Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby gran to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Stephanie J. Smith Date

Mycotoxin exposure of infants through the breastmilk of mothers in western Kenya and associations with maternal and child nutrition.

By

Stephanie J. Smith
Master of Public Health

Global Health Department

_____

Amy Webb-Girard, PhD
Committee Chair

_____

Dana Boyd Barr, PhD
Committee Member

Mycotoxin exposure of infants through the breastmilk of mothers in western Kenya and associations with maternal and child nutrition.

By

Stephanie J. Smith
Bachelor of Science, Chemistry
Furman University
2010

Thesis Committee Chair: Amy Webb-Girard, PhD

A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of

Master of Public Health
in Global Health
2018

ABSTRACT

Malnutrition undermines a child's growth and development with significant lifelong consequences. With 26% of children stunted in Kenya, malnutrition represents a significant public health burden.[15] Recent research implicates environmental enteropathy (EE), a sub-clinical inflammatory condition of the intestinal lining that limits nutrient absorption, in the etiology of stunting. To date, the majority of research on the causes of EE and subsequent malnutrition has focused on the role of infectious pathogens and parasites. However, increasing attention is turning to mycotoxins. Mycotoxins are carcinogenic toxins released by fungi that infect common staple and legume crops. This study quantifies infant exposure to mycotoxins (aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol) through breastmilk and explores the role of mycotoxins, specifically aflatoxin M_1, in the etiology of child malnutrition and maternal mastitis and anemia in western Kenya. Anthropometry, breastmilk and capillary blood samples were collected at 4- and 9-months postpartum from a cohort of 505 women recruited from 8 health facilities in two counties in western Kenya. Breastmilk samples were processed and analyzed for mycotoxins using liquid-chromatography high resolution mass spectrometry. Subclinical mastitis was evaluated using ion selective electrodes for sodium:potassium contents. Hemoglobin from capillary samples were quantified using the HemoCue Hb 201+. All four mycotoxins were found to be present in 34-90% of the breastmilk samples depending on the mycotoxin and visit. There were no statistically significant associations between sampling season or facility. Aflatoxin M_1 was the only mycotoxin to have levels above the European Union (EU) cutoffs for infant milk replacement formula (0.025 ng/mL). At the 4^{th} and 9^{th} month visit 73.85% and 75.82% of samples, respectively were above the EU cutoffs. Further, 47.93% of samples were elevated at both visits and only 5.44% were below cutoffs at both visits. Regression modeling revealed no statistically significant relationship

between elevated aflatoxin levels and child stunting, wasting, underweight, or anemia at 4 or 9 months. There were also no statistically significant associations detected between breastmilk aflatoxin levels and maternal mastitis or anemia. There were statistically significant associations found between chronic aflatoxin exposure and infant WAZ and WHZ, and maternal subclinical mastitis at the 4-month follow-up. This study found widespread Aflatoxin M_1 contamination of breastmilk in western Kenya. There were no significant associations between aflatoxin levels and indicators of maternal or infant nutrition; however, longer term exposure and follow up may be necessary to detect impacts on child growth. Given the known negative impacts of mycotoxins on health, mycotoxin control policies and programs are urgently needed in western Kenya that reduce mycotoxin contamination of the food supply.

Mycotoxin exposure of infants through the breastmilk of mothers in western Kenya and associations with maternal and child nutrition.

By

Stephanie J. Smith
Bachelor of Science, Chemistry
Furman University
2010

Thesis Committee Chair: Amy Webb-Girard, PhD

A thesis submitted to the Faculty of the Rollins School of Public Health of Emory University in partial fulfillment of the requirements for the degree of

Master of Public Health
in Global Health
2018

ACKNOWLEDGEMENTS

Thank you to Amy Webb-Girard for introducing me to the world of mycotoxins and for allowing me to pursue my interests in breastfeeding and maternal and child health. Your support, guidance, and encouragement throughout this process has been so refreshing and I am truly grateful! Also, thank you for introducing me to Taproom Coffee and Dulce Vegan Bakery which have provided many yummy treats for me as I have traversed this thesis adventure.

Thank you to my family – to my Gramps for instilling in me an immense appreciation for education and for demonstrating what it means to be a life-long learner; for teaching me the meaning of hard work and perseverance; and for reminding me to "keep climbing" and "keep your chin up". To my Grammie – for all of the newspaper articles you have sent me throughout the years to continue to grow my interest in this field; for taking the time to really understand my project and asking questions; for your sweet words of encouragement and prayers; and for the many great "Grammie" hugs you gave me while I was working at home over Christmas break. To my Mom – for the many hours you kept me company in the Galleria while I worked; for always being willing to listen to my ideas, questions, and concerns even if you did not necessarily know what I was talking about ;-); for figuring out how to send me groceries from PA when I felt too stressed to go grocery shopping myself; for always thinking my "SUS…no…SIS…no…oh what is it called again?...SAS" skills are awesome; and for your constant encouragement and prayers to keep me going. To Darcy and Jamie – for always checking in via Facetime to keep me in the loop of what is going on, for letting me participate in family dinners from afar, for taking me on virtual hiking adventures with the pups when I can't get out on the trail myself, and for your words of encouragement. To my Dad – thanks for welcoming me in SC over spring break even though I spent the entire time writing; for taking walking breaks with me and Sawyer to the ice machine; for sending snacks to keep me going; and for your words of encouragement. And to all my Aunts, Uncles, and Cousins – thanks for all the encouragement via WhatsApp messages, texts, and snaps! And to Sawyer/SoySoy/Soy Bean/Pupadoo – thanks for making sure I get outside at least for a few minutes every day and for your endless snuggles and puppy kisses while I worked.

Thank you to my friends – Jessie, Laura, Lindz, Katie, Kelsey, Emily, Kat, Maureen – Thanks for all your words of encouragement, texts, letters, prayers, audiobook hookups, and reminders of what is truly important in life. You guys are the best! I can't wait to be able to actually hangout again! ☺

TABLE OF CONTENTS

1. INTRODUCTION

A. Introduction and Rationale

The "First 1,000 Days", the period between conception and two years old, is the most critical growth and development window in a child's life. Ensuring proper nutrition during this time period builds a strong nutritional foundation in a child so as to hopefully prevent malnutrition and its consequences later in life.[1] Malnutrition, often described by indicators such as stunting, wasting, and underweight, has many detrimental consequences for a child. Besides restricted growth, malnutrition contributes to intellectual delays, reduced performance in school, and is a strong predictor of mortality for children within their first five years of life.[2] Malnutrition in childhood also has implications for adulthood, including decreased efficiency, lower salaries, and higher risk of chronic diseases if extreme weight gain occurs later in life.[3] Although the extent to which stunting, wasting, and underweight are caused is not fully understood, it is known that nutritional deficits, along with chronic infection, lack of health care, lack of access to clean and safe water, poor sanitation and hygiene, and environmental factors are major contributors.[4,5,6]

Among the previously mentioned factors along the causal pathway to malnutrition, one newly hypothesized factor affecting malnutrition in children is mycotoxin exposure leading to environmental enteropathy. Environmental Enteropathy (EE) is characterized by inflammation in the small intestine and intestinal villi damage leading to the decreased ability to absorb nutrients properly. EE manifests sub-clinically, so children can suffer the consequences of the intestine's inability to function properly without overt intestinal symptoms.[7,8] Mycotoxins are secondary fungal metabolites that when ingested, absorbed,

or inhaled, can lead to a host of problems within the body, including exacerbating the consequences of malnutrition, depending on the route and amount of exposure.[9]

Breastfeeding is one of the best tools employed to provide proper nutrition to developing infants and to combat malnutrition. Unfortunately, though, when a mother is malnourished herself, she is unable to pass along all of the nutrients a developing infant needs.[10] Likewise, when a mother ingests something toxic, such as mycotoxins, it can be passed through her breastmilk to her child.[11] It is crucial to the continued fight of malnutrition in the world, and specifically in Kenya, to understand the extent to which mycotoxins are passed through breastmilk and the consequences these toxins have on infant malnutrition.

B. Problem Statement

Globally, in 2016, there were 154.8 million children under the age of five stunted, 51.7 million wasted, and 94.5 million underweight.[12-14] In Kenya, in 2014, among children under the age of five, 26% were stunted, 4% were wasted, and 11% were underweight.[15] To tackle this issue, continued research into the causes of malnutrition, including the role of mycotoxin exposure, are crucial. The Food and Agricultural Organization (FAO) estimates that about 25% of crops are contaminated with molds, and of that portion, 100% are contaminated with at least one mycotoxin.[16] Developing countries are disproportionately affected due to their poor agricultural practices, harsh weather environments, and lack of infrastructure. The main crops affected by mycotoxin growth are the same crops that are staple foods in developing countries, namely maize, wheat, groundnuts.[17] This secondary analysis aims to document the extent of mycotoxin exposure through breastmilk and its contributions to malnutrition in infants in western Kenya. In

understanding how mycotoxin exposure plays a role in malnutrition scientists, researchers, community developers, governments, and most importantly mothers and families will be better equipped to fight mycotoxin exposure and malnutrition in their communities.

C. Purpose Statement

This study seeks to evaluate whether mycotoxins are passed through breastmilk, and if so, is there significant associations between the mycotoxins in breastmilk and infant nutritional status. As a secondary objective, this study examines the impacts of mycotoxins on maternal health, including breast health (mastitis) and anemia.

D. Research Questions

- Are mycotoxins (aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol) present in the breastmilk of mothers in western Kenya at 4- and 9-months postpartum?
- At what concentrations are aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol present at 4- and 9-months postpartum?
- Do the concentrations of aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol exceed the European Union cutoffs for infant milk replacement formula? If so, what percentages of the samples are above the cutoffs?
- Is there an association between the concentration of aflatoxin M_1 in breastmilk and stunting, wasting, underweight, and anemia in infants at 4- and 9-months?
- Is there an association between the concentration of aflatoxin M_1 in breastmilk and anemia and mastitis in mothers at 4- and 9-months postpartum?

- Is there an association between chronic aflatoxin exposure through breastmilk and stunting, wasting, underweight, and anemia in infants at 4- and 9-months?
- Is there an association between chronic aflatoxin exposure through breastmilk and maternal anemia and mastitis at 4- and 9-months postpartum?

E. Significance Statement

This research is important to the health and wellbeing of children and mothers of western Kenya. The information gleaned from the results of the study can inform further research as well as policy and programmatic practice. Most importantly it will aid in the fight against mycotoxin contamination and malnutrition in the developing world.

F. Definition of Terms

- *Stunting* – low height-for-age z-score (HAZ); z-score two standard deviations below the median of the World Health Organization reference population
- *Wasting* – low weight-for-height z-score (WHZ); z-score two standard deviations below the median of the World Health Organization reference population
- *Underweight* – low weight-for-age z-score (WAZ); z-score two standard deviations below the median of the World Health Organization reference population
- *First 1,000 Days* – period between conception and two years old; the most critical growth and development window in a child's life and the best time to build a strong nutritional foundation with the hopes of preventing malnutrition and its consequences in the future

- *Environmental Enteropathy (EE)* - characterized by inflammation in the small intestine and the dulling of the intestinal villi causing intestinal permeability and decreasing the small intestine's ability to absorb crucial nutrients and minerals
- *Mycotoxins* - toxic secondary fungal metabolites
- *Mycotoxicosis* – disease caused by exposure (absorption, inhalation, consumption) to mycotoxins
- *Aflatoxin* – Type of mycotoxin produced by *Aspergillus* fungal species; most toxic
- *Fumonisin* – Type of mycotoxin produced by *Fusarium* and *Alternaria* fungal species
- *Ochratoxin A* – Type of mycotoxin produced by *Aspergillus ochraceus* and other *Aspergillus* species
- *Deoxynivalenol* – Type of mycotoxin which falls under the trichothecenes family; mainly produced by *Fusarium* species

2. LITERATURE REVIEW

2.1 Introduction

The nutritional status of a child is one of the most important markers of a child's overall health and development. Three indicators specifically, stunting, wasting, and underweight, are monitored to assess the health of children across the world. Globally, in 2016, there were 154.8 million children under the age of five stunted, 51.7 million wasted, and 94.5 million underweight.[12-14] In Kenya specifically, the demographic health survey of

2014 revealed that among children under the age of five, 26% were stunted, 4% were wasted, and 11% were underweight.[15] With such large numbers of children being affected in the world and in Kenya, the causes and implications of a poor nutritional status need to be bettered studied and understood.

2.1.1 Stunting

Stunting, defined as a height-for-age z-score (HAZ) two standard deviations below the median of the World Health Organization reference population, is a manifestation of chronic malnutrition.[3] Stunting has many detrimental consequences for a child. Besides restricted growth, stunting contributes to intellectual delays, reduced performance in school, and later decreased efficiency and lower salaries in adulthood. It can also lead to chronic diseases, such as diabetes and heart disease, in adulthood if extreme weight gain occurs later in life.[3] It is also a strong predictor of mortality for children within their first five years of life.[2] In women, stunting during childhood can lead to pregnancy complications in adulthood. Once stunted, it is very difficult for a child to catch back up. Stunting, present after two years of life is basically irreversible.[6] Effectively addressing stunting requires an understanding of its causes. However, the etiology of stunting is complex; causal pathways are not fully understood, and the importance of specific causal pathways may be context specific. That said, nutritional deficits, along with chronic infection, and environmental factors are major contributors.

2.1.2 Wasting

Wasting not only takes into account the height of a child, but also the weight. Wasting is classified as a weight-for-height z-score (WHZ) below two standard deviations below the median of the reference population. While stunting is the best indicator for chronic malnutrition, wasting is the best indicator for acute malnutrition.[4] Wasting can leave children more susceptible to death from infectious diseases, such as measles, pneumonia, and diarrheal diseases. Research indicates stunting and wasting have similar causal pathways. Wasting is caused by poor nutrition, either from lack of adequate nutritious foods brought on my suboptimal caring practices and/or food insecurity. It is exacerbated by infection, lack of health care, lack of access to clean and safe water, and poor sanitation and hygiene.[5]

2.1.3 Underweight

Underweight is the third commonly applied indicator for child nutritional status. Measured using the weight of the child relative to one's age, underweight is defined as a weight-for-age z-score (WAZ) below two standard deviations below the median of the reference population. Because this indicator cannot distinguish between a tall thin child and a shorter child of normal body weight it cannot distinguish chronic from acute malnutrition, therefore stunting and wasting are often looked to first. The underweight indicator can be useful, though, in areas where wasting is not as prevalent, especially because it is easier to measure than stunting or wasting which require length measurements. Underweight has similar causal pathways as both stunting and wasting.[4]

2.1.4 First 1,000 Days and Maternal Nutrition

Maternal nutrition is a significant influencer of a child's nutritional status. Inadequate nutrition and growth begins when a child experiences poor nutrition from conception. The period between conception and two years old, known as the "First 1,000 Days", is the most critical growth and development window in a child's life, and is therefore the best time to build a strong nutritional foundation with the hopes of preventing malnutrition and its consequences in the future.[1] Proper nutrition during this time also helps to promote overall health and development as this is a critical time period for many other systems in the body as well, such as the central nervous system and immune system.[18] In high-, middle-, and low- income countries, the nutritional status of mothers have significant implications for the growth of their babies. A malnourished mother is unable to pass along all of the nutrients that a developing fetus or infant needs, risking altered gene expression as well as metabolism and body tissue restructuring that will last for the remainder of the child's life, having detrimental consequences. It is under these conditions that stunting, wasting, and underweight can begin, although with developing fetuses and newborns the terms intrauterine growth restriction and low birth weight are more commonly used.[1] Christian *et. al.* reported that about 20% of stunting begins in the womb.[7]

2.1.5 Environmental Enteropathy

Among the previously mentioned and studied causes of stunting, wasting, and underweight, a newer concept along the causal pathway is environmental enteropathy. Environmental Enteropathy (EE), formerly known as Tropical Enteropathy, is characterized by inflammation in the small intestine and the dulling of the intestinal villi.[7,8] The dulling and the inflammation cause the intestine to become more permeable, thus decreasing the small intestine's ability to absorb important minerals and nutrients from food. Although the cause of EE is not yet fully understood, poor hygiene and sanitation are considered key contributors. One strong piece of evidence for this is infants are not born with EE, but rather acquire it later in life. Studies also show that the presence of EE can be predicted by the number of disease causing organisms living in the small intestine, and that the severity of EE exists along a spectrum.[19] Due to the subclinical nature of EE, many individuals, including children, may suffer from the consequences of the intestine's inability to function properly without overt intestinal symptoms.

As mentioned previously, EE prohibits the intestine from properly absorbing nutrients. As a result, hypotheses have been made about the potential correlation between EE and malnutrition. Previous studies have shown that nutritional interventions struggle to diminish the effects of stunting. They also reveal that while episodes of diarrhea do have an impact on stunting, in between these episodes children experience "catch up" growth, leading researchers to believe there may be a more important pathway yet to be discovered. A pathway that perhaps involves EE.[19]

A few studies have explored the connection between EE and malnutrition. One such study by Prendergast *et. al.* (2014) in Zimbabwe collected both stunted and non-stunted 18-month old infants and tested previously collected blood for biomarkers of intestinal damage and inflammation at 6 weeks and 3, 6, 12, and 18 months. The results revealed that plasma levels of I-FABP, a small protein located at the tips of the villi of small intestines released into the bloodstream when intestinal damage occurs, were not associated with stunting at 18-months. There was, though, an increased odds of stunting with higher levels of AGP and higher $\log_{10}$ levels of CRP, biomarkers of inflammation.[6] Another study by Arndt *et. al.* (2016) in Bangladesh looked at biomarkers (myeloperoxidase, neopterin, and alpha-1-antitrypsin) in stool samples to determine the severity of EE, assigning a composite EE score on a 10-point scale, and identify an association with the length-for-age z-score (LAZ). They determined that while alpha-1-antitrypsin and neopterin were not statistically significantly associated with child growth, children with higher levels of myeloperoxidase were more stunted in growth during each studied 3-month period than those with lower levels. They also found that composite EE scores were negatively associated with LAZ over a 3-month period.[20] This potential association between EE and stunting and malnutrition need to continue to be explored before a comprehensive understanding can be gained. One potential underexplored contributor to Environmental Enteropathy are mycotoxins.

