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The Relationship Between Aflatoxin Exposure and Chronic Malnutrition in Nampula Province, Mozambique

June 10, 2021

Maureen Malave   Sofia Costa   João Salavessa   Katie Appel   Shibani Ghosh

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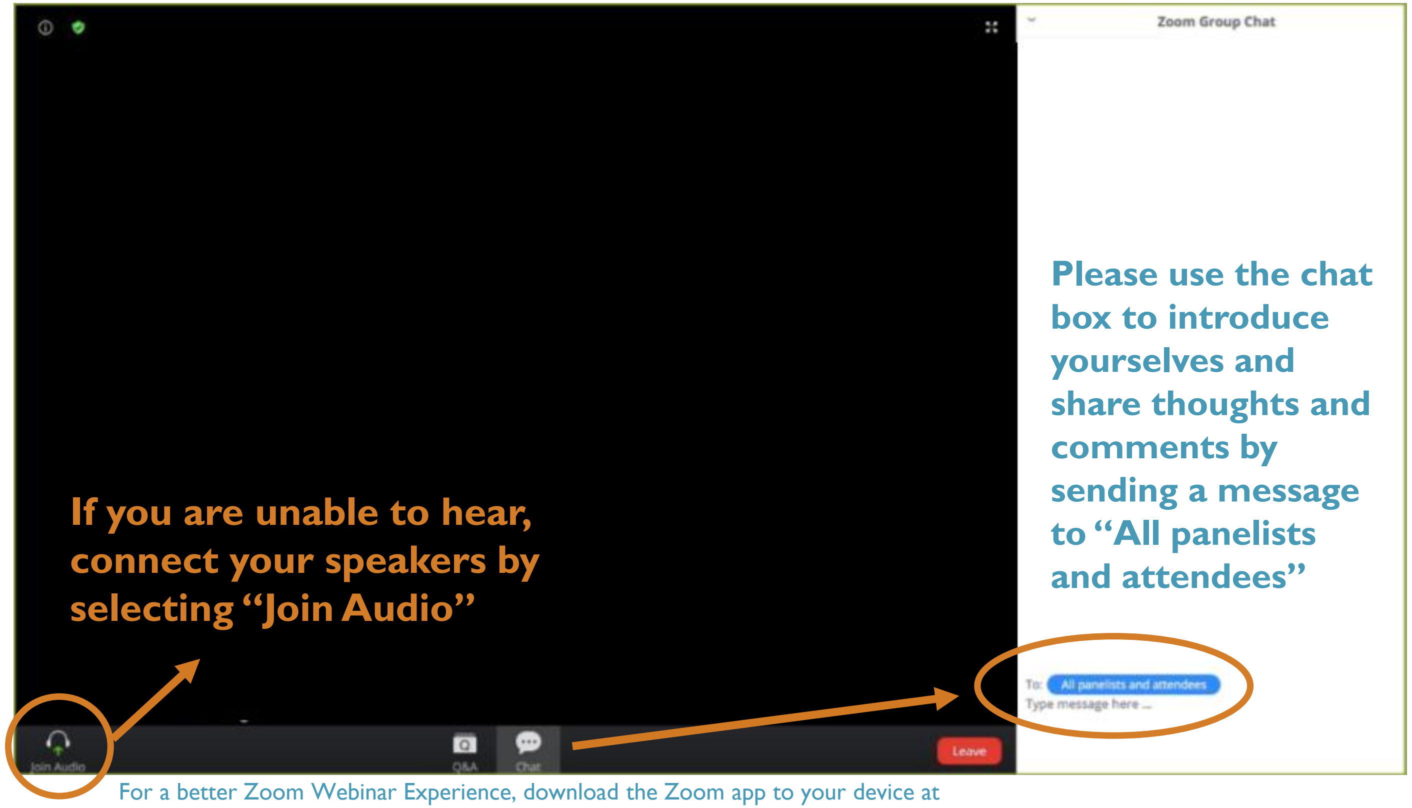

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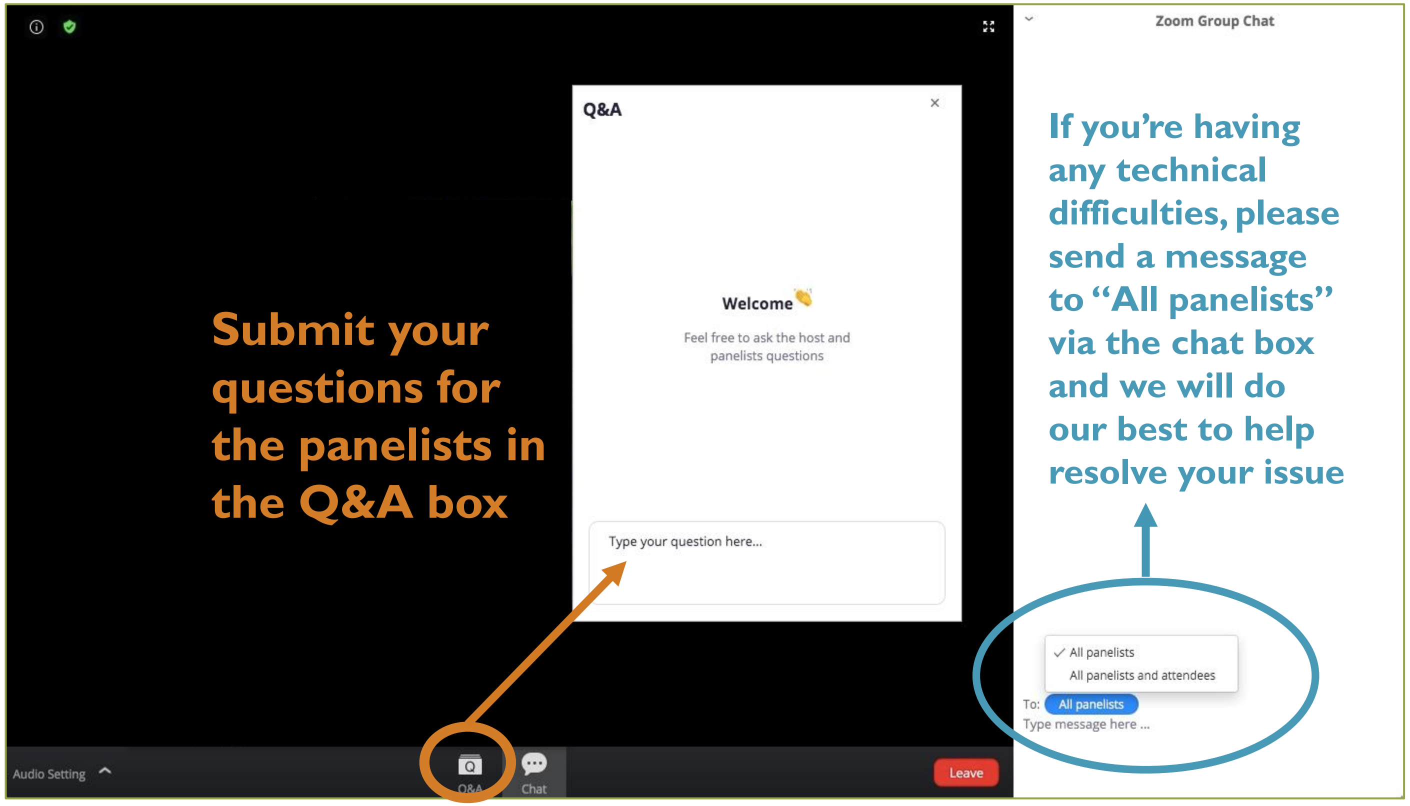

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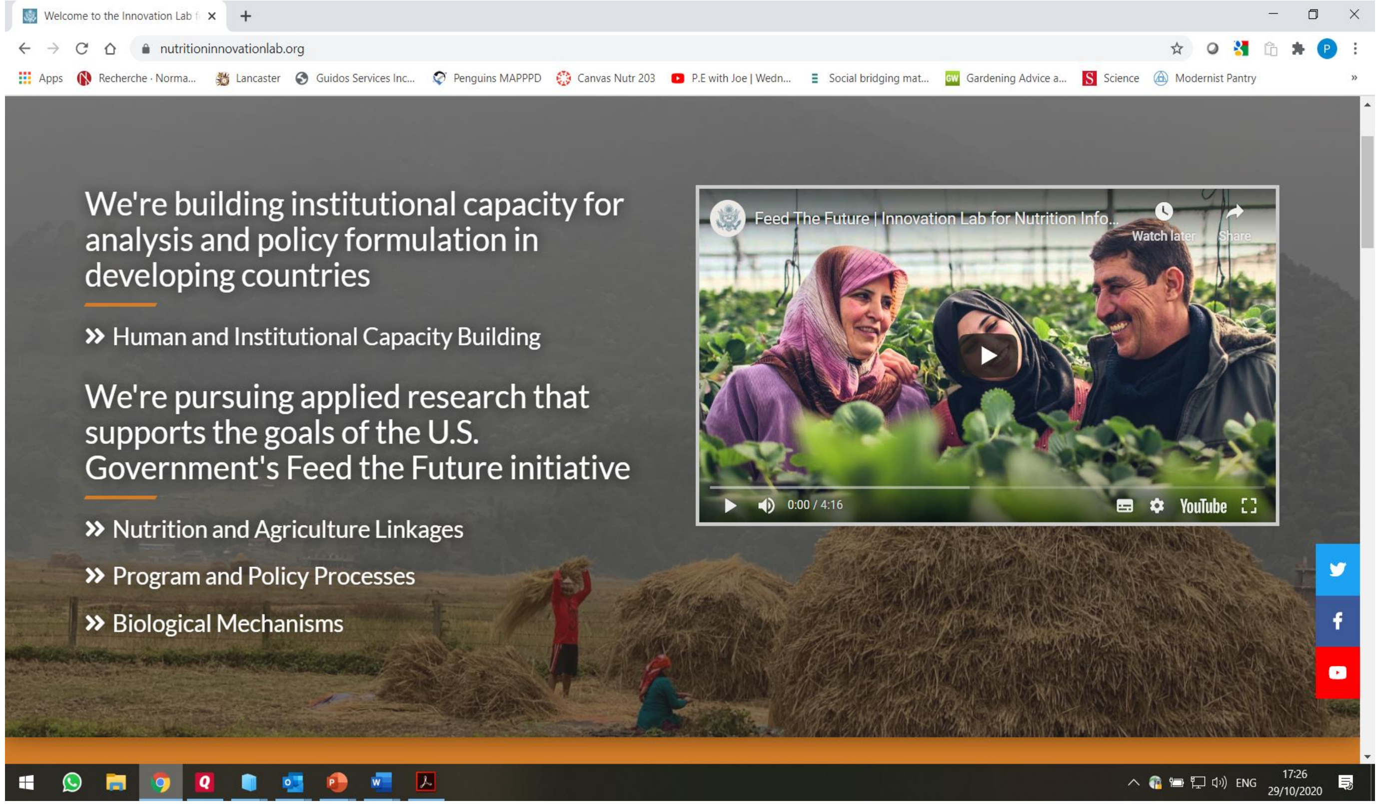

