 79% of the children consumed 'Kita'; a flat bread, prepared from maize, mainly in 2-3 times per day. Fifty-seven percent, had one fourth of a full 'Enjera'; a pancake prepared from maize & teff blend in 2-3 times per day. Fifty percent had Bread; made up of wheat one times in three days. Thirty-seven of the children consumed 'Shiro Wot'; a stew mainly prepared from broad bean in 2-3 times per day. In addition, 23.5% had cow milk one times in three days. Very few or less than 10% of the children consumed the rest assessed food items, mainly consumed food items in the recent three days are listed in Table 3.

Maize is one of the common staple food in the study area, and it was the main (85.5%) stored crop in the studied households, followed by teff (13%). Sixty-one percent of the households use modern silos to store their crops and majority (68%) store for more than five weeks. Fifteen percent of the children in the study consumed diversified (four and above food groups).

Table 2: Type of food mainly consumed by the young children, frequency of consumption, and usual portion size in the recent 3 days (72 hrs) in Butajira District, South-central Ethiopia, 2018

Mainly consumed food items in recent 3 days		**Frequency of consumption**	**Usual portion size**
'Kita'; a flat bread prepared from maize	Yes 262 (79%) No 70 (21%)	(50.4%) 2-3 times per day	(60.3%),84gram
Enjera; pan cake made from teff & maize	Yes 188(56.6% No 144(43.4%)	(36%) 2-3 times per day	(84%), 93gram
Bread, made from wheat	Yes 166 (50%) No 166 (50%)	(42.8%) one times in 3 days	(52%), 89 gram
'Shirowot'; a stew made from broad been	Yes 122(36.7%) No 210(63.3%)	(37.7%) 2-3 times per day	(60.7%), 35gram
Cow milk	Yes 78 (23.5%) No 254(76.5%)	(35.9%) one times in three days	(51.3%), 129ml

5.3 Aflatoxin exposure among young children, level detected in urine and its distribution with socio-demographic characteristics of the study participants

Three-hundred-twenty-seven (327) urine samples from children age 12-59 month were tested for Aflatoxin M1(AFM1), out of which 62.4% (95% CI; 0.56, 0.67) were positive. The level of AFM1 in the urine samples ranged from 0.15ng/ml to 0.4ng/ml.

The distribution of aflatoxin exposure is shown in Table 3. Based on gender, the distribution is almost similar i.e. male (61.7%) and female (63.6%)

Table 3: Distribution of urinary Aflatoxin M1 exposure by age, sex and agro-ecology zone in Butajira District, South-central Ethiopia, 2018

Variables	Number of children urinary AFM1 test done	Number of positive samples (percent)
Total	327	204 (62.4%)
Age-group		
12-23	24	20 (83%)
24-35	79	57 (72%)
36-47	139	79 (56.8%)
48-59	85	48 (56.5%)
Sex		
Boys	206	127 (61.7%)
Girls	121	77 (63.6%)
Agro-ecological zone		
Highland	92	50 (54%)
Midland	123	78 (63%)
Lowland	112	76 (67.9%)

5.4 Prevalence of stunting and its distribution with socio-demographic characteristics of the study participants

The study showed a high prevalence of stunted growth. Of the 322 children, 196 (60.9%, CI:0.55, 0.66) of them were stunted. Among which, 22.7% were severely stunted. With respect to gender, 125(61.9%) of male and 71(59%) of female children were stunted. Height-for-age Z-scores distribution by age-group, sex and agro-ecological zone in the study area is shown in table 4.

Table 4:Distribution of Height-for-age Z-score children by age, sex and agro-ecological zone among 12-59 month in Butajira District, South-central Ethiopia, 2018

Variables	Total number of children	stunted (%)	Mean z-score	*P-value*
Total	322	196 (60.9%)	-2.2±0.95	
Age-group				
12-23	25	9 (36%)	-1.38±0.25	
24-35	79	45 (57%)	-2.19±0.12	P=0.015
36-47	133	82 (61.7%)	-2.31±0.75	
48-59	85	60 (70.6%)	-2.34±0.08	
Sex				
Boy	202	125 (61.9%)	-2.34±0.07	P=0.629
Girl	120	71 (59%)	-2.19±0.08	
Agro-ecological zone				
Highland	91	64 (70%)	-2.38±0.97	
Midland	122	66 (54%)	-2.07±0.09	P=0.056
Lowland	109	66 (60.6%)	-2.25±0.09	

5.5 Association between stunting and aflatoxin exposure

Variables that were statistically significant and clinically important in the bivariate analysis were fitted in to the multiple logistic regression model. The results of the bivariate and multivariate analysis are shown in Table 6.

On the multiple logistic regression model being in the age group 24 to 35 month (95%; 1.24, 11.17), 36 to 47 month (95%; 1.66, 14.97) and 48 to 49 month (2.54, 25.19); having no toilet facility or using the field/bush as a source of toilet (95%; 0.13, 0.64) and storage of crops used for household consumption for more than two weeks (95%; 1.04, 18.28) and more than five weeks (95%; 1.51, 26.48); showed a statistically significant association with stunting.

Children exposed to aflatoxin at a level of 0.4ng/ml were 1.9 times (95% CI; 0.79, 4.46) more likely to develop stunting than those who were not exposed, but this association was not statistically significant at p-value <0.05 and 95% CI.

Table 5: Results of bivariate and multivariate logistic regression of children age 12-59 month in Butajira District, South-central Ethiopia, 2018

Dependent variable:	**Stunted**			
Independent variables	**Yes(%)**	**No (%)**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**
Aflatoxin exposure				
Not exposed	74(61.7%)	46(38.3%)	1	1
Exposed at level 0.15ng/ml	86(57.7%)	63(42.3%)	0.9 (0.56-1.49)	0.8 (0.46, 1.44)
Exposed at level 0.40ng/ml	33(67.3%)	16(32.7%)	2.4 (1.09-5.06)	1.9 (0.79, 4.46)
Age:				
12-23 month	9(36%)	16(64%)	1	1
24-35 month	45(57%)	34(43%)	2.4 (0.93, 5.97)	3.7 (1.24, 11.17) *
36-47 month	82(61.7%)	51(38.3%)	2.9 (1.18, 6.95) *	4.9 (1.66, 14.97) **
48-59 month	60(70.6%)	25(29.4%)	4.3 (1.67,10.93)**	8.1 (2.54,25.19)***
Agro-ecology zone				
Highland	64(70.4%)	27(29.6%)	1	1
Midland	66(54%)	56(46%)	0.5 (0.28, 0.88) *	0.7 (0.28, 1.51)
Lowland	66(60.6%)	43(39.4%)	0.7 (0.36, 1.17)	0.55 (0.25, 25.9)
Maternal occupation:				
Government & private employee	5(42%)	7(58%)	1	1
Merchant	28(59.6%)	19(40.4%)	2.1 (0.57, 7.47)	0.9 (0.17, 4.52)
Housewife	158(62.5%)	95(37.5%)	2.3 (0.72, 7.54)	1.1 (0.23, 4.59)
Other	5(50%)	5(50%)	1.4 (0.26, 7.58)	0.5 (0.06, 3.89)
Illness in the past two weeks				
Malaria/fever	19(67.9%)	9(32.1%)	1	1
Diarrheal	16(59.3%)	11(40.7%)	0.7 (0.23, 2.08)	0.7 (0.19, 2.56)
Flu	36(67%)	18(33%)	0.9 (0.36, 2.5)	1.1 (0.34, 3.55)
Cough & other	15(88%)	2(12%)	1.7 (0.29, 9.64)	6.1 (0.87, 42.29)
Had not been ill	110(56%)	86(44%)	0.6 (0.26-1.4)	0.5 (0.19, 1.37)
Water source for drinking				
Piped to yard/plot	21(51%)	20(49%)	1	1
Public tap	101(59%)	71(41%)	1.4 (0.68, 2.68)	1.2 (0.5, 3.07)
Protected well	19(65.5%)	10(34.5%)	1.8 (0.68, 4.82)	1.9 (0.53, 7.38)
Unprotected well	24(70.6%)	10(29.4%)	2.3 (0.88, 5.96)	1.4 (0.39, 4.77)
Protected spring	10(59%)	7(41%)	1.4 (0.43, 4.27)	1.2 (0.3, 4.81)
Pound/river/lake/stream/dam	21(72%)	8(28%)	2.5 (0.9, 6.93)	1.3 (0.29, 5.76)

Type of toilet				
Pit latrine with slab	158(66%)	80(34%)	1	1
Pit latrine without slab	22(51%)	21(49%)	0.5 (0.28, 1.0)	0.5 (0.24-1.12)
No facility/bush/field	16(39%)	25(61%)	0.3 (0.16, 0.6)***	1.5 (0.13-0.64)**
Number of under-five children in the household				
1	94(57%)	71(43%)	1	1
2	96(65%)	51(35%)	1.4 (0.89, 2.25)	1.3 (0.78, 2.34)
3	6(60%)	4(40%)	1.1(0.31, 4.17)	1.3 (0.31, 5.47)
Duration crops are stored for household consumption:				
One-week maximum	5(42%)	7(58%)	1	1
More than two weeks	53(62%)	33(38%)	2.2 (0.66, 7.67)	4.4 (1.04, 18.28) *
More than five weeks	138(62%)	86(38%)	2.2 (0.69, 7.3)	6.3 (1.51, 26.48)**

*P value <0.05 **P value <0.01 ***P value <0.001

6 DISCUSSION

This study assessed aflatoxin exposure among young children aged 12-59 months by detecting urinary Aflatoxin M1 using ELISA. The prevalence of aflatoxin exposure was 62.4% among the study participants.