2.2 What are Mycotoxins

Mycotoxins are a specific type of fungus that grow on grains, corn, and legumes. Broadly, fungi are understood to be organisms with nuclei which produce spores and grow

in elongated filament-like structures. Since they do not contain any chlorophyll they sustain themselves by either feeding off of dead, decaying materials or living as parasites in animals, plants, or other fungi.[21] When fungi choose animals as hosts and begin to grow, the resulting diseases are categorized as mycoses. Alternatively, diseases known as mycotoxicoses occur within a host when toxic secondary fungal metabolites, mycotoxins, are consumed in the diet, inhaled into the respiratory system, or come in contact with the skin of the host. Neither mycoses nor mycotoxicoses are communicable person-to-person. Mycoses are divided into two different categories, primary pathogens, which affect healthy individuals, and opportunistic pathogens, which affect individuals with weak immune systems. Opportunistic pathogens are the most prevalent mycoses that affect humans and are most commonly found among the developed world in immunocompromised patients. Mycotoxicoses result from an exposure to a natural substance contaminated with mycotoxins. Since mycotoxicoses are a product of an exposure, the symptoms of these diseases depend on many exposure related factors.[22] These factors include the "type of mycotoxin; the amount and duration of the exposure (acute or chronic); the age, health, and sex of the exposed individual; and many poorly understood synergistic effects of involving genetics, dietary status, and interactions with other toxic insults."[22] Other health issues of the host, such as misuse of alcohol, insufficient intake of calories, vitamin deficiency, and infectious disease status can also dictate how the mycotoxicoses affects that individual as well. Not only can mycotoxicoses bring about symptoms of their own but they can also cause the host to be susceptible to other microbial diseases, can create a compound effect with other toxins, and can exacerbate the consequences of malnutrition. Very little is known about treating mycotoxicoses.[22]

Mycotoxins, toxic secondary fungal metabolites, were first named when an unknown disease spread rapidly through a rafter of baby turkeys in 1962. This disease was eventually traced back to ground peanut meal that contained moldy secondary metabolites from *Aspergillus flavus*, now known as aflatoxin, one variety of mycotoxin. A collaboration of numerous scientists between 1960 and 1975 worked to identify other mycotoxins during a period that became known as the "Mycotoxin Gold Rush". As of 2003 there were between 300-400 identified mycotoxins. They can be classified in many different ways depending on the field of study and area of expertise of the classifier. Organic chemists classify mycotoxins based on their structure, physicians by the diseases they cause, and biochemists by their biosynthetic beginnings. While mycotoxins are produced by fungi they are not the only toxic substance produced by fungi. Antibiotics are fungal derivatives that are lethal to bacteria while phytotoxins are fungal products toxic to plants. Mycotoxins on the other hand are only lethal to animals and other vertebrates. Despite there being hundreds of different mycotoxins, there are only a handful that are of concern to animal and human health. These include: aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxin, patulin, trichothecenes, and zearalenone. Many of these different types of mycotoxins also have several different varieties that can be categorized under the larger mycotoxin family. For the purposes of this paper, only aflatoxins, fumonisins, ochratoxin, and a specific type of trichothecenes known as deoxynivalenol will be explored in greater detail as these were the mycotoxins of focus for this secondary study.[22]

2.2.1 Aflatoxins

As mentioned previously, aflatoxins were first identified and named during the unknown disease outbreak among a rafter of baby turkeys in 1962.[22] Aflatoxins

can be produced by a few different fungi strains including *Aspergillus flavus, Aspergillus parasiticus, Aspergillus bombycis, Aspergillus ochraceoroseus, Aspergillus nomius, and Aspergillus pseudotamari*.[22,23] *Aspergillus flavus* and *Aspergillus parasiticus* are the two most common and well known aflatoxin producing fungi strains, however, as the remaining four are not as prevalent.[22] While there are many different types of aflatoxins, the four most common produced by *Aspergillus flavus* and *Aspergillus parasiticus* include B_1, B_2, G_1, and G_2.

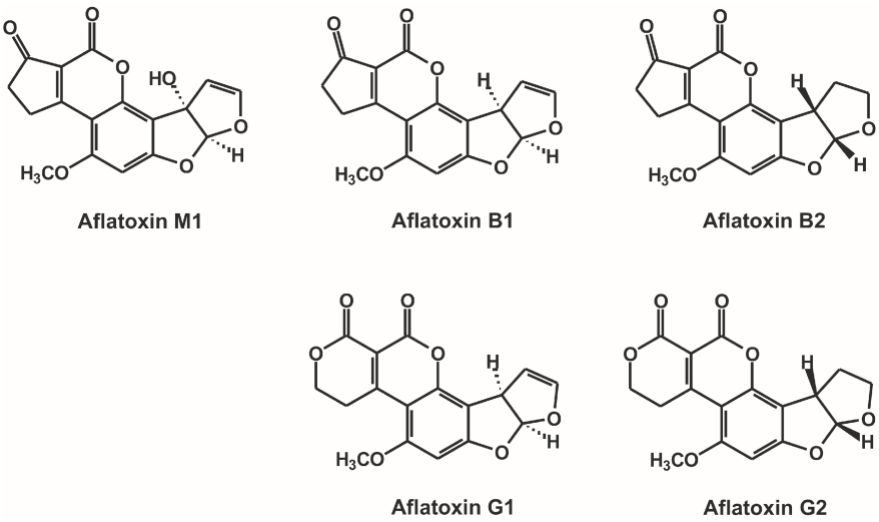


Figure 1: Chemical Structures of Aflatoxin M_1, B_1, B_2, G_1, and G_2.[24]

All four of these aflatoxins can be produced by either of the two main strains mentioned above.[17] Aflatoxin B_1 and B_2 produced by the *Aspergillus flavus* strain can be found in corn (maize), peanuts (groundnuts), cereals, other nuts, seeds, and other commodities.[22,17] Under the *Aspergillus parasiticus* strain all four of the most common aflatoxins can be produced and are often found in corn (maize) and peanuts (groundnuts).[17] Aflatoxins can contaminate crops either before harvest in the fields or after harvest during the storage process.[22] In the field, drought

conditions, high heat and little water, can lessen the strength of corn or other crops during the growth process by causing tiny fractures in the crop which allow *Aspergillus* to enter and grow. Surprisingly, *Aspergillus flavus* can withstand extreme heat, unlike other fungi, surviving, growing, and thriving in temperatures of upwards of 100°F. Aflatoxins have even demonstrated growth at 118°F.[25] Aflatoxin growth during the storage process depends on the moisture content of the contaminated crop and the humidity of the storage area. The greater the moisture and humidity, the more likely aflatoxins will flourish.[22] Since aflatoxins affect many common sources of animal feed, they can also contaminate meat, eggs, milk, and other dairy products. Aflatoxin metabolites tend to be stored in fat, so if an animal consumes them in their feed it can be stored and passed on in other forms.[26,27] Alshannaq et. al. reports that AFM_1 can be detected in cow milk only 12-24 hours after eating feed contaminated with aflatoxin B_1.[28]

Figure 2: Corn contaminated by *Aspergillus* fungi which produces Aflatoxins. Image provided by Joseph Atehnkeng of CGIAR.

Of all of the mycotoxins in existence, aflatoxins are arguably one of the most toxic.[22] Aflatoxins have been recognized by the International Agency for Research on Cancer (IARC) as carcinogenic to humans, falling into the group 1 monograph.[29] Diseases related to aflatoxins are known as aflatoxicoses, and primarily target the liver. Chronic aflatoxin exposure often leads to liver cancer, while acute aflatoxin exposure can lead to death depending on the severity of the exposure. It has been estimated that the acute lethal dose of aflatoxin is about 10-20 milligrams. There is also some research showing that inhalation exposure to aflatoxin can lead to lung cancer. Aflatoxin B_1 is the most toxic of the four most common aflatoxins (B_1, B_2, G_1, and G_2) and therefore is the most widely studied. Based on its structure, Aflatoxin B_1 is also capable of accepting a hydroxyl group and transforming into aflatoxin M_1, which can be found in milk in both in humans and animals after the ingestion of Aflatoxin B_1. Overall, the evidence surrounding aflatoxins and their cancer-causing properties is some of the most well supported of any natural toxin.[22]

2.2.2 Fumonisins

Mycotoxins categorized as fumonisins are a much younger concept as compared to aflatoxins. They were described a little over 20 years later than aflatoxins, in the year 1988 by a team from South Africa.[22,30] Although fumonisins were not characterized until 1988, their presence and concerns about their effects in both animals and humans have been around since the late 1800s. The effects of fumonisins were first noticed in horses who were developing Equine

leucoencephalomalacia (ELEM), also known by the common name "staggers", after consuming corn that was moldy.[30] Symptoms of this disease in horses include lack of appetite with intense thirst, drooping of the head and/or ears, blindness, confusion, loss of consciousness, and death. After autopsies were performed on these horses their brains revealed deterioration of brain matter into white curdled liquid.[30,31] Rabbits also develop a type of leucoencephalomalacia upon fumonisins exposure. Pigs, on the other hand, develop fluid in their pleural cavity and lungs. Disease in rats include programmed cell death in the liver. In humans, though, consumption of and exposure to fumonisins can lead to esophageal cancer. There are also some hypotheses that fumonisins may also lead to neural tube defects in humans as there has been some research that reveal these same defects in experimental animals exposed to fumonisins.[22]

Several fungi strands are capable of producing fumonisins including *Fusarium proliferatum*, *Fusarium nygamai*, *Fusarium verticillioides* (previously *Fusarium moniliforme*), and *Alternaria alternate f. sp. Lycopersici.*[22] The most commonly produced fumonisin is fumonisin B_1 (Figure 3) although there are many other types including an A-series, B-series, and C-series, and P-series.[31] The most commonly discussed fungi in relation to fumonisins is *Fusarium verticillioides.* It is actually quite common for *Fusarium verticillioides* to grow as an endophyte in numerous parts of the corn plant without causing disease. Similar to aflatoxins though, during drought conditions that make crops more susceptible to infection, fungi can grow and cause stalk rot and disease. Other factors that can also promote disease growth are the aligning of specific genotypes of plant and fungi, as well as

damage from insects. Although *Fusarium verticillioides* can cause disease within corn crops this does necessitate fumonisins growth.[22]

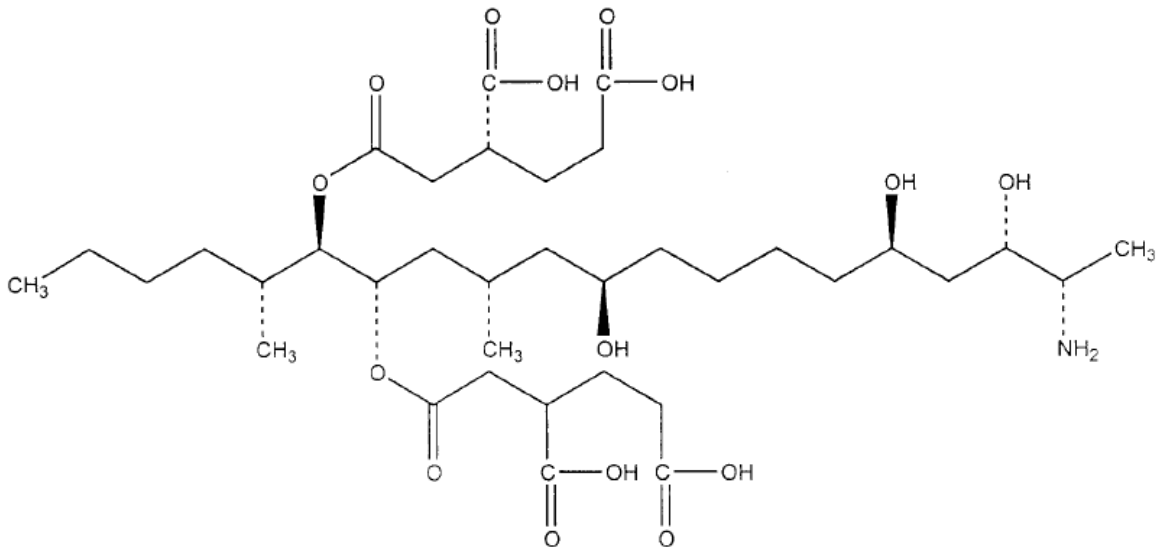


Figure 3: Chemical Structures of Fumonisin B_1.[22]

2.2.3 Ochratoxin A

In 1965, during the "Mycotoxin Gold Rush", Ochratoxin A (OTAA) was learned to be a secondary metabolite of the fungi *Aspergillus ochraceus*. Since then, it has been determined that ochratoxin A can also be produced by a multitude of other species of *Aspergillus* fungi as well, including *Aspergillus alliaceus, Aspergillus auricomus, Aspergillus carbonarius, Aspergillus glaucus, Aspergillus melleus,* and *Aspergillus niger.* It was also initially thought that multiple species of *Penicillium* also produce ochratoxin A, however *Penicillium verrucosum* has since been identified as the only species capable. This specific mycotoxin is more versatile than fumonisins and can grow in oats, grapes, wheat, rye, barley, coffee beans, and other plant products. Barley though is the most susceptible.[22] It can also be transported through meat, milk, and blood.[32] Interestingly, *Aspergillus niger* is

utilized in the creation of citric acid and enzymes therefore monitoring this particular strain during production is critical.[22] Ochratoxin A is the most potent ochratoxin and is perhaps the most toxic type of mycotoxin next to the aflatoxins. Ochratoxin A conforms to the same patterns as most other mycotoxins in that the crop, moisture level of the crop, and the temperatures of the surrounding area affects the ability of ochratoxin A to flourish. [22]

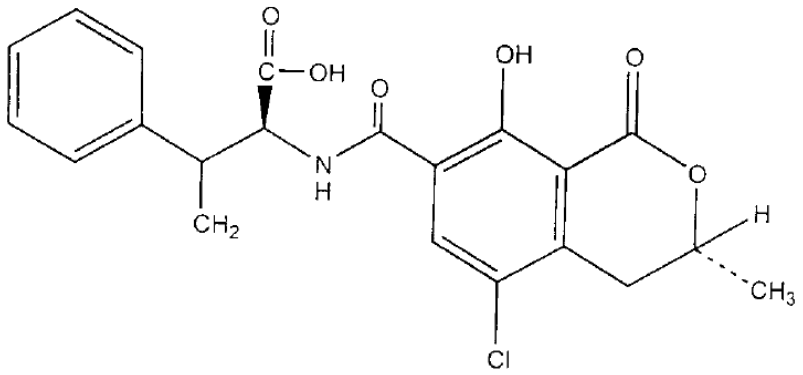


Figure 4: Chemical Structures of Ochratoxin A.[22]

Exposure to ochratoxin A can lead to many issues for plants, animals, and humans. The IARC categorized ochratoxin A into their group 2B monograph which labels it as possibly carcinogenic to humans.[33] It has been identified as both a kidney and a liver toxin, with the kidney being ochratoxin A's primary target.[22] It is also a teratogenic compound, meaning it can affect fetal development, potentially leading to birth defects.[34] Animals that have been exposed to contaminated feed have reduced growth rates. While pigs are the animal most sensitive to OTAA's effects, studies also show that it affects poultry, rabbits, and rats. In pigs, OTAA normally manifests itself in lack of weight gain and kidney and liver disease. In poultry, it results in poor egg production, lack of weight gain, diarrhea,

nephrotoxicity, poor egg shell quality, and decreased immune function among chicks. OTAA in rabbits accumulates in muscle tissue, mammary glands, liver, and their kidneys.[33] Humans require more time to process and get rid of OTAA compare to animals. As a result, it has greater potential to negatively affect human systems. Ochratoxin A affects humans in many of the same ways it does animals. It still mainly targets the kidneys and the liver.[22] However, OTAA is known to be present in the blood of Northern and Eastern American individuals without the emergence of any symptoms.[30]

2.2.4 Deoxynivalenol

Deoxynivalenol (DON) is a member of a group of mycotoxins known as trichothecenes. The common identifier within this family of roughly 140 other metabolites is the backbone consisting of 12,13-epoxytrichothene and a double bond with a side chain substitution.[35] Figure 5 below displays the structure specific to deoxynivalenol.[22] It is the three free hydroxy groups (-OH) that cause DON to be so toxic. The specific genus mainly responsible for the production of DON is *Fusarium*, with *Fusarium graminearum* and *Fusarium culmorum* being the two most common species.[35] While DON is not the most toxic trichothecene it does still have significant health consequences upon exposure. Animals, when exposed to low doses of DON, refuse their food and experience weight loss, whereas high doses bring about diarrhea, nausea, and vomiting. DON is also known more commonly as “food refusal factor” and “vomitoxin”.[22] The crops most commonly contaminated by deoxynivalenol include safflower seeds, wheat, mixed feeds, rye, corn, and barley.[35] Similar to aflatoxins, DON is also very stable in high

temperatures (338-662°F). One study showed that even after 30 minutes of exposure to temperatures of 338°F there was no decrease in DON concentration. Despite DON remaining in food products even after cooking, it does not appear to be a significant threat to human health. It can result in nausea, vomiting, diarrhea, headaches, dizziness, fever, and abdominal pain in humans but that is the extent of the major consequences.[35] Humans are also at risk of secondary exposure to DON from various animal products such as meat, milk, and eggs. This risk though, again, does not pose a significant threat as the levels of DON in these products are minimal.[13] After numerous animal studies, the IARC categorized DON into their group 3 monograph, which defines it as "not classifiable as to its carcinogenicity to humans" as the data was inconclusive.[29,35]

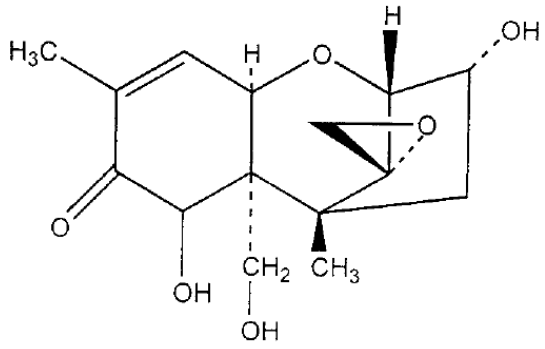


Figure 5: Chemical Structures of deoxynivalenol.[22]

2.3 Agriculture

As mentioned previously, mycotoxins most commonly affect corn, legumes, and grains, and may also subsequently contaminate milk, meat, eggs through contaminated feeds. There are many factors that can affect mycotoxin growth during the cultivation and storage process and they are most commonly found in the developing world. First, the climate has major implications on mycotoxin growth. When drought conditions are present,

crops, like corn, are put under stress which can cause cracking in the grain. These little fractures provide the perfect opportunity for mycotoxins to insert themselves into the crops and flourish. Second, insect damage can also increase the susceptibility of crops to mycotoxins. Again, using corn as an example, insect larvae begin eating the husk silk and then move on to the kernels, damaging the integrity of the kernel. These injuries to the kernels allow mycotoxins to enter the crop and grow in favorable conditions. Typically, the more damage to the crop the higher the concentration of mycotoxin present.[36] With greenhouse gas concentrations on the rise, and the average global surface temperatures increasing, researchers believe it is likely that the occurrence of drought in specific regions will also increase.[37] Therefore, it is likely that climate change may increase mycotoxin concerns globally.[25] Another newer technology that affects mycotoxin growth is the introduction of nonmycotoxigenic fungal strains to crops to attempt to combat the growth of toxic mycotoxins.[38] While these are important factors to address during crop growth, they do not mitigate mycotoxin growth during crop storage. Climate factors into storage as the humidity surrounding the crops affects the moisture within the stored crop. Also, how well the crop was dried before storage affects the moisture inside. Moist conditions favor fungi and therefore mycotoxin growth.[25] Proper sorting of the crop before storage can also affect mycotoxin growth as well. When crops are sorted, mycotoxin-infected kernels, nuts, or other commodities can be removed to prevent the spread of mycotoxin throughout the rest of the harvest. Finally, crops can be treated with ammonia to remove mycotoxins. However, this technique is only suitable for crops designated for animal feed.[39] Attention to mycotoxin contamination and its resulting concerns is crucial as the Food and

Agricultural Organization (FAO) estimates that about 25% of crops are contaminated with molds, and of that portion, 100% are contaminated with one or more mycotoxins.[16]