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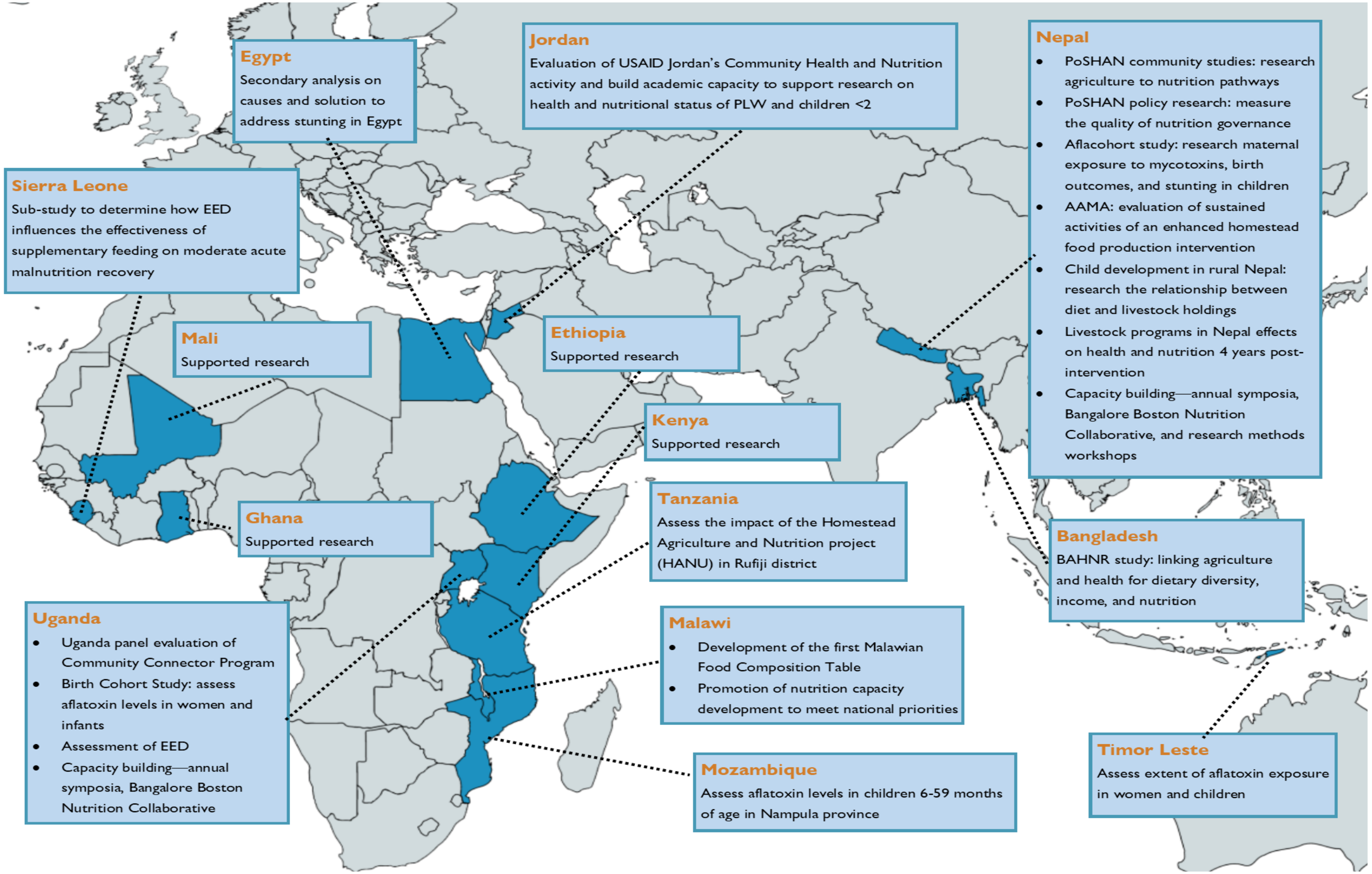

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INNOVATION LAB FOR NUTRITION
WEBINAR

THURSDAY, JUNE 10TH
8:00AM-9:00AM (ET)

The Relationship Between Aflatoxin Exposure and Chronic Malnutrition in Nampula Province, Mozambique

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Assessing the relationship of serum aflatoxin levels and stunting in children 6-59 months of age in 10 districts of Nampula Province, Mozambique

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Study Collaborators

- Universidade Lúrio
- INS
- ANSA
- INE
- Nampula Central Hospital
- University of Georgia
- Tufts University

Dr. Shibani Ghosh, Dr. Patrick Webb, Professor João Salavessa, Ms. Sofia Costa, Dr. Réka Cane, Mr. António Júnior, Mr. Acacio Sabonete, Professor Jia-Sheng Wang, Ms. Lourdes Fidalgo, Ms. Carina Ismael, Ms. Julia Sambo, Ms. Tatiana Marrufo, Ms. Patricia Goncalves, Dr. Basilio Cubula, Mr. Arlindo Charles, Dr. Manuel Lázaro, Dr. Johanna Andrews-Trevino, Dr. Merry Fitzpatrick, Dr. Robin Shrestha, Ms. Grace Namirembe, Dr. Bernadette Chimera, Dr. Lichen Liang, Ms. Katherine Heneveld, Ms. Hannah Koehn, and Ms. Amy Byrne

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Aflatoxin Background

- Type of mycotoxin, produced by the mold *Aspergillus*
- Colorless and odorless (mold is green color)
- Contaminate staple crops such as maize, groundnuts, and cassava
- Cause liver cancer and may be associated with stunting
- May impair absorption of nutrients critical for normal child growth and development

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Research Theme: Neglected Biological Mechanisms

To contribute to a better understanding of the mycotoxin-stunting relationship, the Feed the Future Nutrition Innovation Lab has implemented several studies to explore the relationship to mycotoxins, with a focus on early life nutrition

- Aflatoxin Study (Northern Mozambique- 10 districts of Nampula province)
- Gulu Cohort: HIV, Food Security in pregnancy (Northern Uganda)
- Birth Cohort Uganda (Northern and South West Uganda)
- Alfatoxin in pregnancy and birth outcomes (Greater Kampala area, Uganda)
- AflaCohort Study (Banke district, Terai- Nepal)

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Rationale

- Significant negative association between aflatoxin exposure and linear growth – in Benin and Togo (Gong et al. 2002 BMJ; 325)
- Presence of aflatoxin B1 in pregnancy in Ghana and adverse birth outcomes (Shuaib et al. 2010 Tropical Medicine and International Health, 15:2 160-167)
- Aflatoxin levels in pregnancy associated with adverse birth outcomes in Uganda (Lauer et al, 2019, MCN; 15:2)
- Relationship in pregnancy and birth outcomes even at low levels of aflatoxin observed in Nepal (Andrews-Trevino et al, 2019, *The Journal of Nutrition*, Volume 149, Issue 10, October 2019, Pages 1818–1825)

Rationale

- Relationships observed in Sub-Saharan Africa and South Asia
- Variability of the relationship (in pregnancy, in under 2s, in under five) in different populations
- Conflicting evidence of the relationship in Nepal (Mitchell et al. 2016, Journal of Exposure Science and Environmental Epi, 1-6)
- RCT on maize grain replacement in Kenya: impact being primarily on younger children (Hoffman et al. 2018- BMJ Global Health)

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Rationale for Study in Nampula

- High rate of stunting in Nampula province (55% of children under five)
- Population highly dependent on crops such as maize and groundnuts
- Previous assessment of crops and soil levels in Nampula province has shown aflatoxin contamination

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Study Objectives

1. Assess the mean serum aflatoxin in children 6-23 months and 24-59 months of age

2. Examine differences in serum aflatoxin (mean) by age group

3. Enumerate the association between serum aflatoxin and linear growth (HAZ) adjusting for confounders

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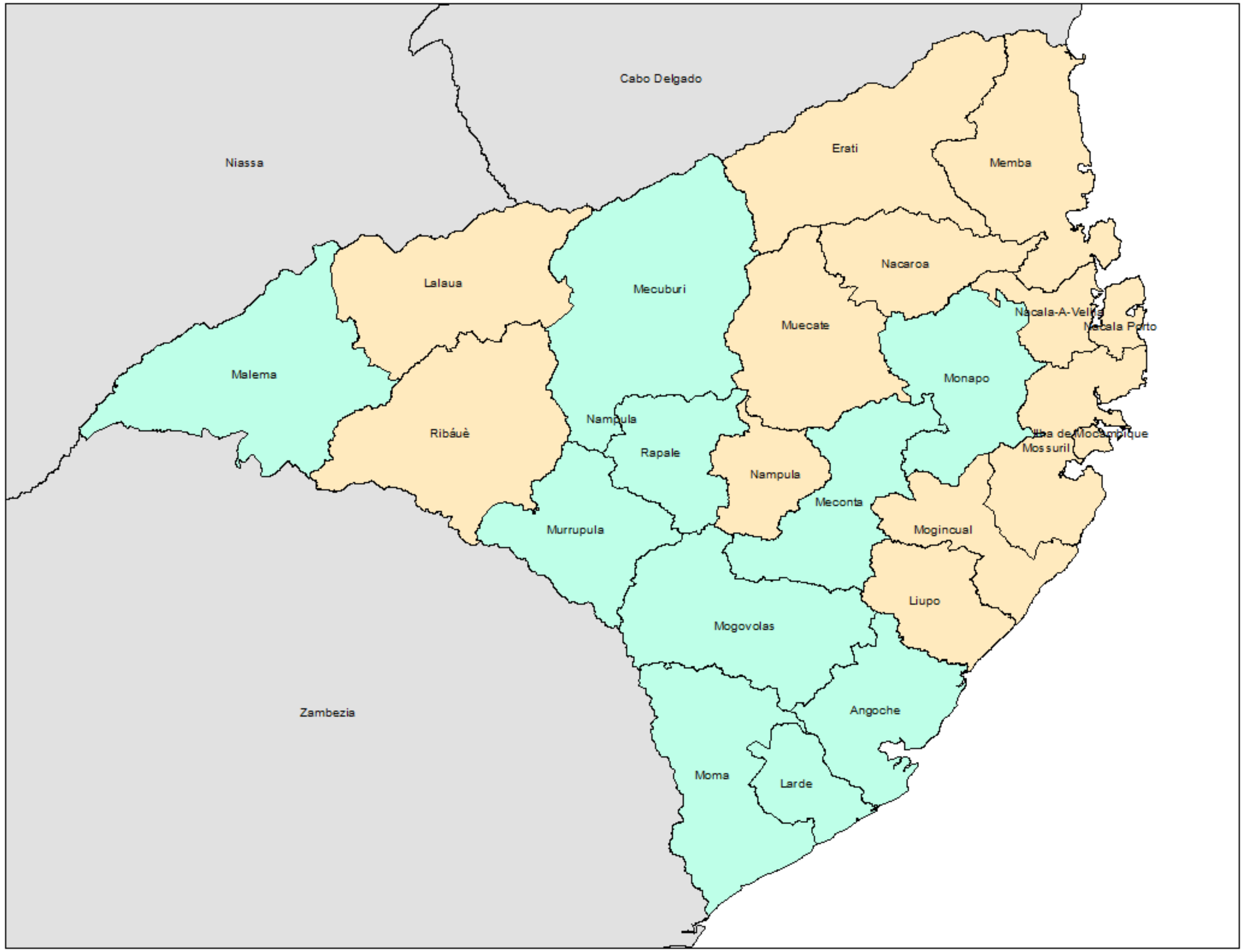
Study Methods

