Maternal Aflatoxin Exposure and Birth Outcomes: A Study of Diets, Agricultural Practices and Nutrition in Rural Nepal

A Thesis Presented to the Faculty of the Friedman School of Nutrition Science and Policy

In partial fulfillment of the requirement for the Degree of Doctor of Philosophy
Tufts University

April 24th, 2018

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To my parents, Jesús and Marcelita.
Los amo.

ACKNOWLEDGEMENTS

I express my sincere gratitude to all those individuals and organizations who have contributed in so many ways to make this journey possible. In particular, I wish to express many thanks to:

My committee members (in alphabetical order) each of whom is unique and provided much support and balance throughout this process*. Bea,* from day one at Friedman you impressed me with your infinite curiosity and objectivity. Through your classroom teaching and as my mentor and supervisor on Exit Strategies you motivated me to strengthen and refine my analytical skills*. Jerry,* you have taught me so much methodologically, helping me through the intricacies of statistical modeling. And more importantly, you have embodied humility and work-life balance. Thank you for always saying something positive during my committee meetings. Your words were the encouragement I needed during some very solitary spells of data analysis and writing. *Patrick*, mein Doktorvater, thank you for taking me under your wing. Your ability to always see the big picture is admirable. I am thankful for your voice of experience and wealth of knowledge. And last but surely not least, *Shibani*, meine Doktormutter, without your infinite support and intense conversations at Traveller's Village, Summit, diverse conferences, Thinking Cup, and the endless hours in your office and on Skype, none of this would have been possible. Thank you for your professional mentoring, encouragement and for continuous trust in my abilities as a researcher (and, for your affinity to discover the best chocolate cake – we still have some important nutritional work to do!)

The United States Agency for International Development (USAID) Feed the Future Innovation Lab for Nutrition for funding this study. And Maura and Ahmed who were always very supportive of our research and of my dissertation. And *Jane Friedman* and the *Friedman Foundation* for honoring me with the Friedman Fellowship to help my way through grad school.

Support for this research was provided by the Feed the Future Innovation Lab for Nutrition, which is funded by the United States Agency for International Development (USAID) under grant ID: AID-OAA-L-10-00006. The authors express special gratitude to USAID/Nepal Mission, Dr. Maura Mack, Dr. Ahmed Kablan, AflaCohort study team and the study participants, without whom this research would not have been possible. The opinions expressed herein are solely those of the authors.

Mothers and infants who participated in the AflaCohort Study. Your time and generosity made this research possible. You all inspire me as mothers. And my AflaCohort babies will forever inspire me professionally and personally.

Our AflaCohort research team. Ashish and Meena, you were so dedicated and committed to making sure the day-to-day data collection, cleaning and sample

processing ran smoothly. Thank you to all the current supervisors, enumerators, nurses, field assistants, lab technologists, AflaCohort office staff, research coordinators, data managers, folks in the HKI-Kathmandu office and their predecessors. I could not have asked for a better, more hardworking, and welcoming group of people to work with on this study. And last but not least, thank you Dale and Dr. Baral and the rest of the investigator team. Your expertise and support has been invaluable to my success and to the study.

My friends, colleagues, and professors (in alphabetical order) who have stood by me along the way: Andrea, Bob, Brooke, Corey, Diane, Esther, Heather, Jamie, Joseph, Katie, Liz, Marian, Melissa, Nicole, Nisha, Olga, Ranjita, Rebecca, Reem, Rita, Robin J., Robin S., and Vicky. Thank you for your advice, support and solidarity. Janet and Marion, you were my rocks during this process. I will be forever thankful.

My parents and my brothers who are my essence and have been with me though my life journey doing everything in their power to help me succeed. Mami, gracias por estar conmigo en las buenas y en las malas. Me has ayudado a alcanzar lo inimaginable. Sin tu amor incondicional (y tu maravillosa comida) no sería la mujer quien soy hoy. FWRP, your eternal optimism and dad humor makes me smile every single day. I will really miss our Osmium dates.

And last but not least, *my beautiful and perfect Marcela and Jesús*. Bello, you have stood by me and encouraged me throughout what seems like an eternity in grad school. From Houston to Boston to Chicago to …? I can't wait for our next adventure together beginning in 2019 when we are both finally done with all our training. And, Marcelita, my little pumpkin, mi bebita, amorcito de mi vida, no te imaginas el amor que siento por ti. Eres tú quien me inspira todos los días. No me acuerdo la última vez que dormí bien pero te amo, guerrera preciosa.

ABSTRACT

Despite significant reductions in child stunting over recent decades, 36% of children remain stunted in Nepal (2016). Poor linear growth can begin *in utero* and continue beyond the age of two years, making the first 1000 days of life a critical period for stunting prevention. Recent epidemiological studies suggest that exposure to aflatoxins could contribute to low weight at birth and postnatal stunting. However, study findings showing linkages between *in utero* aflatoxin exposure and adverse birth outcomes remain inconclusive, and factors contributing to widespread exposure to aflatoxin during pregnancy are inadequately understood.

This dissertation contributes to the evidence-base to inform the design of aflatoxin reduction interventions and a better understanding of the potential influence of aflatoxin exposure on adverse birth outcomes. All three studies used data from 1675 pregnant women and newborns participating in the ongoing USAID-funded Mycotoxin (AflaCohort) Birth Cohort Study in Banke, Nepal.

In Study 1, we estimated pregnant women's frequency of consumption of aflatoxin-prone foods (i.e. maize and groundnuts) and calculated dietary diversity scores. Ordinary Least Squares (OLS) and Quantile Regression models were used to compare the strength of associations between frequencies of consumption of maize and groundnuts and dietary diversity, and serum aflatoxin levels (n=1648). After adjusting for wealth and other covariates, women who had consumed maize and/or groundnuts more frequently showed higher levels of aflatoxin albumin adducts. Findings indicated that dietary diversity was not predictive of aflatoxin exposure. Seasonality was a strong predictor of prenatal aflatoxin exposure, with the highest levels seen in the winter months following maize and groundnut harvest seasons. The second study examined the correlations between food handling procedures and good agricultural practices (GAPs) in maize, groundnut and chili farming households, and aflatoxin exposure as measured by aflatoxin albumin adducts during pregnancy. Multivariate OLS regression modeling revealed no evidence that the GAPs used in a minority of maize farming households (n=392) were associated with reduced exposure of pregnant women to aflatoxin in this sample. The infrequent use of recommended GAPs may have limited our ability to detect such an association. Moreover, off-farm food acquisition was common. Levels of aflatoxin exposure observed in this study likely reflect consumption of various foods susceptible to aflatoxin from multiple sources. Study 3 used linear and logistic regression models to explore the relationship between prenatal aflatoxin levels and selected adverse birth outcomes in a sub-sample of 1621 mother-newborn pairs. Twenty percent of infants were low birth weight, 52% small-for-gestational-age, 16% stunted, and 13% were born prematurely. None of the birth outcomes studied were associated with maternal aflatoxin levels, which were considerably lower than those observed in Africa and the Middle East where a relationship with low birth weight has been previously documented.

Together, the results presented in this dissertation underscore the importance of viewing aflatoxin contamination as a component of food safety within complex food systems. Our study, together with the mixed results from previous studies, reiterates how incomplete the evidence of the relationship between aflatoxin and birth outcomes remains at this point. It also suggests that additional research is necessary to elucidate the aflatoxin-fetal growth relationship, including determination of threshold values.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1. INTRODUCTION

Aflatoxin, a known carcinogen, has received growing attention in the field of nutrition and child health due to its association with poor linear growth [1-9]. High levels of aflatoxin contamination have been found in milk and a variety of groundnuts and maize-based foods in Nepal [10-13]. A study showed a high and almost ubiquitous prevalence of aflatoxin exposure (94%) during pregnancy in the Sarlahi district of Nepal [14]. This study also found aflatoxin B1-lysine adduct/mg albumin in human cord blood samples, suggesting the fetus has an ability to convert aflatoxins into toxicologically active compounds. Exposure levels in pregnant women ranged from 0.45 to 2,939.30 picograms (pg) aflatoxin B1-lysine adduct per milligram (mg) of albumin. A more recent study in Bhaktapur [15], showed aflatoxin exposure is widespread in children.

Despite Nepal's noteworthy progress in poverty reduction over the past decade, Nepal is still considered a low-income country, and a quarter of the population lives under the poverty line. The prevalence of stunting in Nepal has decreased markedly in the last two decades. That said, the latest Demographic Health Survey still showed high rates (36%) of stunting in Nepal [16, 17]. To understand how to prevent aflatoxin exposure during the first 1000 days[1], a period of critical growth, researchers and policymakers need context specific data on factors contributing to aflatoxin exposure in vulnerable populations.

[1] From conception through the first 2 years of life (1000 days)

Dissertation Outline

The primary goal of this dissertation was to gain a better understanding of the potential influence of aflatoxin exposure on adverse birth outcomes and to contribute to the evidence-base to inform the design of aflatoxin reduction interventions. We used in-depth prenatal and birth survey data and maternal aflatoxin biomarker data from an ongoing USAID-funded longitudinal Birth Cohort Study[2] conducted in Banke, Nepal.

This dissertation consists three independent research articles. The first two articles address dietary and agronomic determinants of aflatoxin exposure in pregnancy, a critical and vulnerable period for fetal development. The third article examines how prenatal aflatoxin exposure affects birth outcomes.

Specific aim 1: To examine if consumption of aflatoxin-prone foods and dietary diversity influence prenatal aflatoxin levels

First, we documented exposure patterns and potential determinants of prenatal aflatoxin exposure. The goal of Chapter 4 was to identify food items associated with higher aflatoxin levels during pregnancy and to determine if greater dietary diversity was correlated with lower levels of maternal aflatoxin. Here, we assessed whether frequency of consumption of maize, groundnuts or milk, was associated with maternal serum aflatoxin levels. We also studied the correlation between dietary diversity and maternal aflatoxin levels. Dietary diversity may lower aflatoxin exposure via reduced consumption of contaminated

[2] Undertaken by the Feed the Future Innovation Lab for Nutrition

foods, especially in monotonous diets that are dependent on aflatoxin-prone foods such as maize or groundnuts. This information will be key in drawing attention to the common sources of aflatoxin contamination among pregnant women and advancing awareness on how to reduce aflatoxin contamination during such a critical time of childhood development.

Specific aim 2: To identify food handling and agricultural practices associated with prenatal aflatoxin exposure

In Chapter 5 we explored household food handling behaviors (e.g. sorting and discarding moldy grains) and maize, groundnut and chili farmers' agricultural practices and their association with aflatoxin exposure during pregnancy. Several previous studies have shown potential for hand-sorting visibly diseased maize kernels where contamination with mycotoxins is reduced to acceptable levels [18-20]. Furthermore, the application of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) is considered an acceptable means of primary prevention for aflatoxins [18, 21, 22]. In this study we assessed whether use of GAPs was associated with maternal serum aflatoxin levels. Farmers producing aflatoxin-prone foods were asked about practices from pre-harvest through post-harvest (e.g. drying and storage). This study provided insight into the adoptability of aflatoxin management practices. Our findings may be helpful in designing future aflatoxin reduction studies and optimize public health and agricultural extension interventions.

Specific aim 3: To explore the relationship between prenatal aflatoxin levels and selected adverse birth outcomes (low birth weight, small-for-gestational-age, stunting and prematurity)

As a third component of this dissertation, we investigated associations between *in utero* aflatoxin exposure and selected adverse birth outcomes. Current evidence pertaining to this relationship remains mixed. While a few studies [23, 24] show a link between aflatoxin exposure and poor *in utero* growth, other studies show no link with this toxin [7, 25]. We assessed anthropometric birth outcomes such as low birth weight, small-for-gestational-age, stunting at birth, as well as preterm birth. Investigations concerning maternal aflatoxin exposure and its effects on birth outcomes in South Asia are largely lacking. In addition to contributing to bodies of research regarding aflatoxins and their relation to human health (specifically birth outcomes), this was the first large longitudinal birth cohort investigating maternal aflatoxin exposure in the Asian continent.

These findings will help improve intervention programs, especially those seeking to make the most of the 1000-day window of opportunity to improve maternal and infant health and growth.

CHAPTER 2. LITERATURE REVIEW

Stunting

In low-income countries, nutrition-related issues have been attributed to around a third of the burden of infant and early childhood (under 5) mortality [26]. Stunting is a widely used indicator of chronic malnutrition in early childhood [27]. Stunting has severe short- and long-term consequences. It can result in cognitive impairment, weakened immunity, complications during pregnancy and reduced lifetime earnings [28]. In recent decades, considerable progress has been made in reducing worldwide stunting rates, but in 2011, stunting still affected one-third (162 million) of children under 5 years of age in low-income countries. The causes of stunting are only partly understood, and the rates are highest in South Asia and sub-Saharan Africa [29]. Of the 162 million stunted children around the world 56% live in Asia [30]. A recent analysis showed nutrition-specific strategies could reduce stunting by 20 percent; however, the most successful nutrition interventions only address part of the deficit in poor linear growth [29, 31]. Emerging epidemiological human evidence suggests that nutrition-sensitive issues (e.g. agriculture and food safety), particularity aflatoxin contamination, could be important contributors to the stubbornly high stunting rates [1-9, 32].

Mycotoxins

There are many types of mycotoxins, including aflatoxins, citrinin, ergot akaloids, fumonisins, ochratoxin A, patulin, trichothecenes, and zearalenone [33]. Fusarium mycotoxins, such as fumonisins and Deoxynivalenol (DON), have also

been linked to impaired growth [34, 35]. DON, known as “vomitoxin”, has been shown to impair food intake and weight gain in experimental animals, while fumonisins have also been shown to reduce growth [34, 36-38]. Aflatoxins are common contaminants in staple foods, such as maize and peanuts, in developing countries [31, 39]. They are proven carcinogens, immunotoxins, and growth retardants [40, 41]. *Aspergillus flavus* and *Aspergillus parasiticus* fungi produce four main types of aflatoxins: B1, B2, G1, and G2 [42].

Aflatoxins and Animal Health

An extensive body of literature has documented that high levels of aflatoxin in foods present a threat to the overall health, growth and reproductive outcomes of animals [37, 38, 43-57]. Aflatoxin B1 at high levels has been shown to cause cancer in laboratory animals. Also, a study showed growth retardation, thymic shrinkage, impaired peripheral immune efficiency, and defects related to zinc intestinal absorption in piglets exposed to maternal aflatoxicosis [54].

Aflatoxins and Human Health

The Food and Agriculture Organization (FAO) estimates that aflatoxins contaminate up to 25% of agricultural crops worldwide and represent an important threat to food safety [58]. Aflatoxins are a major public health issue, with approximately 4.5 billion people, mostly in resource-poor countries, exposed to this toxin [41, 59].

Aflatoxins can proliferate during field growth or long-term storage. Long-term storage and inadequate storage infrastructure (especially exposure to moist conditions) are of particular concern, especially in developing nations where these realities are common and can contribute significantly to the burden of food contamination. More often than not, populations that are particularly at high risk of chronic exposure to aflatoxins are those that are poor, have limited dietary variety, store foods for long periods of time, and are reliant on commonly contaminated foodstuffs such as maize and groundnuts [6, 8]. Since aflatoxins are resistant to most processing methods, they are in found in processed as well as unprocessed foods [7].