2.4 Maternal and Child Health

Amongst the health concerns related to mycotoxins, of particular interest is the relation between mycotoxins and the health of women and children. As mentioned previously, mycotoxins have a host of different effects and resulting symptoms depending on the specific mycotoxin. While these effects are of concern, there are also additional concerns when it comes to the interaction of mycotoxins with mother and child, especially during prenatal and postnatal periods. One such concern is whether mycotoxins can be passed through the blood from mother to child. One study analyzing a population of first time mothers in Kenya tested the blood of moms prenatally and at birth. They also took a cord blood sample from the infant. Their results showed that aflatoxins (AFM_1, AFM_2, and/or AFB_1) were present in 43% of their antenatal sample and 53% of the sample at birth, with 34 individuals being present at both times. For the cord blood samples, 37% of the 101 samples contained aflatoxins. Interestingly, most of the cord blood samples that contained aflatoxins came from mothers who tested negative for aflatoxins at delivery. The study by De Vries also identified that aflatoxin prevalence was higher during the warm wet months (March to June), that female babies born to mothers positive for aflatoxins had a lower birth weight than females born to mothers who were not, while male babies born to mothers positive for aflatoxins had a higher birth weight than males born to mothers not positive for aflatoxins.[40] Another more comprehensive review revealed that when mothers' diets are contaminated with aflatoxins, infant exposure in utero is widespread.[41]

A 2017 study out of Turkey addresses the question as to if mycotoxins can be passed from the mother via breastmilk. Out of a collection of 74 breast milk samples, 66 (89.2%) tested positive for AFM_1. It also showed a statistically significant seasonality effect with greater numbers of samples exceeding the European Union's aflatoxin regulated threshold in June than in December.[42] The European Union designates the maximal residual concentrations of the following mycotoxins in "infant formula, follow-on formula, dietary foods for special medical purposes intended specifically for infants or processed cereal-based foods and baby foods for infant and young children" as: AFB_1 (0.1 ng/mL), AFM_1 (0.025 ng/mL), OTA (0.5 ng/mL), FB_1 (200 ng/mL), and DON (200 ng/mL).[43]

While aflatoxins are the most commonly studied mycotoxin because of their toxicity, there have also been a few studies that have also measured the urinary concentrations of deoxynivalenol (DON) and ochratoxin A (OTA) in pregnant women in the UK and Croatia.[44,45] The study in Croatia revealed 75% of the samples contained OTA.[44] In the UK, about 85% of the samples contained DON. There was also a study performed in Guatemala that explored the possible link between a specific type of neural tube defect, frontoethmoidal encephalomeningocele (FEEM), and fumonisins. The study revealed that villages with greater occurrences of FEEM had higher fumonisin exposure levels as measured in maternal blood and urine samples, and villages with no occurrence of FEEM had statistically significantly lower exposure levels.[45]

Finally, of particular concern is how mycotoxins affect environmental enteropathy and the ability for mothers and children to absorb key minerals and nutrients. A healthy gastrointestinal tract can typically filter out most toxins, such as mycotoxins. Fumonisins, Aflatoxins, and Ochratoxins have particularly detrimental effects in the intestines. FB_1 has

been shown to cause the accumulation of sphinganine in intestinal epithelial cells, which in turn causes the inhibition of G_0/G_1 phases of the cell cycle. The accumulation of sphinganine can also change the distribution of glycoproteins within the portion of the small intestine between the duodenum and the ileum known as the jejunum. This rearrangement makes it easier for FB_1 to pass across the epithelial cells. Finally, FB_1 stifles protein expression at the tight junctions and disrupts immune function. OTA has been shown to lessen the amount of glucose absorbed at the SGLT1 transporter. In animal studies, OTA shows similar affects as FB_1 as it increases intestinal permeability, increased susceptibility to parasite infections, suppressed tight junction protein expression, decreased the height of intestinal villi, and increased epithelial cell apoptosis. Aflatoxins again are very comparable to OTA and FB_1. In vitro tests with colon cells showed that AFB_1 triggered genetic damage, hindered growth of cell, and increased the activity of lactate dehydrogenase. Animal studies displayed an increase in apoptosis, a decrease in intestinal villi height, and damage to the intestinal barrier. Overall, the damage all three mycotoxins cause are very similar to each other.[46]

2.5 Public Health Problem

Although documentation of mycotoxins exists from the middle ages and they have been recognized officially since the 1960's, research on mycotoxins in relation to pregnancy and infant development is still greatly lacking.[23] The evidence base currently available highlights the toxicity, carcinogenicity, teratogenicity, and immunotoxicity of mycotoxins to the general population and this ought to spur greater action and research in the realm of maternal and child health.[36] According to the Centers for Disease Control

(CDC), in developing countries, about 4.5 billion people might be chronically exposed to aflatoxins in their diet. Developed countries have many systems in place to monitor their production, processing, and storage of crops to prevent mycotoxin contamination, such as the regulations set by the European Union. The agriculture sector in developing countries, on the other hand, depends largely on subsistence farmers who often do not have access to appropriate storage facilities or testing materials. Similarly, countries may not have or enforce regulations concerning mycotoxin contamination, therefore increasing the likelihood of mycotoxin growth and exposure.[25] Also, mycotoxins affect the main staple crops (corn, wheat, groundnuts) of these developing nations which also makes exposure to these toxins, in animals and humans, highly likely.

The few studies that have analyzed the potential association between mycotoxin exposure and undernutrition have resulted in significant findings that should continue to inform research today. One such study in Tanzania revealed that infants who were exposed to fumonisin amounts greater than the provisional maximum tolerable daily intake of 2 μg/kg in corn during complementary feeding were statistically significantly shorter by 1.3 cm.[2] A cross-sectional study by Gong among children 9 months to 5 years in Togo and Benin revealed a significant association between the amount of aflatoxin bound to albumin in the blood and both stunting and wasting.[47] Turner, in studying children 6 to 9 years old in Gambia, revealed that aflatoxin albumin adduct levels were weakly associated with wasting but had a strong statistical association with lower levels of secretory immunoglobulin A (sIgA). SIgA is crucial to maintaining proper mucosal barriers in the digestive tract.[48] In a later study Turner also discovered a statistically significant association between a reduction in aflatoxin bound to albumin and an increase in height

and weight for children during their first year of life.[49] Sadeghi, in 2009, published findings revealing a significant association between Iranian infants' height at birth and aflatoxins found in their mothers' breast milk.[50] Khlangwiset details several other studies with evidence for aflatoxins effects on women, infants, and children.[51] While these studies are a great beginning to uncovering the extent to which mycotoxins impact the health of mothers and their children, there is still so much more room for the extension of this research.

This need for continued research led to this thesis. Using secondary data collected from mothers in western Kenya, this analysis seeks to evaluate if mycotoxins are present in breastmilk and at what levels. If present, it also seeks to determine whether significant associations between the mycotoxin concentrations in breastmilk and infant nutritional status exist. Also, as a secondary objective, this study examines associations between mycotoxin levels in breastmilk, as a proxy for maternal exposure, on breast health (mastitis) and anemia. Figure 6 lays out a conceptual framework for this work.

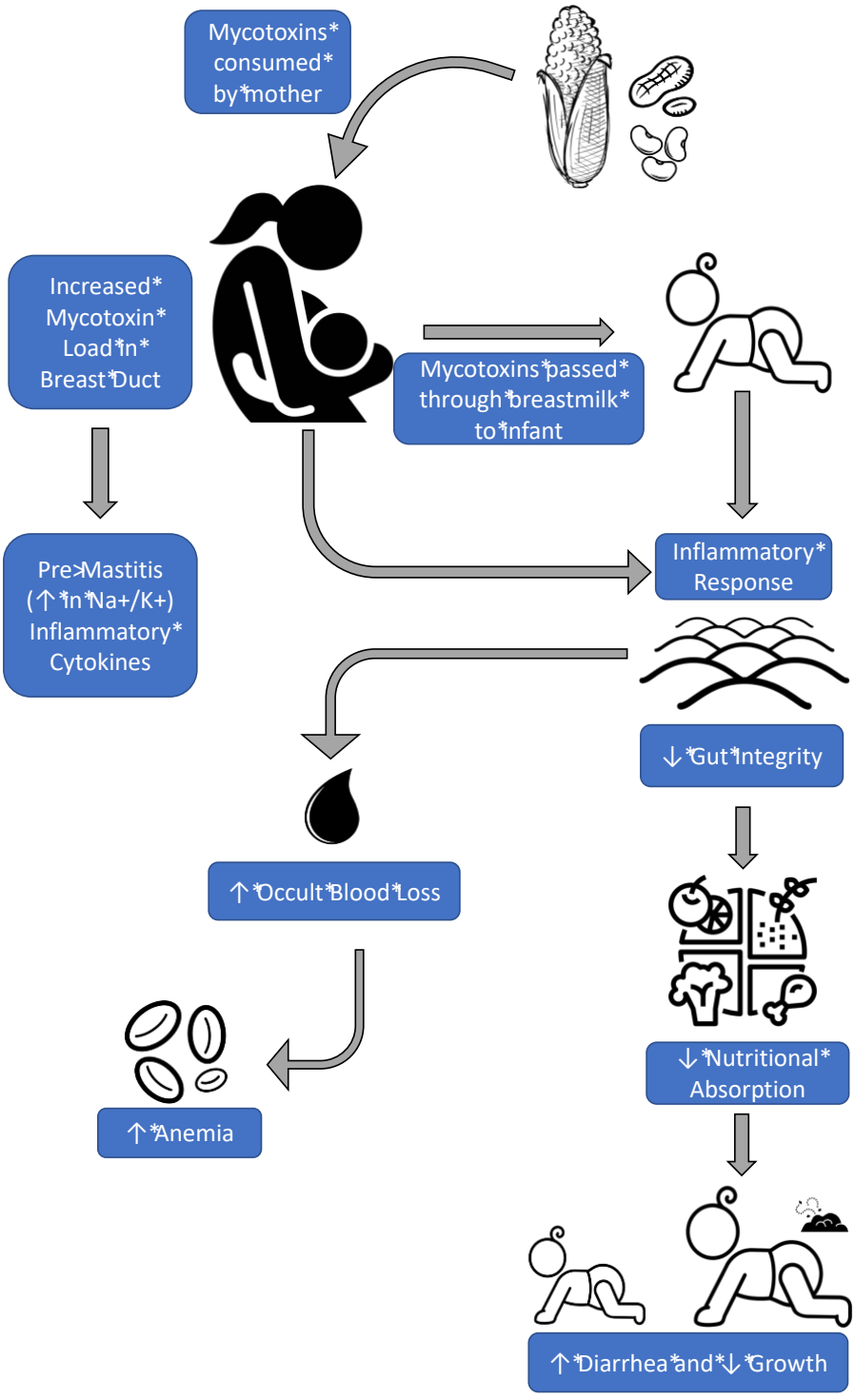


Figure 6: Conceptual framework for mycotoxin exposure leading to infant malnutrition and anemia, as well as maternal mastitis and anemia. Graphics sourced from the Noun Project. Contributing artists include: Denis Sazhin, Tomas Knopp, Icons Producer, Luis Prado, Laymik, Emiliegraphics, Becris, See Link, and Nicolas Vergoz.

3. MANUSCRIPT

Contribution of Student (Required for RSPH Thesis Only; Not Required for Publication):

After obtaining the data, my role in this project included cleaning the data, developing the data analysis plan, performing all data analyses, and writing and preparing the manuscript for publication, including the creation of all figures and tables. The breastmilk analyses and accompanying methods section were provided by Dr. Dana Boyd Barr, Professor, Department of Environmental Health, Rollins School of Public Health.

MANUSCRIPT TITLE: Mycotoxin exposure of infants through the breastmilk of mothers in western Kenya and associations with maternal and child nutrition.

Authors: Stephanie J. Smith[1], Dana Boyd Barr[2], Amy Webb Girard[1]

Affiliations of All Authors:
[1]Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta GA, USA
[2]Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta GA, USA

Corresponding Author:
Stephanie J. Smith
1518 Clifton Rd., Atlanta, GA 30322
412-849-7861
stephanie.j.smith@alumni.emory.edu

Running Title: Mycotoxin exposure through breastmilk and associations

Target Journal: Environmental Health Perspectives

ABSTRACT:

Malnutrition undermines a child's growth and development with significant lifelong consequences. Recent research implicates environmental enteropathy (EE) in the etiology of stunting, with increasing attention turning to mycotoxins. Mycotoxins are carcinogenic toxins released by fungi that infect common staple and legume crops. This study quantifies infant exposure to mycotoxins (aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol) through breastmilk and associations with child malnutrition and maternal mastitis and anemia. Anthropometry, breastmilk, and capillary blood samples were collected at 4- and 9-months postpartum from a cohort of 505 women. Breastmilk samples were analyzed for mycotoxins using liquid-chromatography high resolution mass spectrometry. Subclinical mastitis was evaluated using ion selective electrodes for sodium:potassium contents. Hemoglobin from capillary samples were quantified using the HemoCue Hb 201+. All four mycotoxins were found to be present in 34-90% of the breastmilk samples depending on the mycotoxin and visit. There were no statistically significant associations between sampling season or facility. Aflatoxin M_1 was the only mycotoxin to have levels above the European Union (EU) cutoffs for infant milk replacement formula (0.025 ng/mL). At the 4^{th} and 9^{th} month visit 73.85% and 75.82% of samples, respectively were above the EU cutoffs. Further, 47.93% of samples were elevated at both visits and only 5.44% were below cutoffs at both visits. Regression modeling revealed no statistically significant relationship between elevated aflatoxin levels and child stunting, wasting, underweight, or anemia at 4 or 9 months. There were also no statistically significant associations detected between breastmilk aflatoxin levels and maternal mastitis or anemia. This study found widespread Aflatoxin M_1 contamination of breastmilk in western Kenya. There were no significant associations between aflatoxin levels and indicators of maternal or infant nutrition; however,

longer term exposure and follow up may be necessary to detect impacts on child growth. Given the known negative impacts of mycotoxins on health, mycotoxin control policies and programs are urgently needed in western Kenya that reduce mycotoxin contamination of the food supply.

INTRODUCTION: In 2014, in Kenya, 26% of children under the age of five were stunted, 4% wasted, and 11% underweight.[1] Stunting, wasting, and underweight are all indicators of malnutrition. Malnutrition has many detrimental consequences for a child. Besides restricted growth, malnutrition contributes to intellectual delays, reduced performance in school, and is a strong predictor of mortality for children within their first five years of life.[2] Stunting in childhood also has implications for adulthood, including decreased efficiency, lower salaries, and higher risk of chronic diseases if extreme weight gain occurs later in life.[3] Although the extent to which stunting, wasting, and underweight are caused is not fully understood, it is known that nutritional deficits, along with chronic infection, lack of health care, lack of access to clean and safe water, poor sanitation and hygiene, and environmental factors are major contributors.[4,5,6] Mycotoxins and their effect on environmental enteropathy, characterized by intestinal damage and inflammation preventing proper absorption, are also newly investigated factors on the malnutrition causal pathway.

Maternal nutrition is a significant influencer of a child's nutritional status. Inadequate nutrition and growth begins when a child experiences poor nutrition from conception. The period between conception and two years old, known as the "First 1,000 Days", is the most critical growth and development window in a child's life, and is therefore the best time to build a strong nutritional foundation with the hopes of preventing malnutrition and its consequences in the future.[7] Proper nutrition during this time also helps to promote overall health and development as this is a critical time period for many other systems in the body as well, such as the central nervous system and immune system.[8] In high-, middle-, and low- income countries, the nutritional status of mothers have significant implications for the growth of their babies. A malnourished mother is unable to pass along all of the nutrients that a developing fetus or infant needs, risking altered gene

expression as well as metabolism and body tissue restructuring that will last for the remainder of the child's life, having detrimental consequences. It is under these conditions that stunting, wasting, and underweight can begin, although with developing fetuses and newborns the terms intrauterine growth restriction and low birth weight are more commonly used.[7] Christian *et. al.* reported that about 20% of stunting begins in the womb.[9]

Among the previously mentioned and studied causes of stunting, wasting, and underweight, a newer concept along the causal pathway is environmental enteropathy. Environmental Enteropathy (EE) is characterized by inflammation in the small intestine and the dulling of the intestinal villi.[10,11] The dulling and the inflammation cause the intestine to become more permeable, thus decreasing the small intestine's ability to absorb important minerals and nutrients from food. Hypotheses have been made about the potential correlation between EE and stunting. Previous studies have shown that nutritional interventions struggle to diminish the effects of stunting. They also reveal that while episodes of diarrhea do have an impact on stunting, in between these episodes children experience "catch up" growth, leading researchers to believe there may be a more important pathway yet to be discovered. A pathway that perhaps involves Environmental Enteropathy. Due to the subclinical nature of EE, many individuals, including children, may suffer from the consequences of the intestine's inability to function properly without overt intestinal symptoms.[12] One potential cause of Environmental Enteropathy are mycotoxins.[13]

Mycotoxins are secondary fungal metabolites that cause diseases known as mycotoxicoses after a host is exposed via inhalation, ingestion, or dermal absorption. Mycotoxicoses are most commonly found in the developing world due to poor agricultural practices leading to mycotoxin growth, especially during cultivation and storage.[14,15] First, the climate has major implications on mycotoxin growth. When drought conditions are present, crops, like corn, are put under stress

which can cause cracking in the grain. These little fractures provide the perfect opportunity for mycotoxins to insert themselves into the crops and flourish. Second, insect damage can also increase the susceptibility of crops to mycotoxins. Typically, the more damage to the crop the higher the concentration of mycotoxin present.[15] With greenhouse gas concentrations on the rise, and the average global surface temperatures increasing, researchers believe it is likely that the occurrence of drought in specific regions will also increase.[16] Therefore, it is likely that climate change may increase mycotoxin concerns globally.[17] While these are important factors to address during crop growth, there are also several factors that affect mycotoxin growth during crop storage. Climate factors into storage as the humidity surrounding the crops affects the moisture within the stored crop. Also, how well the crop was dried before storage affects the moisture inside. Moist conditions favor fungi and therefore mycotoxin growth.[17] Proper sorting of the crop before storage can also affect mycotoxin growth as well. When crops are sorted, mycotoxin-infected kernels, nuts, or other commodities can be removed to prevent the spread of mycotoxin throughout the rest of the harvest. Finally, crops can be treated with ammonia to remove mycotoxins. However, this technique is only suitable for crops designated for animal feed.[18] Attention to mycotoxin contamination and its resulting concerns is crucial as the Food and Agricultural Organization (FAO) estimates that about 25% of crops are contaminated with molds, and of that portion, 100% are contaminated with one or more mycotoxins.[19]

Not only can mycotoxicoses bring about symptoms of their own, but they can also cause the host to be susceptible to other microbial diseases, can create a compound effect with other toxins, and can exacerbate the consequences of malnutrition.[14] Despite there being hundreds of different mycotoxins, there are only a handful that are of concern to animal and human health. For the purposes of this paper, only aflatoxins, fumonisins, ochratoxin, and a specific type of

trichothecenes known as deoxynivalenol will be explored in greater detail as these were the mycotoxins of focus for this secondary study.[14]