- Cross-sectional, two groups (under 2, 2-5 years)
- Sample size calculation: 720 per age group (plus 25% attrition)
- Located in 10 Feed the Future Zone of Influence districts of Nampula (green districts in map)
- Sampling Strategy:
  - Population proportional by district, random selection 2017 census INE enumerations areas
  - Random selection of households within EAs
- Representative of children 6-59 months of age in the 10 districts



Ethical Approval, Sensitization, and Mobilization

- The study was approved by UniLurio Bioethics Board and National Bio-ethics committee (CNBS) and by the Institutional Review Board at Tufts University

- Sensitization of Central hospital, provincial (DPS) and district officials (all 10 districts) through:
  - One on one interactions: provincial level
  - District visits
  - Health facility mapping
  - Launch Event
  - Community Sensitization : Lead team visits prior to data collection
  - Community radio

- At data collection: Informed consent process, caregivers requested to participate in study

Household Data Collection (at Household)

Respondent	Module	Topic
Female caregiver or mother of selected child or children	A	Household identification and household member information
	C	Diet of caregiver and child/children, household food security
	D	Child feeding practices and health
	E	Water, hygiene, and sanitation
	H	Social participation
Household head	B	Agricultural production
	F	Socioeconomic characteristics
	G	Access to durable goods

Nutritional and Biomarker Data Collection at Health facility

Exclusion:

- Children with severe acute malnutrition (MUAC<11.5 cm) were not enrolled in the study
- Children with severe anemia (<7 g/dl Hb) did not have blood drawn

Data Collection:

- Anthropometry: weight, length/height, MUAC, head circumference, knee-heel length
- One venous blood draw by Central Hospital technicians
- Anemia (HemoCue 301 analyzer) and malaria (Rapid Diagnostic Test) assessment

Referral: Children with SAM, anemia, and diagnosed with malaria were referred to the health facility staff and treatment was provided

Biomarker processing:

- Sample processing and handling by Central Hospital Nampula technicians
- Storage in -80 freezer in Nampula and transport to the United States
- Serum aflatoxin analysis using HPLC using validated lab protocols (University of Georgia, USA)

Data Management, Calculations, and Analysis

- Data were collected electronically, transferred to database (Tufts server), and cleaned daily
- Data cleaning, sample weight computation
- Variable generation and computations
  - Anthropometric Z-scores computed using WHO reference standards
  - Prevalence of stunting and underweight
  - Diet diversity, household food insecurity
  - Agricultural production and practices
- Statistical analysis
  - Descriptive statistics
  - Bi-variate analysis
  - Multi-variable analysis: ordinary least square and logistic regressions
  - Adjusted for sample weights and clustering (EAs) to make findings representative of children in 10 districts

Study Timeline

2017

- **May**: Scoping visit
- **Nov**: Protocol development

2018

- **Feb**: Tufts IRB submission
- **Mar**: Partner meetings & local IRB submission
- **Jul**: Health center identification & cold chain assessment
- **Aug**: Supervisor training & pretest
- **Oct**: Enumerator training & launch event

Data collection (Nov-Dec)

2019

- **Mar**: Serum sample shipment
- **Mar-Jul**: Data cleaning
- **Mar-Aug**: Aflatoxin analysis

Aflatoxin results (Aug)

Sample Size

	Children 6-23 months	Children 24-59 months	Total
Enrollment and household surveys	493	764	1257
Anthropometry measurements	419	656	1075
Blood draws	317	599	916
Complete records	311	583	894

Results

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Serum Aflatoxin Levels

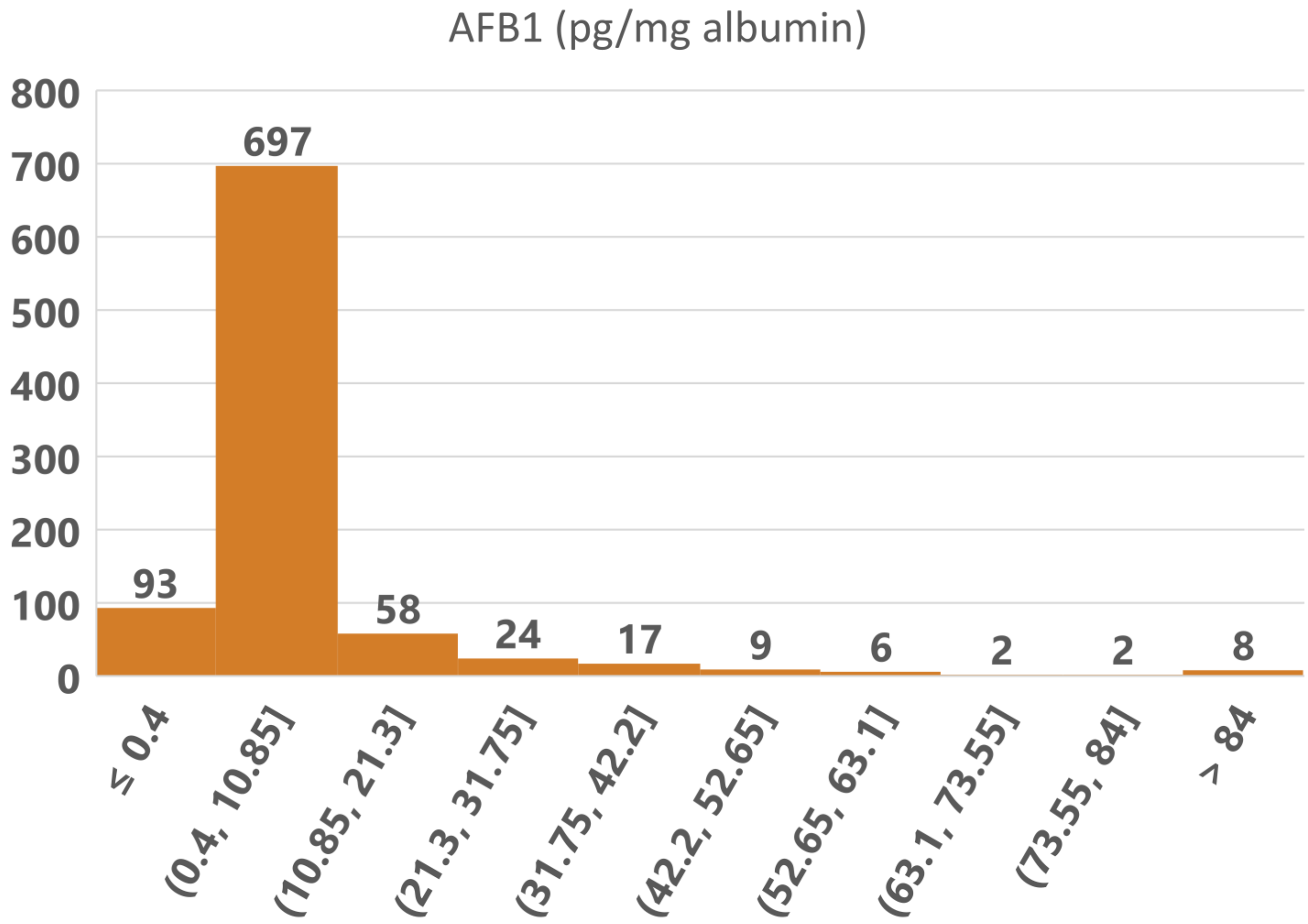
90% samples: detectable levels of aflatoxin