Ethiopia, like other African countries, has hot and humid environments which are conducive for aflatoxin contamination. This study found high (62.4%) prevalence of aflatoxin exposure in the urine of children, with level ranging from 0.15ng/ml to 0.4ng/ml. This indicates that the children were recently exposed to aflatoxins in their diet. As there is no safe threshold for aflatoxin exposure any level of exposure is considered a risk (13). Our study has higher prevalence and concentration of aflatoxin level in contrast to a study done in Ethiopia, were AFM1 was detected in 7% of the study participants with a range 0.064-0.0070ng/ml (31). And similarly a study in Cameroon, reported a prevalence of 14% with concentration ranging from 0.06-4.7ng/ml of AFM1 (32). The above two cross-sectional studies used LC-MS/MS for detection of aflatoxin in urine, which made the possibility for trace detection difficult and the studies have relatively small sample size than the present study, where the available number of samples for the analysis of aflatoxin exposure can limit the statistically power of the result. In another study done in Kenya by using ELISA, AFM1 was detected in 79.2% of the study participants (51). Similarly, a study done in China using ELISA, found that 82% of the participants were exposed to AFM1 in urine (52). This can be attributed to the difference in extent of contamination by the study participants. In a contrast a study done in Ghana using HPLC fluorescent detection, AFM1 was detected in all urine samples at a range of 24.7-8368.9 pg/mg creatinine (4). This shows that, besides the difference in the extent of contamination of the participants, choice of analytical method determines the level of detection. A very sensitive detection system like fluorescence and ELISA allow detection at very low pictogram/ml (32).

This study also showed that ‘Kita’; made from maize, ‘Enjiera’; a pancake, prepared from teff and maize blend; bread, prepared from wheat, ‘Shiro wot’; stew, prepared from broad bean and cow milk were the mainly consumed food types in the three days’ recall. Different studies done in Ethiopia, showed that the ingredients consumed by the study participants are prone to contamination by aflatoxin. A survey done by Ayalew. A in 2010, aflatoxins were detected in 88% of maize samples with a concentration of 4.1µg/kg

(38). A survey by Alemu et al; found contamination of maize with aflatoxin B1 (AFB1) in Southern Ethiopia with concentration of 22.72µg/k (53). According to a report by USAID in 2011, aflatoxin B1 was detected in four major crops of Ethiopia: barley, sorghum, teff and wheat (41).

Statistically significant association was not found between aflatoxin exposure level and stunting. This result is consistent with a study done in Ethiopia , Kenya (51) and Cameroon (32), which reported that no association was found with the different malnutrition categories (stunted, wasted and underweight) and aflatoxin level detected in the urine of young children. In contrast to this, a study done in Benin and Togo among under-five children found an association between aflatoxinB1-albumin adducts and stunting (35). The same researchers subsequently conducted a longitudinal study and found that the highest quartile of aflatoxinB1-albumin adduct was associated with a 1.7cm mean height reduction compared with the lowest quartile (36). This difference in results may be due to the relatively long half-life of the aflatoxin B1-albumin adduct and theoretically aflatoxin B1-albumin adduct accumulate following chronic-exposure to reach levels 30-fold higher than that the level's found after a single dose. In addition to this, need to be urinary aflatoxin exposure only shows a recent exposure. For aflatoxin exposure to result in stunting the children chronically exposed and this again may depend on the exposure dose and duration, genetics, health or nutritional status of the children.

The strength of this study can be seen in terms of using ELISA to analyse the level of AFM1 in urine, which is a highly sensitive analytical method, simple, rapid, preferred to analyse large samples and made trace detection possible as the excretion rate of aflatoxin M1 through kidneys is very low. Even though, ELISA has issues with specificity, where compounds with similar chemical groups as AFM1 can also interact with the antibodies. But this is argued by Groopman et al, in that AFM1 is the most common metabolite of AFB1 in urine, so results are unlikely to be distorted (28).

Absence of data on maternal height, which can affect the prevalence of stunting can be considered as a limitation and the food frequency questionnaires were subjected to recall bias.

7 CONCLUSION

The study showed a high prevalence of aflatoxin exposure in young children with level ranging from 0.15-0.4ng/ml. This clearly indicates that the children were recently exposed to aflatoxin, mainly through their diet. However, the study didn't find a statistically significant association between aflatoxin exposure and stunting.

8 RECOMMENDATIONS

In view of the results of the study the following recommendations are made:

- **For policy makers**: National polices need to be revised, particularly those related to agriculture and food safety in order to take in to account the measures for control of aflatoxin exposure. Regulatory limits for the level of aflatoxins in food items need to be devised.
    - On the agricultural sector, improving the pre- and post- harvest crop management system through measures involving proper drying, storage and food preparation methods, which are effective especially in subsistence farming communities where food crops are produced and consumed within households & communities without prior inspection or control.
    - In the health sector, possible measures need to be set in order to reduce aflatoxin exposure and associated health risks. Such measures may include, promoting dietary diversification and limit quantities of intake of foods, which are susceptible to aflatoxin contamination. Regulations also need to be set targeting food processing industries.
- **For program managers**: Interventions that are focused on reducing the contamination of aflatoxin need to be developed like awareness raising programs among the public about aflatoxin exposure, the health risks and ways of control.
- **For researchers**: Further and more researches are needed to investigate the impact aflatoxin has on growth and development of children throughout the country using a better analytical method (like LC/MS/MS), a strong study design and a long time exposure biomarker, like AFB1-albumin adduct.