Of all of the mycotoxins in existence, aflatoxins are arguably the most toxic. Ochratoxin A is a close second, as the most potent ochratoxin.[14] The remaining two, while not the most potent, still have significant toxic effects. Amongst the health concerns related to mycotoxins, of particular interest is the relation between mycotoxins and the health of women and children, especially during prenatal and postnatal periods. Currently, due to their toxicity, aflatoxins are most commonly studied. It is known that aflatoxins can be passed through blood from mother to infant via the umbilical cord, that aflatoxins levels are affected by seasonality, and that aflatoxin levels can affect infant birth weight, as infant exposures in utero are widespread.[20,21] Of critical importance to this study is whether mycotoxins can be passed via breastmilk. A 2017 study out of Turkey, revealed 89.2% of breastmilk samples tested positive for aflatoxins.[22] Other studied mycotoxins include studies surrounding urinary concentrations of deoxynivalenol (DON) and ochratoxin A (OTA) in pregnant women in the UK and Croatia, and associations between fumonisins and frontoethmoidal encephalomeningocele in Guatemala.[23,24,25] Finally, of particular concern is how mycotoxins affect environmental enteropathy and the ability for mothers and children to absorb key minerals and nutrients. Fumonisins, Aflatoxins, and Ochratoxins have particularly detrimental effects in the intestines. FB_1, a type of fumonisin, has been shown to cause the accumulation of sphinganine in intestinal epithelial cells, which in turn causes the inhibition of G_0/G_1 phases of the cell cycle. The accumulation of sphinganine can also change the distribution of glycoproteins within the jejunum. This rearrangement makes it easier for FB_1 to pass across the epithelial cells. Finally, FB_1 stifles protein expression at the tight junctions and disrupts immune function. OTA lessens the amount of glucose absorbed at the SGLT1 transporter. In animal studies, OTA showed similar effects as

FB_1 as it increased intestinal permeability, increased susceptibility to parasite infections, suppressed tight junction protein expression, decreased the height of intestinal villi, and increased epithelial cell apoptosis. Aflatoxins again are very comparable to OTA and FB_1. In vitro tests with colon cells showed that AFB_1 triggered genetic damage, hindered growth of cell, and increased the activity of lactate dehydrogenase. Animal studies displayed an increase in apoptosis, a decrease in intestinal villi height, and damage to the intestinal barrier. Overall, the damage all three mycotoxins cause in the intestines are very similar to each other and support the hypotheses about EE.[13]

Research on mycotoxins in relation to pregnancy and infant development is still greatly lacking.[26] The knowledge currently available relating to the toxicity, carcinogenicity, teratogenicity, and immunotoxicity of mycotoxin exposure for the general population is alarming and ought to continue to spur on action.[15] According to the Centers for Disease Control (CDC), in developing countries, about 4.5 billion people might be chronically exposed to aflatoxins in their diet. Developed countries have many systems in place to monitor their production, processing, and storage of crops to prevent mycotoxin contamination, such as the regulations set by the European Union. Developing countries, on the other hand, are frequently made up of subsistence farmers who often do not have access to appropriate storage facilities or testing materials, and lack as many regulations, therefore increasing the likelihood of mycotoxin growth and exposure.[17] Also, mycotoxins affect the main staple crops (corn, wheat, groundnuts) of these developing nations which also makes exposure to these toxins, in animals and humans, highly likely.

The few studies that have analyzed the potential association between mycotoxin exposure and undernutrition have resulted in significant findings that should continue to inform research today. One such study in Tanzania revealed that infants who were exposed to fumonisin amounts

greater than the provisional maximum tolerable daily intake of 2 μg/kg in corn during complementary feeding were statistically significantly shorter by 1.3 cm.[2] A cross-sectional study by Gong among children 9 months to 5 years in Togo and Benin revealed a significant association between the amount of aflatoxin bound to albumin in the blood and both stunting and wasting.[27] Turner, in studying children 6 to 9 years old in Gambia, revealed that aflatoxin albumin adduct levels were weakly associated with wasting but had a strong statistical association with lower levels of secretory immunoglobulin A (sIgA). SIgA is crucial to maintaining proper mucosal barriers in the digestive tract.[28] In a later study Turner also discovered a statistically significant association between a reduction in aflatoxin bound to albumin and an increase in height and weight for children during their first year of life.[29] Sadeghi, in 2009, published findings revealing a significant association between Iranian infants' height at birth and aflatoxins found in their mothers' breast milk.[30] Khlangwiset details several other studies with evidence for aflatoxins effects on women, infants, and children.[31] While these studies are a great beginning to uncovering the extent to which mycotoxins impact the health of mothers and their children, there is still so much more room for the extension of this research.

With such large numbers of children being affected by malnutrition in the world and Kenya, the causes and implications of a poor nutritional status needs to be bettered studied and understood. This need for continued research led to this secondary analysis. Using data already gathered from mothers in western Kenya for a previous study surrounding vitamin A and orange flesh sweet potatoes, this analysis seeks to evaluate if mycotoxins are passed through breastmilk, and if so, is there a significant association between the presence of mycotoxins and if infants are stunted, wasted, or underweight. Also, as a secondary objective, to examine impacts of mycotoxins on maternal health, including breast health (mastitis) and anemia.

METHODS: This secondary analysis utilizes data collected from the Mama SASHA program implemented in two counties in western Kenya between 2009 and 2013. A quasi-experimental study, Mama SASHA explored the impacts of Orange Flesh Sweet Potato (OFSP) promotion through the health sector on vitamin A status. A cohort of 505 women, recruited from eight health facilities (four intervention, four control), were followed from early pregnancy through 9-10 months postpartum. Breastmilk samples were collected at 4- and 9-months postpartum. Hemoglobin of mothers at 4- and 9-months, and infants at 9-months was determined from capillary blood samples obtained by finger/heel-pricks using HemoCue Hb 201+. Hemoglobin concentrations were adjusted for altidude.[32] Detailed descriptions on previously studied portions of the program design, activities, and monitoring and evaluation strategy can be referenced in Webb Girard *et. al.* and Cole *et. al.*[33,34] Below are the methods of interest for this study.

Data Collection: For breastmilk collection, research assistants set up a private, low light area within the research office for mothers to use and collection occurred at 4- and 9-months postpartum. The research assistant explained the expression technique to mothers using picture cards and mothers expressed into a sterile open top container. Once mothers were finished, the open top containers were placed into a dark box to preserve retinol. The research assistants homogenized the milk by gently swirling the milk while keeping the container in the dark box. The milk was then transferred in 3mL aliquots into three amber cryovials and stored in a solar powered freezer at -20 °C. Capillary blood samples were drawn by research assistants using purple top microtainer capillary blood collector (Becton Dickinson) with EDTA and single-use sterile micro-lancets (Becton Dickinson). Within three minutes of the blood draw, hemoglobin levels were tested using HemoCue Hb 201+.

Sample Storage: Breastmilk samples were temporarily stored on site using solar freezers and kept at -20 °C until transport. About a week or two after collection, breastmilk samples were transferred on dry ice to Egerton University for storage at -80 °C until transport to Emory University for analysis.[35] Samples were shipped by air on dry ice to Emory University and stored at -80 °C. Samples were thawed and homogenized prior to analysis.

Breastmilk Analysis: Sodium and potassium concentrations were determined using standard ion selective electrodes. Breast milk samples were analyzed for mycotoxins using a modified version of the Sørensen and Elbæk method (2005).[36] Briefly, 2.0 ml milk was incubated overnight with 100 µl β-glucuronidase solution at 37 °C to liberate glucuronide-bound conjugates. The hydrolysate was acidified to pH 2 with sulfuric acid and mixed. A liquid-liquid extraction was performed twice (10 mL; 1:1.5 hexane:acetonitrile) and the hexane layer was removed completely and discarded. The acetonitrile layer was taken to near dryness and 5 mL water was added. The pH of the extract/water solution was taken to 8 using sodium hydroxide. The basic solution was passed through a preconditioned OASIS solid phase extraction column, washed with water, and then the cartridge was dried. The mycotoxins were eluted with 4 mL methanol and the eluate was evaporated to dryness. The residue was redissolved in 500 µl mobile phase (20% methanol in water). The reconstituted extracts were analyzed using liquid-chromatography-high resolution mass spectrometry. Data were collected on an OrbiTrap using data dependent acquisition which collects data on both molecular and fragment (product) ions giving selectivity similar to MS/MS. Calibrants and quality control materials (10%, blanks and positive controls) were analyzed concurrently with samples. Limits of quantification were 2 pg/mL (Aflatoxin M1), 1 pg/mL (Ochratoxin A), 10 pg/mL (Deoxynivalenol), and 30 pg/mL (Fumonisin B1).

Variable Specification: The European Union cutoffs, identified in Warth (2016) were used to create categorical variables for elevated mycotoxin levels: AFM_1 (0.025 ng/mL), OTA (0.5 ng/mL), FB_1 (200 ng/mL), and DON (200 ng/mL).[37] Mastitis was categorized as follows per Arsenault: no mastitis (Na:K < 0.6), moderate mastitis ($0.6 \leq$ Na:K ≤ 1.0), and severe mastitis (Na:K > 1.0).[38] Any levels of Na:K greater than 0.6 were coded as subclinical mastitis. Stunting, wasting and underweight were set at z scores < -2 SD.[39] Hemoglobin was adjusted for altitude using the method described by Nestel; Mothers and infants were considered anemic if altitude adjusted hemoglobin concentrations were < 12 g/dL and 11g/dL, respectively.[32]

Statistical Analysis: First distributions of and correlations among AFM_1, FB_1, OTAA, DON, Na:K ratios, altitude adjusted hemoglobin, HAZ, WAZ and WHZ were examined using SAS 9.4. Distributions of mycotoxin concentrations and Na:K data were explored by visit number, facility, and by month. Socio-demographic characteristics of mothers were analyzed overall and by aflatoxin cutoffs. A bias analysis was performed to assess differences between mothers included in analyses versus those excluded due to missing mycotoxin data. Logistic regression was used to analyze associations between mean mycotoxin levels and intervention/control facilities and seasons. To assess associations between mycotoxins and child nutrition, associations between aflatoxin levels, child HAZ, WHZ, WAZ scores, and altitude adjusted hemoglobin were examined using linear regression analysis. Unadjusted associations between elevated mycotoxin levels, stunting, wasting, underweight, and anemia were assessed using logistic regression. To examine associations between maternal health and mycotoxin levels, the associations between mycotoxin levels, Na/K ratios, and altitude adjusted hemoglobin were examined using linear regression analysis and associations between elevated mycotoxin levels, mastitis and anemia using logistic regression.

RESULTS: A total of 505 mothers were enrolled in the Mama SASHA cohort study. There were 5 mothers removed from the 4-month analysis due to duplication leaving 500 mothers. Of those mothers, 371 provided breastmilk samples at 4-months postpartum and 364 at 9-months postpartum and were included in the mycotoxin analysis. Table 1 displays characteristics of these included mothers. The only significant difference at the 95% confidence level resulted between the education of mothers at the 9-month visit. The bias analysis (Supplemental Table 1) between the mothers included and those excluded revealed statistically significant differences at the 95% confidence level among mothers' interview month during the 4-month visit, and food insecurity score at enrollment and occupation of the head of household at the 9-month visit. The percentage of samples with the four mycotoxins present in the samples ranged from about 37-90% at the 4-month visit and 34-86% at the 9-month visit (Table 2). The mycotoxin present in the highest percentage of samples at the 4- and 9-month visit was Fumonisin B_1. Table 2 displays the mean amount of each mycotoxin present in samples at both visits, as well as the percentage of samples above the EU cutoffs. Aflatoxin M_1 was the only mycotoxin to have levels above EU cutoffs at both visits. At the 4-month visit 73.85% of samples and 75.82% of samples during the 9-month follow-up were above cutoffs. Also, 47.93% of samples were above cutoffs at both visits, and only 5.44% below at both visits. There was no influence of season or site on mycotoxin concentrations (Supplemental Tables 2 and 3). Linear and logistic regression modeling revealed no statistically significant relationship between aflatoxin levels above cutoffs in breastmilk samples and stunting, wasting, underweight, or anemia in infants at 4 or 9 months (Table 3). Linear and logistic regression also revealed no statistically significant relationship between breastmilk aflatoxin levels and mastitis or maternal anemia (Table 3). There was a statistically significant difference between the mean WHZ and mean WAZ of infants chronically exposed to AFM_1 compared to those who

were not chronically exposed at the 4-month follow-up visit (Table 4). Also, mothers who were chronically exposed to AFM_1 were 1.82 times more likely to suffer from subclinical mastitis than mothers who were not chronically exposed at 4-months postpartum (Table 4). There were no statistically significant differences between mothers chronically exposed to AFM_1 compared to those who were not for stunting and anemia in infants, or for mastitis and anemia in mothers at 4-months follow-up. There were also no statistically significant relationships for stunting, wasting, underweight, and anemia in infants of mothers who were chronically exposed compared to those who were not at 9-months follow-up. There were also no statistically significant differences in maternal anemia and mastitis for mothers chronically exposed compared to those who were not.

DISCUSSION: The results of these analyses reveal that aflatoxin contamination of breastmilk is widespread in the study region and that infants are chronically exposed to aflatoxin levels well above the European Union cutoffs in their first year of life. Ochratoxin A, deoxynivalenol, and fumonisin on the other hand, while present in breastmilk, were below cutoffs. Chronic exposure to elevated levels of aflatoxin were associated with mean WHZ and WAZ in infants, and subclinical mastitis in mothers at 4-months follow-up.

This study contributes to documenting the variation of mycotoxin exposure across different contexts. Table 3 reveals the percentages of breastmilk samples with each specified mycotoxin present at the 4- and 9-month visit. Munoz's 2014 study describes 20 studies reporting on the presence of Ochratoxin A (OTA) in breastmilk in a host of European and Non-European countries. The review noted that the proportion of breastmilk samples contaminated with OTA ranged from 2 to 100% .[46] The results for the percentages of ochratoxin A present in breastmilk samples in this study were very similar to amounts listed by Munoz (2014) for studies in Norway, Poland,

Slovakia, Egypt, and Sierra Leone.[46] As for fumonisins, Magoha (2014) reported 44% of 131 samples tested positive for FB_1, and 10% of the samples were above the European Union cutoffs.[47] This study discovered 90% and 86% of mothers' breastmilk tested positive for FB_1, compared to Magoha's study. No breastmilk samples from this study though were above the cutoffs specified by the European Union for fumonisins unlike Mogoha's study.[47] Aflatoxin M_1 in particular, the mycotoxin of focus for further analyses in this study, similarly varies greatly in mothers' breastmilk around the globe. In 1984, Coulter reported that of 99 breastmilk samples from Sudanese mothers, 13% contained aflatoxin M_1.[40] Wild's study in 1987 revealed aflatoxins present in 0% of breastmilk samples (n=42) from France and 11% of breastmilk samples (n=54) from Zimbabwe.[41] Maxwell reported in 1989 that 28% of breastmilk samples from Kenyan mothers, 34% of Ghanaian samples, and 37% of Sudanese samples were positive for aflatoxins.[42] More recently, in 2013, Adejumo reported 82% of breastmilk samples from Nigerian mothers were positive for aflatoxin M_1, and that there was a correlation between the amount of aflatoxin B_1 consumed in the diet and the amount of aflatoxin M_1 present in breastmilk.[43] A study out of Iran in 2017 by Jafari revealed only 2.5% of urban breastmilk samples from Iranian mothers contained AFM_1, while 38.9% rural samples tested positive.[44] Finally, Elaridi's work among lactating mothers in Lebanon revealed that of 111 samples, 93.8% of them were positive for aflatoxins.[45] This study notably found a significant proportion of mothers with concentrations of aflatoxin M_1 above the European Union's cutoff for infant milk replacement formula. This study is also the first to report presence or concentrations of deoxynivalenol in breastmilk of humans and indicated 62-66% of samples were contaminated, though none above EU cutoffs.

Aflatoxin M_1 was chosen for further analyses since such a high proportion of breastmilk samples were above cutoffs. At 4 and 9 months post-partum more than ¾ of samples exceeded the

European Union cutoff and this did not differ significantly by season. In contrast, in a survey of 50 Nigerian mothers, only 16% of samples had aflatoxin levels above the European Union cutoffs.[43] In Lebanon, despite 93.8% of samples testing positive for aflatoxins, none of the samples exceeded European Union cutoffs.[45]

In analyzing infant exposure to aflatoxin, previous studies have measured both aflatoxins in breastmilk and serum-aflatoxin albumin. Studies that have then gone on to analyze associations between aflatoxin concentrations and child nutritional status mainly used serum-aflatoxin albumin to measure exposure and, in the aggregate, findings are inconclusive. In Ghana, being in the top quartile for aflatoxin concentrations in maternal blood collected at delivery was associated with low birthweight but not pre-term birth or small-for-gestational-age babies.[48] In the Gambia, aflatoxin-albumin adduct levels in mothers prior to birth were associated with lower WAZ and HAZ in infants at 1 year after birth. However, aflatoxin concentrations in infant cord blood were not associated with either WAZ or HAZ.[29] In contrast, a study in Togo and Benin by Gong *et. al.* (2002) found a significant association between the amount of aflatoxin bound to albumin in the blood of children 9 months to 5 years of age and both HAZ and WAZ. Since the study also studied children post weaning, it determined that children who were breastfed had lower aflatoxin exposures compared to those eating solid foods.[27] This study is the first to examine breastmilk aflatoxin exposure and child nutritional status and found that chronic exposure was associated with WAZ and WHZ in infants at 4-months follow-up. It is possible that the negative consequences of mycotoxins on child growth require more time to manifest and thus explaining why HAZ was not significant. It is also possible that health promoting constituents in breastmilk mitigated the effects of aflatoxin on growth perhaps via gut integrity promoting properties or buffering the toxin until transformation in the liver to a less toxic state.[50]

In this study, relationships between aflatoxin M_1 in breastmilk and maternal anemia were explored. Mycotoxins may contribute to anemia due to the effect they have on intestinal health. Mycotoxin exposure leads to inflammation and dulling of intestinal villi preventing proper absorption and function, thus leading to a potential increase in occult blood loss and thus anemia.[13] A previous study by Shuaib (2010) in Ghana, found a statistically significant association between aflatoxin-albumin and anemia status.[49] In this study, breastmilk aflatoxin concentrations served as an indicator of maternal aflatoxin exposure. Opposite to the findings by Shuaib, this study found no statistically significant relationship between AFM1 in breastmilk and maternal anemia.

Associations between mycotoxins and mastitis have been reported previously in animals. Research in the dairy cows reveal that exposure to mycotoxins decrease milk production, as well as increases mastitis risks.[51,52] Similarly, mastitis is known to contribute to increased concentrations of immune factors such as IL-8, an inflammatory cytokine, in breastmilk in humans, and lead to sub-optimal growth in infants.[53-56] This is the first study in humans to explore mycotoxin exposure and mastitis. During the 4-month follow-up visits, mothers who were chronically exposed to aflatoxins were 1.82 times more likely to suffer from subclinical mastitis than mothers who were not chronically exposed. Subclinical mastitis was measured as having a Na:K > 0.6.