Mean=7.7 pg/mg albumin

SD=25.3, 95% CI=6.0-9.3

Geometric mean=2.2 pg/mg albumin

SD=4.2, 95% CI=2.0-2.5



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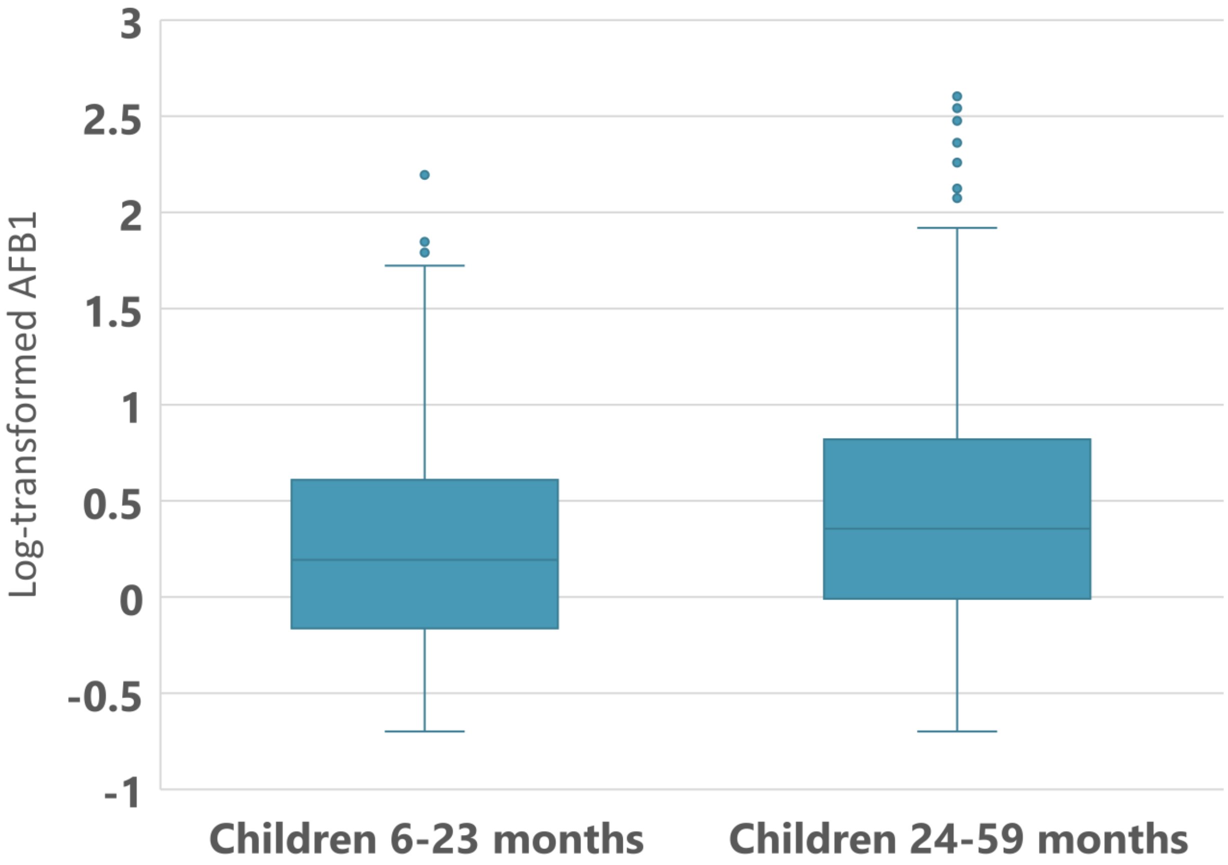
Comparison of Aflatoxin Results to Other Studies

	Mozambique	Uganda	Nepal
	Child	Mothers	Mothers
Sample size	916	220	1479
% detectable	90	100	94
Arithmetic mean ± SD	7.7 ± 25.3	8.9 ± 11.6	3.2 ± 8.3
Geometric mean	2.2	5.9	1.4

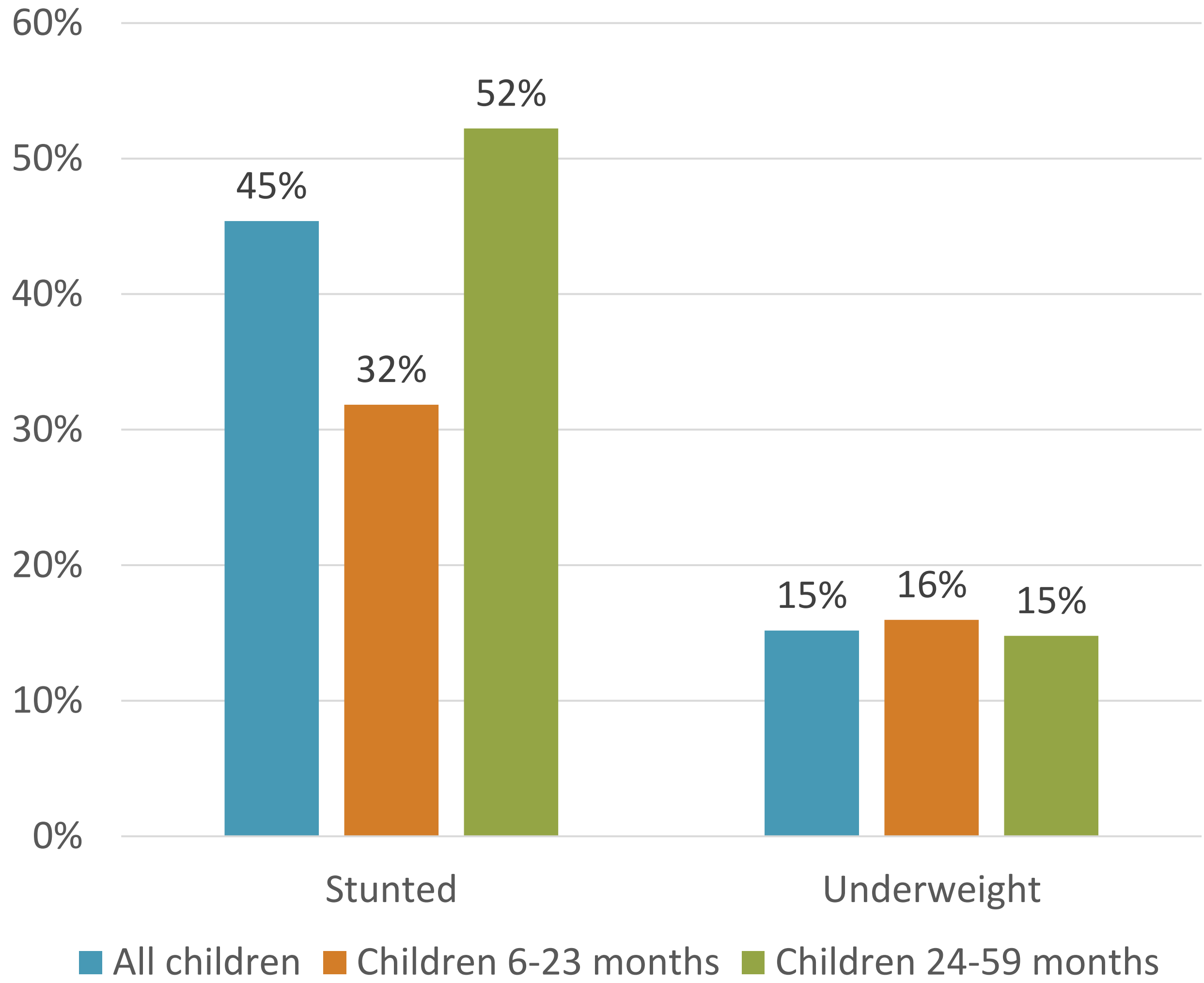
Aflatoxin and Age Group



Age group	N	Mean ± SE	p-value
Children 6-23 months	311	0.24 ± 0.04	0.026
Children 24-59 months	583	0.37 ± 0.05	

Stunting and Underweight

- Defined ≤ 2 SD from the mean (WHO 2006)
- 2% of children had moderate acute malnutrition (>-3 < -2 SD from mean)
- Children with severe acute malnutrition were excluded from the study at enrollment.



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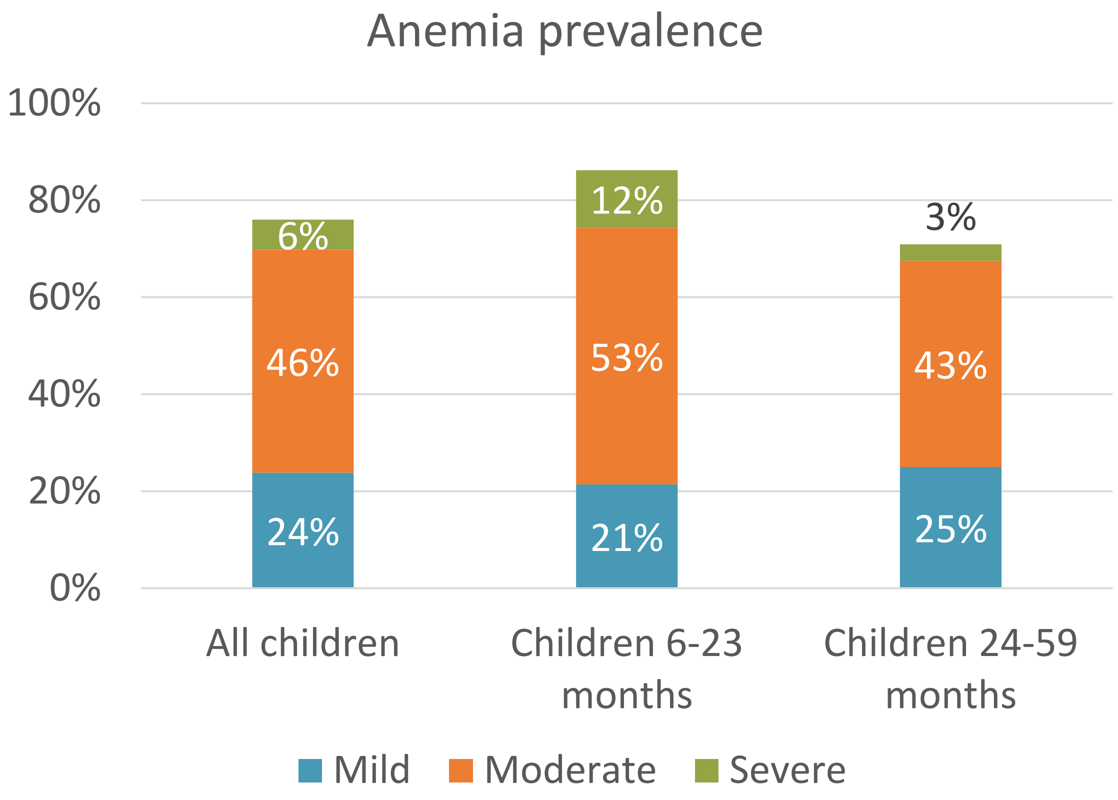
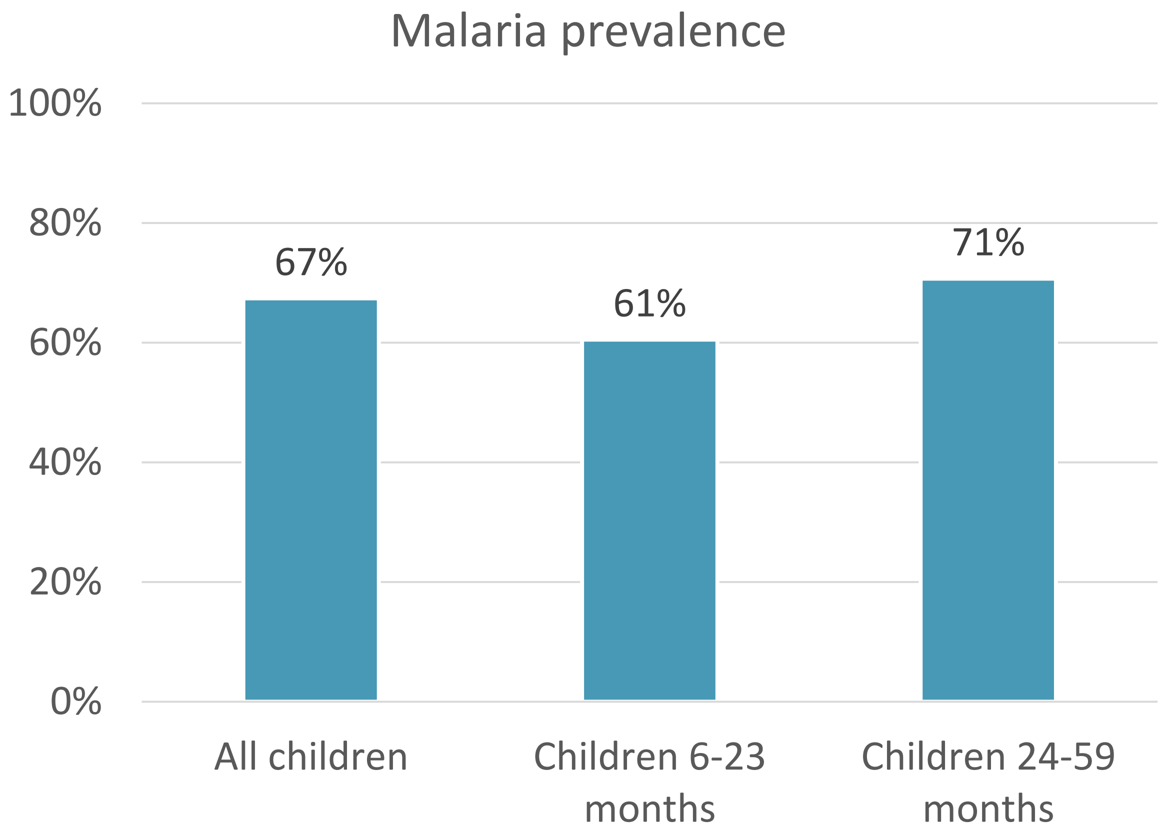
Anemia and Malaria