Aflatoxin B1, the most toxic aflatoxin, is the most studied and has been classified as a natural liver carcinogen [60]. Hepatitis B and aflatoxins synergistically increase the risk of hepatocellular carcinoma (HCC) [42, 61]. Acute aflatoxicosis causes liver damage, vomiting, abdominal pain, pulmonary edema, convulsions, coma and death [62]. Long-term chronic (subsymptomatic) exposure has been linked to chronic hepatocellular injury, impaired linear growth and immunosuppression [6, 63, 64].

The detrimental effects of aflatoxins in terms of maternal and child health, including linear growth of children [64], have not been well studied. To date, the mechanism of growth faltering in relation to aflatoxin exposure during pregnancy and/or early infancy is not clear. One possible pathway is that growth faltering is a consequence of inhibition of protein synthesis resulting from aflatoxin-induced distortion of RNA synthesis [7, 65]. It has also been suggested that intestinal

malabsorption may occur in the fetus following maternal aflatoxin exposure. A conceptual framework published by Smith et al. [66] suggests the potential pathways in which aflatoxin can exert toxic effects on maternal and fetal health: a) generation of environmental enteric dysfunction; b) increase in pro-inflammatory and decrease in anti-inflammatory cytokines and/or c) toxic effects on maternal and fetal organs.

Allowable Limits for Food and Feed

Although it has been known since the 1960s that aflatoxin contamination of maize and groundnuts can be deleterious to health, aflatoxin exposure in humans has recently gained global attention due to the improved knowledge of the detrimental effects on human and animal well-being and the heavy reliance of at-risk populations on these highly contaminated crops. The complete elimination of aflatoxins in agricultural products is extremely unlikely, since aflatoxins are stable in foods and resistant to degradation under normal cooking procedures [7, 62]. Thus, the general recommendation from food safety regulating organizations is that the amounts present in crops and foods should be reduced to the lowest levels that are technologically achievable.

Governments around the world have worked to limit the amount of aflatoxin that finds its way into the food supply. For example, the United States Department of Agriculture (USDA), the European Food Safety Authority (EFSA) and Codex Alimentarius have set limits for the amount of aflatoxin and other mycotoxins that can be present in food products [62, 67, 68]; allowable limits for human foods range from 4-30 ppb aflatoxin, depending on the country [41]. In the

US and other developed countries, citizens rarely become sick as a result of aflatoxin ingestion. Strict regulatory systems ensure contaminated crops are kept out of commercial foods and animal feed [69].

In the United States, the allowable limits are 20 parts per billion (ppb) for foods, peanuts, peanut products, Brazil and pistachio nuts and 0.5 ppb for milk destined for human consumption. Aflatoxin levels allowed for animal feed range from 20 ppb for immature animals and dairy animals to 300 ppb for meal destined for finishing beef cattle [69]. In Europe, stricter regulations exist; maximum levels are 4 ppb for ready-to-eat grains, 10 ppb for grains destined for further processing, 10 ppb for ready-to-eat nuts, and 0.05 ppb for milk destined for human consumption [70]. In Nepal, the Ministry of Agriculture and the Department of Food Technology and Quality Control (DFTCC) have set official limits to 20 ppb for food and cereals and 50 ppb for feed [71]. Although allowable limits have been defined, low resources [17] can limit the government's ability to monitor and regulate markets, making food quality uncertain.

Measuring Aflatoxin Exposure

The fastest and most non-invasive approach to measuring aflatoxin exposure in humans involves an analysis of food samples. A more accurate approach involves biological markers [41]. Human blood, milk, or urine samples can be tested for the presence of aflatoxin derivatives. Aflatoxin M_1 in human urine reflects exposure over the previous 24 hours while serum aflatoxin B1-lysine

adducts per mg albumin reflect blood levels of the toxins over a period of 2-3 months [41, 60, 72, 73].

To date, no exposure duration, critical onsets for exposure, nor a specific serum aflatoxin threshold dose for immunotoxic effects have been established, making it difficult to draw definitive clinical conclusions regarding authoritative safe levels of aflatoxin exposure in humans.

Consumption of Aflatoxin-prone Foods, Dietary Diversity and Aflatoxin Exposure

Since aflatoxin-contaminated foods constitute a potentially large portion of daily dietary intake for many pregnant women and children, it is important to assess not only what the commonly contaminated foods are, but also where these foods are most likely being produced and/or purchased. Often physical (e.g. distance), social and financial factors restrict access to safe foods. In the Nepalese context, where staple foods such as maize, groundnuts, rice, and chili peppers are frequently contaminated with aflatoxins, human exposure is likely to occur at high levels [74].

Two previous studies showed dangerous levels of aflatoxin in food in Nepal. One study by Koirala et al. [10] investigated the levels of aflatoxin in common foods and feed. This study sampled foods from retailers and wholesalers in 16 eastern Nepalese districts from 1995-2003. Their findings showed that a third of these samples were contaminated with aflatoxins, and the highest percentage was shown in peanut butter/vegetable oils (43%). Cornflakes had the highest proportion of contamination that was higher than the recommended value of 30

ppb. This study showed chronic consumption in Nepal may be a public health concern. Kafle et al. [10, 11] showed 44% of the milk samples were contaminated with aflatoxin M1 (the principal hydroxylated form of aflatoxin B1 found in milk) in raw and processed milk marketed in the Kathmandu valley [11] and 22% of these had levels higher than the maximum tolerable limits set by most European countries (0.05 ppb). None of the contaminated samples exceeded the maximum tolerable limit set in the United States (0.5 ppb).

Greater dietary diversity can potentially reduce foodborne toxin exposure, particularly in persons consuming monotonous diets based on maize, groundnuts, cassava and rice [75, 76]. Lessening the dependence on aflatoxin-ridden foods can lower exposure in the diet, and added nutrients in the diet can help counteract effects of the toxin [42, 77].

Good Agricultural Practices (GAPs) and Aflatoxin Exposure

One widely accepted means of primary prevention for aflatoxin is the application of Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) [18, 21, 22]. Although primary prevention via use of GAPs is unlikely to eliminate exposure to aflatoxin entirely, research has shown best management practices can prevent and decrease aflatoxin contamination in food [22, 78, 79]. GAPs, applied throughout pre-harvest and post-harvest, serve various purposes, from improving overall food safety [80] and farm worker health to increasing farmers' profitability [81].

During pre-harvest, crop stress such as drought, insect damage and deficient irrigation can leave crops more vulnerable to *Aspergillus* fungi and aflatoxin contamination. Poor drying and post-harvest practices can result in increased accumulation of aflatoxin. It is important to harvest when the crop matures. During harvest and drying it is also important to protect the outer coats of grain or nuts. Damaged outer coats make kernels more susceptible to mold invasion [82]. Breathable, raised physical barriers between the bare ground and the crop help avoid ground moisture seeping into the crop. After reducing moisture levels to a safe level, food crops should be stored in a dry, cool, shaded and ventilated area to avoid aflatoxin buildup. Storage sacks can be raised on platforms to avoid direct contact with the moist ground.

Various field studies have examined how application of GAPs when growing groundnuts or maize can help reduce aflatoxin in food. A recent study in India [83] showed reduced aflatoxin levels in groundnuts grown in plots where GAPs were applied. A study in Benin [79] showed that aflatoxin levels in maize increased with longer duration in storage, in maize with insect damage, and in maize where local plants were used as protectants during storage. A recent study by Smith et al. [84] examining determinants of aflatoxin exposure in pregnant women in Zimbabwe showed household level dietary coping practices can influence aflatoxin levels. Women practicing one risky practice (i.e. borrowing food, relying on less expensive foods, harvesting immature crops or sending family members to beg) had higher odds of elevated aflatoxin M1.

Training on manual sorting and grading of grains and nuts appears to be a worthy investment for aflatoxin reduction in food, especially in communities with limited resources [85]. A study in Ghana [5] measured levels of aflatoxin B1-lysine adduct/mg albumin in blood and associations with food handling and consumption practices. All 140 adult participants in this study tested positive for aflatoxin B1-lysine adducts. Although almost 90% of respondents reported growing their own food, most (92%) had not heard of aflatoxins and did not know whether they caused illnesses or which foods were associated with aflatoxins. While 85% of the respondents reported throwing away spoiled grains, 8% reported feeding them to animals, and 7% reported eating the spoiled grains [5]. Several experimental studies have shown sorting to be a relatively effective, low-cost method of removing aflatoxin-contaminated nuts. In the Philippines, Galvez et al. [19] showed how sorting groundnuts reduced aflatoxin contamination levels from 300 ng/g to less than 15 ng/g. A study of groundnuts in Haiti and Kenya [20] showed hand sorting resulted in a reduction of 98% in aflatoxin levels. There is limited information on the linkages between food handling practices and mycotoxin biomarker data. A study of South African subsistence farmers [86] showed hand-sorting maize can reduce urinary fumonisin levels.

In Utero Aflatoxin Exposure and Birth Outcomes

While research in Asia is limited, several epidemiological studies in West Africa have demonstrated significant associations between high levels of serum aflatoxin in childhood and growth faltering in infants and young children [1-4, 7].

These studies overwhelmingly support a negative association between maternal and child serum aflatoxin B1-lysine adduct levels and linear growth in children. For example, a study in Benin and Togo showed strong inverse relationships between serum aflatoxin B1-lysine adduct levels and height-for-age z-scores and weight-for-age z-scores in children 1-5 years of age, indicating a role in stunting and underweight [3, 4, 64]. The multivariate analyses used in this study accounted for socio-economic status, village and sex. Children categorized as stunted or underweight had 30-40% higher mean serum aflatoxin B1-lysine adduct levels than the remainder of the children, and strong dose-response relationships were observed between serum aflatoxin B1-lysine adducts per mg of albumin and the extent of stunting and underweight.

An important concern in Nepal is the alarming rate (18%) of low birth weight in infants, which is linked to increased risk of postnatal stunting and morbidity [87-90].

Aflatoxin albumin adducts have been found in cord blood samples, suggesting aflatoxin crosses placental barriers [7, 23, 91]. Results from studies examining the correlation between maternal aflatoxin exposure and birth outcomes are inconclusive (**Table 1**).

Three studies have found a significant association between birth weight and aflatoxin exposure. One study used data from Ghana to investigate the association between birth outcomes and serum aflatoxin B1-lysine adduct levels in pregnant women [24]. This study took place in a clinical setting with a total sample size of 785 women. Aflatoxin B1-lysine adduct/mg albumin in maternal

blood ranged from 0.44 - 268.73 pg/mg albumin. After controlling for socio-demographic variables and potential confounders such as malaria parasitemia, anemia and worm infections, women in the very high aflatoxin quartile (>11.34 pg⁄mg albumin) were more likely to have low birth weight infants (OR, 2.09; 95% CI, 1.19–3.68), and infants from these mothers showed a trend of increasing risk for low birth weight compared to infants of mothers in the lowest exposure quartile. Results from this study showed a dire need for research to evaluate subtler, yet significant impacts of maternal aflatoxin exposure and birth outcomes. A study in the United Arab Emirates also showed high *in utero* aflatoxin exposure (n=201) [32]. In this study, 53% of maternal serum samples and 37% of cord serum samples taken at birth tested positive for serum aflatoxin B1-lysine adducts. Like the study conducted in Ghana, this study also showed a significant negative correlation ($p<0.001$) between birth weight and aflatoxin M1. An older study in Kenya had shown half of the pregnant women tested had been exposed, and also found mean birth weights of females born to aflatoxin positive mothers (n=125) were significantly lower than those born to mothers free of aflatoxins (255 g) [23].

On the other hand, two studies showed no link between prenatal aflatoxin and birth weight. Maxwell et al. [25] collected cord blood samples from 625 babies in Nigeria and tested them for various aflatoxins. Findings from that study did not show a link between any of the aflatoxins in cord blood and birth weight in Nigeria. A more recent study in The Gambia by Turner et al. [7] investigated the effect of maternal *in utero* aflatoxin exposure on infant growth in the first year of

life. This study showed no significant association between maternal aflatoxin exposure and lower birth length. However, this study showed a strong effect of maternal aflatoxin exposure during pregnancy on growth during the first year of life. Results showed a reduction in serum aflatoxin B1-lysine adduct levels from 100pg/mg to 10 pg/mg would lead to a 2 centimeter increase in height in the first year of life. This Gambian study controlled for sex, age, placental weight, maternal weight, gestation duration and season. However, it did not control for potentially confounding factors such as income or diet.

Table 1.Compilation of previous studies examining prenatal exposure to aflatoxin and birth outcomes

Authors	Year	Country	Design	Aflatoxin	n	(Geometric) mean and range	Birth outcomes	Significant association / direction
DeVries et al.	1989	Kenya	Longitudinal (pregnancy and birth)	B1,Bl, MI, M2,GI,G2	125	*AFMl and AFM2:* Mean data not available Range: 12-1689 pg/ml *AFB1:* Mean data not available Range: 87-11574 pg/ml	Weight	Yes/negative, (girls only)
Maxwell et al.	1993	Nigeria	Cross-sectional prospective	B1, Bl, MI, M2, GI, G2	625	Mean data not available Ranges (ng/l): *AFMl*: 32-11 354 *AFM2*: 14-3644 *AFB1*:168-69973 *AFB2*:15-144 *AFG1*:97-16543 *AFG2*:15-275	Weight	No (cord)
Abdulrazzaq et al.	2002	UAE	Cross-sectional prospective	M1, M2 and B1	201	*AFM1:* 1108pg/ml Range 110–4060 pg/ml *AFM2:*	Weight	Yes/negative (with cord AFM1)

						geometric mean 854 pg/ml Range 210–3700pg/ml *AFB1:* 2040 pg/ml Range: 228–15225 pg/ml		
Turner et al.	2007	The Gambia	Longitudinal (pregnancy birth and up to 1 year of life)	B1	138	*AFB1* Geo mean: 40.4 pg/mg Range: 4.8–260.8	Length Weight	No No
Shuaib et al.	2010	Ghana	Cross-sectional	B1	785	Mean: 10.9 pg／mg Range: 0.44–268.73 pg／mg	SGA LBW Preterm delivery Stillbirth	No Yes/negative No No
Andrews-Trevino et al.	2018	Nepal	Longitudinal (pregnancy birth and up to 2 years of life)	B1	1621	*AFB1* Geo mean: 1.37 Range: undetectable (<0.4)-147 pg／mg	LBW SGA Stunting at birth Stillbirth	No No No No

Note: Sorted by date of publication

CHAPTER 3. METHODS

This section describes in detail the data sources and methods used in this dissertation. To address the aforementioned specific aims, this dissertation used quantitative data from the USAID-funded AflaCohort Study in Banke, Nepal.