Limitation of the breastmilk analysis include that although a highly selective mass spectrometer was used to collect data, it is possible that other co-extracted analytes may have had the same exact mass resulting in overestimation of mycotoxin levels in the milk. Also, no reference material was available against which to calibrate the analytic measurement method although all means of method validation possible were conducted in accordance with most biomonitoring methods. As for the study design and statistical analysis, there was children were only able to be

followed up until 9 months postpartum. It is plausible that mycotoxin impacts on growth require time to accumulate and may not be visible until later in the 1000-day window; thus, associations may be underestimated. That said, this study is one of the few longitudinal studies exploring chronicity of exposure in the first year and findings suggest that exposure is indeed, chronic and substantial. Follow up of infants into their second and third year of life is merited. Second, since AFM_1 is derived from AFB_1, the aflatoxin actually consumed in contaminated food, measurement of AFM_1 is not a direct measurement of consumption, rather it is an indicator of exposure. As a result, AFM_1 may underestimate AFB_1 consumption as it takes 12-24 hours for AFB_1 to appear as AFM_1 in breastmilk after conversion.[44] Because no associations were significant in unadjusted models for the aflatoxin cutoff analysis, estimating effects from models adjusted for potentially influential covariates were not pursued. Also, time constraints did not permit for significant findings from the chronic exposure regression analysis to be modeled more in-depth including potential influential covariates. Despite the limitations, this study also presents many strengths. First, the number of samples of breastmilk were relatively large compared to previous studies. Second, this study also examined the presence of a mycotoxin, deoxynivalenol, never measured before in human breastmilk. It also examined several new relationships between aflatoxin exposure and infant and maternal health.

In the future, the knowledge gained from this study can be used to design a study solely focused on mycotoxin analysis, perhaps measuring mycotoxin albumin levels instead as it is a better measure of dietary intake. Also, it would be interesting to explore more thoroughly, perhaps through a longitudinal study, the differences in exposure during gestation, during the breastfeeding period, the weaning period, and post-weaning period. Since there is such little research

surrounding the association between mycotoxin exposure and a child's nutritional status, and since the research that does exist is conflicting, this topic needs to continue to be explored.

CONCLUSION: The analyses described here surrounding mycotoxin exposure in infants through the breastmilk of mothers in western Kenya provided a comprehensive understanding of the presence of and the mean levels of the tested mycotoxins (aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol) in breastmilk. Comparison of these results to previous studies revealed both similarities and differences. Aflatoxin contamination is widespread among countries around the world and within the study region of western Kenya. While all studied mycotoxins were found to be present in breastmilk samples, only aflatoxin levels were above European Union cutoffs for infant milk replacement formula. Analysis of all four mycotoxins based on season and on control vs. intervention site revealed no statistically significant differences. Finally, the more in-depth analyses with aflatoxins revealed no statistically significant associations between aflatoxin levels above cutoffs and infant malnutrition and anemia, nor with maternal anemia and mastitis at 4- and 9-month visits. It did though reveal statistically significant associations between chronic aflatoxin exposure and WAZ and WHZ in infants, and maternal subclinical mastitis during the 4-month follow-up.

REFERENCES:

1. Statistics, K. N. B. of, Health/Kenya, M. of, Council/Kenya, N. A. C., Institute, K. M. R., & Development/Kenya, N. C. for P. and. (2015). Kenya Demographic and Health Survey 2014. Retrieved from http://dhsprogram.com/publications/publication-FR308-DHS-Final-Reports.cfm
2. Etzel RA. Reducing malnutrition: time to consider potential links between stunting and mycotoxin exposure? Pediatrics. 2014 Jul;134(1):4-6. doi: 10.1542/peds.2014-0827. Epub 2014 Jun 2.
3. WHO | Stunting in a nutshell. (n.d.). Retrieved March 12, 2018, from http://www.who.int/nutrition/healthygrowthproj_stunted_videos/en/
4. WHO | Description. (n.d.). Retrieved March 13, 2018, from http://www.who.int/nutgrowthdb/about/introduction/en/index2.html
5. WHO | Global Nutrition Targets 2025: Wasting policy brief. (n.d.). Retrieved March 13, 2018, from http://www.who.int/nutrition/publications/globaltargets2025_policybrief_wasting/en/
6. Prendergast AJ, Rukobo S, Chasekwa B, Mutasa K, Ntozini R, Mbuya MN, Jones A, Moulton LH, Stoltzfus RJ, Humphrey JH. Stunting is characterized by chronic inflammation in Zimbabwean infants. PLoS One. 2014 Feb 18;9(2):e86928. doi: 10.1371/journal.pone.0086928.
7. Wrottesley, S. V., Lamper, C., & Pisa, P. T. (2016). Review of the importance of nutrition during the first 1000 days: maternal nutritional status and its associations with fetal growth and birth, neonatal and infant outcomes among African women. *Journal of Developmental Origins of Health and Disease*, *7*(2), 144–162. https://doi.org/10.1017/S2040174415001439
8. Martorell, R. (2017). Improved Nutrition in the First 1000 Days and Adult Human Capital and Health. *American Journal of Human Biology : The Official Journal of the Human Biology Council*, *29*(2). https://doi.org/10.1002/ajhb.22952
9. Prendergast, A. J., & Humphrey, J. H. (2014). The stunting syndrome in developing countries. *Paediatrics and International Child Health*, *34*(4), 250–265. https://doi.org/10.1179/2046905514Y.0000000158
10. Prendergast, A. J., & Humphrey, J. H. (2014). The stunting syndrome in developing countries. *Paediatrics and International Child Health*, *34*(4), 250–265. https://doi.org/10.1179/2046905514Y.0000000158
11. Korpe, P. S., & Petri, W. A. (2012). Environmental Enteropathy: Critical implications of a poorly understood condition. *Trends in Molecular Medicine*, *18*(6), 328–336. https://doi.org/10.1016/j.molmed.2012.04.007
12. Prendergast A, Kelly P. Enteropathies in the developing world: neglected effects on global health. Am J Trop Med Hyg. 2012 May;86(5):756-63. doi: 10.4269/ajtmh.2012.11-0743.
13. Liew, W.-P.-P., & Mohd-Redzwan, S. (2018). Mycotoxin: Its Impact on Gut Health and Microbiota. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00060
14. Bennett, J.W. and Klich, M. (2003). Mycotoxins. *American Society for Microbiology*, *16*, *3*, 497-516. DOI: 10.1128/CMR.16.3.497-516.2003
15. Niu, Guodong. (2010). *Toxicity of mycotoxins to insects and underlying molecular and biochemical mechanisms* (Doctoral dissertation). Retrieved from ResearchGate. (46090626)

16. National Drought Mitigation Center. (n.d.). *Climate Change*. Retrieved January 13, 2018, from http://drought.unl.edu/DroughtBasics/ClimateChange.aspx
17. Schmidt, C. W. (2013). Breaking the Mold: New Strategies for Fighting Aflatoxins. *Environmental Health Perspectives*, *121*(9), A270–A275. https://doi.org/10.1289/ehp.121-a270
18. Bhat, R. V., & Miller, J. D. (n.d.). Mycotoxins and food supply. Retrieved January 13, 2018, from http://www.fao.org/docrep/U3550t/u3550t0e.htm
19. De Saeger, S., Audenaert, K., & Croubels, S. (2016). Report from the 5th International Symposium on Mycotoxins and Toxigenic Moulds: Challenges and Perspectives (MYTOX) Held in Ghent, Belgium, May 2016. *Toxins*, *8*(5). https://doi.org/10.3390/toxins8050146
20. De Vries, H.R., Maxwell, S.M., & Hendrickse, R.G.. (1989). Feotel and Neonatal Exposure to Aflatoxins. *Acta Paediatrica Scandinavica* 78: 373-378.
21. Smith, L. E., Prendergast, A. J., Turner, P. C., Humphrey, J. H., & Stoltzfus, R. J. (2017). Aflatoxin Exposure During Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. *The American Journal of Tropical Medicine and Hygiene*, *96*(4), 770–776. https://doi.org/10.4269/ajtmh.16-0730
22. Altun, S. K., Gürbüz, S., & Ayağ, E. (2017). Aflatoxin M_1 in human breast milk in southeastern Turkey. *Mycotoxin Research*, *33*(2), 103–107. https://doi.org/10.1007/s12550-016-0268-4
23. Klapec, T., Šarkanj, B., Banjari, I., & Strelec, I. (2012). Urinary ochratoxin A and ochratoxin alpha in pregnant women. *Food and Chemical Toxicology*, *50*(12), 4487–4492. https://doi.org/10.1016/j.fct.2012.09.030
24. Wells, L., Hardie, L., Williams, C., White, K., Liu, Y., De Santis, B., … Sathyapalan, T. (2016). Determination of Deoxynivalenol in the Urine of Pregnant Women in the UK. *Toxins*, *8*(11). https://doi.org/10.3390/toxins8110306
25. Marshall, A.-L., Venuti, D. J., & Eastman, D. J. (2017). Fumonisin Exposure in Guatemalan Women of Child-Bearing Age: A Potential Link to the Observed High Incidence of Frontoethmoidal Encephalocele. *Annals of Global Health*, *83*(1), 9. https://doi.org/10.1016/j.aogh.2017.03.018
26. Steyn, P. S. (1995). Mycotoxins, general view, chemistry and structure. *Toxicology Letters*, *82–83*(Supplement C), 843–851. https://doi.org/10.1016/0378-4274(95)03525-7
27. Gong, Y. Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P. C., Hall, A. J., & Wild, C. P. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *BMJ : British Medical Journal*, *325*(7354), 20–21.
28. Turner, P. C., Moore, S. E., Hall, A. J., Prentice, A. M., & Wild, C. P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*, *111*(2), 217–220.
29. Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y., Hall, A. J., Prentice, A. M., & Wild, C. P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, *36*(5), 1119–1125. https://doi.org/10.1093/ije/dym122
30. Sadeghi, N., Oveisi, M. R., Jannat, B., Hajimahmoodi, M., Bonyani, H., & Jannat, F. (2009). Incidence of aflatoxin M1 in human breast milk in Tehran, Iran. *Food Control*, *20*(1), 75–78. https://doi.org/10.1016/j.foodcont.2008.02.005

31. Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment: A review. *Critical Reviews in Toxicology*, *41*(9), 740–755. https://doi.org/10.3109/10408444.2011.575766
32. Nestel P (2002) Adjusting Hemoglobin Values in Program Surveys. Washington, DC: INACG.
33. Girard, A. W., Grant, F., Watkinson, M., Okuku, H. S., Wanjala, R., Cole, D., … Low, J. (2017). Promotion of Orange-Fleshed Sweet Potato Increased Vitamin A Intakes and Reduced the Odds of Low Retinol-Binding Protein among Postpartum Kenyan Women123. *The Journal of Nutrition*, *147*(5), 955–963. https://doi.org/10.3945/jn.116.236406
34. Cole, D. C., Levin, C., Loechl, C., Thiele, G., Grant, F., Girard, A. W., … Low, J. (2016). Planning an integrated agriculture and health program and designing its evaluation: Experience from Western Kenya. *Evaluation and Program Planning*, *56*, 11–22. https://doi.org/10.1016/j.evalprogplan.2016.03.001
35. Webb-Girard, A., Grant, F, Wanjala, R, Okuku, HS, Deneen, M, Kowalski, A. (2015). *Final Report to the International Potato Center and the Bill and Melinda Gates Foundation: Cohort study of the impact of an integrated agriculture, nutrition and health intervention on the Vitamin A and health status of mothers and their infants from pregnancy through 9 months postpartum: The Mama SASHA COVA study*. (Unpublished)
36. Sørensen LK, Elbaek TH. Determination of mycotoxins in bovine milk by liquid chromatography tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2005 Jun 25;820(2):183-96. Epub 2005 Apr 26. PubMed PMID: 15899372.
37. Warth, B., Braun, D., Ezekiel, C. N., Turner, P. C., Degen, G. H., & Marko, D. (2016). Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. *Chemical Research in Toxicology*, *29*(7), 1087–1097. https://doi.org/10.1021/acs.chemrestox.6b00125
38. Arsenault, J. E., Aboud, S., Manji, K. P., Fawzi, W. W., & Villamor, E. (2010). Vitamin Supplementation Increases Risk of Subclinical Mastitis in HIV-Infected Women. *The Journal of Nutrition*, *140*(10), 1788–1792. https://doi.org/10.3945/jn.110.122713
39. WHO | Description | Cutoff Points and Summary Statistics. (n.d.). Retrieved March 17, 2018, from http://www.who.int/nutgrowthdb/about/introduction/en/index5.html
40. Coulter, J. B., Lamplugh, S. M., Suliman, G. I., Omer, M. I., & Hendrickse, R. G. (1984). Aflatoxins in human breast milk. *Annals of Tropical Paediatrics*, *4*(2), 61–66.
41. Wild Christopher P., Pionneau Florence A., Montesano Ruggero, Mutiro Crispen F., & Chetsanga Christopher J. (2006). Aflatoxin detected in human breast milk by immunoassay. *International Journal of Cancer*, *40*(3), 328–333. https://doi.org/10.1002/ijc.2910400308
42. Maxwell, S. M., Apeagyei, F., Vries, H. R. D., Mwanmut, D. D., & Hendrickse, R. G. (1989). Aflatoxins in Breast Milk, Neonatal Cord Blood and Sera of Pregnant Women. *Journal of Toxicology: Toxin Reviews*, *8*(1–2), 19–29. https://doi.org/10.3109/15569548909059735
43. Adejumo, O., Atanda, O., Raiola, A., Somorin, Y., Bandyopadhyay, R., & Ritieni, A. (2013). Correlation between aflatoxin M1 content of breast milk, dietary exposure to aflatoxin B1 and socioeconomic status of lactating mothers in Ogun State, Nigeria. *Food and Chemical Toxicology*, *56*, 171–177. https://doi.org/10.1016/j.fct.2013.02.027
44. Tina Jafari, Aziz A. Fallah, Soleiman Kheiri, Abdolmajid Fadaei &

Sayed Asadollah Amini (2017) Aflatoxin M1 in human breast milk in Shahrekord, Iran and association with dietary factors, Food Additives & Contaminants: Part B, 10:2, 128-136, DOI: 10.1080/19393210.2017.1282545

45. Elaridi, J., Bassil, M., Kharma, J. A., Daou, F., & Hassan, H. F. (2017). Analysis of Aflatoxin M^sub 1^ in Breast Milk and Its Association with Nutritional and Socioeconomic Status of Lactating Mothers in Lebanon. *Journal of Food Protection; Des Moines*, *80*(10), 1737–1741. http://dx.doi.org.proxy.library.emory.edu/10.4315/0362-028X.JFP-17-083
46. Muñoz, K., Blaszkewicz, M., Campos, V., Vega, M., & Degen, G. H. (2014). Exposure of infants to ochratoxin A with breast milk. *Archives of Toxicology*, *88*(3), 837–846. https://doi.org/10.1007/s00204-013-1168-4
47. Magoha, H., De Meulenaer, B., Kimanya, M., Hipolite, D., Lachat, C., & Kolsteren, P. (2014). Fumonisin B1 contamination in breast milk and its exposure in infants under 6 months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology*, *74*, 112–116. https://doi.org/10.1016/j.fct.2014.09.008
48. Shuaib Faisal M. B., Jolly Pauline E., Ehiri John E., Yatich Nelly, Jiang Yi, Funkhouser Ellen, … Williams Jonathan H. (2010). Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana. *Tropical Medicine & International Health*, *15*(2), 160–167. https://doi.org/10.1111/j.1365-3156.2009.02435.x
49. Shuaib, F. M. B., Jolly, P. E., Ehiri, J. E., Jiang, Y., Ellis, W. O., Stiles, J. K., … Williams, J. H. (2010). Association between Anemia and Aflatoxin B1 Biomarker Levels among Pregnant Women in Kumasi, Ghana. *The American Journal of Tropical Medicine and Hygiene*, *83*(5), 1077–1083. https://doi.org/10.4269/ajtmh.2010.09-0772
50. Walker, W. A., & Iyengar, R. S. (2014). Breast milk, microbiota, and intestinal immune homeostasis. *Pediatric Research*, *77*(1–2), 220–228. https://doi.org/10.1038/pr.2014.160
51. Fink-Gremmels, J. (2008). The role of mycotoxins in the health and performance of dairy cows. *The Veterinary Journal*, *176*(1), 84–92. https://doi.org/10.1016/j.tvjl.2007.12.034
52. Brown, R. W., Pier, A. C., Richard, J. L., & Krogstad, R. E. (1981). Effects of dietary aflatoxin on existing bacterial intramammary infections of dairy cows. *American Journal of Veterinary Research*, *42*(6), 927–933.
53. Filteau, S. M., Lietz, G., Mulokozi, G., Bilotta, S., Henry, C. J. K., & Tomkins, A. M. (1999). Milk cytokines and subclinical breast inflammation in Tanzanian women: effects of dietary red palm oil or sunflower oil supplementation. *Immunology*, *97*(4), 595–600. https://doi.org/10.1046/j.1365-2567.1999.00834.x
54. Filteau, S. M., Rice, A. L., Ball, J. J., Chakraborty, J., Stoltzfus, R., de Francisco, A., & Willumsen, J. F. (1999). Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or β-carotene. *The American Journal of Clinical Nutrition*, *69*(5), 953–958. https://doi.org/10.1093/ajcn/69.5.953
55. Willumsen, J. F., Filteau, S. M., Coutsoudis, A., Newell, M., Rollins, N. C., Coovadia, H. M., & Tomkins, A. M. (2003). Breastmilk Rna viral load in Hiv-infected South African women: effects of subclinical mastitis and infant feeding. *Aids*, *17*(3), 407–414.
56. Gomo, E., Filteau, S. M., Tomkins, A. M., Ndhlovu, P., Michaelsen, K. F., & Friis, H. (2003). Subclinical mastitis among HIV-infected and uninfected Zimbabwean women participating in a multimicronutrient supplementation trial. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *97*(2), 212–216.

TABLES:

Table 1: Characteristics of mothers from western Kenya included in mycotoxin analysis. Data are presented stratified by visit and by aflatoxin cutoffs.