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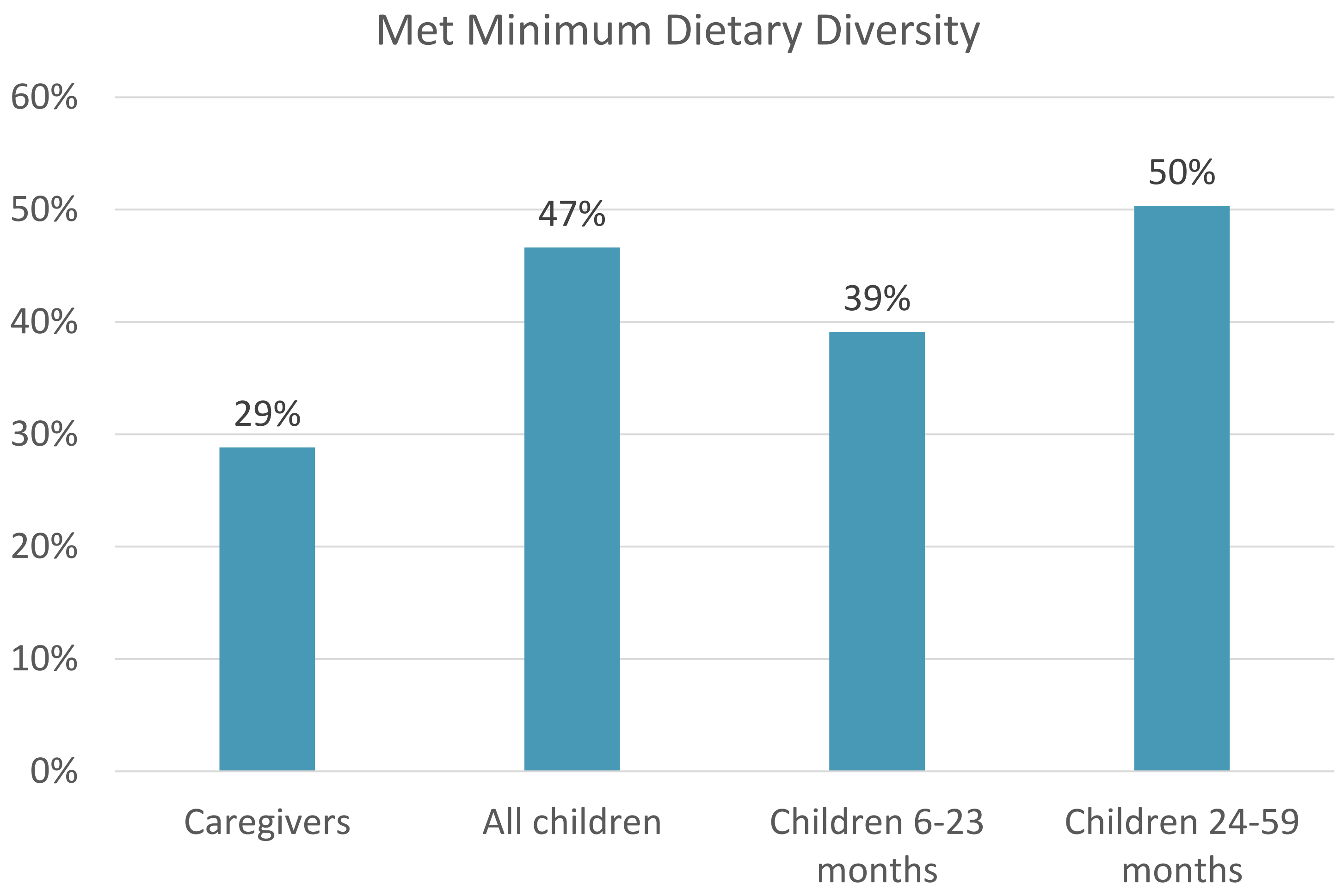
Dietary Diversity

Caregivers:

FAO Minimum Dietary Diversity in Women (5 out of 10 food groups)

Children:

IYCF Minimum Dietary Diversity (4 out 7 food groups)



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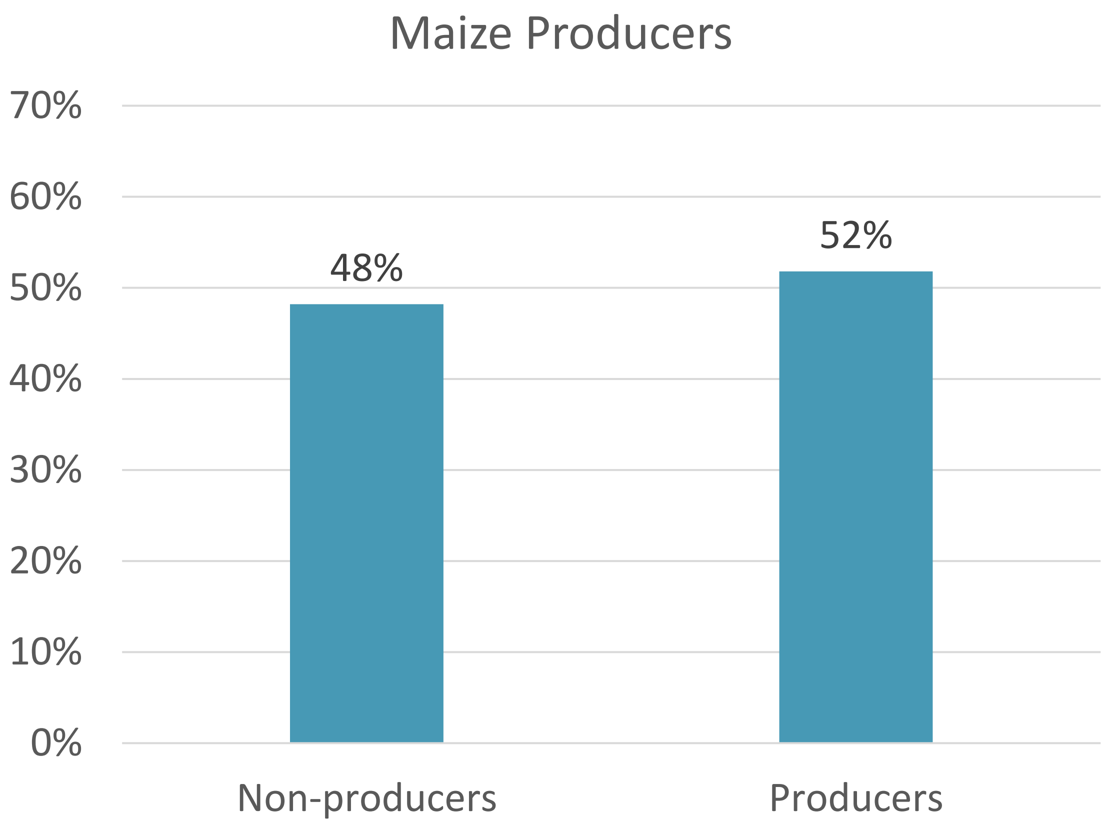
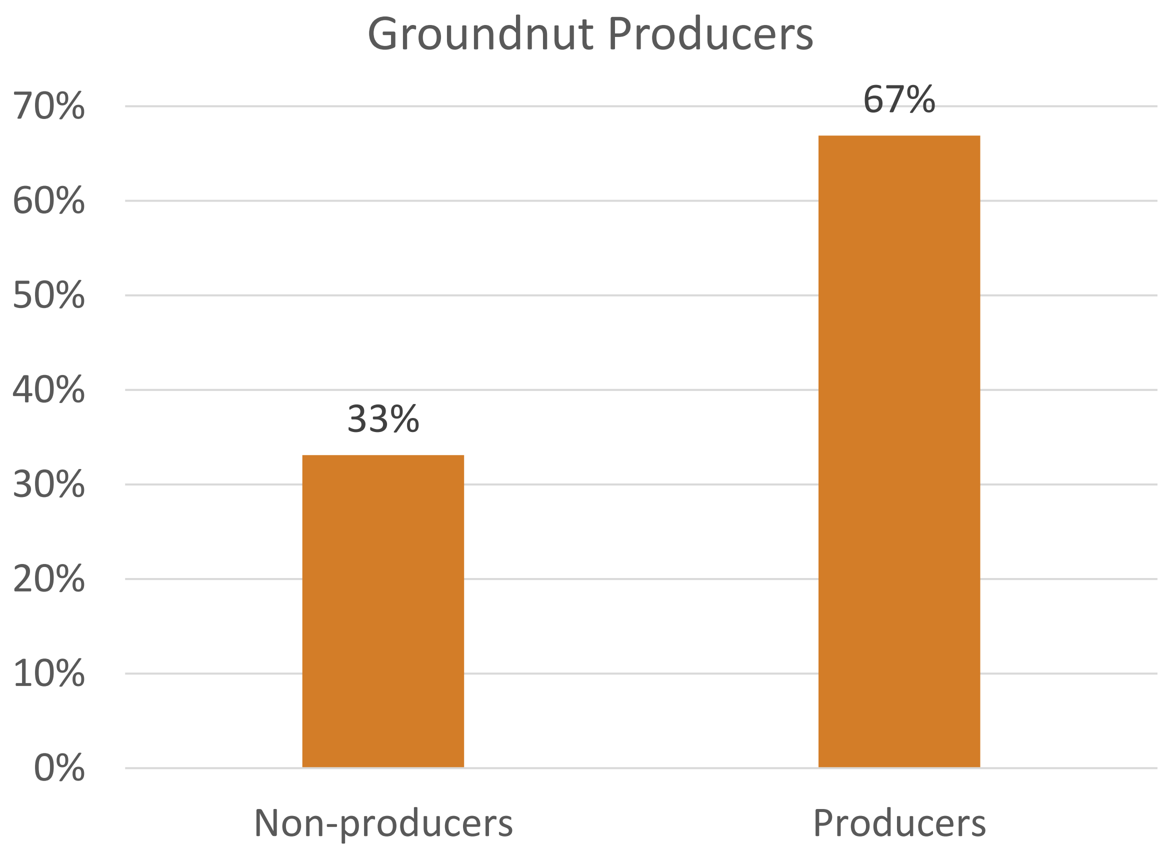
Household Agricultural Production