Research Setting

All research in this dissertation was conducted in the tropical, rural district of Banke in Nepal. Banke is situated in the mid-western southern plains (Terai) region, along the border of the Indian state of Uttar Pradesh. Stunting rates are 39% in this part of the terai [16]. These rates are higher than the national average. Adult literacy rates are 49% for women and 66% for men [92]. While Nepali is the main language spoken in Banke district, other languages such as Awadhi, Tharu, Urdu, and Hindi are also common [92].

According to a report [92] by the Ministry of Agricultural Development's (MoAD) Agriculture Management Information System in Nepal, Banke is a highly productive low-altitude agricultural region. It is one of the top 20 highest rice producing districts in the country. Wheat, maize, lentils, fruits (e.g. mangoes, bananas) and herbs (e.g. chamomile, menthe) are also commonly cultivated in the area. Agriculture is predominantly rain-fed. Smallholder farming is the most common type of farming in the area, with 75% of farmers having less than 1 hectare of land. An average of two crops are cultivated in rain-fed lands while farmers with irrigation systems typically grow 3 crops a year.

Rainfall is highest in the Terai during the monsoon season. April, May, and June are the hottest months with high maximum daily temperatures of 35-40°C. December to February are the coldest, driest months with average daily temperatures ranging from a low of 6 to a high of 30°C [92].

AflaCohort Study

The AflaCohort Study[3] is an ongoing longitudinal observational birth cohort that started in July 2015. The study follows 1675 mother-infant pairs during the first 100 days - from conception through two years of age. The AflaCohort Study is ongoing and is expected to be complete in March 2019, when all enrolled infants have reached two years of age. This research is an ongoing collaboration between the Innovation Lab for Nutrition (led by Tufts University), the Patan Academy of Medical Sciences and Helen Keller International-Nepal. Other partners include the Child Health Division, Ministry of Health of the Government of Nepal, Purdue University, University of Georgia, Kathmandu Medical College and the Nepalgunj Medical College Hospital.

The main aims of the AflaCohort Study are the following: a) examine the relationship of maternal aflatoxin exposure in pregnancy and birth outcomes, including infant birth weight; b) examine the relationship of exposure to mycotoxin of infants through breast milk and their linear growth; c) examine the relationship of exposure to mycotoxin through complementary feeding and linear

[3] This trial was registered at clinicaltrials.gov as

growth; d) enumerate the relative contributions of maternal and infant mycotoxin exposures in impairing linear growth, controlling for other potential explanatory factors; and e) examine dietary exposure to mycotoxins from a sub-sample of households via collection and analysis of commonly consumed crops such as maize, chilies, rice and groundnuts[4].

The research team worked closely with a network of Female Community Health Volunteers (FCHVs) to recruit pregnant women in the communities. The FCHV directed the study team to participating households. Study personnel also frequently interacted with mothers' groups made up of female volunteers at a village level during the pregnancy census, enrollment and recruitment periods. Given the prevalence of Awadhi in the area, all study tools were translated from English into both Nepali and Awadhi and back translated to ensure accurate translation.

All AflaCohort research was conducted in 17 Village Development Committees of the Banke district in the mid-western development region of Nepal between February 2015-March 2017. The 17 VDCs included in the study were: Basudevpur, Bageswari, Bankatawa, Belahari, Ganapur, Khaskarkando, Khajurakhurda, Kohalpur, Manikapur, Puraina, Puraini, Rajhena, Samsherganj, Sonpur, Tithiria, Udharapur and Udayapur. In 2017, the Nepali Ministry of Federal Affairs and Local Development dissolved VDCs and replaced them with

[4] 2017-2019 Partnership with the Future Innovation Lab for the Reduction of Post-Harvest Loss

rural municipalities[5]. Since the research was conducted prior to the disbanding of VDCs, the old structures were maintained throughout the analyses.

Inclusion criteria for the birth cohort included: a) healthy pregnancy; b) aged 16-49; c) less than 30 weeks of gestation; d) planning to deliver and remain in the study area during the duration of the study; e) singleton pregnancy. Exclusion criteria included: a) severe anemia (<7 g/dL), pregnancy induced hypertension (blood pressure >140/90 mm Hg), severe malnourishment (MUAC <17.5 cm).

This longitudinal study involves several time points: 1) prenatal; 2) birth; 3) child 3 months of age; 4) child 6 months of age; 5) child 9 months of age; 6) child 12 months of age; 7) annual follow-up; 8) child 18 months of age; and 9) child 24 months of age (**Figure 1**).

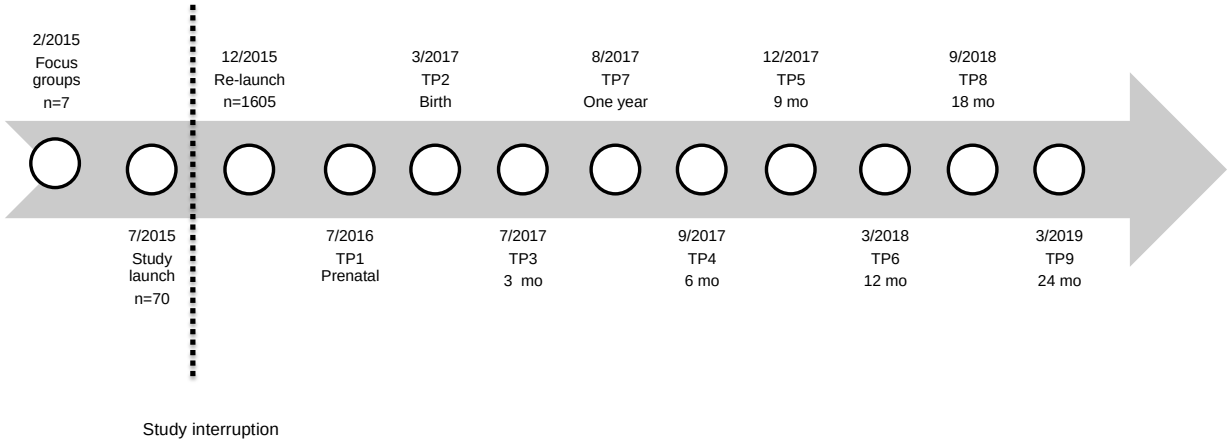


Figure 1. AflaCohort timeline

Research Team

A total of 6 supervisors, 17 enumerators, 17 field assistants and 5 nurses

[5] The Himalayan Times, March 2017

were hired for the study. Each enumerator oversaw data collection for one VDC. Field assistants were locals who served as liaisons between the community and the research personnel.

To ensure uniformity of training and fieldwork procedures, all field staff were hired and trained in January - March 2015. Given the ethnic and linguistic diversity in Banke, field team members were hired locally and were fluent in both Nepali and Awadhi, the predominant languages in the area. The original training, given in Nepali, was held from February to March 2015.Supervisors, enumerators and nurses were given theoretical and practical training using lectures, exercises and field practice. Field assistants were trained to assist enumerators during anthropometric data collection and nurses during venous blood draws. During data collection training, supervisors, enumerators and nurses were assessed for proper scoring and data response entry. A final assessment of each trainee's understanding of the study protocol, ethical standards and data entry procedures took place at the end of the training period and ensured proper understanding of the research methods and purpose. Given the longitudinal nature of the study refresher training courses were provided to all team members on a regular basis.

Ethical Approval

The Nepal Health Research Council (NHRC) Ethical Review Board (ERB) and the Tufts Health Sciences (HSC) Institutional Review Board (IRB) approved both the qualitative study and the birth cohort study. The research team also

obtained permission from local authorities, such as District Public Health Officers (DPHO) before commencing research activities.

All men and women who met enrollment criteria for the focus group study were asked to give verbal consent for participation prior to their participation. All pregnant women who met enrollment criteria for the birth cohort study were asked to give verbal and written consent for participation in the study prior to their participation. Each pregnant woman, and in some cases legal guardian, was informed of the nature of the study and written consent was obtained.

All respondents were de-identified prior to data acquisition, and IRB approval was obtained prior to receiving data. Data were stored in a restricted-access folder on a shared drive managed by the study team at Tufts University and was accessible through a password-protected portal. Data were checked for accurate coding and consistency between follow-up measures.

Qualitative Focus Groups to Guide Quantitative Studies

As part of the planning phase for the birth cohort study, seven semi-structured focus groups (4 female, 3 male) were conducted in Nepali, Awadhi and Tharu with Banke district farmers. Participants included women (n=40) and men (n=34) ages 18 and above, representing a mix of ethnic groups - Brahmin, Muslim, Madhesi, Dalit, Magar, Janjati, Chhetri and Tharu. All participants either were farmers or had some training in farming. The setting included two rural Village Development Committees (Bankatawa and Sonpur) and two urban municipalities (Kohalpur and Manikapur) in the Banke district. Participants were asked to define

food safety, factors influencing food safety in their home and community, and perceptions and effects of mold/fungus infestations. Inductive methods were used to code and identify themes in the study transcripts.

Recruitment took place at local health posts by study moderators, local health personnel, and through door-to-door visits by Female Community Health Volunteers (FCHVs) and agricultural extension workers, who serve their neighbors as primary points of contact for health and agriculture. Verbal recruitment sessions included basic information about the focus group including: time, date, and location of focus group, compensation provided, structure of focus group, and topics to be covered. After providing verbal consent, respondents received printed copies of the consent form to take home. Focus groups were convened a few days after recruitment.

Questions were asked about what food safety means, how to keep food safe, and local problems with food safety. Respondents were asked about mold and aflatoxins. Responses were prompted in relation to certain stages of food production or consumption (e.g. pre-harvest, post-harvest, storage and consumption).

Focus group transcripts were translated into English and analyzed using NVIVO software. Two coders coded the English focus groups, with 50% double coding (3 focus groups). The coders first independently coded three common focus groups. Data were coded using the key word approach, which consists of reading the focus group notes thoroughly and generating and assigning numerous categorical codes to each comment to identify recurring themes and

ideas within the focus group text. More specifically, each coder created codes by labeling words, phrases, sentences or sections; by looking for repetitions, surprising remarks or statements; by identifying similar findings that had been found before; or by identifying a theory or a concept that was relevant. Both analysts brought together similar codes to create categories and themes. Once the independent coding was complete, the two coders discussed each code and adjudicated differences. Comments were rearranged accordingly as new insights and patterns emerged.

The qualitative data were used to assess local definitions and current understanding of harmful effects that mold, fungus and/or aflatoxins can have on human health. The analysis provides a thematic analysis of farmers´ agricultural, food safety and crop storage knowledge and practices in Banke. The findings were used to improve the birth cohort study questionnaires.

Data Collection

Questionnaire

Once enrolled, each participant was interviewed individually in her home. Enrollment and prenatal data collection started in July 2015, but was halted for 3 months due to nation-wide strikes in August-October and resumed in December 2015. Survey data were collected electronically using an ODK Collect application on Android tablets. Data were transferred daily to a secure central database either by wireless technology directly or by the transfer of SD storage cards to laptop computers with a wireless connection.

Capillary and Venous Blood Sample Collection

Within a week of the prenatal interview, a nurse visited each pregnant woman in her home during the morning hours to collect a blood sample. The nurse collected fingerprick (capillary) blood for anemia testing (using HemoCue® Hb 301 Systems) and a venous blood sample for aflatoxin B1 testing. Participants were immediately notified of their hemoglobin levels and were referred to the nearest government health facility if hemoglobin levels were below 11 g/dL (moderate/severe anemia). Women with severe anemia (7 g/dL) were referred to the closest health clinic and the clinical research team members visited women continually to encourage proper and timely treatment. Once the nurse had verified the participant was neither undernourished (MUAC <17.5) or severely anemic, she proceeded to collect a single 3-5 mL of blood from the antecubital vein using 21 Gauge disposable needles and 5ml BD Vacutainer® blood collection tubes.

To ensure sample integrity, samples were stored in cool boxes filled with wet ice and transported to the laboratory for processing within 5 hours of collection. Blood was allowed to clot for half an hour at room temperature and subsequently centrifuged at less than 5000 RPM for 10 minutes. Serum was frozen at -20 degrees Celsius or lower and within a week air-shipped to a -80 degrees Celsius freezer located at the Patan Academy for Health Sciences in Kathmandu. Once in Kathmandu, samples were frozen at -80°C until ready for further analysis in the United States.

Determination of Aflatoxin B1 in Maternal Sera Samples

A 400-microliter aliquot for each pregnant woman was air-shipped on dry ice to the University of Georgia (UGA) Mycotoxin Laboratory for aflatoxin B_1-albumin adducts analysis. A high-performance liquid chromatography (HPLC) with fluorescence detection method was used to assay serum samples for the presence of aflatoxin-albumin conjugate [93-95]. Samples were analyzed for aflatoxin covalently bound to blood albumin via lysine. Aflatoxin results were adjusted for serum albumin concentration. This aflatoxin B1 measurement reflects exposure during the previous 2-3 months [96]. In accordance with standard practice, values under the lower limit of detection (LOD) of <0.4 pg aflatoxin-lysine/mg albumin were substituted with a constant value of half the LOD [97].

Variable Definitions and Derivations (Chapters 4-6)

Below are descriptions of all derived variables used in analyses throughout the three dissertation chapters.

Maternal Characteristics

Logged Aflatoxin Level

Given the positive-skewed nature of the maternal aflatoxin albumin adduct data (the outcome variable for Chapters 4 and 5 and main predictor in Chapter 6), aflatoxin levels were natural log transformed prior to statistical analyses. The

logged functional form is commonly used to transform serum aflatoxin levels to handle the long right tail of the data caused by participants who have high levels. Original values ranged from undetectable (<0.4) to 147 pg/mg AFB1-lysine albumin adducts and logged values ranged from -1.61 to 4.99. There is no established safe level of exposure [98]; therefore, for these analyses any detected level of aflatoxin was considered potentially harmful.

Age

An age variable was created to reflect 5 age categories in years: below 20, 20-24, 25-29, 30-35 and 35 or above.

Education

Maternal number of years of education completed was categorized into four groupings meaningful in Nepal [99]: no education, some primary education (1-5 years), some secondary education (6-10 years) and completed secondary or more (more than 10 years).

Anemia

As per the WHO recommendations [100], hemoglobin values of less than 11 g/dL were defined as anemia (included mild, moderate and severe anemia). Women with hemoglobin levels lower than 7 g/dL were classified as severely anemic.

MUAC

A wide range of MUAC cut-offs have been used in studies among pregnant women [101]. The most commonly used cut-offs were between 22-24 cm. Therefore, for this study we defined low MUAC during pregnancy as less than or equal to 23 cm.

Low Stature

In line with previous literature showing a link between maternal stunting and SGA [102, 103] short maternal stature was defined as height of <145 cm.

Minimum Dietary Diversity-Woman (MDD-W)

Participants were asked about consumption of 49 pre-determined food items in the past seven days and 24 hours (**Table 2**). Data from the Food Frequency Questionnaire (FFQ) were used to calculate dietary diversity (DD) scores, a commonly used proxy for diet quality [104]. Minimum diversity measurements were calculated using FAO Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [104]. Food items from the 24-hour dietary intake recall were categorized into one of the 10 FAO MDD-W food groups: (1) grains/roots/tubers, (2) pulses, (3) nuts and seeds, (4) dairy, (5) meats, (6) eggs, (7) dark green leafy vegetables, (8) other vitamin A sources, (9) other vegetables, (10) other fruits. Scores were analyzed both continuously and as a dichotomous indicator for whether participants achieved minimum dietary diversity (defined as consuming at least 5 of the 10 food groups in the previous

24 hours (day or night)). Women were coded as 0 if they consumed fewer than 5 food groups and 1 if they had consumed at least 5 food groups in the past day.