	4-month visit				9-month visit			
	All	Above AFM_1 Cutoff	Below AFM_1 Cutoff	p-value	All	Above AFM_1 Cutoff	Below AFM_1 Cutoff	p-value
Intervention vs. Control Facilities, n (%)								
Intervention	181 (48.79)	132 (72.93)	49 (27.07)	0.69	182 (50.00)	139 (76.37)	43 (23.63)	0.81
Control	190 (51.21)	142 (74.74)	48 (25.26)		182 (50.00)	137 (75.27)	45 (24.73)	
Mother's Age at Enrollment in Years, mean (SD)	24.45 (5.46)	24.51 (5.59)	24.28 (5.08)	0.72	24.47 (5.48)	24.24 (5.33)	25.20 (5.88)	0.15
Mother's Age at Enrollment in Quartiles, n (%)								
17-20	113 (30.46)	85 (75.22)	28 (24.78)	0.98	115 (31.59)	92 (80.00)	23 (20.00)	0.39
21-23	76 (20.49)	55 (72.37)	21 (27.63)		67 (18.41)	49 (73.13)	18 (26.87)	
24-28	106 (28.57)	78 (73.58)	28 (26.42)		106 (29.12)	82 (77.36)	24 (22.64)	
28-44	76 (20.49)	56 (73.68)	20 (26.32)		76 (20.88)	53 (69.74)	23 (30.26)	
Mother's Education – Completed Primary School or Higher, n (%)								
Yes	257 (69.27)	187 (72.76)	70 (27.24)	0.47	251 (68.96)	181 (72.11)	70 (27.89)	0.01
No	114 (30.73)	87 (76.32)	27 (23.68)		113 (31.04)	95 (84.07)	18 (15.93)	
Occupation of Head of Household, n (%)								
Doesn't Work	42 (11.35)	31 (73.81)	11 (26.19)	0.87	39 (10.74)	27 (69.23)	12 (30.77)	0.71
Agriculture	73 (19.73)	57 (78.08)	16 (21.92)		65 (17.91)	49 (75.38)	16 (24.62)	

Salaried Employee	59 (15.95)	44 (74.58)	15 (25.42)		58 (15.98)	47 (81.03)	11 (18.97)	
Casual Labor	88 (23.78)	62 (70.45)	26 (29.55)		91 (25.07)	71 (78.02)	20 (21.98)	
Other	108 (29.19)	79 (73.15)	29 (26.85)		110 (30.30)	82 (74.55)	28 (25.45)	
Wealth Tertile, n (%)								
First	118 (31.89)	84 (71.19)	34 (28.81)	0.69	120 (33.15)	88 (73.33)	32 (26.67)	0.30
Second	126 (34.05)	95 (75.40)	31 (24.60)		119 (32.87)	96 (80.67)	23 (19.33)	
Third	126 (34.05)	95 (75.40)	31 (24.60)		123 (33.98)	90 (73.17)	33 (26.83)	
Food Insecurity Enrollment, n (%)								
Mild or None	200 (55.56)	152 (76.00)	48 (24.00)	0.19	189 (53.39)	147 (77.78)	42 (22.22)	0.53
Moderate	78 (21.67)	59 (75.64)	19 (24.36)		84 (23.73)	60 (71.43)	24 (28.57)	
Severe	82 (22.78)	54 (65.85)	28 (34.15)		81 (22.88)	61 (75.31)	20 (24.69)	
Food Insecurity Visit 4, n (%)								
Mild	---	---	---	---	222 (60.99)	164 (73.87)	58 (26.13)	0.51
Moderate	---	---	---	---	85 (23.35)	68 (80.00)	17 (20.00)	
Severe	---	---	---	---	57 (15.66)	44 (77.19)	13 (22.81)	
Interview Month, n (%)								
Dry Season Jan-Feb & June-Sept	278 (74.93)	204 (73.38)	74 (26.62)	0.68	151 (41.48)	116 (76.82)	35 (23.18)	0.93
Rainy Season (Long) March-May	2 (0.54)	2 (100.00)	0 (0.00)		80 (21.98)	60 (75.00)	20 (25.00)	

Rainy Season (Short) Oct-Dec	91 (24.53)	68 (74.73)	23 (25.27)	133 (36.54)	100 (75.19)	33 (24.81)
Total	371 (100.00)			364 (100.00)		

Table 2: Quantification of mycotoxins in breastmilk samples of women in western Kenya at four and nine months postpartum.

	4-month visit			**9-month visit**		
	samples above LOQ**, n (%)	mean (SD)	above EU cutoff*, n (%)	samples above LOQ**, n (%)	mean (SD)	above EU cutoff,* n (%)
Aflatoxin M_1 (AFM_1)	289 (77.90)	0.89 (1.07)	274 (73.85)	284 (78.02)	1.00 (1.12)	276 (75.82)
Fumonisins (FB_1)	334 (90.03)	9.48 (9.08)	0 (0)	312 (85.71)	8.80 (9.21)	0 (0)
Ochratoxin A (OTAA)	136 (36.66)	0.03 (0.05)	0 (0)	122 (33.52)	0.03 (0.05)	0 (0)
Deoxynivalenol (DON)	243 (65.50)	0.05 (0.05)	0 (0)	225 (61.98)	0.05 (0.05)	0 (0)
Total Number of Mothers	371			364		
Mothers above cutoffs at both 4- and 9-month visit						185 (47.93)
Mothers below cutoffs at both 4- and 9-month visit						21 (5.44)

*European Union Cutoffs: AFM_1 (0.025 ng/mL), FB_1 (200 ng/mL), OTA (0.5 ng/mL), and DON (200 ng/mL)
**Limits of Quantification: AFM_1 (0.001 ng/mL), FB_1 (0.01 ng/mL), OTA (0.002 ng/mL), and DON (0.01 ng/mL)

Table 3: Relationship between stunting, wasting, underweight, and anemia in infants, and anemia and mastitis in mothers and aflatoxins in breastmilk. European Union cutoffs for the maximum amount of aflatoxin permitted in infant milk replacement formula is 0.025 ng/mL.

	4-month visit				9-month visit			
	All	Above AFM_1 Cutoff	Below AFM_1 Cutoff	Parameter Estimate/ Odds Ratio (95 % CI)	All	Above AFM_1 Cutoff	Below AFM_1 Cutoff	Parameter Estimate/ Odds Ratio (95 % CI)
Infant HAZ, mean (SD)	-0.59 (1.12)	-0.62 (1.14)	-0.49 (1.07)	-0.13 (-0.39, 0.13)	-0.71 (1.07)	-0.67 (1.06)	-0.84 (1.08)	0.17 (-0.09, 0.43)
% Stunted, n (%)	32 (8.63)	26 (9.49)	6 (6.19)	1.59 (0.63, 3.99)	37 (10.25)	24 (8.79)	13 (14.77)	0.56 (0.27, 1.15)
Infant WHZ, mean (SD)	0.58 (1.39)	0.61 (1.42)	0.52 (1.29)	0.09 (-0.23, 0.41)	0.29 (1.14)	0.28 (1.11)	0.32 (1.22)	0.04 (-0.31, 0.24)
% Wasted, n (%)	10 (2.70)	7 (2.55)	3 (3.09)	0.82 (0.21, 3.24)	7 (1.92)	7 (2.54)	0 (0.00)	NA†
Infant WAZ, mean (SD)	-0.04 (1.05)	-0.05 (1.09)	-0.01 (0.94)	-0.04 (-0.28, 0.21)	-0.22 (0.96)	-0.20 (0.95)	-0.28 (1.01)	0.08 (-0.15, 0.31)
% Underweight, n (%)	11 (2.96)	10 (3.65)	1 (1.03)	3.64 (0.46, 28.78)	12 (3.32)	9 (3.30)	3 (3.41)	0.97 (0.26, 3.65)
Infant Hb, mean (SD)	---	---	----	---	9.82 (1.33)	9.85 (1.32)	9.74 (1.37)	0.11 (-0.21, 0.43)
% Anemic, n (%)	---	---	---	---	285 (78.73)	211 (77.01)	74 (84.09)	0.63 (0.34, 1.20)
Maternal Na:K, mean (SD)	0.91 (0.48)	0.93 (0.51)	0.83 (0.38)	0.10 (-0.01, 0.21)	0.87 (0.40)	0.87 (0.42)	0.85 (0.35)	0.03 (-0.07, 0.13)
% Subclinical Mastitis, n (%)	287 (77.36)	216 (78.83)	71 (73.20)	1.36 (0.80, 2.33)	292 (80.22)	219 (79.35)	73 (82.95)	0.79 (0.42, 1.48)
Maternal Hb, mean (SD)	12.28 (1.81)	12.35 (1.77)	12.09 (1.93)	0.25 (-0.17, 0.68)	12.83 (1.60)	12.77 (1.57)	13.04 (1.67)	-0.27 (-0.66, 0.11)
% Anemic, n (%)	141 (38.01)	100 (36.50)	41 (42.27)	0.79 (0.49, 1.26)	90 (24.79)	74 (26.91)	16 (18.18)	1.66 (0.91, 3.03)
Total	371 (100.00)	274 (100.00)	97 (100.00)		364 (100.00)	276 (100.00)	88 (100.00)	

† Insufficient cases of wasting to estimate

Table 4: Relationship between stunting, wasting, underweight, and anemia in infants, and anemia and mastitis in mothers and mothers chronically exposed to aflatoxin. Chronic exposure was defined as being above EU cutoffs at both the 4-month visit and the 9-month visit and not exposed was defined as being below cutoffs at both visits.

	4-month visit				**9-month visit**			
	All	Chronic	Not	Parameter Estimate/ Odds Ratio (95 % CI)	All	Chronic	Not	Parameter Estimate/ Odds Ratio (95 % CI)
Infant HAZ, mean (SD)	-0.62 (1.18)	-0.66 (1.29)	-0.78 (0.86)	0.05 (-0.29, 0.39)	-0.64 (1.01)	-0.63 (1.00)	-0.72 (1.14)	0.04 (-0.19, 0.28)
% Stunted, n (%)	21 (13.29)	20 (13.99)	1 (6.67)	1.51 (0.53, 4.28)	18 (8.74)	15 (8.11)	3 (14.29)	0.73 (0.37, 1.42)
Infant WHZ, mean (SD)	0.58 (1.34)	0.57 (1.27)	1.43 (1.40)	-0.43* (-0.77, -0.08)	0.15 (1.06)	0.18 (1.06)	-0.17 (0.99)	0.17 (-0.07, 0.41)
% Wasted, n (%)	3 (1.90)	3 (2.10)	0 (0.00)	NA†	5 (2.43)	5 (2.70)	0 (0.00)	NA†
Infant WAZ, mean (SD)	-0.06 (1.02)	-0.12 (1.03)	0.50 (0.91)	-0.31* (-0.58, -0.04)	-0.29 (0.96)	-0.27 (0.95)	-0.59 (1.00)	0.16 (-0.06, 0.38)
% Underweight, n (%)	6 (3.80)	6 (4.20)	0 (0.00)	NA†	10 (4.85)	8 (4.32)	2 (9.52)	0.66 (0.29, 1.47)
Infant Hb, mean (SD)	---	---	---	---	9.80 (1.29)	9.79 (1.28)	9.93 (1.33)	-0.07 (-0.36, 0.22)
% Anemic, n (%)	---	---	---	---	166 (80.98)	149 (80.98)	17 (80.95)	0.00 (0.56, 1.78)
Maternal Na:K, mean (SD)	0.90 (0.46)	0.92 (0.46)	0.83 (0.36)	0.04 (-0.07, 0.17)	0.87 (0.42)	0.88 (0.44)	0.78 (0.27)	0.05 (-0.04, 0.15)
% Subclinical Mastitis, n (%)	128 (81.01)	119 (83.22)	9 (60.00)	1.82* (1.04, 3.19)	162 (78.64)	145 (78.38)	17 (80.95)	0.92 (0.52, 1.64)
Maternal Hb, mean (SD)	12.36 (1.83)	12.39 (1.75)	12.50 (1.87)	-0.06 (-0.53, 0.42)	12.80 (1.59)	12.78 (1.59)	12.85 (1.72)	-0.03 (-0.40, 0.33)
% Anemic, n (%)	58 (36.71)	52 (36.36)	6 (40.00)	0.93 (0.54, 1.60)	56 (27.18)	49 (26.49)	7 (33.33)	0.85 (0.52, 1.38)
Total	245 (100.00)	143 (100.00)	15 (100.00)		211 (100.00)	185 (100.00)	21 (100.00)	

† Insufficient cases of wasting to estimate

*Statistically significant at 95% Confidence Level

4. IMPLICATIONS:

The findings of this study have the potential to make strong impacts in the field of public health in relation to maternal and child health. They contribute to documenting the variation of mycotoxin exposure across different contexts. Testing the mean mycotoxin levels for aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol in breastmilk revealed new insights into the prevalence of mycotoxin contamination. While the ochratoxin A concentrations were very similar to the few studies previously reporting contamination levels, new information was gleaned from the remaining three tested. This study discovered 86-90% mothers' breastmilk tested positive for FB_1, compared to only 44% in Magoha's study in Tanzania, a significantly greater percentage.[52] It was also the first study to quantify the concentration of deoxynivalenol in breastmilk. AFM_1 concentrations in this study again revealed higher concentrations of contamination than previously published studies.[53-58] Another newer contribution this study made was to also report the proportion of samples above the EU cutoffs. While a few previous studies have also reported proportions, this study's proportions above cutoffs are significantly higher with about 75% of samples above the cutoff.[56,58] This knowledge of the prevalence of mycotoxin contamination paired with the knowledge of the health consequences of mycotoxin exposure should help drive change within agricultural practices and policy. In relation to agriculture, the findings contributed by this study should prompt the creation of new storage practices to aid in the prevention of mycotoxin growth. Since mycotoxins are invisible to the naked eye, perhaps knowing the severity of contamination will also help prompt the invention of an easy-to-use, cost-efficient device that could be made available to individuals to test their personal crop stores. Local governments should be paying attention to these high numbers as well, using them to inform policies surrounding crop storage, treatment, and distribution.

Adding the results from this study to the few studies in existence surrounding aflatoxin's association with the nutritional status of children reveal inconsistencies requiring further study. Studies in Ghana, the Gambia, Togo, and Benin reveal a few associations regarding aflatoxin-albumin levels and stunting, wasting, and underweight. Ghana revealed an association with aflatoxin-albumin levels and low birthweight babies while Gambia, Benin, and Togo with low WAZ and HAZ for children of varying age.[59,48,47] This study is the first to examine breastmilk aflatoxin exposure and child nutritional status and found that chronic exposure was negatively associated with WAZ and WHZ in infants at 4-months follow-up. It is possible though that the negative consequences of mycotoxins on child growth require more time to manifest and thus explaining why HAZ was not significant. These new contributions should continue to spur on research surrounding this topic in an effort to better understand the implications of mycotoxin exposure on child malnutrition. This understanding is crucial as the implication for childhood malnutrition, as described earlier, have many lasting consequences well into adulthood. Potential pathways to continued research might include key features such as: a longitudinal study design to ensure participant tracking over longer periods of time; utilization of blood biomarkers, like aflatoxin-albumin, to quantify exposure more directly related to mycotoxin consumption; utilization of biomarkers related to EE so that the relationship between mycotoxins, EE, and malnutrition can be better understood; and diversity among study sites and countries to gain a wider understanding of the burden across the globe.

Finally, relationships between aflatoxin M_1 in breastmilk and maternal anemia and mastitis were explored. While there were no statistically significant associations between maternal anemia and aflatoxin concentrations, there was a statistically significant association between chronic aflatoxin exposure at 4-months postpartum and maternal subclinical mastitis. This is the first study

to explore the association between mycotoxin exposure and mastitis. This again, is an area of research that needs to continue to be explored.

The analyses described here document mycotoxin exposure in infants through the breastmilk of mothers in western Kenya. In doing so they provide a comprehensive understanding of the presence of and the mean levels of mycotoxins (aflatoxin M_1, fumonisin B_1, ochratoxin A, and deoxynivalenol) in breastmilk. Comparison of these results to previous studies revealed both similarities and differences. Aflatoxin contamination is widespread among countries around the world and within the study region of western Kenya. While all studied mycotoxins were found to be present in breastmilk samples, only aflatoxins were above European Union cutoffs for infant milk replacement formula. Analysis of all four mycotoxins based on season and on control vs. intervention site revealed no statistically significant differences. Finally, the more in-depth analyses with aflatoxins revealed no statistically significant associations between aflatoxin levels above cutoffs and infant malnutrition and anemia, nor with maternal anemia and mastitis at 4- and 9-month visits. It did though reveal statistically significant associations between chronic aflatoxin exposure and WAZ and WHZ in infants, and maternal subclinical mastitis during the 4-month follow-up.

REFERENCES:

1. Wrottesley, S. V., Lamper, C., & Pisa, P. T. (2016). Review of the importance of nutrition during the first 1000 days: maternal nutritional status and its associations with fetal growth and birth, neonatal and infant outcomes among African women. *Journal of Developmental Origins of Health and Disease*, *7*(2), 144–162. https://doi.org/10.1017/S2040174415001439
2. Etzel RA. Reducing malnutrition: time to consider potential links between stunting and mycotoxin exposure? Pediatrics. 2014 Jul;134(1):4-6. doi: 10.1542/peds.2014-0827. Epub 2014 Jun 2.
3. WHO | Stunting in a nutshell. (n.d.). Retrieved March 12, 2018, from http://www.who.int/nutrition/healthygrowthproj_stunted_videos/en/
4. WHO | Description. (n.d.). Retrieved March 13, 2018, from http://www.who.int/nutgrowthdb/about/introduction/en/index2.html
5. WHO | Global Nutrition Targets 2025: Wasting policy brief. (n.d.). Retrieved March 13, 2018, from http://www.who.int/nutrition/publications/globaltargets2025_policybrief_wasting/en/
6. Prendergast AJ, Rukobo S, Chasekwa B, Mutasa K, Ntozini R, Mbuya MN, Jones A, Moulton LH, Stoltzfus RJ, Humphrey JH. Stunting is characterized by chronic inflammation in Zimbabwean infants. PLoS One. 2014 Feb 18;9(2):e86928. doi: 10.1371/journal.pone.0086928.
7. Prendergast, A. J., & Humphrey, J. H. (2014). The stunting syndrome in developing countries. *Paediatrics and International Child Health*, *34*(4), 250–265. https://doi.org/10.1179/2046905514Y.0000000158
8. Korpe, P. S., & Petri, W. A. (2012). Environmental Enteropathy: Critical implications of a poorly understood condition. *Trends in Molecular Medicine*, *18*(6), 328–336. https://doi.org/10.1016/j.molmed.2012.04.007
9. Bennett, J.W. and Klich, M. (2003). Mycotoxins. *American Society for Microbiology, 16, 3*, 497-516. DOI: 10.1128/CMR.16.3.497-516.2003
10. Bravi, F., Wiens, F., Decarli, A., Dal Pont, A., Agostoni, C., & Ferraroni, M. (2016). Impact of maternal nutrition on breast-milk composition: a systematic review. *The American Journal of Clinical Nutrition*, *104*(3), 646–662. https://doi.org/10.3945/ajcn.115.120881
11. Mead, M. N. (2008). Contaminants in Human Milk: Weighing the Risks against the Benefits of Breastfeeding. *Environmental Health Perspectives*, *116*(10), A426–A434.
12. GHO | By category | Global and regional trends by WHO Regions, 1990-2016 - Underweight. (n.d.). Retrieved March 12, 2018, from http://apps.who.int/gho/data/view.main.NUTWHOUNDERWEIGHTv?lang=en
13. GHO | By category | Global and regional trends by WHO Regions, 1990-2016 - Stunting. (n.d.). Retrieved March 12, 2018, from http://apps.who.int/gho/data/view.main.NUTWHOSTUNTINGv?lang=en
14. GHO | By category | Global and regional trends by WHO Regions, 1990-2016 - Wasting. (n.d.). Retrieved March 12, 2018, from http://apps.who.int/gho/data/view.main.NUTWHOSEVWASTINGv?lang=en
15. Statistics, K. N. B. of, Health/Kenya, M. of, Council/Kenya, N. A. C., Institute, K. M. R., & Development/Kenya, N. C. for P. and. (2015). Kenya Demographic and Health Survey