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10 ANNEXES

10.1 Annex: Conceptual frame work

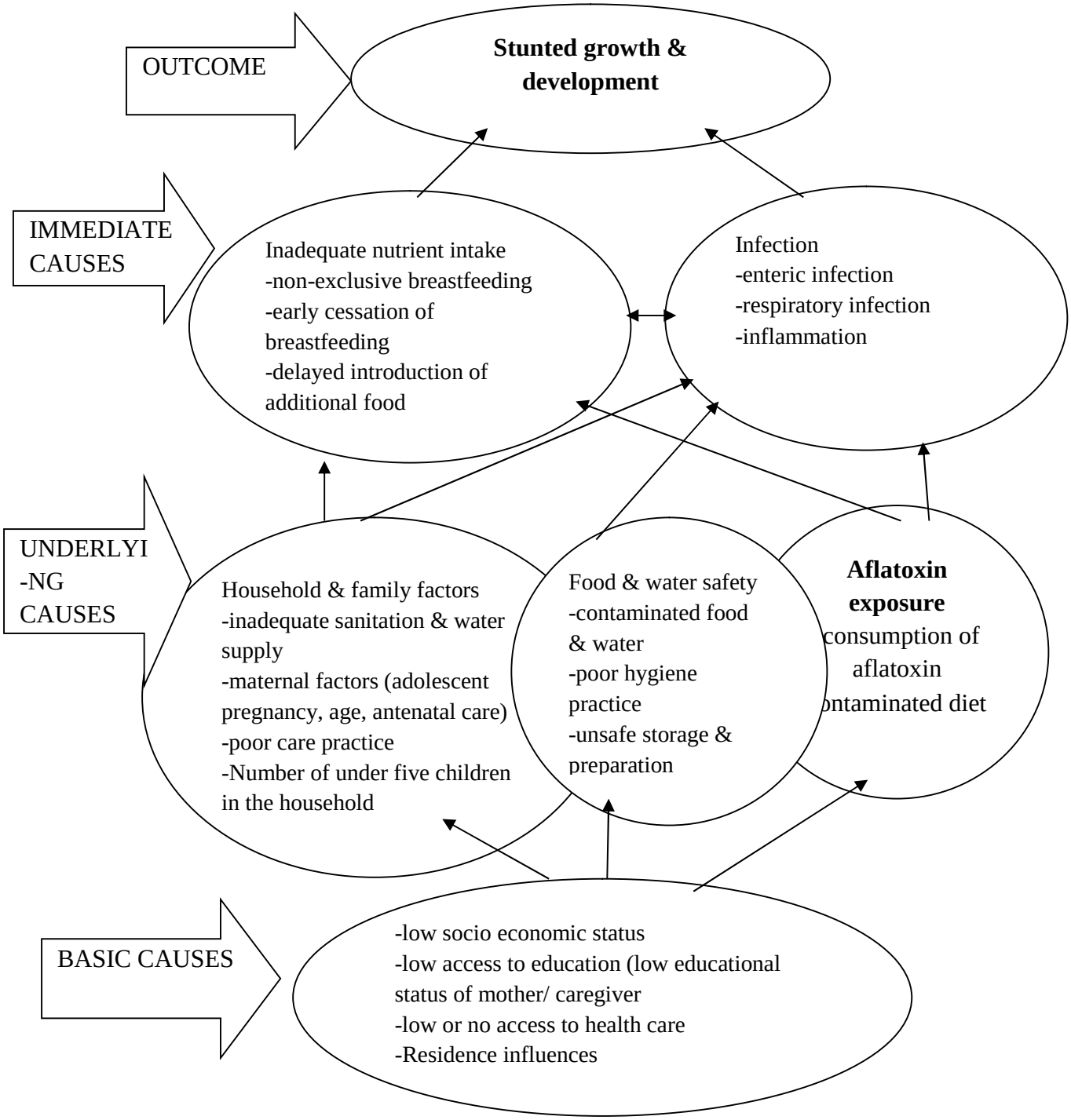

Figure 2: Adopted from WHO Conceptual framework for childhood stunting, 2013

10.2 Annex: Subject information sheet and Informed consent form

Subject information sheet

This sheet is to be read for the participants of the study before collecting any information from them.

Hello. My name is _____I am here on behalf of Mary Ayele, student of Addis Ababa University, College of Health Sciences and School of Public Health. She is conducting a research on 'Aflatoxin exposure level and its association with stunting in young children' in Butajira, for the partial fulfilment of master's in public health. She received permission from Addis Ababa University, School of public health and Gurage zone regional health bureau to conduct this study. You are selected by chance to participate in this study, because you are a mother/care giver of a child 12-59 month old. You will participate if you give me consent after you have understood the following information:

What the study is about: The purpose of the study is to know whether young children are exposed to Aflatoxin and if this exposure is related with stunting.

Aflatoxins are naturally occurring toxic by-products that contaminate staple foods and cause different health consequences on humans and animals. Evidences suggest several mechanisms by which aflatoxin can impair growth and development in children.

Design of the study: The study is a cross-sectional study among children age 12-59 month (12-59 month).

What I will ask you to do: If you agree to participate in this study, I will conduct an interview that will take about 15-20 minutes. Your child's height or length according to his/her age will be measured. I will collect 5-10ml of urine sample from your child. I would very much appreciate, your collaboration and participation in this study.

Risks and Benefits: The result of the study helps to develop strategies and interventions targeting children in order to reduce aflatoxin exposure and to prevent the health consequences following it. In this way your child may benefit from the intervention policy. There is no risk you should fear as a result of participating in this study. And again there is no payment or compensation you will receive due to your participation on this research.

Confidentiality: All information given by you will be kept confidential. Any of yours and your child's personal information will not register. The records of the study will be kept private, only the researchers will have access to the records. Your child name will not be included in the urine sample taken, which will protect his/her identity and preserve anonymity. In any sort of report, we make public we will not include any information that will make it possible to identify you.

Taking part is voluntary: Your participation is purely based on your willingness. You have the right to choose not to take part in the study. If you choose to take part, you have the right to stop at any time. If you are willing to participate or refuse or decide to withdraw later, you will not be subjected to any ill-treatment.

If you have questions: If you have questions regarding this study, you can contact the principal investigator and if you need to clarify the question, you can ask me at any time of the interview.

Address of the principal investigator:

Name: Mary Ayele

Phone: 0921452310/0967213849

Email: maryayele4@gmail.com

Informed consent

Are you willing to participate in this study?

1. Yes (take informed consent and continue the interview)
2. No (write the reason for refusal and continue with the next participant) _____

I, the selected participant of the study has understood the information sheet and I am willing to participate in this study.

Informed consent certified by:

Respondent's signature_____ Date_____

Data collector: Name_____ Signature_____

The date of data collection_____Time started__End time_____

The status of the questionnaire:

1. Completed ☐
2. Partially completed ☐
3. Not filled ☐

The supervisor, Name _____ Signature_____, checks the questionnaire ☐

10.3 Annex: Questionnaire

a. English Version

Questionnaire code: _____

Instruction: for open questions, fill the correct response on the space provided and for questions with choices, circle the correct response from the choice provided.

Section 1: Identification

Date of data collection_____ Starting time _____ Time ended_____

S.N	Questions	Response and coding	Skip
101	Questionnaire code	_____	
102	House hold number	_____	
103	Name of Woreda	_____	
104	Name of Kebele	_____	
105	Relation of the respondent with the child	_____	

Section 2: Information on the child

Now I would like to ask some questions about your child recruited for this study

S.N	Questions	Response and coding	Skip
201	What is the sex of the child?	Male .....1 Female..... 2	
202	When was the date of birth? ***(Use maternal memory, birth certificate, vaccination or other card that have age of child or local calendar)*** ***If she don't know the birth date ask month & year***	_____ (date/month/year) _____ (month/year)	
203	How old is the child in month?	_____	
204	In the two preceding weeks, what illness did your child suffer from?	Had not been ill…….1 Malaria/fever…….2 Diarrhea…….3 Flu……...4 Coughing…….5 Other (specify) _____	If (1), skip 212

205	Did you seek advice or treatment for the above illness from any source?	Yes.....1 No..... 2	

Section 3: Mothers socio-demographic and economic characteristics

Now I would like to ask some information's about you (the mother) and your family

S.N	Questions	Responses and coding	Skip
301	What is your current age?	_____	
302	Marital status	Single .....1 Married .....2 Divorced .....3 Widowed.....4	
303	Religion	Orthodox.....1 Muslim .....2 Protestant.....3 Catholic.....4 Other_____	
304	Have you ever attended school ?	Yes..... 1 No 2	If No (2), skip to 307
305	What is the highest level of education you attended?	Primary education.....1 Secondary education.....2 Higher/tertiary.....3 Technical/vocational.....4	
306	What do you do for living?	Government employee.....1 Private employee.....2 Merchant.....3 Daily laborer.....4 Farmer.....5 Housewife.....6 Other (specify) _____	
307	How many children age 5 and below live in this household? (**including the participant child)**	_____	

Section 4: Household characteristics

Now I would like to ask you about the house you are currently living.

Instruction: If the interviewee is not comfortable to answer or too young to answer, you may request other older member of the household to help you get the necessary information.

S.N	Questions	Responses and coding	skip
401	Owner ship of the house?	Private……..…1 Government house……2 Rent……..3 Relatives/others house…..4 Other (specify) _____	
402	Main construction material used for the roof	Thatch/leaf/mud..... 1 Plastic..... 2 Bamboo.....3 Wood planks..... 4 Corrugated iron/metal.....5 Wood.....6 Cement/concrete..... 7 Other (specify) _____	
403	Main material of the wall	No walls.....1 Wood with Mud .....2 Cane/Trunks/Bamboo/Reed .....3 Dirt.....4 Stone with mud .....5 Card board .....6 Stone with lime/cement .....7 Bricks .....8 Wood planks/shingles .....9 Corrugated iron/metal……10 Other(specify) _____	
404	Does your household have the following material that is functioning?	Yes NO 1. Electricity..... 1 2 2. Radio..... 1 2 3. Television..... 1 2 4. non-mobile..... 1 2 telephone 5. Refrigerator..... 1 2 6. Sofa ..... 1 2 7. Electric mitad..... 1 2 8. Kerosene lamp/.... 1 2 Pressure lamp 9.Solar……. 1 2	
405	Does any member of this household own:	Yes NO 1.Mobile phone..... 1 2	