Table 2. Food items included in the Food Frequency Questionnaire

	Food Item(s)	How many times have you eaten __ in the past 24 hours?	FAO MDD-W food groups code
3.2.1	Rice bhat/riceroti		1
3.2.2	Corn dhido/bhat/roti		1
3.2.3	Wheat/buckwheat dhido/roti		1
3.2.4	Millet dhido/roti		1
3.2.5	Daal (any)		2
3.2.6	Maseura		2
3.2.7	Other legumes (including chickpeas, dried peas, lima beans and soybeans)		2
3.2.8	Groundnuts		3
3.2.9	Milk		4
3.2.10	Curds/whey		4
3.2.11	Milk tea		4
3.2.12	Vegetable oil (any)		-
3.2.13	Ghee		-
3.2.14	Hydrogenated oil (Banaspati Ghee)		-
3.2.15	Eggs (any)		6
3.2.16	Chicken/duck		5
3.2.17	Goat		5
3.2.18	Buff		5
3.2.19	Pork		5
3.2.20	Large fish (fresh)		5
3.2.21	Small fish (fresh)		5
3.2.22	Dried fish		5
3.2.23	Snails		-
3.2.24	Dark green leafy vegetables		7
3.2.25	Carrots		8
3.2.26	Gundruk		9
3.2.27	Ripe pumpkin		8
3.2.28	Green beans (Bodi, simi)		9
3.2.29	Green peas (mutterkosa)		9
3.2.30	Gourd (lauka, ghiraula, bitter, titekarela, jhimni)		9

3.2.31	Okra/Ladies finger/Bhindi		9
3.2.32	Eggplant		9
3.2.33	Potatoes		1
3.2.34	Tomato		9
3.2.35	Cauliflower		9
3.2.36	Cabbage		9
3.2.37	Drumstick (sajCHan)		9
3.2.38	Green jackfruit		10
3.2.39	Ripe mango		8
3.2.40	Jackfruit (ripe)		10
3.2.41	Guava		10
3.2.42	Orange/tangerine		10
3.2.43	Ripe papaya		8
3.2.44	Apple		10
3.2.45	Pineapple		10
3.2.46	Banana		10
3.2.47	Jaard/Rakshi		-
3.2.48	Instant Noodles (packet, e.g. Wai Wai)		1
3.2.49	Snacks (beaten rice, puffed rice, sweets, biscuits, dalmot, popcorn)		-

Food group	**Code**
Grains/roots/tubers	1
Pulses	2
Nuts and seeds	3
Dairy	4
Meats	5
Eggs	6
DGLV	7
Other vitamin A sources	8
Other vegetable	9
Other fruit	10

Household and Environmental Characteristics

Wealth Index

Public health and policy research is often concerned with differential impacts of socioeconomic status (SES) on health determinants or outcomes. However, SES is often difficult to identify, especially when numeric measurements of

welfare such as income or consumption and expenditure data are unavailable or unreliable (common in developing contexts) [105]. An alternative to numeric welfare measurements is the construction of a wealth index using Principal Components Analysis (PCA) [105-107]. Previous researchers have undertaken validation of PCA-based SES indices using Demographic Health Survey (DHS) data [107, 108].

This proxy measure, based on measures of living standards, offers numerous advantages to numeric welfare measures such as a shorter data collection period, lower participant burden, and less recall bias and non-response [105, 107]. Direct observation of certain household characteristics (e.g. materials for walls, floors, roof) and straightforward questions with clear response categories reduce both enumerator and participant burden. Although still subject to measurement error, wealth indexes suffer fewer reporting errors than numeric welfare measurements [105, 107] when measuring SES.

For this dissertation, the PCA technique was used to construct a wealth index for the AflaCohort Study. The first step was to determine the assets and household data to be included in the PCA. Durable assets like those used in the DHS wealth index calculations such as radio, television, mobile phone, bicycle, motorcycle and electric fans were used in the AflaCohort wealth index calculation. Other household data such as household members per room, infrastructure materials (walls, roofs and floors), access to electricity, cooking fuel, home ownership, access to piped water, toilet (shared/not) and livestock ownership were also used in the PCA. Selection of assets was based on whether

ownership of the household item was associated with greater purchasing power as measured by ownership of other items. Fewer than 1% of the participants were missing data for one or more of the indicator variables; in those cases, missing values were replaced with the mean/mode of the variable.

Subsequently, frequencies and descriptive data were run on all variables to be considered for the PCA. Dichotomous variables were created for each category of all categorical variables while the number of household members was coded as a continuous variable. Indicator variables with little or no variation (zero standard deviation) were eliminated from the PCA. A wealth index score was created using a summation of individual weighted factor scores (**see Appendix 1**). A variable with a higher score is generally associated with higher SES, and negative scores are associated with lower SES [107]. Wealth index scores for this sample ranged from -9.11 to 10.16. Based on these values, households were categorized into one of five wealth quintiles. Internal coherence was examined by comparing housing characteristics and mean asset ownership by wealth index quintile. **Table 3** shows how the housing characteristics and mean assets ownership varied by socioeconomic group. Housing characteristics and asset ownership improved or increased by socioeconomic group.

Table 3. Housing characteristics and ownership of durable assets by wealth index quintile

	Poorest	Poor	Middle	Rich	Richest
Household members per room	3.5	3.2	3.1	2.8	2.6
Finished Wall	32	49	69	80	84
Finished Roof	43	66	75	83	89
Cement Floor	10	11	22	37	50
Electricity	61	87	92	98	100
Improved Cooking Fuel	15	20	25	34	46
Own Home	83	94	96	98	100
Piped Water	3	9	19	34	62
Treated Drinking Water	4	3	3	1	2
Improved Toilet	46	61	70	71	84
Do Not Share Toilet	27	41	54	57	72
Own Livestock	34	51	59	65	74
Radio	2	5	8	15	39
TV	17	44	64	77	89
Mobile	85	95	94	97	96
Bicycle	62	85	87	89	95
Motorcycle	2	5	12	23	49
Electric Fan	44	78	87	93	98

All numbers are percentages except number of household members

Seasonality

Season was initially classified into 6 seasonal categories: spring (Baisakh and Chaitra), summer (Jestha and Ashar), rainy (Shrawan and Bhadra), autumn (Ashwin and Kartik), pre-winter (Mangsir and Poush), and winter (Magh and Falgun) [109]. Data were captured during all seasons except for autumn. Given that the highest aflatoxin levels were seen in women measured in pre-winter and winter these seasons were merged into a dichotomous winter variable for the multivariate regression models.

Chapter 4 Methods

The first study aimed to identify food items associated with higher aflatoxin levels during pregnancy and to determine if greater dietary diversity was correlated with lower levels of maternal aflatoxin.

Research Subjects

A household survey was successfully administered to 1665 of the 1675 enrolled women. Of those, 1648 had both dietary and serum aflatoxin data.

Study Design and Variable Computations

The primary outcome was maternal serum aflatoxin level, measured as pg of aflatoxin B1 lysine adducts per mg of albumin. Maize consumption the past week, groundnut consumption in the past week, and dietary diversity were the primary explanatory variables of interest. Maize and groundnuts were chosen as foods of interest because existing literature showed they were likely to be contaminated with aflatoxin [10, 41]. Other foods of interest were chilies and milk. Chili consumption data were not available in the food frequency questionnaire.

A trained enumerator administered food frequency questionnaires to each pregnant woman. Data were collected on the frequency of consumption of foods that are likely to be contaminated with aflatoxin. Respondents were asked about all foods they consumed the previous day, and in the previous 7 days, inside and outside the home. A nurse visited each woman in her household for the venous blood draw no later than one week after collecting the dietary data to ensure

comparability between the dietary and serum aflatoxin data. Aflatoxin-prone food consumption variables were entered as in their original continuous formats (i.e. number of occasions) for statistical analysis.

Enumerators also administered yearly household food consumption questionnaires. The questionnaire covered a list of 75 food items, including rice, chilies, groundnuts, milk, and maize. Consumption of these foods was entered as binary variables (i.e. consumed yes or no in the past year for statistical analysis. For some women, data for yearly consumption values were randomly missing. Values for these respondents were replaced with the mode of the observed values for variables that showed significance in the bivariate analysis and had less than 1% missing values.

Statistical Analysis

Descriptive statistics were used to describe participant characteristics. Exploratory analysis included box plots and scatter plots to detect potential outliers. Levels of aflatoxin exposure were categorized into quintiles based on the observed distribution of data for descriptive purposes. The highest quintile included women with aflatoxin B1 levels of greater than or equal to 2.9 pg/mg. The aflatoxin albumin adducts data (the outcome variable) had a strong positive skew and were therefore natural log transformed for the bivariate statistical analyses. To avoid loss of information, all variables were first entered in a continuous format and subsequently converted into categorical variables as needed. Bivariate analyses included Student’s t-tests, Pearson’s correlations and

analysis of variance (ANOVA) for continuous variables and chi-squared tests for binary variables. All statistical tests were two-sided. P-values of less than 0.05 were considered statistically significant. All statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX).

Covariate-adjusted parameter estimates with 95% confidence intervals were computed using two multivariate regression techniques - ordinary least squares (OLS) and quantile (median) regression (QR) [110] to model the association between diet and maternal aflatoxin exposure. QR allowed us to better understand the relationship at different points in the conditional distribution of serum aflatoxin levels and was more robust to outliers in the data. Instead of merely studying the relationship to the conditional mean of (logged) aflatoxin levels, QR provided a more comprehensive characterization of the aflatoxin data across different points of the distribution (i.e. 10^{th}, 30^{th}, 50^{th}, 70^{th}, and 90^{th} quantiles). Following the QR, restricted cubic splines (RCS) and non-parametric smoothing curves were incorporated to test for an unadjusted non-linear relationship between aflatoxin levels and weekly maize and groundnut consumption.

Covariates in the full model were selected based on their potential for confounding the relationships of interest in this study. Models were constructed using backward elimination, where all reasonable confounders were initially included and removed if they were neither statistically significant at $p<0.05$ nor biologically relevant. Participants with missing data (n=27) were dropped from the models. Maternal age, education and wealth index were explored as both

continuous and categorical variables in the regression models. The final OLS and QR models included continuous age, education, wealth index, and MUAC.

$$Log\ (maternal\ aflatoxin\ levels) = \beta_0 + \beta_1\ (frequency\ of\ maize\ consumption\ in\ the\ previous\ week) + \beta_2\ (frequency\ of\ groundnuts\ consumption\ in\ the\ previous\ week) + \beta_3\ (frequency\ of\ milk\ consumption\ in\ the\ previous\ week) + \beta_4\ (dietary\ diversity\ score) + \beta_5\ (age) + \beta_6\ (years\ of\ education) + \beta_7\ (wealth\ index\ score) + \beta_8\ (MUAC) + \beta_9\ (winter\ season) + \beta_{10}\ (location) + u$$

Chapter 5 Methods

In Chapter 5 we used data from the prenatal time point to examine the relationship between household sorting practices and selected GAPs and serum aflatoxin levels in pregnant women.

Application of Focus Group Findings to Birth Cohort Study Tools

Participants perceived food safety as a problem and expressed concern regarding the challenges they encounter during production and storage of food crops. Food safety problems identified were related to: pests and diseases, molds, overuse of pesticides, unpredictable weather, infrastructure (irrigation, storage facilities), lack of agricultural inputs (especially seeds) and lack of knowledge on proper farming techniques. Although some participants mentioned harmful effects of mold/fungus on food, participants had not heard of aflatoxins.

When participants were shown pictures of foodstuffs infested with *A. flavus* (one type of mold that produces aflatoxins) some identified rice, potatoes, maize, wheat, peanuts and chilies as crops particularly susceptible to these types of molds/fungal infestations. The participants identified several food safety mitigation strategies: 1) ensuring crop safety from pre-harvest to storage (improved knowledge and inputs, natural and synthetic protection from pests and diseases, appropriate drying and storage behaviors and infrastructure), 2) safeguarding crops before consumption (covering, rinsing, cleaning), and 3) processing moldy crops by re-drying in the sun, discarding, selling or producing alcohol.

Research Subjects

Of the 1675 women enrolled in this study, 1648 with data aflatoxin biomarker and survey data were included in analyses relating to aflatoxin awareness, livestock ownership, households' sorting practices and food acquisition. Analyses regarding GAPs were limited to sub-sets of women from those agriculturally productive households producing each of the aflatoxin-prone crops (i.e. maize, groundnuts, chilies [10, 41]). To avoid attenuating the relationship between GAPs and levels of aflatoxin in the women's serum, households that did not consume any of the crop in question or who purchased or received the crop in addition to home-producing it, were excluded from the analyses. A total of 421 women were from maize-producing households; of these, 396 households exclusively consumed home-produced maize. Of the 37 women in groundnut-producing

households, 25 reported consuming home-produced groundnuts only. There were 95 chili producers, and of these 37 reported eating only chilies they had grown themselves.

Study Design and Variable Computations

Maternal serum aflatoxin level was the primary outcome in Chapter 5. Women in farming households were asked a series of questions regarding pre-harvest, harvest, and post-harvest practices. Enumerators administered the agricultural questions during the prenatal visit. Among other things, respondents were asked about the length of storage of the crop. A storage period variable was created to reflect 5 categories based on length of storage: did not store the crop, 1-3 months, 4-6 months, 7-9 months, or 10 or more months.