2014. Retrieved from http://dhsprogram.com/publications/publication-FR308-DHS-Final-Reports.cfm
16. De Saeger, S., Audenaert, K., & Croubels, S. (2016). Report from the 5th International Symposium on Mycotoxins and Toxigenic Moulds: Challenges and Perspectives (MYTOX) Held in Ghent, Belgium, May 2016. *Toxins*, *8*(5). https://doi.org/10.3390/toxins8050146
17. Mycotoxins in Grain - What are mycotoxins? - Food-borne mycotoxins - Fungal ecology and mycotoxin production in food - Prevention and control of mycotoxins in stored grains and seeds - Detecting mycotoxins - Summary - Further information. (n.d.). Retrieved January 7, 2018, from http://www.fao.org/wairdocs/x5008e/x5008e01.htm
18. Martorell, R. (2017). Improved Nutrition in the First 1000 Days and Adult Human Capital and Health. *American Journal of Human Biology : The Official Journal of the Human Biology Council*, *29*(2). https://doi.org/10.1002/ajhb.22952
19. Prendergast A, Kelly P. Enteropathies in the developing world: neglected effects on global health. Am J Trop Med Hyg. 2012 May;86(5):756-63. doi: 10.4269/ajtmh.2012.11-0743.
20. Arndt, M. B., Richardson, B. A., Ahmed, T., Mahfuz, M., Haque, R., John-Stewart, G. C., … Walson, J. L. (2016). Fecal Markers of Environmental Enteropathy and Subsequent Growth in Bangladeshi Children. *The American Journal of Tropical Medicine and Hygiene*, *95*(3), 694–701. https://doi.org/10.4269/ajtmh.16-0098
21. Taylor, John W., Raper, John R., and Williams, Marvin C. (2014). Fungi. In AccessScience. Mc Graw-Hill Education. https://doi-org.proxy.library.emory.edu/10.1036/1097-8542.275800.
22. Bennett, J.W. and Klich, M. (2003). Mycotoxins. *American Society for Microbiology*, *16, 3*, 497-516. DOI: 10.1128/CMR.16.3.497-516.2003
23. Steyn, P. S. (1995). Mycotoxins, general view, chemistry and structure. *Toxicology Letters*, *82–83*(Supplement C), 843–851. https://doi.org/10.1016/0378-4274(95)03525-7
24. Poór, M., Bálint, M., Hetényi, C., Gődér, B., Kunsági-Máté, S., Kőszegi, T., & Lemli, B. (2017). Investigation of Non-Covalent Interactions of Aflatoxins (B1, B2, G1, G2, and M1) with Serum Albumin. *Toxins*, *9*(11), 339. MDPI AG. Retrieved from http://dx.doi.org/10.3390/toxins9110339
25. Schmidt, C. W. (2013). Breaking the Mold: New Strategies for Fighting Aflatoxins. *Environmental Health Perspectives*, *121*(9), A270–A275. https://doi.org/10.1289/ehp.121-a270
26. Herzallah, S. M. (2009). Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. *Food Chemistry*, *114*(3), 1141–1146. https://doi.org/10.1016/j.foodchem.2008.10.077
27. Peraica, M., Domijan, A., Jurjević, Ž., & Cvjetković, B. (2002). Prevention of exposure to mycotoxins from food and feed. *Arhiv Za Higijenu Rada i Toksikologiju*, *53*, 229–237.
28. Alshannaq, A., & Yu, J.-H. (2017). Occurrence, Toxicity, and Analysis of Major Mycotoxins in Food. *International Journal of Environmental Research and Public Health*, *14*(6). https://doi.org/10.3390/ijerph14060632
29. IARC Monographs- Classifications. (n.d.). Retrieved January 8, 2018, from http://monographs.iarc.fr/ENG/Classification/
30. Pitt, J. I., & Miller, J. D. (2017). A Concise History of Mycotoxin Research. *Journal of Agricultural and Food Chemistry*, *65*(33), 7021–7033. https://doi.org/10.1021/acs.jafc.6b04494

31. Schmale III, David G., Munkvold, Gary P.. *Fumonisins*. (n.d.). Retrieved January 11, 2018, from https://www.apsnet.org/edcenter/intropp/topics/Mycotoxins/Pages/Fumonisins.aspx
32. Schmale III, David G., Munkvold, Gary P.. *Ochratoxins*. (n.d.). Retrieved January 12, 2018, from https://www.apsnet.org/edcenter/intropp/topics/Mycotoxins/Pages/Ochratoxins.aspx
33. Tao, Y., Xie, S., Xu, F., Wang, Y., Chen, D., Pan, Y., … Yuan, Z. (n.d.). Ochratoxin A: Toxicity, oxidative stress and metabolism. *Food and Chemical Toxicology*. https://doi.org/10.1016/j.fct.2018.01.002
34. Teratogenic | Define Teratogenic at Dictionary.com. (n.d.). Retrieved January 12, 2018, from http://www.dictionary.com/browse/teratogenic
35. Sobrova, P., Adam, V., Vasatkova, A., Beklova, M., Zeman, L., & Kizek, R. (2010). Deoxynivalenol and its toxicity. *Interdisciplinary Toxicology*, *3*(3), 94–99. https://doi.org/10.2478/v10102-010-0019-x
36. Niu, Guodong. (2010). *Toxicity of mycotoxins to insects and underlying molecular and biochemical mechanisms* (Doctoral dissertation). Retrieved from ResearchGate. (46090626)
37. National Drought Mitigation Center. (n.d.). *Climate Change*. Retrieved January 13, 2018, from http://drought.unl.edu/DroughtBasics/ClimateChange.aspx
38. Bhat, R.V., and Visanthi, S. (2003). Food Safety in Food Security and Food Trade: mycotoxin food safety risk in developing countries. (International Food Policy Research Institute – Vision 2020; Brief 10, issue 3. Available at http://ageconsearch.umn.edu/bitstream/16571/1/fo030010.pdf)
39. Bhat, R. V., & Miller, J. D. (n.d.). Mycotoxins and food supply. Retrieved January 13, 2018, from http://www.fao.org/docrep/U3550t/u3550t0e.htm
40. De Vries, H.R., Maxwell, S.M., & Hendrickse, R.G.. (1989). Feotel and Neonatal Exposure to Aflatoxins. *Acta Paediatrica Scandinavica* 78: 373-378.
41. Smith, L. E., Prendergast, A. J., Turner, P. C., Humphrey, J. H., & Stoltzfus, R. J. (2017). Aflatoxin Exposure During Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. *The American Journal of Tropical Medicine and Hygiene*, *96*(4), 770–776. https://doi.org/10.4269/ajtmh.16-0730
42. Altun, S. K., Gürbüz, S., & Ayağ, E. (2017). Aflatoxin M_1 in human breast milk in southeastern Turkey. *Mycotoxin Research*, *33*(2), 103–107. https://doi.org/10.1007/s12550-016-0268-4
43. Warth, B., Braun, D., Ezekiel, C. N., Turner, P. C., Degen, G. H., & Marko, D. (2016). Biomonitoring of Mycotoxins in Human Breast Milk: Current State and Future Perspectives. *Chemical Research in Toxicology*, *29*(7), 1087–1097. https://doi.org/10.1021/acs.chemrestox.6b00125
44. Klapec, T., Šarkanj, B., Banjari, I., & Strelec, I. (2012). Urinary ochratoxin A and ochratoxin alpha in pregnant women. *Food and Chemical Toxicology*, *50*(12), 4487–4492. https://doi.org/10.1016/j.fct.2012.09.030
45. Wells, L., Hardie, L., Williams, C., White, K., Liu, Y., De Santis, B., … Sathyapalan, T. (2016). Determination of Deoxynivalenol in the Urine of Pregnant Women in the UK. *Toxins*, *8*(11). https://doi.org/10.3390/toxins8110306
46. Liew, W.-P.-P., & Mohd-Redzwan, S. (2018). Mycotoxin: Its Impact on Gut Health and Microbiota. *Frontiers in Cellular and Infection Microbiology*, *8*. https://doi.org/10.3389/fcimb.2018.00060

47. Gong, Y. Y., Cardwell, K., Hounsa, A., Egal, S., Turner, P. C., Hall, A. J., & Wild, C. P. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *BMJ : British Medical Journal*, *325*(7354), 20–21.
48. Turner, P. C., Moore, S. E., Hall, A. J., Prentice, A. M., & Wild, C. P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*, *111*(2), 217–220.
49. Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y., Hall, A. J., Prentice, A. M., & Wild, C. P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, *36*(5), 1119–1125. https://doi.org/10.1093/ije/dym122
50. Sadeghi, N., Oveisi, M. R., Jannat, B., Hajimahmoodi, M., Bonyani, H., & Jannat, F. (2009). Incidence of aflatoxin M1 in human breast milk in Tehran, Iran. *Food Control*, *20*(1), 75–78. https://doi.org/10.1016/j.foodcont.2008.02.005
51. Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment: A review. *Critical Reviews in Toxicology*, *41*(9), 740–755. https://doi.org/10.3109/10408444.2011.575766
52. Magoha, H., De Meulenaer, B., Kimanya, M., Hipolite, D., Lachat, C., & Kolsteren, P. (2014). Fumonisin B1 contamination in breast milk and its exposure in infants under 6 months of age in Rombo, Northern Tanzania. *Food and Chemical Toxicology*, *74*, 112–116. https://doi.org/10.1016/j.fct.2014.09.008
53. Coulter, J. B., Lamplugh, S. M., Suliman, G. I., Omer, M. I., & Hendrickse, R. G. (1984). Aflatoxins in human breast milk. *Annals of Tropical Paediatrics*, *4*(2), 61–66.
54. Wild Christopher P., Pionneau Florence A., Montesano Ruggero, Mutiro Crispen F., & Chetsanga Christopher J. (2006). Aflatoxin detected in human breast milk by immunoassay. *International Journal of Cancer*, *40*(3), 328–333. https://doi.org/10.1002/ijc.2910400308
55. Maxwell, S. M., Apeagyei, F., Vries, H. R. D., Mwanmut, D. D., & Hendrickse, R. G. (1989). Aflatoxins in Breast Milk, Neonatal Cord Blood and Sera of Pregnant Women. *Journal of Toxicology: Toxin Reviews*, *8*(1–2), 19–29. https://doi.org/10.3109/15569548909059735
56. Adejumo, O., Atanda, O., Raiola, A., Somorin, Y., Bandyopadhyay, R., & Ritieni, A. (2013). Correlation between aflatoxin M1 content of breast milk, dietary exposure to aflatoxin B1 and socioeconomic status of lactating mothers in Ogun State, Nigeria. *Food and Chemical Toxicology*, *56*, 171–177. https://doi.org/10.1016/j.fct.2013.02.027
57. Tina Jafari, Aziz A. Fallah, Soleiman Kheiri, Abdolmajid Fadaei & Sayed Asadollah Amini (2017) Aflatoxin M1 in human breast milk in Shahrekord, Iran and association with dietary factors, Food Additives & Contaminants: Part B, 10:2, 128-136, DOI: 10.1080/19393210.2017.1282545
58. Elaridi, J., Bassil, M., Kharma, J. A., Daou, F., & Hassan, H. F. (2017). Analysis of Aflatoxin M^sub 1^ in Breast Milk and Its Association with Nutritional and Socioeconomic Status of Lactating Mothers in Lebanon. *Journal of Food Protection; Des Moines*, *80*(10), 1737–1741. http://dx.doi.org.proxy.library.emory.edu/10.4315/0362-028X.JFP-17-083
59. Shuaib Faisal M. B., Jolly Pauline E., Ehiri John E., Yatich Nelly, Jiang Yi, Funkhouser Ellen, … Williams Jonathan H. (2010). Association between birth outcomes and aflatoxin

B1 biomarker blood levels in pregnant women in Kumasi, Ghana. *Tropical Medicine & International Health*, *15*(2), 160–167. https://doi.org/10.1111/j.1365-3156.2009.02435.x

SUPPLEMENTAL MATERIAL:

Supplemental Table 1: Bias analysis of mothers included and excluded in mycotoxin analysis

	4-month visit				9-month visit			
	All	Excluded	Included	p-value	All	Excluded	Included	p-value
Intervention vs. Control Facilities, n (%)								
Intervention	194 (48.99)	13 (6.70)	181 (93.30)	0.76	193 (50.26)	11 (5.70)	182 (94.30)	0.66
Control	202 (51.01)	12 (5.94)	190 (94.06)		191 (49.74)	9 (4.71)	182 (95.29)	
Interview Month, n (%)								
Dry Season Jan-Feb & June-Sept	294 (74.24)	16 (5.44)	278 (94.56)	< 0.001	159 (41.41)	8 (5.03)	151 (94.97)	0.34
Rainy Season (Long) March-May	6 (1.52)	4 (66.67)	2 (33.33)		87 (22.66)	7 (8.05)	80 (91.95)	
Rainy Season (Short) Oct-Dec	96 (24.24)	5 (5.21)	91 (94.79)		138 (35.94)	5 (3.62)	133 (96.38)	
Mother's Age at Enrollment in Years, mean (SD)	24.31 (5.47)	23.90 (5.49)	24.45 (5.46)	0.33	24.31 (5.47)	23.90 (5.43)	24.47 (5.48)	0.29
Mother's Age at Enrollment in Quartiles, n (%)								
17-20	161 (32.20)	48 (29.81)	113 (70.19)	0.53	163 (32.28)	48 (29.45)	115 (70.55)	0.26
21-23	102 (20.40)	26 (25.49)	76 (74.51)		102 (20.20)	35 (34.31)	67 (65.69)	
24-28	138 (27.60)	32 (23.19)	106 (76.81)		139 (27.52)	33 (23.74)	106 (76.26)	
28-44	99 (19.80)	23 (23.23)	76 (76.77)		101 (20.00)	25 (24.75)	76 (75.25)	
Mother's Education – Completed Primary School or Higher, n (%)								
Yes	349 (69.80)	92 (26.36)	257 (73.64)	0.66	352 (69.70)	101 (28.69)	251 (71.31)	0.56

No	151 (30.20)	37 (24.50)	114 (75.50)		153 (30.30)	40 (26.14)	113 (73.86)	
Occupation of Head of Household, n (%)								
Doesn't Work	67 (13.45)	25 (37.31)	42 (62.69)	0.19	68 (13.52)	29 (42.65)	39 (57.35)	0.02
Agriculture	94 (18.88)	21 (22.34)	73 (77.66)		94 (18.69)	29 (30.85)	65 (69.15)	
Salaried Employee	81 (16.27)	22 (27.16)	59 (72.84)		81 (16.10)	23 (28.40)	58 (71.60)	
Casual Labor	114 (22.89)	26 (22.81)	88 (77.19)		116 (23.06)	25 (21.55)	91 (78.45)	
Other	142 (28.51)	3 (27.27)	8 (72.73)		144 (28.63)	34 (23.61)	110 (76.39)	
Wealth Tertile, n (%)								
First	160 (32.13)	42 (26.25)	118 (73.75)	0.96	162 (32.21)	42 (25.93)	120 (74.07	0.75
Second	168 (33.73)	42 (25.00)	126 (75.00)		169 (33.60)	50 (29.59)	119 (70.41)	
Third	170 (34.14)	44 (25.88)	126 (74.12)		172 (34.19)	49 (28.49)	123 (71.51)	
Food Insecurity Enrollment, n (%)								
Mild	274 (56.15)	74 (27.01)	200 (72.99)	0.67	276 (55.98)	87 (31.52)	189 (68.48)	0.03
Moderate	101 (20.70)	23 (22.77)	78 (77.23)		102 (20.69)	18 (17.65)	84 (82.35)	
Severe	113 (23.16)	31 (27.43)	82 (72.57)		115 (23.33)	34 (29.57)	81 (70.43)	
Food Insecurity Visit 4, n (%)								
Mild	---	---	---	---	234 (61.26)	12 (5.13)	222 (94.87)	0.43
Moderate	---	---	---		87 (22.77)	2 (2.30)	85 (97.70)	

Severe	---	---	---	61 (15.97)	4 (6.56)	57 (93.44)
Total	500 (100.00)			505 (100.00)		

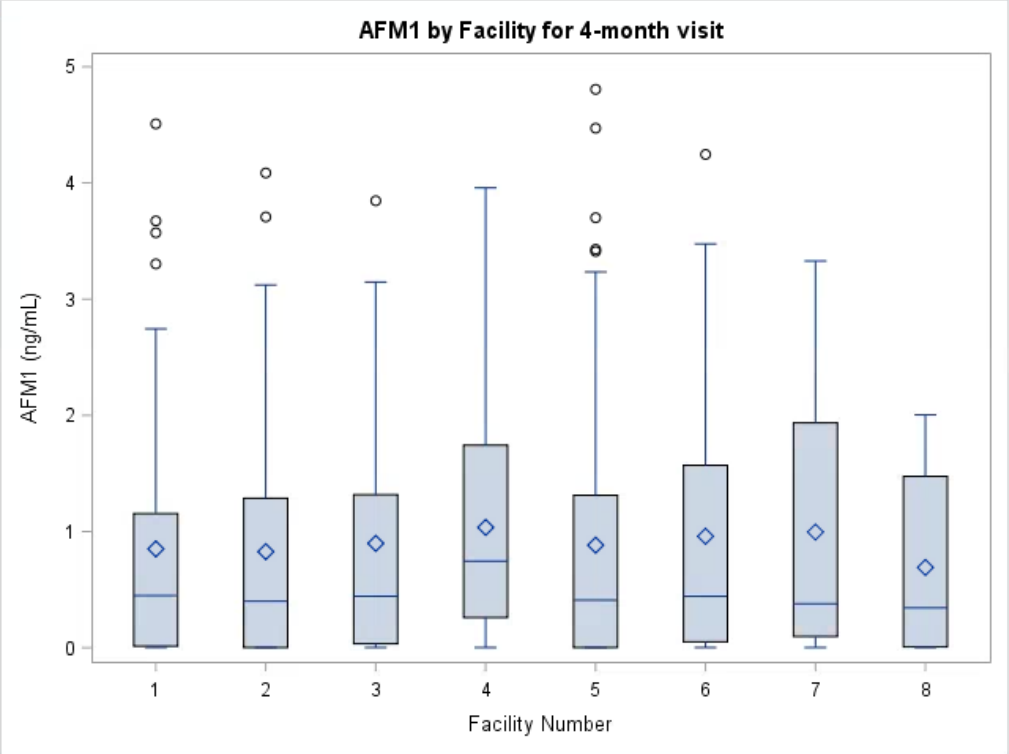
Supplemental Table 2: Mean (SD) mycotoxin concentrations in maternal breastmilk samples by Intervention/Control Sites.