		2. Bicycle..... 1 2 3. Motorcycle..... 1 2 4. animal-drawn cart 1 2 5. Car/truck..... 1 2	
406	How many rooms does your house have?	_____	
407	Do you have separate room, used as a kitchen?	Yes.....1 No.....2	
408	What type of fuel does your household mainly use for cooking?	Electricity.....1 Natural gas.....2 Charcoal..... 3 Wood..... 4 Agricultural crop..... 5 Animal dung..... .6 Other(specify) _____	
409	What kind of toilet facility do members of your household usually use?	Flush or pour flush toilet.....1 Pit latrine with slab.....2 Pit latrine without slab.....3 Ventilated improved pit latrine.....4 bucket toilet.....5 No facility/Bush/field..... 6 Other (specify) _____	
410	What is the main source of drinking water for members of your household?	Piped inside dwelling .....1 Piped to yard/plot.....2 Public tap .....3 Protected well .....4 Unprotected well.....5 Protected spring .....6 Unprotected spring.....7 Pond/lake/River/stream/spring/Dam.8 Rain water .....9 Tanker truck……10 Bottled water…….11 Other(specify) _____	
411	Does any member of this household own any agricultural land?	Yes.....1 No.....2	If no (2), skip to question 413
412	How many (local units) of agricultural land do members of this household own?	Local units_____	
413	Does this household own any livestock, herds, other farm animals or	Yes.....1 No.....2	If no (2), skip to question 415

	poultry?		
414	How many of the following animals do this household own?	Milk cow or bulls / / Livestock/other cattle / / Horse, donkey, mules / / Sheep / / Goats / / Chicken / / Bee hives / /	
415	What is the main source of water used by your household for cooking and hand washing?	Piped inside dwelling .....1 Piped to yard/plot.....2 Public tap .....3 Protected well/spring .....4 Unprotected well/spring.....5 Protected well .....6 Unprotected well.....7 Pond/lake/River/stream/spring/Dam.8 Rain water .....9 Tanker truck…….10 Bottled water……11 Other(specify) _____	
416	Do you do anything to the water to make it safer to drink?	Yes.....1 No.....2 Don't know.....3	
417	What do you usually do to make the water safer to drink?	Boil.....1 Add bleach/chlorine.....2 Strain through a cloth.....3 Use water filter (ceramic/sand/composite/etc) .....4 Solar disinfection.....5 Let it stand & settle.....6 Other (specify) _____	
418	Where do you usually store your crops (grains & legumes) for home consumption	Gota, made from teff straw & mud...1 Gotera, made from wood or bamboo……...2 In underground pits.....3 In plastic bags/ containers.....4 In modern silos.....5 Other (specify) _____	
419	For how long do you store the crop (s)?	1-week maximum……1 More than 2 weeks……...2 More than 5 weeks……...3 Other (specify) _____	
420	What is the main crop in your store used for home consumption?	Teff.....1 Maize.....2 Wheat .....3 Barley.....4 Sorghum.....5	

		Other (specify) _____	

Section 5: Now I would like to ask you the type of food items your child has eaten yesterday (from yesterday 12:00 to today 12:00 in the morning)

S.n	Question	Response
501	Cereal containing food items like, bread, pasta, rice, biscuit, enjiera, cookies etc.	Yes ……1 No……2
502	Roots and tubers like, potato, cassava, beet-root, 'kocho' (false banana) etc.	Yes ……1 No……2
503	Colored fruits and vegetables like, orange, carrot, mango, papaya etc.	Yes ……1 No……2
504	Dark green vegetables, like spinach, cabbage etc.	Yes ……1 No……2
505	Meat, it can be from ox, sheep, goat, camel	Yes ……1 No…… 2
506	Organ meats, liver, kidney etc.	Yes ……1 No……2
507	Poultry, chicken, duck etc.	Yes ……1 No……2
508	Egg	Yes ……1 No……2
509	Fish	Yes ……1 No……2
510	Legumes, pea, broad-pea, chicken-pea etc.	Yes ……1 No……2
511	Nuts	Yes ……1 No…… 2
512	Dairy products; milk, cheese, yogurt etc.	Yes ……1 No…… 2
513	Fats and oils	Yes ……1 No…… 2
514	Sweet foods or drinks, like honey	Yes ……1 No…… 2
515	Tea or coffee	Yes ……1 No…… 2

Section 6: Type of food eaten by the child in the previous 3 days and frequency of consumption.

Now I would like to ask you the type of food items your child has eaten in the previous 3 days from now & how many times he had that specific food in that 3 days.

Select only one frequency and one portion size per row. Tick on the appropriate response.

S.N	How often, did you eat the following food items to your child for the last 3 days		Never	1 times per 3 days	2-3 times per 3 days	1 times per day	2-3 times per day	What was your usual portion size? *Show & select the gram from photograph provided*
601	'Enjera'- pan cake, made from:	Teff						full, 3/4, 1/2, 1/4, Enjera
		Maize						
		Teff and maize blend						
		Sorghum and maize blend						
		Sorghum and teff blend						
602	Bread, made from:	Wheat						¼, ½ slice of bread or full
		Wheat and Maize blend						
		Maize and sorghum						
603	Kitta, a form of Pan cake, made from:	Maize						*Choose the gram from the photograph*
		False banana ('Kocho')						
604	Porridge, made from:	Maize						*Choose the gram from the*
		Maize and Wheat blend						

		Maize, Wheat and barley blend						*photograph*
		Maize, wheat and teff blend						
		Maize, wheat, barley and teff blend						
		Maize and barley blend						
		False banana (Bulla)						
605	‘Kollo’, roasted, made from:	Barley (‘gebus kollo’)						1, 2 or more than two handful
		‘Shimbura’, roasted, made from chick pea						
		‘Ater’, roasted, made from pea						
		Peanut roasted						
606	‘Shiro wot’, stew made from	Pea, broad bean & chicken pea blend						*Choose the gram from the photograph*
		Pea and chicken pea blend						
		Pea, chicken pea, broad bean & gwaya blend						
		Pea only						
		Broad bean						
		Chicken pea						

607	Cereal products	pasta						*Choose the gram from the photograph*
		rice						
		Mokorony						
		Endomin						
608	Milk and Dairy products	Cow Milk						*Choose the gram from the photograph*
		Powdered Milk						
		Agwat						
		Yogurt						
		Cheese						
609	Egg							1, 2, >2 eggs
610	Peanut butter							½, 1, >1 table spoon of peanut butter
611	Meat Poultry							½, 1, >1 slice beef, ½, 1, >1 best portion chicken

Section 7: Height measurement

Instruction: *follow the height measurement protocol you are given*

S.N	Question	Response
601	Age in month	_____
602	Measurement taken	1. Length (lying down) 2. Height (standing up)
603	Measurement in centimeter (cm), to the nearest 0.1cm	□□□.□

If the selected participant is not willing to get measured, write the reasons for his/her refusal.

_____

Section 7: Urine collection

Instruction: *refer to the urine collection guideline you are given*

S.N	Question	Response
701	Questionnaire number	_____
702	House hold number	_____
704	Date of urine collection	_____
705	Amount of Urine collected in milliliters (54)	_____

If the selected participant is not willing to give urine, write the reasons for his/her refusal. -

_____

b. Amharic version

ለተጠያቂው መረጃ መስጫ እና የስምምነት መጠየቂያ ቅፅ

የተጠያቂው / መላሾች መረጃ ቅፅ/

እንደምን አደሩ / ዋሉ፡፡ ስሜ_____ይባላል፡፡ የመጣሁት ከኢ.አ ዩኒቨርስቲ ህብረተሰብ ጤና ሳይንስ ሁለተኛ ዲግሪ ተማሪ የሆነችው ሜሪ አየለን ወክዬ ነው፡፡ የሁለተኛ ድግሪ ትምህርት ማሞያ ለሚሆን ጥናት መረጃ ሰብሳቢ ነኝ፡፡ የሁለተኛ ድግሪዋን ለመመረቅ በቡታጅራ ክልል ህፃናት በምን ያክል መጠን ለአፍላቶክሲን እንደተጋለጡና አፍላቶክሲን ከልጆች የቁመት እድገት ጋር ያለዉን ግንኙንት ለማወቅ ከኢ.አ ዩኒቨርስቲና ከክልል ጤና ቢሮ ፈቃድ አግኝታ እየሰራች ነው፡፡

እርስዎ የተመረጡት በዚህ ክልል ነዋሪና እድሜው ከአንድ አመት እስከ አምስት አመት የሆነ ህፃን ልጅ እናት/አሳዳጊ ስለሆኑ ነው፡፡ የእርስዎ ተሳትፎ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት ላይ የተመሰረተ ነው፡፡ የጥናቱ ሙሉ መረጃ ሰምተው ከተረዱ በዋላ በጥናቱ ለመሳተፍ ከተስማሙ የስምምነት ፍርማዎን ይሰጡኛል፡፡