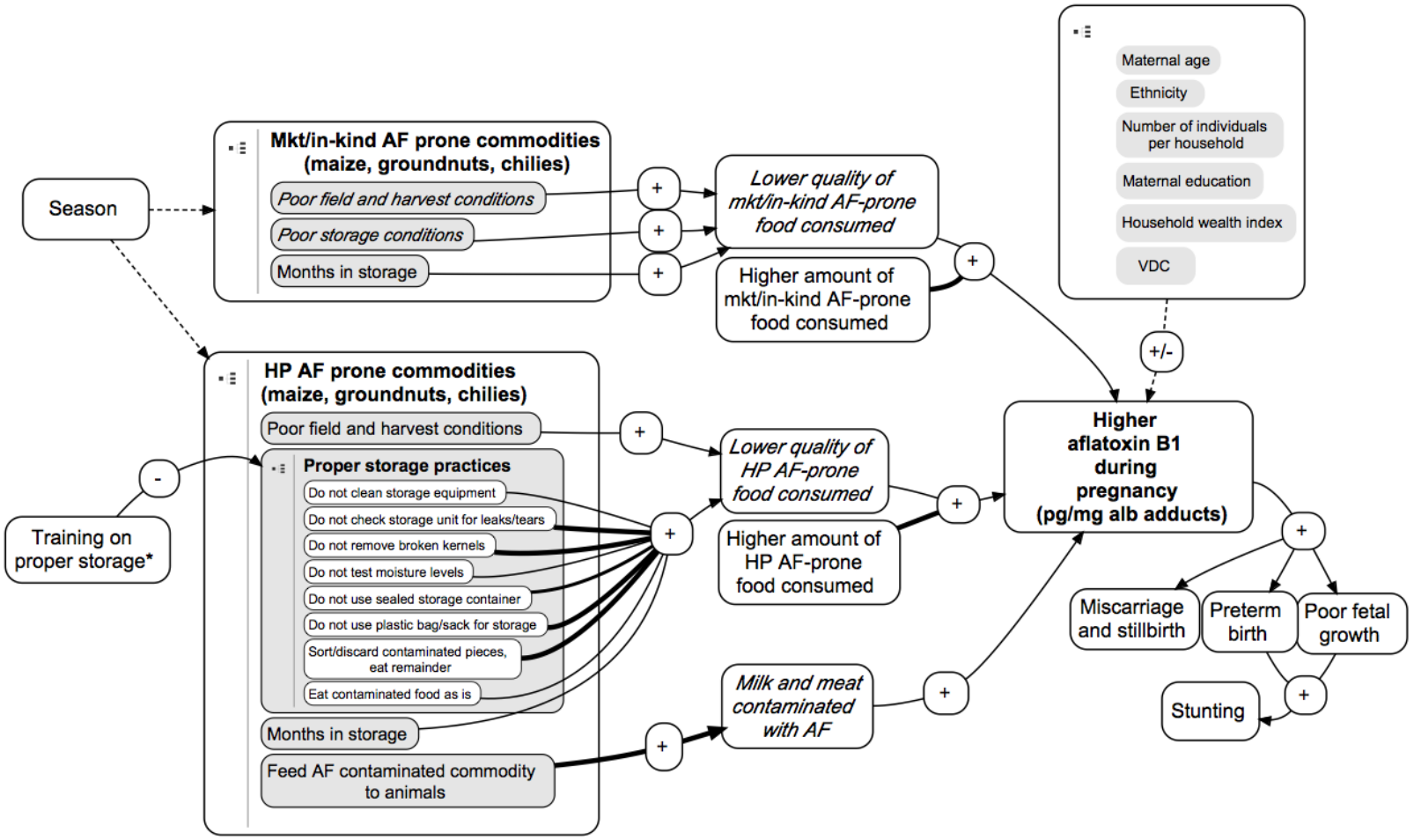
Statistical Analysis

Statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX). Descriptive statistics were calculated for the sample and for GAPs among households producing maize, groundnuts or chilies. Exploratory analysis included visualizing the data via histograms, box, or scatter plots. Inconsistencies in the data were flagged and corrected with the field team, and missing data were imputed for a few cases (<1% missing or don't know responses).

Aflatoxin levels were natural log transformed due to their right-skewness of the data. To avoid loss of information, continuous variables were initially entered

in their original format and subsequently converted into categorical variables on an as needed basis (i.e. aflatoxin levels, months of storage, and days drying in the field). Bivariate associations were evaluated using Student's t-tests, Pearson's correlations and ANOVA for continuous variables and chi-squared tests for binary variables. Two-tailed p-values less than 0.05 were considered statistically significant.

We originally attempted to construct a GAP index using PCA, which creates an index by summing the weights of individually weighted variables. We explored multiple combinations of GAPs known to help prevent aflatoxin contamination; however, individual GAPs did not show correlation to each other (Cronbach's alpha=0.1065). Given the complexity of aflatoxin contamination, we also used Path Analysis to determine potential pathways by which GAPs could influence maternal aflatoxin levels. This analysis did not identify significant pathways by which GAPs were found to affect maternal aflatoxin levels. **Figure 2** represents the hypothesis about the relations among variables.



Commodities include 3 high aflatoxin-prone foods: maize, groundnuts, chilies;
HP, home-produced; mkt, market-sourced; AF, aflatoxin; pg, picograms; mg, milligrams; alb, albumin; VDC, Village Development Committee
Italix: data not available; Black solid arrows, major pathways; Dotted arrows, control pathways; Arrows proportional to sample size
* Including aflatoxins as well as other pest, disease and abiotic problems of stored grains

Figure 2. Conceptual framework GAP and serum aflatoxin levels

The unanticipated small sample size for groundnut and chili farmers limited our ability to run multivariate logistic models examining the relationship between GAPs and serum aflatoxin levels in the women. Covariates in the OLS model for maize producers were selected based on their potential for confounding the relationships of interest in this study. Four women from maize farming households with missing data were dropped from the model.

$$Log\ (maternal\ aflatoxin\ levels) = \beta_0 + \beta_1\ (plastic\ sheets/tarps\ for\ drying\ maize) + \beta_2\ (months\ of\ maize\ storage) + \beta_3\ (method\ for\ determining\ maize\ dryness) + \beta_4\ (rodent\ attacks\ during\ maize\ storage) + \beta_5\ (storage\ problems) + \beta_6\ (frequency\ of\ maize\ consumption\ in\ the\ previous\ week) + \beta_7\ (frequency\ of\ groundnut\ consumption\ in\ the\ previous\ week) + \beta_8\ (age) + \beta_9\ (winter\ season) + \beta_{10}\ (location) + \beta_{11}(wealth\ index\ score) + \beta_{12}\ (years\ of\ education) + u$$

Chapter 6 Methods

Chapter 6 aimed at identifying the correlation between *in utero* aflatoxin exposure and adverse birth outcomes.

Research Subjects

Field Assistants played a crucial role in informing enumerators when a mother had delivered her child. A total of 1675 pregnant women were enrolled in the study from July 2015 to July 2016. Ninety-five percent (n=1634) of mothers were

visited as soon as the team was informed of the child's birth and within one week after the birth of their child (between August 2015 and March 2017). Those who did not receive post-partum visits had either chosen to end their participation in the study or been lost-to-follow-up prior to their child being born (n=41). An additional 13 women were missing serum samples and were excluded from analyses. Due to the fact that infants tend to lose weight during the first few days of life [111], 77 infants measured more than 72 hours after birth were omitted from the analyses.

Study Design and Variable Computations

The main outcomes of interest for this study were low birth weight (LBW), small-for-gestational-age (SGA), stunting at birth, and preterm birth. Data from the first two time-points (prenatal and birth) of the AflaCohort Study were used for this study.

Sample size estimates for this aim were based on findings from a study in the UAE [32] that assessed the association between serum cord aflatoxin levels and birth weight. Results from the UAE showed the mean birth weights of females born to aflatoxin M1 positive mothers were 225 g lower than those born to mothers free of this aflatoxin. To detect 225 g or larger difference in birth weight, with 80% power, a sample size of 56 pregnant women was necessary for this study.

During the prenatal visit, the in-depth household questionnaire collected information on the following: age, ethnicity, education, smoking habits, nutritional

knowledge, dietary diversity, and last menstrual period (LMP). Principal Component Analysis (PCA) was used to calculate socioeconomic status [16, 106-108, 112]. The FAO Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [104] were used to calculate minimum diversity measurements. Foods consumed in the past seven days were categorized into one of the 10 FAO MDD-W food groups[6]. Minimum dietary diversity was defined as having consumed ≥ 5 of the 10 food groups in the previous 24 hours (day or night) [104].

Maternal hemoglobin levels were measured using HemoCue® Hb 301 systems, and anemia was defined as hemoglobin levels of less than 11 g/dL, as per the WHO guidelines [100]. Maternal height, weight and mid-upper arm circumference (MUAC) were measured using Seca scales, ShorrBoard® Stadiometers, and adult (65 cm) MUAC measuring tapes, respectively, and recorded to the nearest 0.1 kg or 0.1 cm. Very short maternal stature was defined as ≤145 cm [90, 103, 113].

Gestational age was estimated by LMP. Study team members were trained to use a standard protocol when prompting and recording information on LMP. Women were prompted to recall their LMP at the time of recruitment (usually around mid-pregnancy). Calendars were used to ask the women to recall the LMP. Holidays were often used as references. In rare cases when the participant was unable to remember the exact date of her LMP, she was asked if it was at

[6] (1) grains/roots/tubers, (2) pulses, (3) nuts and seeds, (4) dairy, (5) meats, (6) eggs, (7) dark green leafy vegetables, (8) other vitamin A sources, (9) other vegetables, (10) other fruits

the beginning, middle or end of the month. Beginning of the months was coded as day 1, middle as 15 and end as day 30. A portable ultrasound was used to collect gestational age information in some of the women, but given the large numbers of missing ultrasound data and the inaccuracy of ultrasound measurements later in pregnancy [114], LMP was used as the gestational age measurement.

Seca scales and ShorrBoard® Stadiometers were used to measure infant weight and height. To ensure accuracy, each measurement was taken three times and tared weight was recorded to the nearest 0.1 kg and recumbent height to the nearest 0.1 cm. During the birth visits, enumerators also collected data on the child's sex, the number of antenatal care (ANC) visits, location of delivery and complications at birth.

High or Low Aflatoxin Levels

A binary variable was defined to indicate levels either below or at/above the geometric mean of 1.37 pg/mg albumin adducts.[7]

Gestational Age at Delivery

Gestational age at delivery was determined by estimating the duration between the first day of the LMP and the birth date.

[7] Levels of exposure were also categorized into tertiles and quartiles based on the observed distribution of data. However, the lower quantiles had very low variability due to the right-skewed nature of the aflatoxin data. Therefore, this type of categorization was not used in the analyses.

Z-Scores

Infants' length, weight, and age data were used to calculate weight-for-age (WAZ), length-for-age (LAZ), and weight-for-length (WLZ) z-scores using the 2006 WHO Growth Standards [115] as the reference. Z-scores were calculated in Stata using WHO Child Growth Standard Macros [101, 116, 117]. Infants with z-scores that diverged by more than six standard deviations from the WHO reference median were excluded prior to analysis [118]. If infants had age, height, or weight values outside the range of reference values, a value of 99 was assigned for the corresponding z-score. Two infants were excluded for WLZ values less than -6 and one was excluded for a z-score of more than 6. An additional 92 infants were excluded for WLZ analyses for having WHZ score values of 99. Two infants were excluded for HAZ scores of less than -6.

Low Birth Weight (LBW)

As per the WHO definition [119], low birth weight was defined as weighing less than 2.5 kilograms at birth, regardless of gestational age.

Small-for-gestational-age (SGA)

Small-for-gestational-age was defined as born with a weight below the 10th percentile for gestational age and sex. Previous literature has shown how the reference population chosen can strongly affect SGA prevalence [120]. Our study first applied the WHO's recently published multinational fetal growth charts [121]

to estimate SGA prevalence. The INTERGROWTH-21st Project fetal growth standards [122] were also applied to estimate SGA rates in this sample.

The primary purpose of applying both sex-specific growth charts to the data was to identify to what extent the choice of cut-offs influences the rates of SGA and the associations between aflatoxin and odds of SGA. Rates of SGA varied depending on the reference population used (30% with INTERGROWTH and 50% using the WHO charts). Given that the INTERGROWTH-21st Project fetal growth standards are recommended for monitoring of fetal growth via routine ultrasound measurements, we present the SGA prevalence based on the WHO's multinational fetal growth charts. Choice of reference population did not affect the final bivariate or multivariate results.

Stunting at Birth

Stunting at birth was defined as less than or equal to -2 standard deviations below the length-for-age mean standardized z-scores [123], irrespective of gestational age.

Preterm Birth, Stillbirth, and Miscarriage

Additional birth outcomes included preterm birth (PTB) (born before 37 weeks of gestation), stillbirth (born with no signs of life at or after 28 weeks gestation), and miscarriage (fetal loss before 28 weeks of gestation).

Statistical Analysis

Descriptive statistics were used to describe anthropometric and adverse birth outcomes in the sample. Exploratory analysis included visualizing the data via histograms, box, or scatter plots.

Potential confounding variables considered in the data analysis were infant's sex, household socioeconomic status, and maternal characteristics such as schooling, hemoglobin levels, height, parity, MUAC, dietary diversity, antenatal visits and smoking during pregnancy [124]. Models were run separately for preterm and full-term infants to detect possible differences by gestational period. Given that no difference was detectable, final models include both preterm and full-term infants.

We used multivariate Ordinary Least Squares (OLS) regression models to test the significance of prenatal aflatoxin correlates on WAZ, WLZ and LAZ scores. Associations between prenatal aflatoxin exposure and low birth weight (LBW), small-for-gestational-age (SGA), stunting at birth, and preterm birth were analyzed with four separate multivariate logistic regression analyses. Crude unadjusted and adjusted odds ratios (OR) and 95% CIs were calculated for each of the birth outcomes. All models were also adjusted for VDC and month of birth [125, 126]. Low birth weight and stunting models were adjusted for gestational age. Models were also adjusted for trimester in which the antenatal visits data were collected.

$$LBW/SGA/Stunting/Preterm = \beta_0 + \beta_1 (maternal\ aflatoxin) + \beta_2 (infant\ sex) + \beta_3 (wealth\ index\ quintile) + \beta_4 (years\ of\ education) + \beta_5 (hemoglobin\ levels) + \beta_6 (low\ maternal\ height) + \beta_7 (parity) + \beta_8 (MUAC) + \beta_9 (dietary\ diversity\ score) + \beta_{10} (number\ of\ antenatal\ visits) + \beta_{11} (smoking) + \beta_{12} (VDC) + \beta_{13} (trimester) + \beta_{14} (month\ of\ birth) + u$$

A two-tailed p-value of < 0.05 was considered significant in all analyses. Statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX).

CHAPTER 4. Consumption of maize and groundnuts linked to higher aflatoxin levels in pregnant women consuming a rice-dominated diet in Nepal

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Acknowledgements
Support for this research was provided by the Feed the Future Innovation Lab for Nutrition, which is funded by the United States Agency for International Development (USAID) under grant ID: AID-OAA-L-10-00006. The authors express special gratitude to USAID/Nepal Mission, Dr. Maura Mack, Dr. Ahmed Kablan, AflaCohort study team and the study participants, without whom this research would not have been possible. The opinions expressed herein are solely those of the authors.

Authors' contributions

JYAT contributed to the design of the study and was responsible for the analysis and writing of the manuscript. PW and SG were responsible for the overall design and planning of the study and contributed to the statistical analysis and writing of the paper. KB was responsible for the overall design and planning of the study. GS and BLR contributed to the statistical analysis of the study and its interpretation and contributed to the writing of the paper. DD and RS contributed to the design and implemented the study. AP supervised the fieldwork and coordinated acquisition of data. KP contributed to the design of the study. JW conducted the analysis of aflatoxin-exposure markers. All authors reviewed the manuscript for accuracy and approved the final manuscript.

Number of tables: 4
Number of supplemental tables: 0
Number of figures: 2
Target journal: Nutrition (IP 3.42) or British Journal of Nutrition (IP 3.302)

Abstract

Background: Aflatoxin contamination is known to have serious health consequences. Aflatoxins are found in a range of foods widely consumed in the tropics, including maize, chilies, spices and oilseeds. The aims of this study were to investigate: a) the association between consumption of specific foods and levels of serum aflatoxin B_1 (AFB_1) in pregnant women, and b) the correlation between dietary diversity and levels of aflatoxin exposure.