	4-month visit			9-month visit		
	Intervention, mean (SD)	Control, mean (SD)	OR (95% CI)	Intervention, mean (SD)	Control, mean (SD)	OR (95% CI)
Aflatoxin M_1 (AFM_1)	0.88 (1.04)	0.91 (1.09)	1.03 (0.85, 1.24)	1.08 (1.13)	0.93 (1.10)	0.89 (0.74, 1.07)
Fumonisins (FB_1)	9.40 (9.32)	9.56 (8.87)	1.02 (0.98, 1.03)	8.86 (8.80)	9.15 (9.61)	1.01 (0.99, 1.03)
Ochratoxin A (OTAA)	0.03 (0.05)	0.03 (0.06)	0.25 (0.01, 11.69)	0.02 (0.05)	0.03 (0.05)	62.10 (0.89, >999.99)
Deoxynivalenol (DON)	0.05 (0.05)	0.05 (0.06)	3.37 (0.07, 161.64)	0.05 (0.05)	0.05 (0.05)	0.90 (0.02, 49.23)

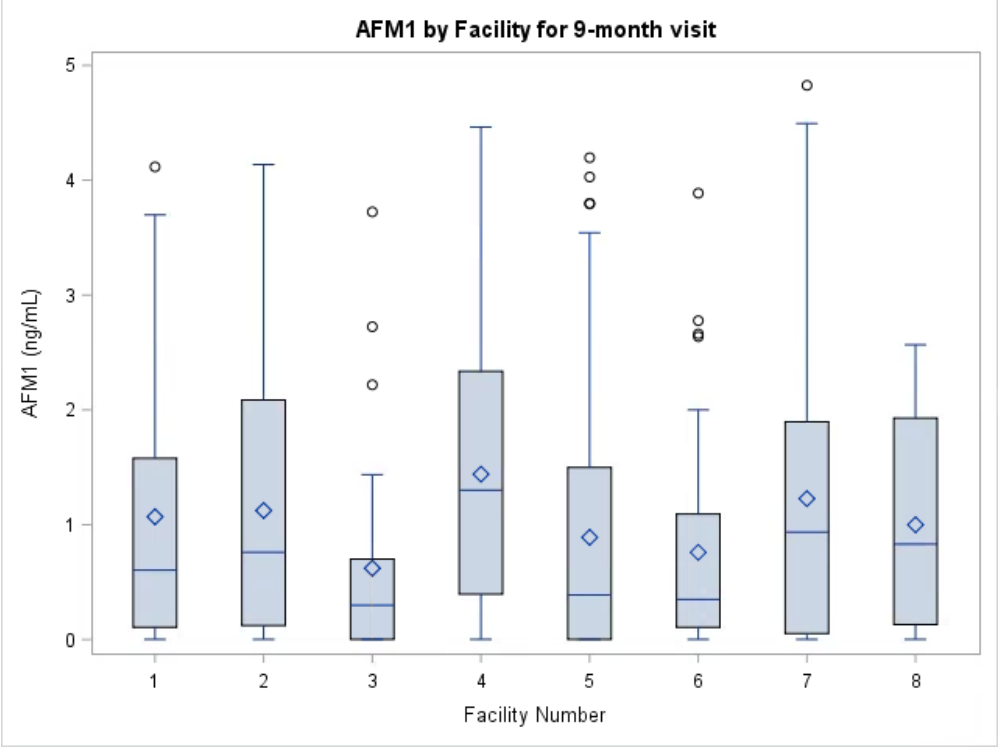
Supplemental Table 3: Mean (SD) mycotoxin levels in maternal breastmilk samples by season.

	4-month visit				9-month visit			
	Dry Season, mean (SD)	Rainy Season (Long), mean (SD)	Rainy Season (Short), mean (SD)	OR (95% CI)	Dry Season, mean (SD)	Rainy Season (Long), mean (SD)	Rainy Season (Short), mean (SD)	OR (95% CI)
Aflatoxin M_1 (AFM_1)	0.91 (1.07)	0.73 (0.99)	0.83 (1.05)	1.08 (0.86, 1.35)	1.18 (1.27)	0.79 (0.84)	0.94 (1.06)	1.20 (1.01, 1.43)
Fumonisins (FB_1)	9.58 (9.24)	3.33 (2.43)	9.33 (8.68)	1.00 (0.98, 1.03)	8.96 (9.44)	8.45 (9.05)	8.83 (9.10)	1.00 (0.98, 1.02)
Ochratoxin A (OTAA)	0.03 (0.05)	0.03 (0.03)	0.03 (0.06)	0.30 (0.00, 21.05)	0.02 (0.04)	0.03 (0.05)	0.03 (0.06)	0.03 (<0.001, 1.49)
Deoxynivalenol (DON)	0.05 (0.05)	0.10 (0.10)	0.05 (0.05)	16.48 (0.15, >999.99)	0.05 (0.05)	0.06 (0.06)	0.05 (0.05)	0.35 (0.01, 14.46)

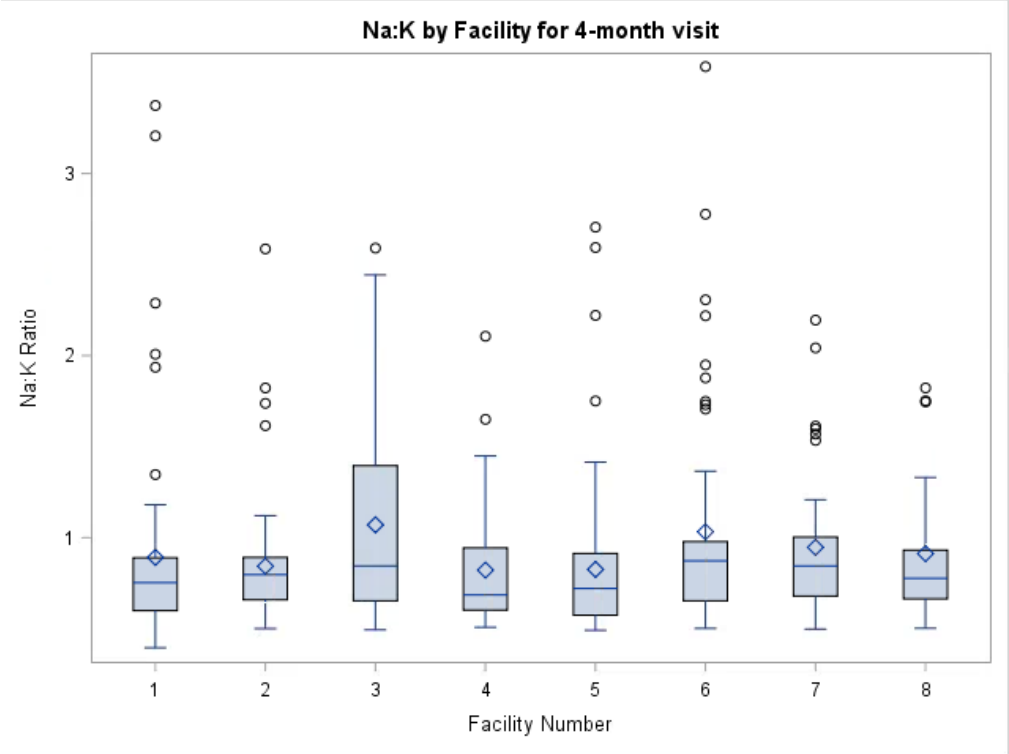
Dry Season (Jan-Feb, Jun-Sept); Rainy Season - Long (Mar-May); Rainy Season – Short (Oct-Dec)



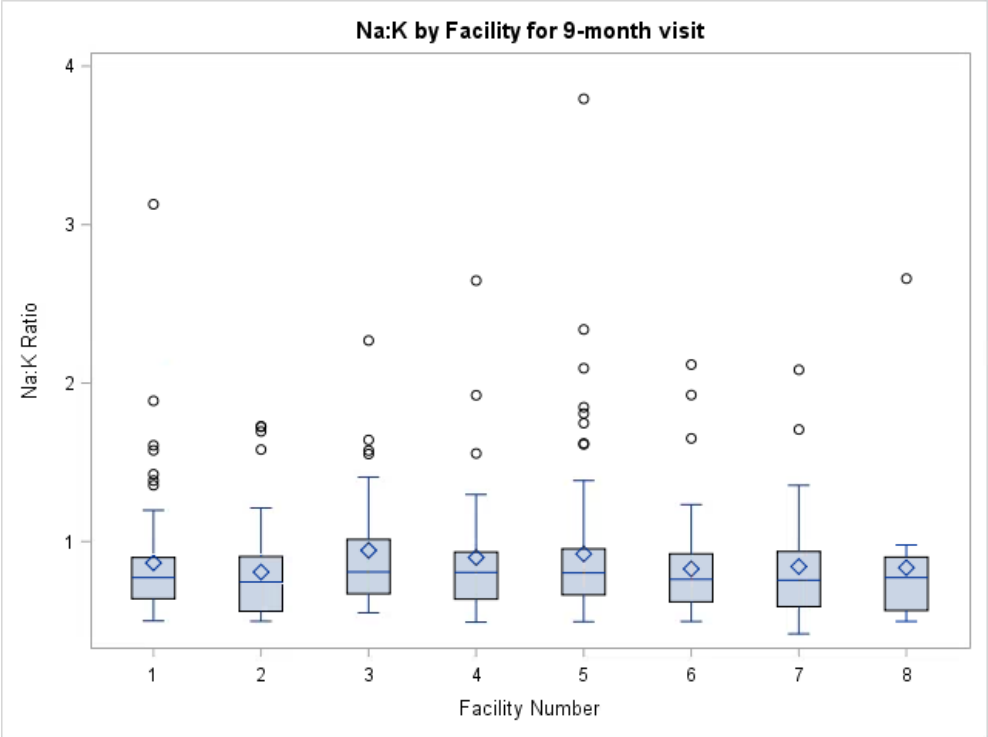
Supplemental Figure 1: Aflatoxin M_1 concentration stratified by facility for the 4-month follow-up visit.



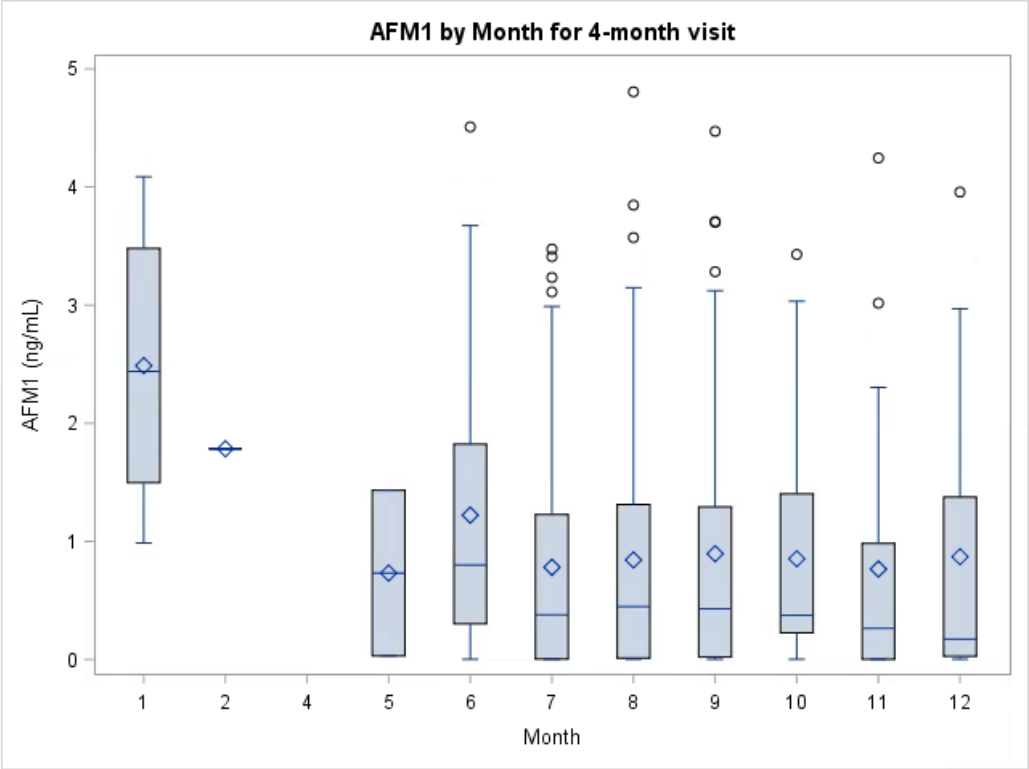
Supplemental Figure 2: Aflatoxin M_1 concentration stratified by facility for the 9-month follow-up visit.



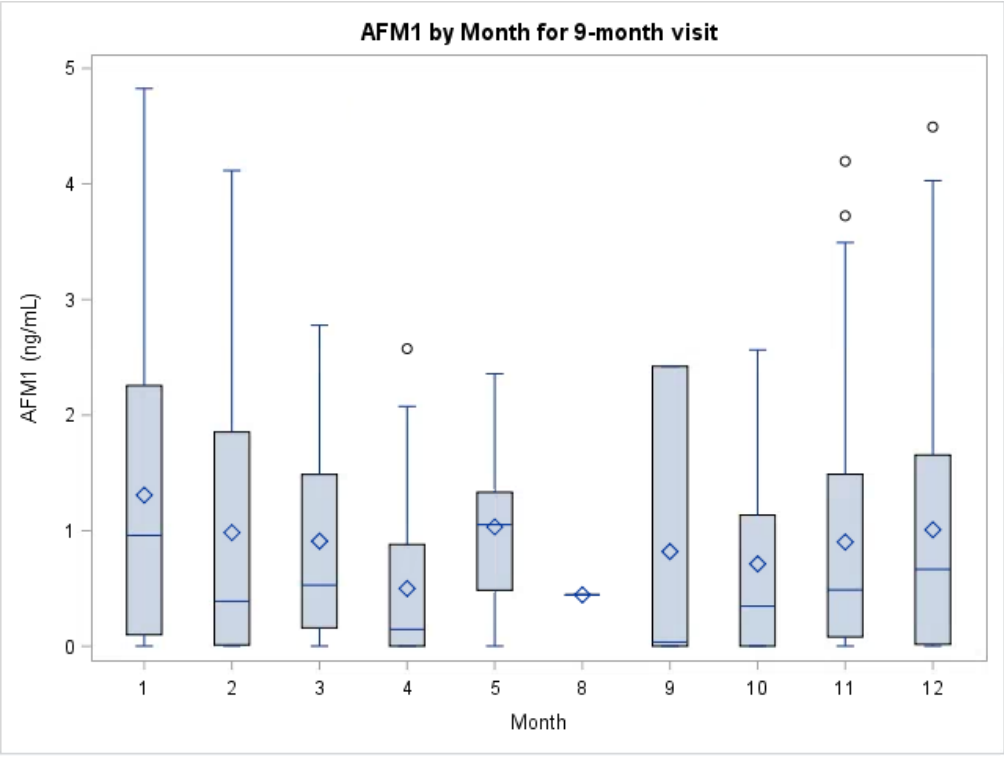
Supplemental Figure 3: Sodium:Potassium ratio stratified by facility for the 4-month follow-up visit.



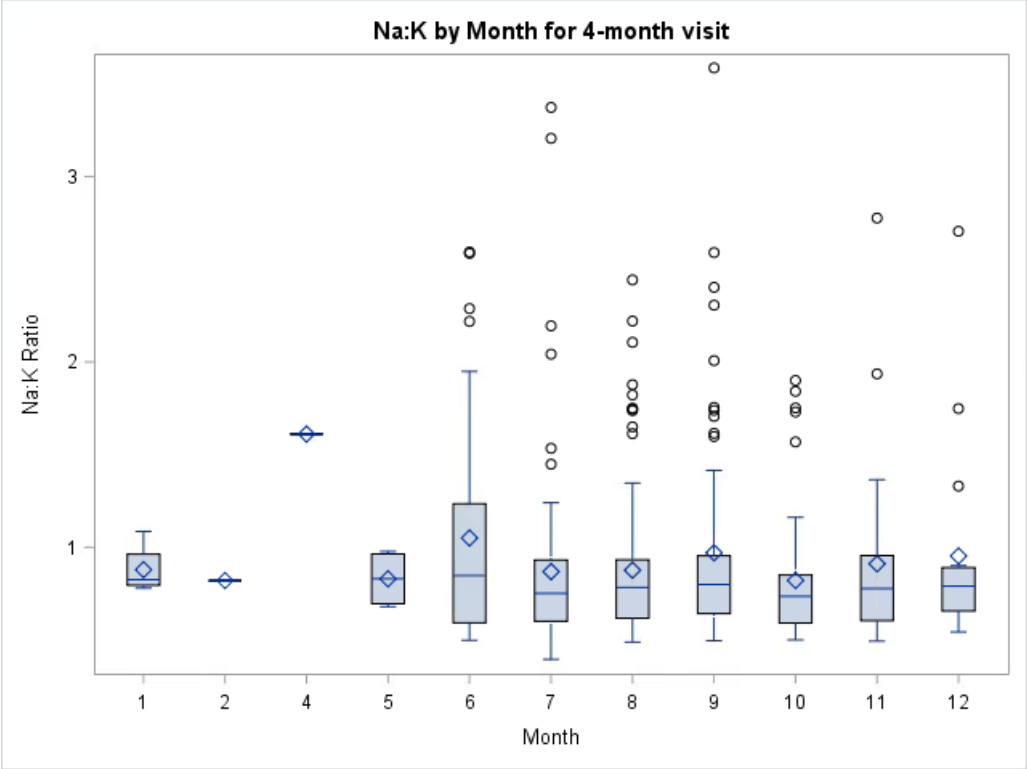
Supplemental Figure 4: Sodium:Potassium ratio stratified by facility for the 9-month follow-up visit.



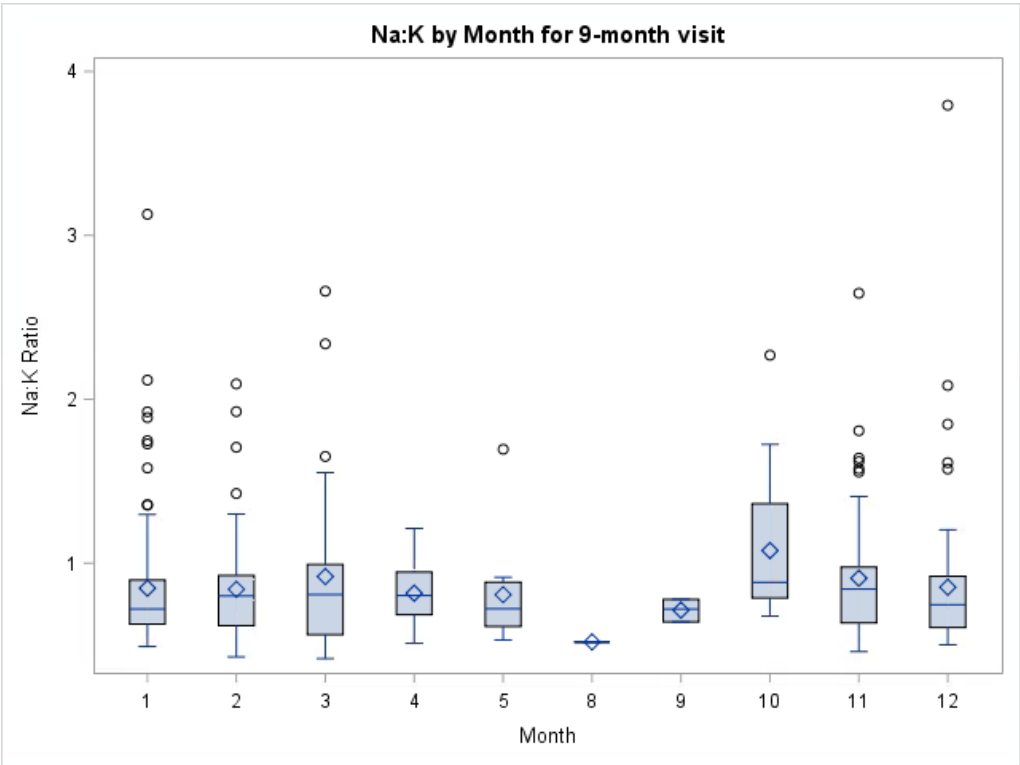
Supplemental Figure 5: Aflatoxin M_1 concentration stratified by month for the 4-month follow-up visit.



Supplemental Figure 6: Aflatoxin M_1 concentration stratified by month for the 9-month follow-up visit.



Supplemental Figure 7: Sodium:Potassium ratio stratified by month for the 4-month follow-up visit.



Supplemental Figure 8: Sodium:Potassium ratio stratified by month for the 9-month follow-up visit.