የጥናቱ አላማ፤ የዚህ ጥናት ዋና አላማ ህፃናት በምን ያክል መጠን ለአፍላቶክሲን እንደተጋለጡና አፍላቶክሲን ከልጆች የቁመት እድገት ጋር ያለዉን ግንኙንት ለማወቅ ነው፡፡

አፍላቶክሲን ማለት በተፈጥሮ የሚገኝ ምግብን የሚበክል ሻጋታ ሲሆን፤ በአብዛኛው ሰብል፣ ጥራጥሬ እና ለውዝ የመሳሰሉ እህሎችን ያጠቃል፡፡ ይህ በሽታ አምጪ ሻጋታ በሰውና በእንስሳት ላይ የጤና እክሎችን ያመጣል፡፡ ከዚህም አንደኛው በህፃናት እድገት ላይ ተጽኖ እንደሚያመጣ ጥናቶች ይጠቁማሉ፡፡

የምጠይቆት ነገሮች፤ ጥናቱ ውስጥ ለመሳተፍ ከተስማሙ ከእርሶ ጋር ከ15-20 ደቂቃ ቃለመጠይቅ አደርጋለዉ፣ የልጆ ቁመትን እለካለው እና ከልጆ 5-10 ሚሊልትር የሚያክል ሽንት እወስዳለው ፡፡ ተሰታፊነቶ በጣም ይበረታታል፡፡

የጥናቱ ጥቅምና ጉዳት፤ የህዚህ ጥናት ውጤት በህጻናት ጤና አጠባበቅ ላይ የሚሰሩ አካላት ህጻናት ለአፍላቶክሲን እንዳይጋለጡ መመሪያ እና እርምጃ ለመቅረጽ ይረዳል፡፡ በዚህም ምክኒያት ልጆቾ ተጠቃሚ ይሆናሉ፡፡ በጥናቱ በመሳተፎ የሚደርስቦት/የሚሰጦት ምንም አይነት ጉዳት ወይም ክፍያ የለም፡፡

ሚስጥራዊነት፤ እርሶ የሚሰጡን መረጃ ሁሉ በሚስጥር ይያዛል፡፡ አንድም የግሎ መረጃ አየጻፍም፤ የተሰበሰበዉን መረጃ ከአጥኞቹ በስተቀር ማንም አያገኘውም፡፡ በሚሰበሰበው መረጃ ላይ የልጆ ስም አይጠቀስም፡፡

ተሳትፎ ፍቃደኝነት ላይ የተመሠረተ ነው፤ጥናቱ ዉስጥ ለመሳተፍም ሆነ መመለስ የማይፈልጉትን ጥያቄ ለመመለስ አይገደዱም፡፡ እንዲሁም ቃለመጠይቁን በፈለጉ ጊዜ የማቆም መብትዎ የተጠበቀ ነው፡፡

በጥናቱ ላይ ጥያቄ ካሎት፤ አስጠኚውን ማግኘት ይችላሉ፡፡ እናም ግልፅ ያልሆነና እንዲብራራሎት የሚፈልጉት ጥያቄ ካለ በየትኛውም ሰዓት መጠየቅ ይችላሉ፡፡

የአስጠኚው አድራሻ፤

ስም፡ ሜሪ አየለ

ስልክ: 0967216849 ፤ ኢሜይል: maryayele4@gmail.com

<u>የስምምነት መጠየቂያ /ማረጋገጫ ቅፅ</u>

ከላይ በተሰጡት መረጃ መሰረት በዚህ ጥናት ለመሳተፍ ፈቃደኛ ኖት?

1 አዎ (የስምምነት ማረጋገጫ ከተወሰደ በኃላ ቃለመጠይቁ ይቀጥል)

2 አይደለሁም (ምክንያቱን ፅፈህ/ሽ ወደ ሚቀጥለው ተሳታፊ እለፍ)

ከዚህ በላይ ስለጥናቱ የተፃፈውን መግለጫ በሚገባኝ ቃላት አንብቤ ወይንም ተነቦልኝ ተረድቻለው፡ በመሆኑም በዚህ ጥናት ለመሳተፍ ፍቃደኝነቴን ከዚህ በታች ባስቀመጥኩት ፍርማዬን አረጋግጣለው፡፡

የተሳታፊው ፍርማ _____ቀን _____

የመረጃ ሰብሳቢው ስም _____ፍርማ_____

መረጃው የተሰበሰበበት ቀን_____የተጀመረበት ሰዓት_____ያለቀበት ሰዓት___

የቃለመጠይቁ ውጤት

1. ሙሉ በሙሉ የተሟላ 2. በከፊል የተሟላ 3. ምንም ያልተሟላ

በተቆጣጣሪዎች ተረጋግጣል ስም_____ፊርማ _____

የጥያቄ መለያ ቁጥር _____

መመሪያ፡- ለሚከተሉት ጥያቄዎች ባዶ ቦታ ለተዘጋጀላቸው የተጠያቂውን ትክክለኛ ምላሽ ባዶ ቦታው ላይ ይሙሉ፤ ምርጫ ላላቸው ጥያቄዎች ከተሰጡጥ አማራጮች ውስጥ ትክክለኛው ላይ ያክቡ

ክፍል አንድ፡ መለያ

የመጠይቁ ቀን_____መጠይቁ የተጀመረበት ሰዓት_____መጠይቁ የተጠናቀቀበት ሰዓት ____

ተ.ቁ	ጥያቄ	መልስ	ወደ ተጠቀሰው ጥያቄ ይሂዱ
101	የጥያቄው መለያ ቁጥር	_____	
102	የቤት ቁጥር	_____	
103	የወረዳ ስም	_____	
104	የቀበሌ ስም	_____	
105	ተጠያቂው ከህፃኑ ጋር ያላቸው ግንኙነት	_____	

ክፍል ሁለት - የሕጻኑ መረጃ

አሁን ልጅን በተመለከተ አንዳንድ ጥያቄዎች እጠይቆታለዉ

ተ.ቁ	ጥያቄ	መልስ	ወደ ተጠቀሰው ጥያቄ ይሂዱ
201	የህፃኑ ፆታ ምንድ ነው ?	ወንድ.....1 ሴት.....2	
202	የትውልድ ቀን ***(እናቱ ካላስታወሰች የልደት ካርድ፣ የክትባት ካርድ ወይም ሌላ የልጁን ዕድሜ የያዘ ካርድ መጠየቅ ይችላሉ)*** ***እናትየዋ ቀኑን ካላስታወሰች ወሩን እና ዓ.ም ይጠይቁ***	_____ (ቀን/ወር/ዓመት) _____ (ወር/ ዓመት)	
203	የህፃኑ ዕድሜ በወር ?	_____	
204	ባለፉት ሁለት ሳምንታት ዉስጥ፣ ህፃኑ በምን አይነት ህመም ታሞ ነበር ?	አልታመመም ነበር.....1 ወባ/ ትኩሳት..... 2 የተቅማጥ በሽታ.....3 ጉንፋን..... 4 ያስለው ነበር.....5 ሌላ ካለ ይጠቀስ _____	መልሱ 1 (አልታመመም) ከሆነ ጥያቄ 212ን ይዝለሉ
205	ከላይ ከተጠቀሱት ህመሞች ውስጥ ህፃኑ ታሞ ከነበረ፣ ህክምና ወይም ምክር አግኝቶ ነበር ?	አዎ.....1 አይ.....2	

ክፍል ሶስት - የእናት እና የቤተሰብ አጠቃላይ መረጃ

ከዚህ በመቀጠል ስለ ልጁ እናት እና ቤተሰቡን በተመለከተ ጥያቄዎችን እጠይቆታለው

ተ.ቁ	ጥያቄ	መልስ	ወደ ተጠቀሰው ጥያቄ ይሂዱ
301	የእናት ዕድሜ (በአሁን ሰዓት)	_____	
302	የጋብቻ ሁኔታ	ያላገባች.....1 ያገባች..... 2 የተፋታች..... 3 የሞተባት 4	
303	ሀይማኖት	ኦርቶዶክስ .....1 ሙስሊም..... 2 ፕሮቴስታንት ..... 3 ካቶሊክ ..... 4 ሌላ ካለ ይጠቀሱ_____	
304	ትምህርት ተምረሽ ታውቂያለሽ	አዎ ..... 1 አይ 2	መልሱ 2 ከሆነ ወደ ጥያቄ 307 ይለፉ
305	ከፍተኛ የትምህርት ደረጃሽ ከሚከተሉት ውስጥ የትኛው ነው	የመጀመሪያ ደረጃ ..... 1 ሁለተኛ ደረጃ ..... 2 ከፍተኛ ደረጃ ..... 3 ሠርተፍኬት 4	
306	የስራ ዘርፍሽ	የመንግስት ሠራተኛ ..... 1 የግል ቅጥረኛ ..... 2 ነጋዴ ..... 3 የቀን ሠራተኛ ..... 4 ግብርና ..... 5 የቤት እመቤት ..... 6 ሌላ ካለ ይገለጽ _____	
307	በቤታችው ውስጥ አምስትና ከአምስት አመት በታች ዕድሜ ያላቸው ስንት ህጻናት ይገኛሉ (**ለጥናቱ ከተመረጠው ህጻን ጨምሮ**)	_____	