Methods: A total of 1,675 women were enrolled in a longitudinal birth cohort study in Banke, Nepal. Of these, 1,648 had both serum aflatoxin values and complete survey data. Ordinary least squares (OLS) and quantile regression (QR) were used to examine relationships between maternal food consumption and serum AFB_1-albumin levels, adjusting for covariates.

Results: Roughly 94% of the women had detectable AFB_1-levels, ranging from 0.4 to 147 picograms aflatoxin B_1-lysine/milligram of albumin. An observed right-skewed distribution was consistent with previous studies. The mean serum AFB_1 was 3.2 ± 8.3 pg/mg albumin (geometric mean 1.37 (CI: 1.3-1.4) pg/mg albumin). Serum aflatoxin levels were not correlated with mothers' dietary diversity. After controlling for wealth, mid-upper arm circumference (a proxy for the nutritional status), and location, only maize and groundnut consumption in the past week and winter season remained significantly associated with maternal aflatoxin levels.

Discussion: Exposure of pregnant women to diet-associated aflatoxin in this rural population of Nepal seems to be driven by groundnut and maize consumption. Possible explanations for the observed patterns include increased consumption of aflatoxin-prone foods after harvest and during the autumn and winter months and/or consumption of contaminated foods during those seasons resulting from prolonged and improper post-harvest storage of these crops at home or in markets. Intervention strategies in the area should focus on reducing dietary exposure to these toxins via prevention, and early detection and removal of contaminated commodities from the food system.

Keywords: aflatoxin, dietary diversity, groundnuts, maize, Nepal, pregnancy

Introduction

Naturally occurring environmental toxins, such as aflatoxin, are known to be threats to human health. Humans are primarily exposed to aflatoxin via dietary intake [127-130]. *Aspergillus flavus* and *Aspergillus parasiticus* fungi, the primary sources of aflatoxin, grow on damaged foods and rapidly multiply under humid, hot, poorly ventilated and unhygienic conditions. Acute aflatoxicosis causes vomiting, abdominal pain, pulmonary edema, convulsions, coma and death [62]. Chronic exposure to aflatoxin, even at low levels, has been found to be harmful to human health [64, 131-136]. For example, long-term chronic (subsymptomatic) exposure has been linked to liver damage, chronic hepatocellular injury and immunosuppression [137, 138]. Furthermore, animal studies have consistently found that aflatoxin can harm a fetus, impair growth, and contribute to long-term health problems after birth [85].

Whereas aflatoxin-producing molds can be visible, the aflatoxins they produce are difficult to detect and remove because they are colorless, odorless, tasteless, and relatively resistant to thermal inactivation [139]. These hidden toxins can occur throughout the food value chain and in a wide spectrum of foods, from staple crops, such as maize and rice, to foods consumed in smaller quantities (e.g. roasted groundnuts and spices). Commonly consumed commodities such as maize, chilies, spices, oilseeds and nuts are especially susceptible to aflatoxin in tropical conditions of high heat and humidity as well as when certain pests damage crops [140-143]. When ruminants ingest feed contaminated with aflatoxin they can metabolize and excrete aflatoxin M1 in their

milk [144]. More often than not, populations that are at particularly high risk of chronic exposure to aflatoxin are those that are poor, have limited dietary variety, store foods for long periods, and rely on highly susceptible food items such as maize and groundnuts [6, 8].

Concern regarding exposure to mycotoxins has grown in recent years because of evidence showing placental transfer of aflatoxin in humans [2, 7, 32, 145, 146]. Recent literature also suggests a link between aflatoxin and impaired linear growth in children, which is related to child stunting [6, 63, 64]. Cross-sectional evidence from Benin and Togo showed strong inverse relationships between serum aflatoxin B1-lysine adduct levels and height-for-age z-scores and weight-for-age z-scores in children 1-5 years of age, suggesting a role in stunting and underweight [3, 4, 64]. Data from The Gambia similarly showed a strong effect of aflatoxin exposure during pregnancy on growth during the first year of life [7]. Previous epidemiological data demonstrating a link between a high rate of aflatoxin exposure among women and impaired linear growth in their children suggest a need to further explore and measure dietary sources of aflatoxin in vulnerable populations [7, 14, 32].

Previous findings suggest improved dietary diversity can reduce foodborne toxin exposure, particularly in those consuming monotonous diets based on maize, groundnuts, cassava and rice [75, 76]. Access to a greater variety of foods can lower the exposure to aflatoxin by lessening the dependence on aflatoxin-prone foods and counteracting the toxicity [42, 77].

Studies examining food and feed in Nepal have shown aflatoxin contamination may pose a public health risk. A study published in 2005 by Koirala et al. [10] measured the occurrence of aflatoxin in food and feed in Nepal. This study showed food items such as maize and groundnut products commonly exceeded the permissible limit for aflatoxin. A study by Kafle et al. [11] examined raw and pasteurized milk samples in the Kathmandu valley, finding that 44% of samples were contaminated with aflatoxin M1. Due to the established carcinogenic properties of aflatoxin B1 and highly toxic health effects of all aflatoxin, legal aflatoxin tolerance levels have been set for foods destined for human consumption. In Nepal, the Ministry of Agriculture and the Department of Food Technology and Quality Control have set official limits of 20 ppb for food and cereals and 50 ppb for animal feed [71].

Human studies in Nepal have shown evidence of aflatoxin exposure during pregnancy as well as among children younger than 3 years of age, two critical periods of child growth [14, 15, 147]. Groopman et al. [14] showed exposure to aflatoxin in samples collected between 1999-2001 in rural, Nepalese women from the Sarlahi district during their first and third trimesters of pregnancy. In this sample, 94% of the women were exposed to aflatoxin (geometric mean of 25.28 picogram (pg) aflatoxin B_1-lysine/milligram (mg) of albumin). A separate study by Mitchell et al. [15] assessed aflatoxin exposure in children in Bhaktapur, Nepal at 15, 24 and 36 months of age. Levels in these children were similar to those in African populations where serum aflatoxin levels have been associated with poor linear growth. Ninety-one percent of children tested positive for aflatoxin

exposure, and the geometric mean was 3.6 (0.53–149) pg AFB_1-lysine/mg albumin.

It is plausible that greater dietary diversity may lower exposure to aflatoxin even in women whose caloric intake is not prominently dependent on maize and groundnuts, the two commodities with the highest risk of aflatoxin contamination. This study was therefore conducted to: a) assess whether consumption frequency of maize and/or groundnuts was associated with aflatoxin levels in pregnant women with a rice-dominated diet, and b) test if increased dietary diversity was associated with lower levels of aflatoxin exposure.

Methods

Study Design and Setting

This study examined data from a longitudinal rolling birth cohort (AflaCohort) in the Banke district in the tropical mid-western southern plains (Terai) region of Nepal. The birth cohort study is composed of 1,675 woman/infant pairs living in 17 Village Development Committees (VDCs[8]), which are secondary administrative units (above the ward level). A rolling recruitment strategy was used to recruit healthy pregnant women aged 16-49, less than 30 weeks into their pregnancy. Each woman needed to be planning to deliver and remain in the study area. Exclusion criteria included a mid-upper arm circumference (MUAC)

[8] In 2017, the Ministry of Federal Affairs and Local Development dissolved VDCs and replaced them with rural municipalities (The Himalayan Times, March 2017). Since this research was conducted prior to the disbanding of VDCs, the old structures were used throughout the analyses.

less than 17.5 cm, severe anemia (less than 7 g/dL), and hypertension (more than or equal to 140/90 mm Hg). Women meeting any of the exclusion criteria were counseled and referred to the closest government health facility for appropriate health care. A total of 1648 women had both survey and serum aflatoxin data available for analysis.

Protection of Human Subjects

The Nepal Health Research Council (NHRC) Ethical Review Board (ERB) and the Tufts Health Sciences (HSC) Institutional Review Board (IRB) approved this study. The research team also obtained permission from District Public Health Officers (DPHO) before commencing research activities. All pregnant women who met enrollment criteria and wanted to participate in the study were informed of the nature of the study. The women, and in some cases their legal guardians, were asked to give verbal and written consent for participation in the study prior to their participation.

Data Collection

In-depth survey data and venous blood samples were concurrently collected from pregnant women in July-August of 2015 and December 2015-August 2016. Data collection was suspended between August and December 2015 due to nation-wide strikes. Data collection resumed in December 2015. There were several types of data collection.

Household Survey. A household survey was administered to 1665 of the 1675 enrolled women. The survey questionnaire was used to collect information on personal characteristics of the respondent, her health, and diet characteristics of her household. More specifically, data collected from the household survey consisted of information pertaining to: 1) household characteristics (infrastructure, assets, size and composition), 2) maternal characteristics (education, diet/food consumption frequencies in the past week, recent illness, birthing history, empowerment), and 3) agricultural production and food purchases in the past year. The household survey also included maternal anthropometric measurements: MUAC, height and weight. Maternal height and weight were measured using a Shorr Board and a Seca scale, respectively. All data were entered electronically using an ODK Collect application on Android tablets. Data were stored on the tablet and directly transferred daily to a secure central database by wireless technology. The data management team verified data quality and corrected discrepancies on a weekly basis.

Blood Sample Collection. Upon completing the household questionnaires with the data collector, a nurse visited the respondent in her household to collect between 3-5 ml of blood from the antecubital vein of the arm using 5ml BD Vacutainer® blood collection tubes. To ensure sample integrity, samples were stored on wet ice in cool boxes and transported on the same day to the laboratory in Kohalpur, Banke. There the blood was allowed to clot for half an hour at room temperature and subsequently centrifuged at less than

5000 RPM for 10 minutes. The serum was separated into 3 separate aliquots and frozen at -20 degrees Celsius or lower. Samples were subsequently air-shipped on a weekly basis to a -80 degrees Celsius freezer at the Patan Academy for Health Sciences (PAHS) in Kathmandu. Once all maternal serum samples were collected, the 400-microliter aliquots destined for aflatoxin analysis were air-shipped, on dry ice, to the University of Georgia for aflatoxin B_1-albumin adducts analysis.

Measures

Aflatoxin B_1 Exposure Assessment. In total, 1650 serum samples were analyzed for AFB_1-albumin levels. Serum samples were assayed for the presence of aflatoxin-albumin conjugate using a validated high-performance liquid chromatography (HPLC) with fluorescence detection method [93-95]. Samples were analyzed for aflatoxin covalently bound to blood albumin via lysine. Results were adjusted for serum albumin concentration; exposure was presented as picograms of aflatoxin B_1 lysine adducts per mg of albumin, and this measurement reflects AFB_1 exposure during the previous 2-3 months [96]. In accordance with standard practice, samples were analyzed with a lower limit of detection (LOD) of <0.4 pg aflatoxin-lysine/mg albumin and values under the LOD were substituted with a constant value of half the LOD [97]. It is difficult to draw definitive conclusions regarding safe levels of aflatoxin exposure in humans. To date, no exposure duration, critical onset for exposure, or specific serum aflatoxin threshold dose for immunotoxic effects have been established.

Therefore, for this analysis, any detected level of aflatoxin is considered potentially harmful.

Food Consumption in the Past Week. Trained data collectors administered food frequency questionnaires to the pregnant woman to collect data on how frequently she recalled eating foods that are likely to be contaminated with aflatoxin, such as maize, wheat, milk, or groundnuts, in the week prior to the blood collection. Nepal is a rice-consuming country; in this sample, 99.8% of women reported consuming rice in the past week. The average consumption of rice was more than twice a day (mean frequency of rice consumption 16 ± 4.5 times a week). The consumption of aflatoxin-susceptible foods was entered as a continuous variable (i.e. number of occasions) for statistical analysis.

Food Consumption in the Past Year. Trained data collectors also administered household food consumption and purchasing questionnaires to collect data on household purchasing practices. The questionnaire covered a list of 75 food items, including chilies, groundnuts, milk, rice, wheat and maize. Consumption of these foods was entered as binary variables (i.e. consumed yes or no) for statistical analysis. For some women, data for yearly consumption values were randomly missing. Values for these respondents were replaced with the mode of the observed values for variables that showed significance in the bivariate analysis and have less than 1% missing values.

Dietary Diversity. Food frequency data were used to calculate dietary diversity (DD) scores, a commonly used proxy for diet quality [104]. Participants were asked about consumption of 49 pre-determined food items in the past seven days and 24 hours. Minimum diversity measurements were calculated using FAO Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [104]. Food items from the 24-hour dietary intake recall were categorized into one of the 10 FAO MDD-W food groups: (1) grains/roots/tubers, (2) pulses, (3) nuts and seeds, (4) dairy, (5) meats, (6) eggs, (7) dark green leafy vegetables, (8) other vitamin A sources, (9) other vegetables, (10) other fruits. Scores were analyzed both continuously and as a dichotomous indicator for whether or not participants achieved minimum dietary diversity (defined as consuming at least 5 of the 10 food groups in the previous 24 hours (day or night)).

Covariates. Statistical analyses included other covariates: age, maternal education, socioeconomic status, MUAC, season and VDC. Maternal age, education and wealth index were explored as both continuous and categorical variables in the regression models. An age variable was created to reflect 5 age categories: below 20, 20-24, 25-29, 30-34 and 35 or above. A variable with four categories was created for education: no school education, some or completed primary school (1-6 years), some or completed secondary school education (7-10 years) or more than secondary (>10 years) for descriptive purposes. Principal Component Analysis (PCA) was used to create a composite proxy measure for household wealth. Previous PCA literature [106-108] and Nepal Demographic

Health Survey [112] guidelines were used to construct the wealth index and subsequently wealth quintiles for descriptive purposes. Data on household characteristics (type of roof, floor, walls, toilet, cooking fuel, piped water, number of household members) and asset ownership (livestock, radio, TV, mobile phone, bicycle, motorcycle, electric fan) were used in the PCA to construct a single wealth quintile variable. Variables comprising less than 1% of the sample were excluded from the analysis. All variables, except number of household members, were dichotomized (yes/no) for the PCA.

MUAC was collected using adult (65 cm) MUAC measuring tapes and was entered as a continuous variable for statistical analysis. Data were captured during all seasons except for autumn. Rainfall and humidity are highest during the rainy (monsoon) season. April-June are the hottest months with a maximum daily temperature of 35-40 degrees Celsius, and December-February are the coldest months with average daily temperatures ranging from a low of 6 to a high of 30 degrees Celsius [92]. Season was initially classified into 6 seasonal categories: spring (Baisakh and Chaitra), summer (Jestha and Ashar), rainy (Shrawan and Bhadra), autumn (Ashwin and Kartik), pre-winter (Mangsir and Poush), and winter (Magh and Falgun) [109]. Spring and summer are typically dry with the hottest and most humid months in the summer and monsoon season from July-August, which is also the cropping season for rice and maize. Autumn is characterized by wet, cool weather, while the pre-winter and winter months tend to be cooler and drier. Pre-winter and winter seasons were merged into a binary winter variable for the multivariate regression models.