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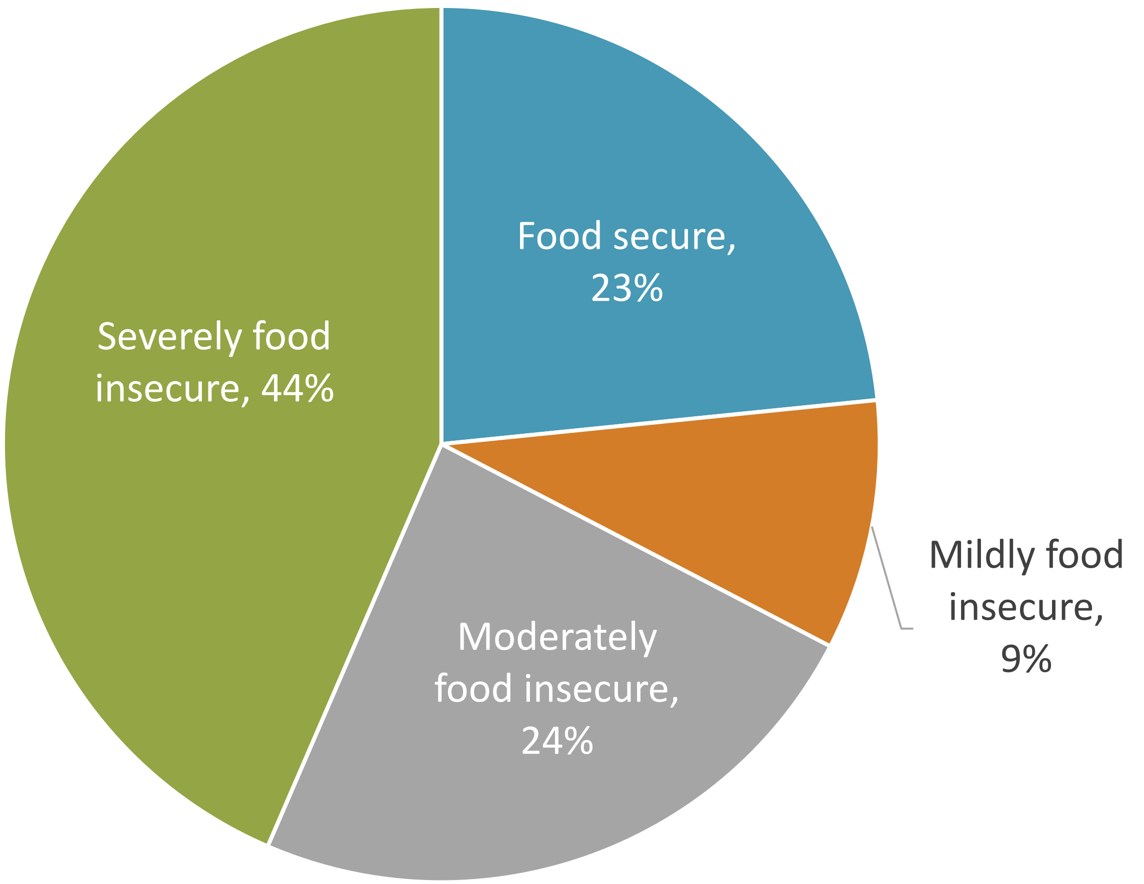
Household Food Security

Assessed using the Household Food Insecurity Access Scale



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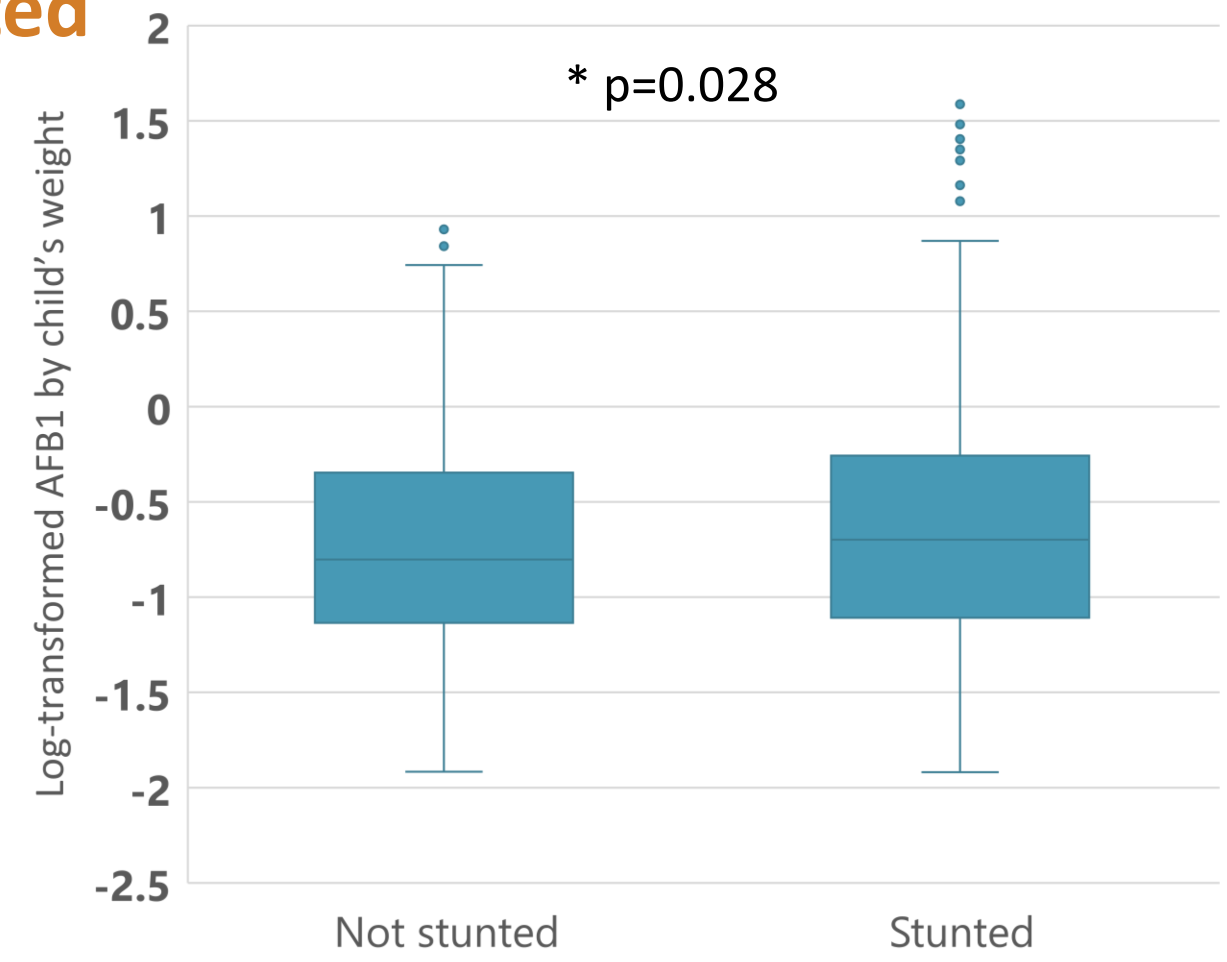
Stunting and Aflatoxin are Associated

	Odds Ratio
Log-transformed AFB1 by child's weight	1.60 *
n	894

A child was **60% more likely to be stunted** with every unit increase in logged aflatoxin level standardized by child's weight.

Logistic regression model adjusted for clustering, anemia, WHZ, age, age^2, sex, and detectable AFB1.