ክፍል 4፡ የኑሮ ሁኔታ

አሁን ስለ ቤተዎ ባህረዮዎችን አስመልክቶ የተወሰኑ ጥያቄዎችን እጠይቆታለው፡፡

ከዚህ በታች ያሉትን ጥያቄዎች የልጁ እናት መመለስ ካልቻለች ወይም ካላወቀች በቤት ውስጥ የሚገኝ እና ስለ ቤቱ አሰራር እውቅት ያለውን ሰው መጠየቅ ይችላሉ፡፡

ተ.ቁ	ጥያቄ	መልስ	ወደ ተጠቀሰው ጥያቄ ይሂዱ
401	የመኖርያ ቤት ይዞታ	የግል..... 1 የቀበሌ..... 2 ኪራይ..... 3 የዘመድ/ የሌላ ሰው..... 4 ሌላ (ግለጽ)_____	
402	የቤቱ ጣሪያ የተሰራበት ዋነኛ የግንባታ ቁላቁስ ምንድን ነው	የሳር ክዳን.....1 ፕላስቲክ.....2 ሸንበቆ.....3 ጣውላ.....4 ቆርቆሮ.....5 እንጨት.....6 ሲሚንቶ.....7 ሌላ ካለ ይጠቀስ _____	
403	የቤቱ ግድግዳ የተሰራበት ዋነኛ የግንባታ ቁሳቁስ ምንድነው?	ግድግዳ የለዉም.....1 እንጨትና ጭቃ..... 2 ዘንባባ/ሸንበቆ/ቀርከሃ/ሣር.....3 ጭቃ..... 4 ድንጋይ ናጭቃ.....5 ካርቶን.....6 ድንጋይና ሲሚንቶ.....7 ጡብ..... 8 ጣውላ.....9 ብሎኬት.....10 ቆርቆሮ.....11 ሌላካለ ይገለጽ _____	
404	ከሚከተሉት ውስጥ በቤት ውስጥ የሚገኘውና አገልግሎት የሚሰጥ የትኛው ነው	አዎ አይ 1.ኤሌክትሪክ..... 1 2 2.ሬዲዮ..... 1 2 3.ቴሌቪዥን..... 1 2 4.የቤት ስልክ..... 1 2 5.ማቀስቀዣ/ፍሪጅ..... 1 2 6.ሶፋ..... 1 2 7.የኤሌክትሪክ ምጣድ.... 1 2 8.የጋዝ መብራት..... 1 2 9.በፀሃይ ሀይል የምሰራ መብራት/ሶላር... 1 2	
405	ከሚከተሉት ውስጥ የቤተሰቡ አባላት ያላቸው የትኛውን ነው	አዎ አይ 1.ሞባይል..... 1 2 2.ብስክሌት..... 1 2 3.ሞተር ሳይክል..... 1 2 4.የእንጨት ጋሪ..... 1 2 5.መኪና..... 1 2	
406	ቤታቹ ዉስጥ ስንት ክፍሎች አሉ	_____	
407	ለኩሽናነት (ምግብ ለማብሰል) የምትጠቀሙት የተለየ(የብቻ) ክፍል አለ	አዎ.....1 አይ.....2	

408	ለማብሰያ የምትጠቀሙት የትኛውን ነው	ኤሌክትሪክ.....1 የተፈጥሮ ጋዝ.....2 ከሰል.....3 እንጨት.....4 የግብርና እፅዋት.....5 ኩበት.....6 ሌላ ካለ ይጠቀስ _____	
409	የምትጠቀሙት የመጸዳጃ አይነት	የሚሰራ በውሃ የሚወረድ መጸዳጃ ቤት ..... 1 የሚሰራ የጉድጓድ መጸዳጃ..... 2 የማይሰራ የጉድጓድ መጸዳጃ ቤት ..... 3 ንፋስ ማውጫ ያለው የጉድጓድ መጸዳጃ ቤት.....4 በባልድ/ጆርካን መጸዳጃ ቤት..... 5 ሜዳ ላይ..... 6 ሌላ ካለ ይገለጽ _____	
410	ውሃ ከየት ነው ምትቀዱት	የቤት ዉስጥ ቧንቧ..... 1 የግቢ ዉስጥ ቧንቧ..... 2 የሰፈር ዉስጥ ቧንቧ/ቦኖ..... 3 የተከለለ የጉድጓድ ዉሃ ..... 4 ያልተከለለ የጉድጓድ ዉሃ ..... 5 የተከለለ የምንጭ ዉሃ..... 6 ያልተከለለ የምንጭ ዉሃ..... 7 የወራጅ ወነዝ/ኩሬ/ሀይቅ..... 8 የዝናብ ውሃ..... 9 የቦቴ/ታንከር ዉሃ..... 10 የታሸገ ውሃ ..... 11 ሌላ ካለ ይገለጽ _____	
411	የእርሻ መሬት አላችሁ	አለን.....1 የለንም..... 2	የለንም ከሆነ ወደ ጥያቄ ቁጥር 413 ይሂዱ
412	ምን ያህል/መደብ መሬት ነው ያላችሁ	_____	
413	በቤት ውስጥ ከብቶች አላችሁ	አለን.....1 የለንም.....2	የለም ከሆነ ወደ ጥያቄ ቁጥር 415 ይሂዱ
414	ከሚከተሉት ውስጥ ቤተሰቡ ስንት የቤት እንስሳት አሉት? በቁጥር	1.ላም / / / 2.በሬ/ኩበት / / / 3.አህያ/ፈረስ/በቅሎ / / / 4.በግ / / / 5.ፍየል / / / 6.ዶሮ / / / 7.የንብ ቀፎ / / /	
415	ለማብሰልና ለእጅ መታጠቢያነት የምትጠቀሙት የውሃ ምንጭ ምንድ ነው	የቤት ዉስጥ ቧንቧ.....1 የግቢ ዉስጥ ቧንቧ..... 2 የሰፈር ዉስጥ ቧንቧ/ቦኖ..... 3 የተከለለ የጉድጓድ ዉሃ ..... 4 ያልተከለለ የጉድጓድ ዉሃ ..... 5 የተከለለ የምንጭ ዉሃ..... 6 ያልተከለለ የምንጭ ዉሃ..... 7 የወራጅ ወነዝ/ኩሬ/ሀይቅ..... 8	

		የዝናብ ውሃ.....9 የቦቴ/ታንከር ዉሃ..... 10 የታሸገ ውሃ ..... 11 ሌላ ካለ ይገለጽ _____	
416	የምትጠቀሙትን ዉሃ ንህጹ ለማድረግ (ለማጣራት) የምትጠቀሙት ዘዴ አለ	አዎ.....1 የለም.....2 አላውቅም.....3	መልሱ 2 ወይም 3 ከሆነ ወደ ጥያቄ 418 ይለፉ
417	በአብዛኛው ጊዜ የምትጠቀሙት ዘዴ ምንድ ነው	ማፍላት.....1 ከሎሪን መጨመር..... 2 በጨርቅ ማሳለፍ..... 3 በ አሸዋ/ሸክላ/ወዘት ማጣራት..... 4 በጸሀይ አይል የሚሰራ ማጣሪያ.....5 አስቀምጦ እንዲዘቅጥ ማድረግ..... 6 ሌላ ካል ይገለጽ _____	
418	ለምግብነት የምትጠቀሙትን ሰብል/ጥራጥሬ በምንድ ነው የምታከማቹት	ጎታ፣ከሳር/ከሸንበቆ የተሰራ.....1 ጎተራ፣ከ እንጨት/ከሸንበቆ የተሰራ .....2 ጉድጋድ ውስጥ..... 3 በፕላስቲክ በተሰራ ቦርሳ..... 4 በማዳበርያ ..... 5 ሌላ ካለ ይገለጽ _____	
419	ለምን ያህል ጊዜ ነው የምታከማቹት	ቢበዛ ለአንድ ሳምንት.....1 ከሁለት ሳምንት በላይ.....2 ከአምስት ሳምንት በላይ.....3 ሌላ ካለ ይገለጽ _____	
420	በአብዛኛው ጊዜ በማከማቻቸው የሚገኘው ሰብል/ጥራጥሬ ምንድ ነው	ጤፍ.....1 ቦቆሎ.....2 ስንዴ.....3 ገብስ.....4 ማሽላ.....5 ሌላ ካለ ይገለጽ _____	