Data Analysis

Descriptive statistics were computed for the participants. The aflatoxin albumin adducts data (the outcome variable) were not normally distributed and therefore natural log transformed for bivariate statistical analyses. Bivariate analyses were conducted using Student's t-tests and ANOVA for continuous variables and chi-squared tests for binary variables. All tests were two-sided. Covariate-adjusted parameter estimates with 95% confidence intervals were computed using two multivariate regression techniques - ordinary least squares (OLS) and quantile (median) regression (QR) [110]. Variance inflation factors (VIF) were calculated in order to diagnose multicollinearity among the predictor variables in the regression models. Associations with diet in the quantile regression models were examined at the 10^{th}, 30^{th}, 50^{th}, 70^{th} and 90^{th} percentiles of maternal aflatoxin levels. Following the QR, restricted cubic splines (RCS) and non-parametric smoothing curves were incorporated to test for an unadjusted non-linear relationship between aflatoxin levels and weekly maize and groundnut consumption. Covariates in the full model were selected based on their potential for confounding the relationships of interest in this study. All statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX).

Results

Aflatoxin B1 lysine albumin adducts

Detectable levels (albumin adducts ≥0.4 pg/mg) were found in 1553/1648 (94%) of the pregnant women with complete blood samples and questionnaire

data. The mean serum AFB_1 concentration was 3.2 ± 8.3 pg/mg albumin with a geometric mean concentration of 1.37 (CI: 1.3-1.4) pg/mg albumin. Levels ranged from undetectable to 147 pg/mg albumin (**Figure 3**).

Unadjusted Analyses

Maternal age and mean mid-upper arm circumference (MUAC) were significantly negatively associated with maternal AFB_1-albumin levels in bivariate analysis. Hemoglobin level, winter season, maize and groundnut consumption in the preceding week, and milk consumption in the past year, on the other hand, were significantly positively associated with maternal aflatoxin levels (**Table 4**).

Dietary Diversity. Grains and tubers were consumed in the past 24 hours by almost all of the respondents, and 62% (1029) and 12% (202) of respondents reported consuming pulses and nuts/seeds, respectively (data not shown). Dairy was more commonly consumed than meat at 43% (710) and 25% (416) respectively. Egg consumption was very low in this sample (12% (202)). Dark green leafy vegetables (DGLV) and vitamin A rich sources and other vegetables were consumed by 28% (458) and 20% (323) of the sample respectively. More than 80% (1337) of respondents reported consuming vegetables in the past 24 hours, and about a third reported consuming other fruits. The proportion of respondents who achieved minimum dietary diversity (≥5 of the 10 food groups in the previous 24 hours, day or night) was 39% **(Figure 4)**. Dietary diversity scores and MDD-W were positively associated with both socioeconomic status and

maternal education ($p<0.0001$ for both, data not shown). In this unadjusted analysis, MDD-W was not significantly associated with maternal aflatoxin levels during pregnancy.

Food Consumption in the Previous Week. Consumption of commodities such as wheat and milk in the past week showed no significant association with maternal AFB_1-albumin levels, whereas maize and groundnut consumption in the past week were positively associated with higher aflatoxin exposure ($p<0.0001$) (wheat data not shown).

Groundnut consumption in the week preceding the prenatal visit was common and more variable than maize consumption. Thirty one percent (518) of the respondents reported groundnut consumption, while only 3% (49) and 2% (31) of respondents reported consuming only maize, or both groundnuts and maize, respectively **(Table 5)**. The mean number of occasions consuming maize was 2.4±2.2 among the very few respondents (80, <5%) who reported consuming maize in the past week. Among the 549 (33%) respondents who reported consuming groundnuts during the past week, the average number of occasions consuming groundnuts was 2.8±2.2.

AFB_1-albumin levels varied across groundnut and maize consumers and non-consumers. Exposure levels were significantly higher for maize and groundnut consumers than for those who ate neither maize nor groundnuts ($p<0.0001$). Mean levels were highest in those reporting eating both maize and groundnuts in the previous week (7.9 pg/mg albumin adducts) and lowest in those who reported

not eating either of these foods (2.4 pg/mg albumin adducts).

Food Consumption in the Past Year. A large majority of respondents reported consuming a combination of three high-risk foods for aflatoxin contamination during the past year – chilies (100%), maize (83%) and groundnuts (97%) (**Table 5**). The majority (93%) of respondents also reported consuming milk in the past year.

Unlike the strong relation in the bivariate analysis between frequency of maize and groundnut consumption in the past week and aflatoxin levels, no association was detected between consuming maize or groundnuts in the past year and aflatoxin levels. Consumption of other low aflatoxin risk commodities such as wheat, fine rice, or coarse rice in the past year was also not significantly associated with maternal aflatoxin exposure, while consumption of milk in the past year was positively associated with AFB_1-albumin levels ($p<0.05$) (rice and wheat data not shown). Consumption of chilies (a common carrier of aflatoxin [148, 149]) in the past year did not show a significant association with aflatoxin level. However, certain precautions should be taken when interpreting this finding. Instead of a lack of association with chilies, the findings may be a reflection of the lack of variation in the binary data (the majority of participants reported consuming chilies in the past year) and the fact that details about the quantities of consumption were not included in the questionnaire. The lack of association may also partly be explained by the fact that aflatoxin adducts in serum only reflect exposure in the last 2-3 months.

Maize and Groundnut Consumption by Socioeconomic Quintiles, Education and Season. **Table 6** shows that maize consumption in the past week was similar across socioeconomic quintiles and was positively associated with maternal education ($p<0.01$). Groundnut consumption, in contrast, was not associated with maternal education; however, it was positively associated with socioeconomic status ($p<0.01$). Frequency of maize consumption was significantly higher in the women visited in the winter months ($p<0.05$). Furthermore, groundnut consumption was considerably more common in the women measured in the winter months ($p<0.001$).

Multivariate Analyses

After adjusting for other socioeconomic factors in the OLS model, groundnut consumption in the past week (0.730 pg/mg, $p<0.001$) and winter season (2.339 pg/mg, $p<0.001$) were still significant predictors of maternal AFB_1-albumin levels. In contrast, dietary diversity and milk consumption in the past year were not significantly associated with maternal aflatoxin levels after adjusting in the multivariate analysis. **Table 7** presents the results of the multivariate OLS and QR analyses.

Quantile regression models were used to quantify the associations of frequency of consumption of aflatoxin-prone foods and dietary diversity at different points of the aflatoxin distribution. The QR results revealed notable heterogeneity in the size of the associations of maize, groundnut consumption

and seasonality with different quantiles of maternal aflatoxin exposure levels. Maize and groundnut consumption were heterogeneously positively associated with higher aflatoxin levels among most aflatoxin-albumin adduct quantiles. For example, every additional occasion of maize consumption reported in the past week was associated with higher AFB_1-albumin levels for women in the 30th aflatoxin quantile (0.094, $p<0.05$). Similarly, women in the 50^{th} and 70^{th} quantiles reporting one more occasion of maize consumption in the past week experienced higher AFB_1-albumin levels (0.112 ($p<0.05$) and 0.109 ($p<0.05$) respectively). On the other hand, frequency of maize consumption was not significantly associated with aflatoxin levels in the OLS nor for the women in the 10^{th} and 90^{th} quantiles.

Women who reported one more occasion of groundnut consumption in the past week experienced significantly higher aflatoxin levels: 0.058 ($p<0.001$), 0.085 ($p<0.01$) and 0.133 ($p<0.001$) pg/mg AFB_1-albumin levels for women in the 30^{th}, 50^{th} and 70^{th} percentiles, respectively. Similarly, women in the 90^{th} quantile reporting one more occasion of groundnut consumption in the past week showed significantly higher AFB_1-albumin levels (0.133 pg/mg, $p<0.001$). However, groundnut consumption was not associated with aflatoxin levels for women in the 10^{th} percentile; this may be a function of the lower groundnut consumption in women with lowest aflatoxin levels.

Moreover, women who reported consuming milk in the past year had higher aflatoxin levels (0.63 ($p<0.01$) and 0.23 ($p<0.05$) in the 10^{th} and 50^{th} quantiles, respectively) than those who did not consume milk in the past year. Restricted cubic spline analyses did not show any evidence of a threshold effect between

either maize or groundnut consumption in the past week and aflatoxin levels, suggesting a linear relationship between consumption of the two foods in the past week and aflatoxin levels – i.e. more frequent consumption results in a proportionately higher level in the blood.

Dietary diversity score showed no significant association with average maternal aflatoxin exposure in the OLS regression, or at most aflatoxin quantiles in the QR regressions. However, dietary diversity score was significantly positively associated with maternal aflatoxin exposure for women in the 10^{th} percentile (0.064 pg/mg, $p<0.05$).

Associations between winter season and AFB_1-albumin levels were positive across all quantiles. Women in the highest aflatoxin quantile showed the strongest association (1.101 pg/mg, $p<0.001$) with winter months. Mean consumption levels of both groundnuts and maize tend to be higher after the September/October harvests.

Discussion

This study investigated associations between the consumption of aflatoxin-prone foods, dietary diversity and maternal aflatoxin exposure during pregnancy in Banke, Nepal. The biomarker data show that 94% of women were exposed to aflatoxin during pregnancy. Exposure to diet-associated aflatoxin in this rural group of pregnant women of Nepal seems to be driven by groundnut and maize consumption, and results showed no association between dietary diversity and maternal aflatoxin levels. There was a strong correlation between seasonality

and maternal exposure to aflatoxin, with women measured in the winter season showing significantly higher levels of aflatoxin.

Findings from this study showing positive associations between maize and groundnut consumption and maternal AFB_1-albumin levels build on, and are consistent with, previous research showing aflatoxin contamination of cereal grains and groundnut products in various parts of Nepal and worldwide [1, 10, 41, 150]. Most previous aflatoxin studies have taken place in countries where maize tends to be consumed both more frequently and in larger quantities [1, 3, 7, 151]. Still, maize seems to be an important source of aflatoxin exposure even in populations in the Terai region of Nepal with a rice-dominated diet. Moreover, groundnuts were confirmed as important contributors to exposure in this study. Roasted groundnuts are a common snack in Nepal, and groundnut products have recently been gaining popularity through government promotion programs around the country [152].

A study by Koirala et al. [10] in Nepal showed contamination in feed and fodder in multiple districts of Nepal. This study found that 42% of groundnut butter, 34% of groundnut samples, and about a third of maize grits and flour and cornflake samples were contaminated with aflatoxin. The study also showed that 18% of the food items sampled, many of them maize and groundnut products, commonly exceeded the permissible limit for aflatoxin (30 ppb). In addition to maize and groundnuts as potential sources of aflatoxin contamination, our study showed positive associations between reported milk consumption in the past year and increased aflatoxin levels. Human exposure to aflatoxin can occur via

milk when cows are fed aflatoxin-contaminated feed. There is evidence that aflatoxin M_1, a hydroxylated AFB_1 metabolite, can be excreted in cow milk [144]. Consumption of such contaminated milk adds to the potential contamination channels of human exposure to aflatoxin. Our findings strongly support further consideration of targeted regulatory, agricultural and food safety interventions across the value chain and at the household level to reduce aflatoxin exposure.

Given that maize, groundnut and milk consumption only explain part of the exposure in this population, other sources must be considered. Sources such as dried chilies, a common spice used in Nepali cuisine, likely present another route for exposure and might explain the remaining variability in maternal aflatoxin levels. For example, spicy pickled sauce (*achar*), made with chilies and spices, is a ubiquitous side dish in Nepali cuisine. Chronic exposure to aflatoxin in such side dishes could also partly explain some of the variation in observed aflatoxin levels.

Other commonly contaminated spices, such as black pepper, nutmeg, cumin, coriander, garlic [153, 154], and other dairy products (e.g. curd) are also typically consumed in small quantities in Nepal and should be considered in future studies as possible routes of dietary exposure. Although this study did not show an association between rice consumption and aflatoxin levels, rice is an intrinsic component of the Nepali diet and can harbor low levels of aflatoxin [134, 136, 155, 156] and can therefore not be discarded as a conceivable source of low doses of aflatoxin. Finally, some of the variation in levels may be explained by other factors we did not account for, such as quality of the aflatoxin-prone foods

consumed, food preparation methods (e.g. sorting) or actual quantity consumed, rather than frequency of consumption.

Previous research suggests that dietary diversity may help reduce intake of aflatoxin by reducing the amount of aflatoxin-prone foods consumed [75]. Increasing diversity in the diet can both lower the dependence on aflatoxin susceptible foods and help counteract adverse effects of aflatoxin [75]. This study, which took place in a population highly reliant on rice as the staple food, found no association between higher dietary diversity and lower aflatoxin exposure. Findings suggest that those who diversified their diets with groundnuts or maize increased their exposure to aflatoxins. While this study did not detect a significant correlation between diet diversity and maternal aflatoxin levels, dietary diversity promotion should not be overlooked in nutrition interventions. Focused actions seeking to lower contamination risk in these two key foods should be prioritized in nutrition strategies designed to promote dietary diversity.

Although women are exposed year-round, the highest levels of exposure were seen during the dry, cool winter season, suggesting higher aflatoxin consumption in the preceding autumn and/or early winter months. The strong association between AFB_1-albumin levels and winter season is consistent with previous literature [14, 64, 146, 157] showing higher levels in the winter or dry seasons. Higher consumption of contaminated foods can come from either increased quantity consumed after harvest (maize and groundnuts are harvested at the beginning of autumn around August/September) and/or consumption of

lower quality, more contaminated foods that have been stored for long periods of time and been exposed to aflatoxin.

In Nepal, maize and groundnuts are typically harvested during August and September when optimum conditions for *Aspergillus* growth (high temperatures and precipitation) prevail. In Banke, temperatures average 25-35 degrees Celsius during these two months, and the mean precipitation is 346 and 222 mm, for August and September respectively [92]. Fully drying recently harvested crops can be challenging under these humid conditions. Prolonged, multi-month post-harvest storage and sub-optimal storage conditions in hot and humid areas such as Banke can lead to increased aflatoxin production, resulting in increased exposure during the winter months. Increased maize and groundnut consumption after harvest, right before winter could also possibly explain the higher levels in the winter months. While this study was not able to determine changes in diets during the autumn and winter months, maternal dietary data are currently being collected on a quarterly basis for a period of one year after the child is born. This periodic post-partum dietary data collection, while not synched with the maternal blood collection during pregnancy, can to some extent help elucidate the relationship between maize, groundnut consumption and season and is the focus of future research with this birth cohort data.

This study had numerous strengths. It contributed to the limited information on aflatoxin exposure of humans in developing countries and is the first to measure the association of maize and groundnut consumption and dietary diversity with maternal aflatoxin levels in pregnant women. The large sample size

reflected the communities from which the sample was drawn and increased the probability of detecting otherwise small associations. The sample was drawn from a population of women with varied socio-demographic and economic circumstances, promoting the generalizability of our results to the population of women in Banke, Nepal. Moreover, the outcome variable, maternal AFB_1-albumin level, was objectively measured using HPLC. The use of quantile regression in the analysis was an important methodological contribution not found in previous research, which has mostly relied on OLS and logistic regression. Unlike OLS, QR does not assume normality or homoscedasticity and is much less influenced by extreme values of serum aflatoxin (outliers). QR enabled the research team to get a more nuanced picture of the effects of maize and groundnut consumption patterns and dietary diversity on maternal aflatoxin levels.