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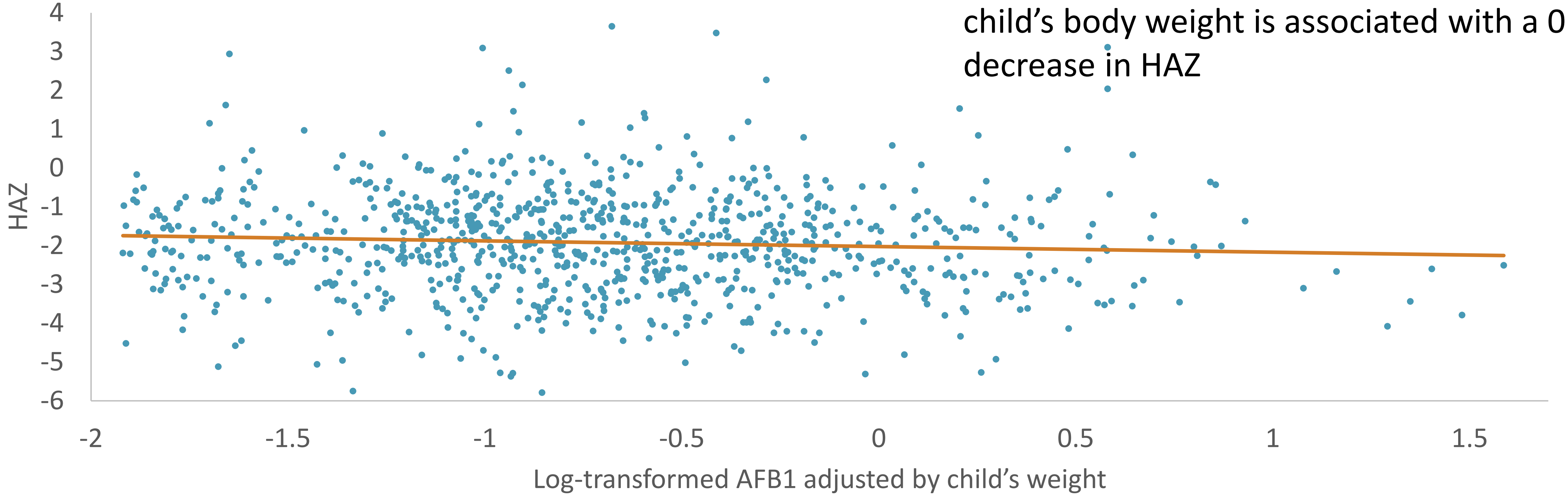
HAZ and Aflatoxin are Associated

β=-0.234, p=0.029

An increase in one unit of AFB1 adjusted for child's body weight is associated with a 0.23 decrease in HAZ



Ordinary Least Squares model adjusted for clustering, anemia, WHZ, age, age^2, sex, and detectable AFB1.

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Aflatoxin Associations by Age Group

- HAZ is associated
  - with AFB1 in children 24 to 59 months of age (β=-0.29, p=0.007)
  - but not children 6 to 23 months of age

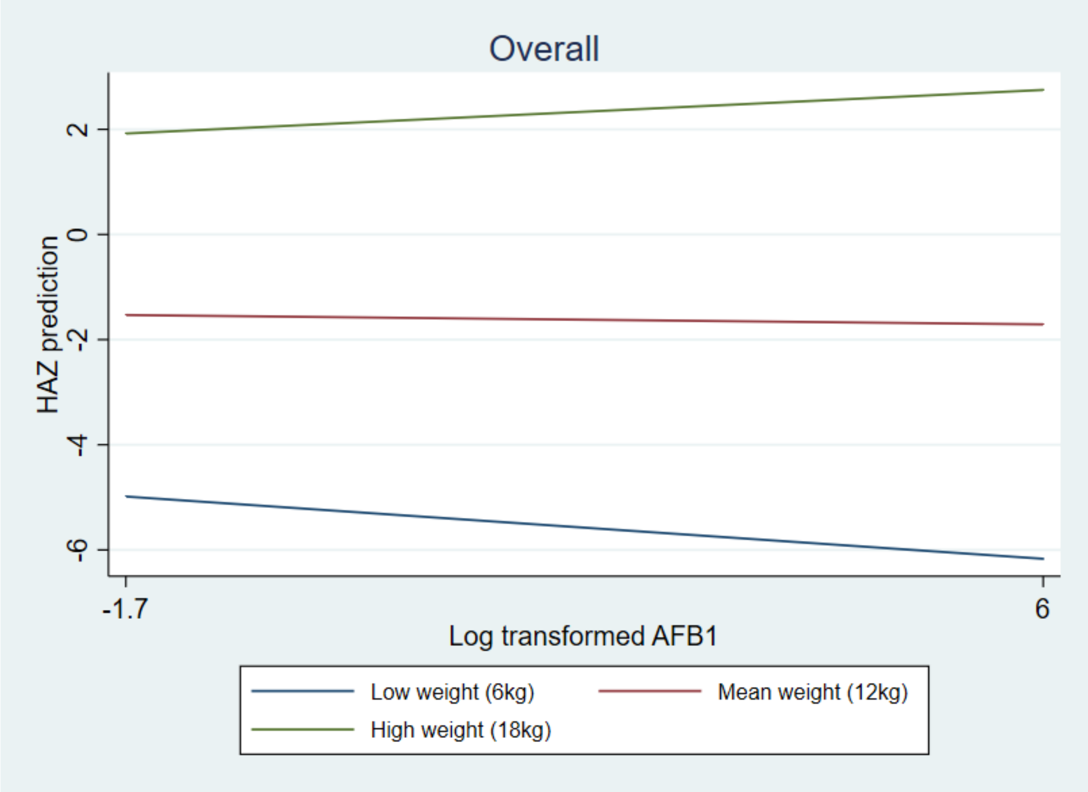
- Stunting is associated
  - with AFB1 in children 24 to 59 months of age (OR=1.67, p=0.011)
  - but not children 6 to 23 months of age

HAZ-Aflatoxin Relationship Varies by Child's Weight

Different relationships are predicted at different weights (on entire study sample):

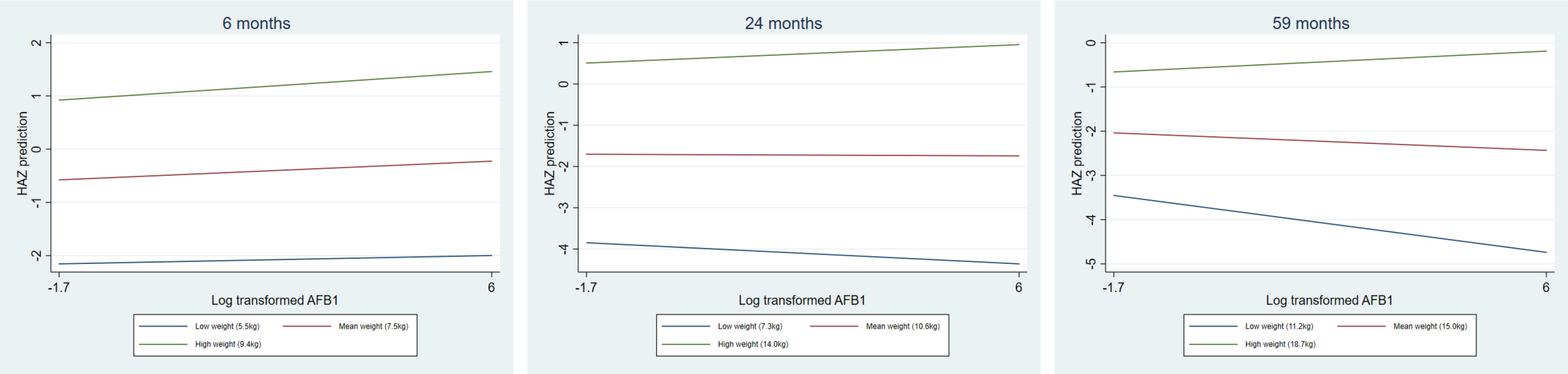
- Positive HAZ-AFB1 association for children with high weight
- No association at average weight
- Negative HAZ-AFB1 association for children with low weight

However, this relationship is further complicated by age

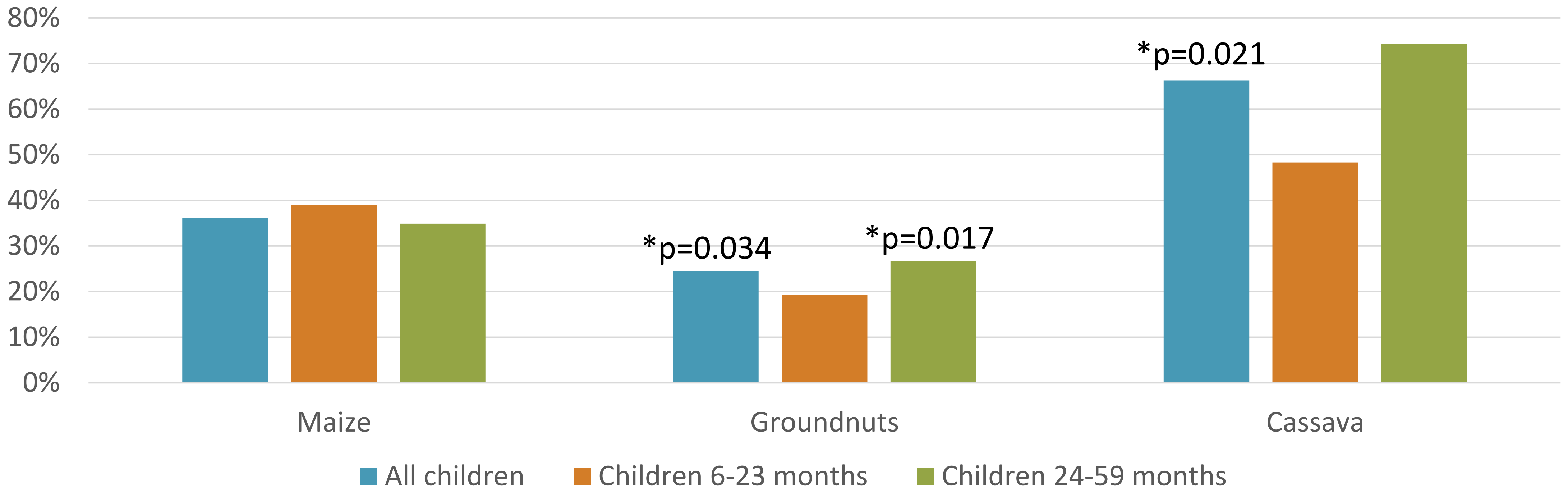


HAZ-Aflatoxin Relationship Varies by Weight and Age

Different relationships are observed when the predictions are conducted at specific ages