ክፍል 5: ልጅዎ ትናንትና ፀሀይ ወጥታ ደግማ እስከምትወጣ (ትናንት ጠዋት 12: 00 ጀምሮ እስከ ዛሬ ጠዋቱ 12:00) ድረስ የተመገባቸዉን ምግቦች ከዚህ ቀጥሎ ይነግሩኛል

ተ.ቁ	ጥያቄ	መልስ
501	ከማንኛዉም ክክ ከሌላቸዉ ጥራጥሬ ማለትም,በቆሎ, ገብስ, ስንዴ, ማሽላ, ዳጉሳ, የተሰራ ዳቦ, ፓስታ, ሩዝ, ያለ እርሾ የተጋገረ ዳቦ, ብስኩት, ኩኪስ ወይም ከአጃ የተሰራ ደረቅ ምግብ	ተመግቧል..... 1 አልተመገበም..... 2
502	ማንኛውም አይነት ድንች, ጎደሬ, ቡላ, ቆጮ, ካዛቫ, ሌሎች ስራስሮች	ተመግቧል..... 1

		አልተመገበም 2
503	ማንኛውም አይነት አትክልትና ፍራፍሬ ዱባ, ካሮት, በውስጡ ቢጫ/ብርቱካናማ ቀለም ያለው ስኳር ድንች ,የበሰለ ማንጎ, የበሰለ ፓፓያ?	ተመግቧል..... 1 አልተመገበም..... 2
504	ደማቅ አረንጓዴ ተክሎች, ሌሎች ቅጠላማ አትክልቶች ማለትም ስላጣ, ቆስጣ, ጥቅል, ጎመን የሀበሻ ጎመን	ተመግቧል..... 1 አልተመገበም..... 2
505	የበሬ ሥጋ, የጥጃ ስጋ, የፍየል ሥጋ ?	ተመግቧል ..... 1 አልተመገበም 2
506	ጉበት, ኩላሊት, ልብና ሌሎች የውስጥ አካሎች?	ተመግቧል..... 1 አልተመገበም..... 2
507	ዶሮ, ጅግራ ወይም ሌሎች አእዋፋት ሥጋ?	ተመግቧል..... 1 አልተመገበም..... 2
508	ማንኛውም አይነት እንቁላል?	ተመግቧል..... 1 አልተመገበም..... 2
509	ማንኛውም አይነት የአሳ ሥጋ?	ተመግቧል..... 1 አልተመገበም..... 2
510	ክክ ካላቸዉ ጥራጥሬዎች ከባቄላ, አተር, ምስር ወይም ሌሎች የተዘጋጀ ምግብ?	ተመግቧል ..... 1 አልተመገበም 2
511	ለዉዝ /ኦቾሎኒ, ሰሊጥ/ሱፍ?	ተመግቧል..... 1 አልተመገበም..... 2
512	አይብ, እርጎ, ወተትና ሌሎች የወተት ተዋፆዎች?	ተመግቧል..... 1 አልተመገበም..... 2
513	ማንኛውም ከዘይት, ስብ, ቅቤ ያለበት ምግብ	ተመግቧል..... 1 አልተመገበም..... 2
514	ማንኛዉም አይነት ማር ወይም ጣፋጭና ለስላሳ መጠጦች	ጠጥቶል..... 1 አልጠጣም..... 2
515	ሻይ ወይንም ቡና	ጠጥቶል..... 1 አልጠጣም..... 2
516	ልጅዎ ትናንትና በቀን ስንት ግዜ ተመግቡአል?	_____ ቁጥር

ክፍል 6፡ የአመጋገብ መጠየቂያ

ልጅዎ ባለፉት ሶስት ቀናት የተመገባቸው ምግቦችና ድግግሞሽ ለማወቅ የተደረገ መጠይቅ

አሁን ልጅ ባለፉት 3ቀናት ውስጥ የተመገበውን/ የጠጣውን ምግቦችን እና ምን ያህል ጊዜ እንደተመገበ እጠይቆታለው ከተሰጡት አማራጮች ውስጥ አንድ ድግግሞሽ እና አንድ የምግብ መጠን ብቻ ይምረጡ

ተ.ቁ	ከተዘረዘሩት ምግቦች ውስጥ ልጅ የትኞቹን ምግቦች በምን ያህል ጊዜ ውስጥ እንደተመገበ ይነግሩኛል		ጭራሽ አልተመገበ ም	በ 3 ቀን ውስጥ ነ ግዜ	በ 3 ቀን ውስጥ ከ 2-3 ግዜ	በቀን ነ ጊዜ	በቀን ከ2-3 ጊዜ	ልጅ በአብዛኛው ጊዜ የተመገበውን መጠን ይነግሩኛል *የተመገበውን መጠን ከ ፎቶግራፉ ላይ ያሰመርጡ*
501	**እንጀራ** የተዘጋጀው ከ፡ -	ጤፍ						¼፣ 1/2፣ ¾፣ ነ ቁርጥ እንጀራ
		ቦቆሎ						
		ጤፍ እና ቦቆሎ ድብልቅ						
		ማሽላ እና ቦቆሎ						
		ማሽላ እና ጤፍ ድብልቅ						
502	**ዳቦ**፤ የተዘጋጀው ከ፡ -	ስንዴ(ፉርኖ ዱቄት)						1/4፣ ½፣ ቁርጥ ወይም ነ ሙሉ ዳቦ
		ስንዴ እና ቦቆሎ ድብልቅ						
		ቦቆሎ እና ማሽላ ድብልቅ						
503	ቂጣ ወይም ቀጭን ዳቦ፣ የተዘጋጀው ከ፡ -	ቦቆሎ						ከ *ፎቶግራፉ ላይ ያሰመርጡ*
		እንሰት ስር (ቆጮ)						
504	ገንፎ፤ የተዘጋጀው ከ፡ -	ቦቆሎ						ከ *ፎቶግራፉ*
		ቦቆሎ እና ስንዴ ድብልቅ						
		ቦቆሎ፤ ስንዴ እና ገብስ እና ድብልቅ						
		ቦቆሎ፤ ስንዴ እና						

		ጤፍ ድብልቅ						ላይ ያስመርጡ
		ገብስ፤ ስንዴ፤ ጤፍ እና ቦቆሎ ድብልቅ						
		ቦቆሎ እና ገብስ ድብልቅ						
		የእንሰት ውጤት (ቡላ)						
505	ቆሎ፤ የተዘጋጀው ከ፡ -	ስንዴ(ቆሎ)						ነ፣ 2 ወይም ከ2 በላይ ሙሉ እጅ ጭበጥ
		ቦቆሎ እሸት						
		ሽንቡራ						
		ለውዝ						
506	ሽሮ ወጥ፤ የተዘጋጀው ከ፡ -	አተር፤ ባቄላ እና ሽንቡራ						ከ ፎቶግራፉ ላይ ያስመርጡ
		አተር እና ሽንቡራ						
		አተር፤ ባቄላ፤ ሽንቡራ እና ጋውያ						
		አተር ብቻ						
		ባቄላ						
		ሽንቡራ						
507	የስንዴ ምርቶች	ፓስታ						ከ ፎቶግራፉ ላይ ያስመርጡ
		ሩዝ						
		መኮሮኒ						
		ኢንዶሚን						
508	ወተት እና የወተት ተዋህጾ	የላም ወተት						ከ ፎቶግራፉ ላይ ያስመርጡ
		የዱቄት ወተት						
		አግዋት						
		እርጎ						
		አይብ						
509	እንቁላል							ነ፣ 2፣ >2 እንቁላል
510	የለውዝ ቅቤ							½፣ ነ፣ >ነ ማንኪያ የለውዝ

							ቅቤ
511	ስጋ						½፣ 1፣ >1 ቁርጥ ስጋ ወይም የዶሮ ስጋ

ክፍል 7- ቁመት መለካት

መመሪያ፡ የህፃኑን ቁመት ለመለካት የተሰጡትን የቁመት መለኪያ መመሪያ ይከተሉ

ተ.ቁ	ጥያቄ	መልስ
601	የህፃኑ ዕድሜ በወር	_____
602	የተለካው	አግድም/ተኝቶ.....1 ቆሞ.....2
603	የህፃኑ ቁመት በ ሳ.ሜ	☐☐☐ ☐

ህፃኑ ወይም አሳዳጊው ፈቃደኛ ከልሆኑ፤ ፍቃደኛ ያለሆኑበት ምክኒያት እዚህ ጋር ይፃፉ _____

ክፍል 8 - የሽንት ናሙና መሰብሰብ

ተ.ቁ	ጥያቄ	መልስ
701	የጥያቄ መለያ ቁጥር	_____
702	የቤት ቁጥር	_____
704	ሽንት ናሙና የተሰበሰበበት ቀን	_____
705	የተሰበሰበው ሽንት ናሙና ብዛት በኤምኤል	_____

ህፃኑ ወይም አሳዳጊው ናሙና ለመስጠት ፈቃደኛ ከልሆኑ፤ ፍቃደኛ ያለሆኑበት ምክኒያት እዚህ ጋር ይፃፉ _____

10.4 Annex: Photographs of selected food items in gram

እንጀራ

A B C D




ሙሉ እንጀራ.....311 ግራም ¾.....224 ግራም ½.....178 ግራም ¼.....93 ግራም

ዳቦ

A B C



89 ግራም 159 ግራም 150 ግራም

ቂጣ

A B C



158 ግራም 84 ግራም 68 ግራም

ቆጮ

A B C



81 ግራም 70 ግራም 152 ግራም

ገንፎ

A B C



216 ግራም 175 ግራም 106 ግራም

ቆሎ

A

አንድ እጅ ጭበጥ.....26 ግራም

B

ሁለት እጅ ጭበጥ.....53 ግራም

ቦቆሎ እሸት

A

አንድ እጅ ጭበጥ.....42 ግራም

B

ሁለት እጅ ጭበጥ.....74 ግራም

ሽንቡራ

A

አንድ እጅ ጭበጥ.....25 ግራም

B

ሁለት እጅ ጭበጥ.....42 ግራም

C

ሶስት እጅ ጭበጥ.....61 ግራም

ለውዝ ተቆልቶ

A

አንድ እጅ ጭበጥ.....21 ግራም

B

ሁለት እጅ ጭበጥ.....46 ግራም

የወጥ ጭልፋ

A

10 ግራም

B

35 ግራም

C

50 ግራም

D

52 ግራም

E

80 ግራም

ፓስታ

A

260 ግራም

B

183 ግራም

መኮሮኒ

A

331 ግራም

B

227 ግራም

ሩዝ

A

ሁለት ሾርባ ማንኪያ....86 ግራም

B

ሶስት ሾርባ ማንኪያ.....125 ግራም

C

አራት ሾርባ ማንኪያ....172 ግራም

የወተት ኩባያ

A

129 ሚሊሊትር

B

300 ሚሊሊትር

C

86 ሚሊሊትር

አይብ

A

28 ግራም

B

57 ግራም

እንቁላል

A

አንድ እንቁላል.....57 ግራም

B

ሁለት እንቁላል.....107 ግራም

C

ሶስት እንቁላል.....159 ግራም

የለውዝ ቅቤ

A

21 ግራም

B

12 ግራም

10.5 Annex: Height and Length Measurement Protocol

❖ **General instructions**

- Measure a child's length:

  - In a recumbent (lying down) position

  - If a child is less than 2 years (24 month) old

  - If a child is aged 2 years (24 month) or older and unable to stand, measure length and subtract 0.7cm to convert to height

- Measure a child's height:

  -In a standing position

  -If a child is 2 years (24 month) or older

  -If a child is less than 2 years old will not lie down to measure length, measure standing height and add 0.7 cm to convert to length

- The data collectors have to check daily whether the measuring board is working properly or not. In case the board is broken during transportation or due to other reasons inform the supervisor at hand immediately.

- Care for the instrument:

  -Keep the equipment clean

  -protect the equipment from humidity and wetness

- Before proceeding to measurement, make sure there is enough light and space for measurement

- Ensure the privacy and comfort of the measured child.

- In order to avoid distractions, allow only the assistance and the mother or care giver of the child to the measuring area/room.

10.6 Annex: Urine Collection Protocol

General instructions

- Explain procedure to child and family as relevant, and obtain informed consent.
- Make sure you have the equipment's needed
- Don't forget to label the sample collected with the right information
- Wear gloves to avoid contamination

Equipment's needed

- Urine collection bag
- Sample container
- Cleaning wipe
- Gloves

Procedures to collect urine from children

1. Clean the pubic and perineal areas with the antiseptic wipe. You can ask the parent/carers for help.
2. Place the urine collection bag under the child's genitalia. If the child is potty trained, place the urine collection bag into a potty and collect the sample in this. An older child can urinate directly in to the container.
3. Transfer urine in to the sample container and screw the lid tightly.
4. Label the sample container with the right **household number**, **questioner code**, **date** and **time** of collection.
5. Place the collected urine sample in to the portable freezer.

10.7 Curriculum Vitae (CV)

Curriculum Vitae (CV): **Mary Ayele**

1. **Personal information**:
    - Name: Mary Ayele Ashko
    - Age: 25 years
    - Sex: Female
    - Date of birth: July 23, 1993 G.C
    - Place of birth: Hawassa, SNNPR, Ethiopia
    - Nationality: Ethiopian
    - Health condition: Normal/healthy
    - Marital status: Single
    - Tel: (+251921452310 /+251944724867)
    - Email: maryayele4@gmail.com
2. **Educational background**:
    - Elementary (1-8): from 2000-2007 G.C @ BNB learning center. (in English)
    - High school (9-10): 2008-2011 G.C @ St. Daniel Comboni secondary and preparatory school. (in English)
    - Higher Education (2012-2015 G.C): Hawassa University College of Medicine and Health science.
    - Bachelor of Science Degree in Public Health
3. **Language**- Amharic and English: speak, read and write
4. **Hobbies**:
    - Reading books like medical books, fictions and bible
    - Leasing to slow music and songs
    - Inspired by art and nature
5. **Work Experience**
    - Voluntary service in VSO (voluntary service oversea) for 4 months (from January to April) in Hawassa.
    - 2-years' experience at Ashko Medium clinic at outpatient department.
    - Worked as data collector for 3month for Paster Institute on the project Service Availability and Readiness Assessment (SARA).

Curriculum Vitae: **Bilal Shikur**

1. **Personal Information**
   - First name: Bilal Shikur Endris
   - Age: 29 years
   - Sex: Male
   - Marital status: Married and father of two
   - Place of Birth: Addis Ababa, Ethiopia
   - Date of Birth: 1 October, 1986
   - Nationality: Ethiopian
   - Language: Amharic, English and Arabic: Speak, Read and Write
   - Email: lebiluka@yahoo.com
   - Phone number (mobile): +251911-47-53-75
2. **Academic Qualification**
   - **Masters of Public Health in Epidemiology (MPH): Nutrition research**

     Period of study: September 2012- July 2014

     Program: Masters of Public Health, Epidemiology Specialty Track

     Institution: Addis Ababa University, School of Public Health, Addis Ababa, Ethiopia
   - **Degree of Doctor of Medicine (MD):**

     Period of study: January 2006-September 2011

     Program: Medicine

     Institution: Addis Ababa University, School of Medicine, Addis Ababa, Ethiopia
   - **Ethiopian Higher Education Entrance Certificate:**

     Period of study: September 2003-July 2005

     Program: Preparatory Program

     Institution: Medhaniyalem, Addis Ababa, Ethiopia
   - **Ethiopian General Secondary Education Certificate:**

     Period of study: September 2001-July 2003 G.C

     Program: High School

     Institution: Dilachin, Addis Ababa, Ethiopia

3. **Work Experience**

- **Assistant Professor of Public Health**

  Duration of employment: Since July, 2014

  Institution: Addis Ababa University, School of Public Health, Addis Ababa, Ethiopia

- **Lecturer**

  Duration of employment: September, 2011 – June, 2014

  Institution: Addis Ababa University, School of Public Health, Addis Ababa, Ethiopia

- **Co-PI** of Addis Ababa Mortality Surveillance Program: June, 2014 -July,2015
- **PI** of Addis Ababa Mortality Surveillance Program-Since July,2015
- **Clinical Intern**

  Duration of Employment: July, 2010 – July, 2011

  Institution: Tikur Anbessa Specialized Teaching Hospital

Declaration

I hereby declare that this MPH thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis have been duly acknowledged.

Name: _____

Signature: _____

Date: _____

This thesis has been submitted for examination with my approval as thesis advisor.

Name: _____

Signature: _____

Date: _____