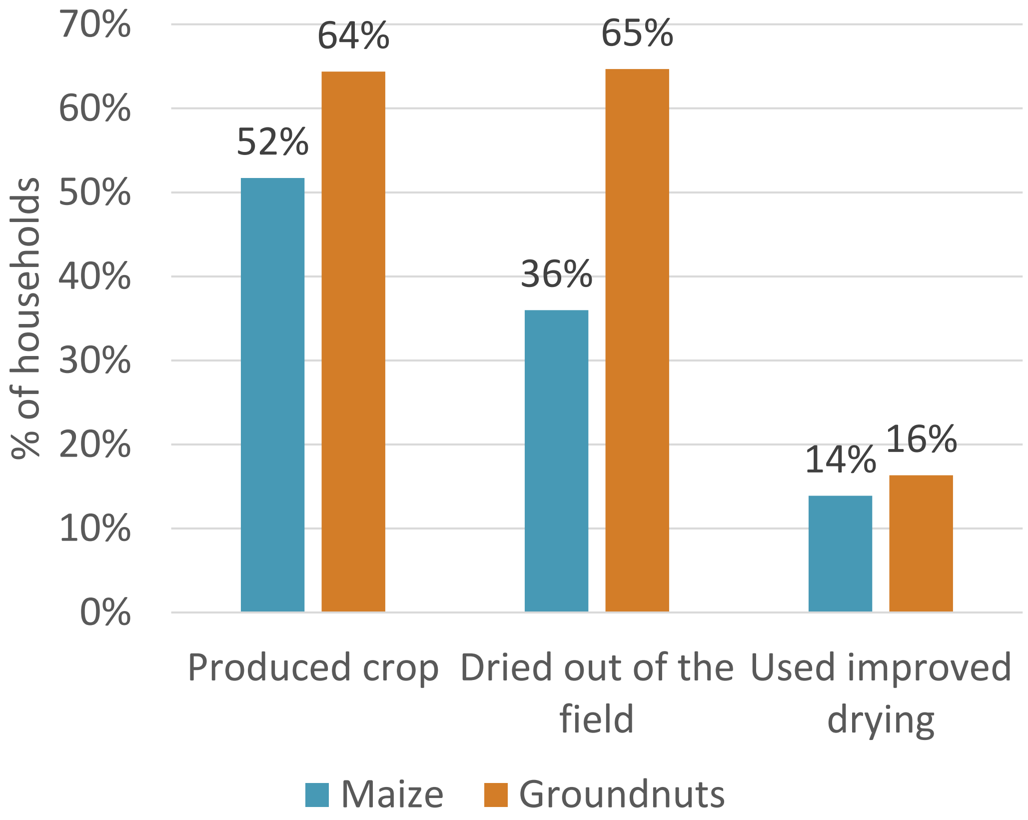
Consuming Aflatoxin-Prone Foods is Associated with Increased Blood Aflatoxin Level



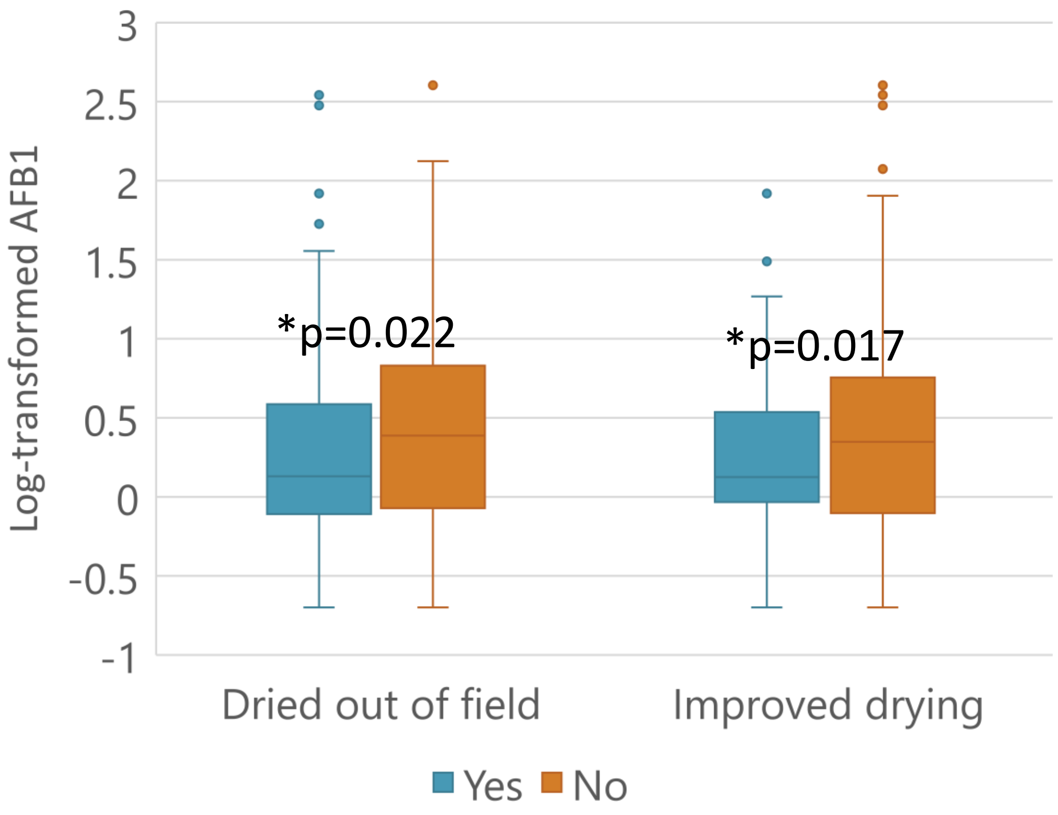
Consumption in past 24 hours of all three foods.
Ordinary Least Squares model adjusted for clustering, WHZ, age, meeting minimum dietary diversity, wealth, and detectable AFB1.

Agricultural Practices

- **Drying location**
  - In the field only
  - Outside of the field (includes those that also dried in the field)
- **Drying method**
  - **Improved methods**: with fans, on platforms or plastic sheets, hung under roof or in kitchen
  - **Unimproved methods**: drying only in the field, spreading directly on dirt, cement, or brick floor, or on roof



Aflatoxin Levels and Maize Agricultural Practices



In maize-producing households, **lower aflatoxin levels were associated with**:

- Drying maize outside of the field (0.59 pg/mg albumin, p=0.022)
- Using improved drying methods (0.55 pg/mg albumin, p=0.017)

OLS models adjusted for clustering, age, household head's education level, and inter-cropping.

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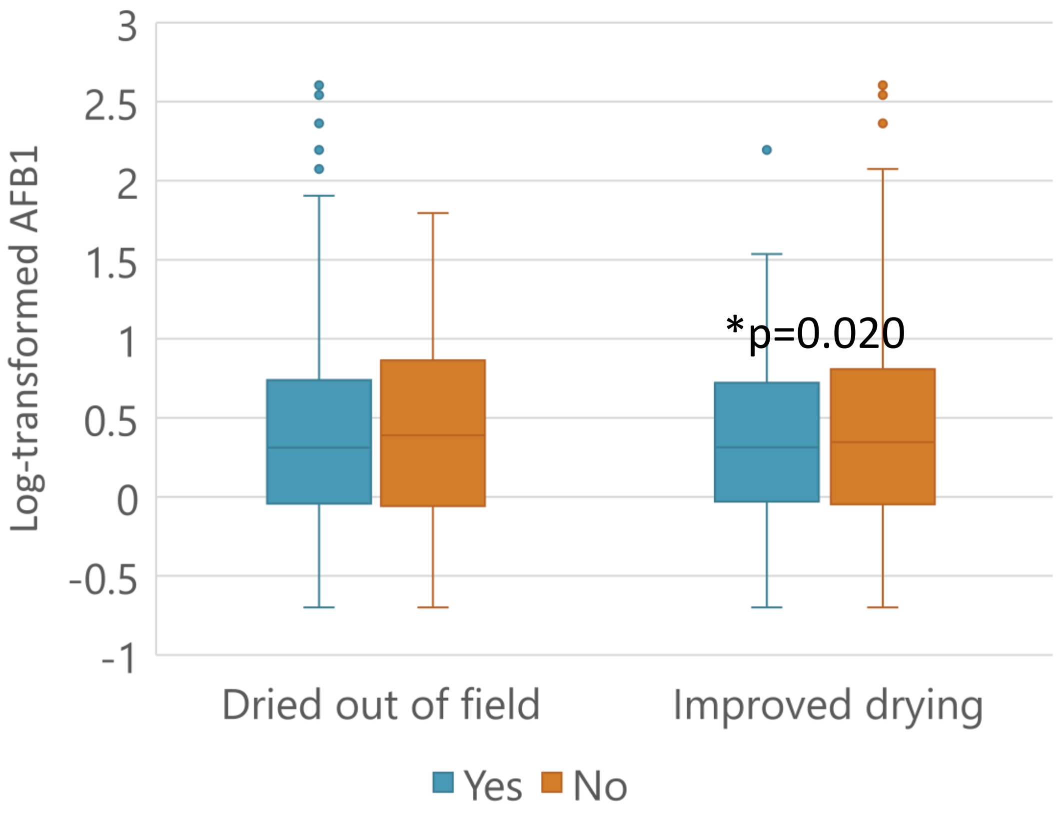
Aflatoxin Levels and Groundnut Agricultural Practices



Children in groundnut-producing households with **improved drying methods had lower aflatoxin levels** (0.64 pg/mg albumin, p=0.020) compared to those in households with unimproved methods

OLS models adjusted for clustering, age, household head's education level, and inter-cropping.

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Conclusions

- Detectable levels of aflatoxin in 90% of children in our study
- Both children 6-23 months and 24-59 months have detectable aflatoxin
- High levels of anemia, malaria, and stunting
- Association between stunting and aflatoxin
    - Stronger relationship in older children
    - Complex relationship (age and body weight)
- Dietary consumption of groundnuts and cassava linked to aflatoxin
- Households with improved agricultural practices (maize and groundnuts) = lower level of aflatoxin)

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Strengths and Limitations

- Representative study of children under five in 10 districts of Nampula
- Use of robust markers and tools for the assessment of aflatoxin, growth, household characteristics
- Sample size not achieved due to unexpected circumstances in the field
- Our study did not have data available on cassava production and processing practices

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Policy Implications and Future Research

- Several initiatives targeting aflatoxin in different crops in Mozambique (particularly groundnut)
- Agricultural, post harvest interventions and policies targeting aflatoxin may want to consider the health effects in a vulnerable population such as infants and young children
- Study focused on rural areas- better understanding of exposure in urban areas is needed
- Emphasizes the need for enhancing multi-sectoral collaboration (agriculture, health and nutrition) around aflatoxin mitigation to improve health and well being, economy, productivity and self reliance
- Discussion around the potential of cassava being a source of contamination

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Study Report

To learn more, view and download the study report

https://www.nutritioninnovationlab.org/where-we-are/mozambique

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