The study had some limitations. First, by using a food frequency questionnaire to measure diet, the study did not measure actual quantity consumed. Nor did the study measure consumption over the previous 3-month period that is characteristic of aflatoxin half-life in the body. Frequency of consumption was instead used as a proxy for quantity consumed. Second, the study did not collect food samples, which would have allowed for an empirical assessment of the level of contamination of food consumed. Finally, due to the rolling nature of the recruitment process, prenatal aflatoxin data were not available in the autumn, making it difficult to get an idea of aflatoxin exposure levels throughout the whole year.

This study was the first to provide detailed quantitative findings on maternal exposure to aflatoxin in a large cohort of pregnant women with a rice-based diet in South Asia. Results confirmed widespread aflatoxin exposure in pregnancy, a critical and vulnerable period for fetal development, and showed that maize and groundnut consumption were important dietary contributors of aflatoxin even in areas with rice-based diets. Prolonged exposure to aflatoxin, particularly during a critical period in fetal development in which cross-placental transmission is possible [32, 146] can result in adverse nutritional and immunological consequences. The impact of this on linear growth is the focus of forthcoming papers based on this research.

Given the known adverse health effects and the widespread exposure among this vulnerable population, future studies need to evaluate the efficacy of preventive measures specific to prevailing diets in the area, and with special attention paid to aflatoxin prone foods such as maize and groundnuts. Efforts should seek to identify and promote the most cost-effective household interventions and strategies. Aflatoxin reduction campaigns should inform pregnant women and their families not only of the nutritional value of consuming maize and groundnuts but also of the special precautions that should be taken when purchasing, storing, and consuming such aflatoxin-prone foods, particularly before and during the dry winter season when contamination levels seem to be high. Such campaigns can train women and their families to identify poor quality maize or groundnuts and provide families with information on low-cost processing and handling methods (e.g. sorting and removal of unfit maize

kernels and groundnuts) to decrease aflatoxin contamination. A combination of such proven practical, low-cost aflatoxin reduction techniques at the household level and market level regulation of aflatoxin-prone foods can help reduce exposure to aflatoxin in vulnerable populations.

Tables and Figures

Table 4. Aflatoxin levels by sociodemographic and health characteristics of pregnant Nepalese women enrolled in the AflaCohort Study

	n	n %	Mean AFB1	SD	Geo mean AFB1	95% CI		Highest AFB1 quintile n	Highest AFB1 quintile %
Age Category							**		
<20	347	21	4.4	11.2	1.6	1.4-1.9		77	22
21-24	627	38	3.3	7.6	1.4	1.3-1.5		117	19
25-29	470	29	2.4	4.4	1.2	1.1-1.4		93	20
30-34	135	8	3.0	12.9	1.2	1.0-1.4		26	19
35+	69	4	2.2	2.6	1.3	1.0-1.6		16	23
Schooling									
None	606	37	2.5	5.1	1.3	1.2-1.4		121	20
Some primary (1-5)	321	19	3.1	5.6	1.0	1.3-1.6		68	21
Some secondary (6-10)	577	35	4.2	12.0	1.4	1.3-1.6		117	20
More than secondary (10+)	144	9	2.6	5.1	1.3	1.1-1.5		23	16
Wealth Index Quintile									
Poorest	328	20	3.3	9.0	1.4	1.3-1.6		73	22
Poor	330	20	3.0	7.2	1.2	1.1-1.4		58	18
Middle	330	20	2.8	6.3	1.3	1.2-1.5		64	19
Rich	329	20	3.9	9.1	1.5	1.4-1.7		74	23
Richest	331	20	3.0	9.3	1.3	1.2-1.5		60	18
Religion									
Hindu	1257	76	3.2	8.5	1.3	1.3-1.4		244	19
Buddhist	5	0	3.4	5.3	1.2	0.0-9.3		1	20
Muslim	363	22	3.1	7.2	1.4	1.2-1.6		78	22
Christian	23	1	5.4	11.2	1.8	1.0-3.2		6	26
Ethnicity									
Brahmin	77	5	3.5	9.1	1.5	1.2-2.0		21	27
Chettri	299	18	3.7	9.2	1.4	1.3-1.6		59	20

Tharu	168	10	2.6	7.1	1.0	0.8-1.2		27	16
Muslim	360	22	3.0	7.1	1.4	1.3-1.6		76	21
Dalit	380	23	2.9	8.6	1.3	1.2-1.5		73	19
Other	364	22	3.5	8.5	1.5	1.4-1.7		73	20
Anemia (<11 g/dL)									
No	977	59	3.5	9.3	1.4	1.3-1.5		207	21
Yes	671	41	2.7	6.4	1.3	1.2-1.4		121	18
Maternal Stature									
Short/Average (>145 cm)	1422	86	3.2	8.3	1.4	1.3-1.4		284	20
Very short (≤145 cm)	224	14	3.2	8.3	1.5	1.3-1.7		45	20
MUAC									
Average	1099	67	3.2	8.5	1.4	1.3-1.4		216	20
Low (≤23 cm)	549	33	3.3	7.9	1.4	1.3-1.5		113	21
Minimum Dietary Diversity (FAO MDD-W)									
No	1003	61	3.0	7.3	1.3	1.2-1.4		200	20
Yes	645	39	3.6	9.6	1.4	1.3-1.6		129	20
Season							***		
Spring	513	31	2.2	5.1	1.2	1.1-1.3		73	14
Summer	392	24	1.3	2.8	0.8	0.8-0.9		31	8
Rainy	32	2	1.2	1.3	0.8	0.6-1.1		2	6
Autumn	0	0	n/a	n/a	n/a	n/a		n/a	n/a
Pre-winter	238	14	6.6	14.1	2.6	2.2-3.1		92	39
Winter	473	29	4.2	9.6	2.6	1.6-2.0		131	28

Highest quintile ≥2.9 pg/mg. AFB1 (pg/mg) values were log-transformed before analysis. Geo, geometric; MUAC, mid-upper arm circumference; FAO, Food and Agriculture Organization of the United Nations. *p<0.05; **p<0.01; ***p<0.001. Maternal mean MUAC borderline significantly negative association with maternal AFB1. Mean hemoglobin of 11.2±1.2; mean mid-upper arm circumference of 24.1±2.5; mean dietary diversity score of 4.2±1.5, minimum dietary diversity defined as ≥ 5 of the 10 food groups in the previous 24 hours (day or night). Numbers do not always add up due to missing responses.

Table 5. Maize, groundnut and chili consumption in the past week and year

	n	% or mean ± SD		Mean AFB1	SD	Geo mean AFB1	95% CI		Highest AFB1 quintile n	Highest AFB1 quintile %	
MAIZE AND/OR GROUNDNUTS											
Percentage of women reporting maize and/or groundnut consumption in the past week											
Neither (REF)	1050	63.7		2.4	6.0	1.1	1.1-1.2		156	14.9	
Maize only	49	3.0		5.7	13.3	1.8	1.2-2.6	**	14	28.6	**
Groundnuts only	518	31.4		4.4	10.6	1.8	1.7-2.0	***	147	28.4	***
Both	31	1.9		7.9	13.3	3.2	2.0-5.1	***	12	38.7	***
MAIZE											
Percentage of women reporting maize consumption in the past week											
No	1568	95.1		3	7.9	1.3	1.3-1.4		303	19.3	
Yes	80	4.9		6.5	13.3	2.2	1.7-3.0	***	26	32.5	***
Average maize consumption frequency in the past week	80	2.4±2.3	***								
Percentage of women reporting maize consumption in the past year											

No	282	17.2	3.2	7.4	1.5	1.3-1.7		62	22.0	
Yes	1362	82.9	3.2	8.5	1.4	1.3-1.4		267	19.6	
GROUNDNUTS										
Percentage of women reporting groundnut consumption in the past week										
No	1099	66.7	2.5	6.6	1.2	1.1-1.2		170	15.5	
Yes	549	33.3	4.5	10.8	1.9	1.7-2.1	***	159	29.0	***
Average groundnut consumption frequency past week	549	2.8±2.2								
Percentage of women reporting groundnut consumption in the past year										
No	45	2.7	2.3	4.0	1.2	0.9-1.7		6	13.3	
Yes	1601	97.3	3.2	8.4	1.4	1.3-1.5		323	20.2	
CHILIES										
Percentage of women reporting chili consumption in the past week			Data not available							
No										
Yes										
Percentage of women reporting chili consumption in the past year										
No	4	0.2	0.7	0.3	0.6	0.3-		0	0.0	

Yes	1642	99.8	3.2	8.3	1.4	1.3-1.4		329	20.0	
MILK										
Percentage of women reporting milk consumption in the past week										
No	983	59.65	3.3	8.9	1.4	1.3-1.4		189	19.2	
Yes	665	40.35	3	7.2	1.4	1.3-1.5		21	20.0	
Average milk consumption frequency past week	665	5.5±4.0								
Percentage of women reporting milk consumption in the past year										
No	117	7.1	2.2	3.5	1.1	0.9-1.3		19	16.2	
Yes	1531	92.9	3.3	8.5	1.4	1.1-1.5	*	310	20.3	*

*AFB1, aflatoxin B1; SD, standard deviation; Geo, geometric; CI, confidence interval; REF, reference category. *p<0.05; **p<0.01; ***p<0.001. Highest quintile ≥2.9 pg/mg. AFB1 (pg/mg) values were log-transformed before analysis. Numbers do not always add up due to missing responses. Data on weekly consumption of chilies not available.*

Table 6. Average frequencies of maize and groundnut consumption in the previous week by wealth, education levels, and season

	Maize			Groundnut		
	Frequency	SD		Frequency	SD	
Schooling						
None	0.1	0.5	**	0.9	1.8	
Some primary (1-5)	0.1	0.6		1.0	2.0	
Some secondary (6-10)	0.2	1.0		0.8	1.8	
More than secondary (10+)	0.1	0.7		0.9	1.7	
Wealth Index						
Poorest	0.1	0.4		0.7	1.4	**
Poor	0.1	0.8		0.8	1.8	
Middle	0.1	0.5		1.1	2.0	
Rich	0.1	0.9		1.0	1.7	
Richest	0.2	0.9		1.1	2.0	
Season						
Winter	0.2	1.0	*	1.4	2.0	***
Non-winter	0.1	0.6		0.8	1.7	

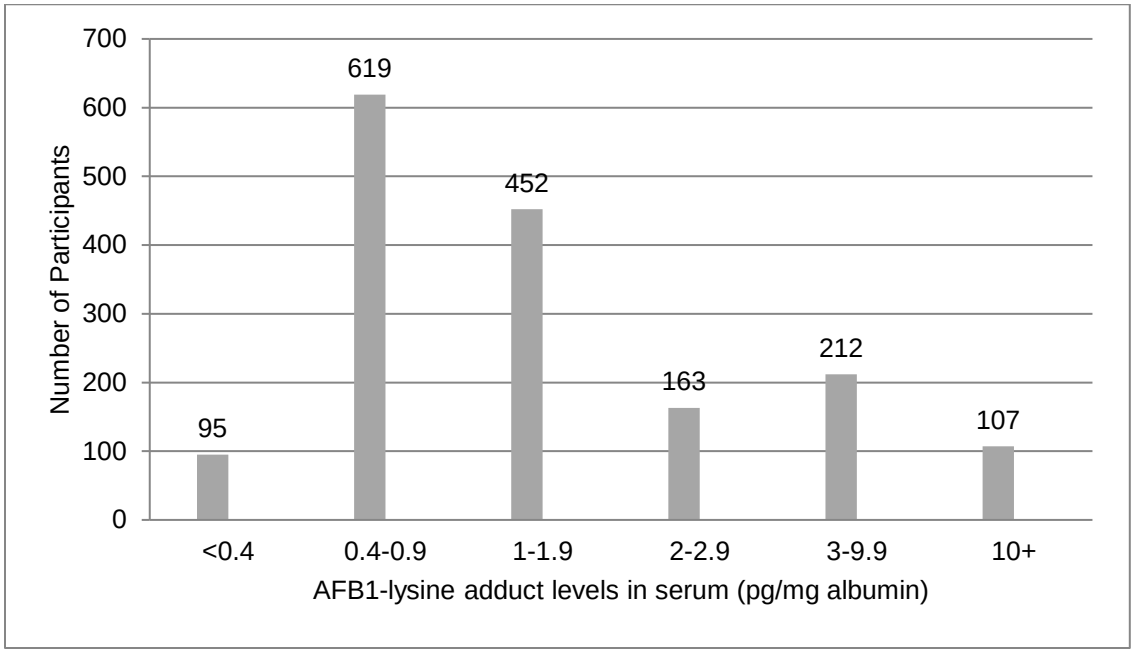
SD, standard deviation; *p<0.05; **p<0.01; ***p<0.001.

Table 7. Multivariate ordinary least squares and quantile regression analysis of the association between weekly maize and groundnut consumption and maternal aflatoxin levels

	OLS[a]		Q10[b]		Q30[c]		Q50[d]		Q70[e]		Q90[f]	
Frequency of maize consumption (times/week)	0.549 (0.281)		0.091 (0.054)		0.094 (0.041)	*	0.112 (0.051)	*	0.109 (0.048)	*	0.147 (0.111)	
Frequency of groundnut consumption (times/week)	0.730 (0.121)	** *	0.037 (0.027)		0.058 (0.016)	** *	0.085 (0.026)	**	0.133 (0.026)	** *	0.133 (0.030)	** *
Consumed milk in the past year	0.906 (0.799)		0.630 (0.221)	* *	0.194 (0.108)		0.230 (0.106)	*	0.173 (0.128)		0.066 (0.244)	
Dietary diversity score	-0.229 (0.149)		0.064 (0.029)	*	0.004 (0.020)		0.008 (0.018)		-0.012 (0.026)		-0.057 (0.053)	
Age (years)	-0.079 (0.045)		-0.011 (0.008)		-0.009 (0.007)		-0.008 (0.006)		-0.009 (0.007)		-0.022 (0.013)	
Schooling (years)	0.092 (0.060)		0.000 (0.009)		-0.003 (0.008)		0.003 (0.008)		0.004 (0.009)		0.023 (0.020)	
SES (wealth index score)	-0.041 (0.072)		-0.009 (0.013)		-0.006 (0.008)		-0.013 (0.009)		-0.007 (0.027)		-0.018 (0.027)	
MUAC (cm)	-0.053 (0.084)		-0.024 (0.017)		0.002 (0.009)		-0.009 (0.011)		-0.016 (0.014		-0.012 (0.028)	
Winter season	2.339 (0.430)	** *	0.313 (0.091)	* *	0.460 (0.059)	** *	0.552 (0.066)	** *	0.623 (0.085)	** *	1.101 (0.130)	** *

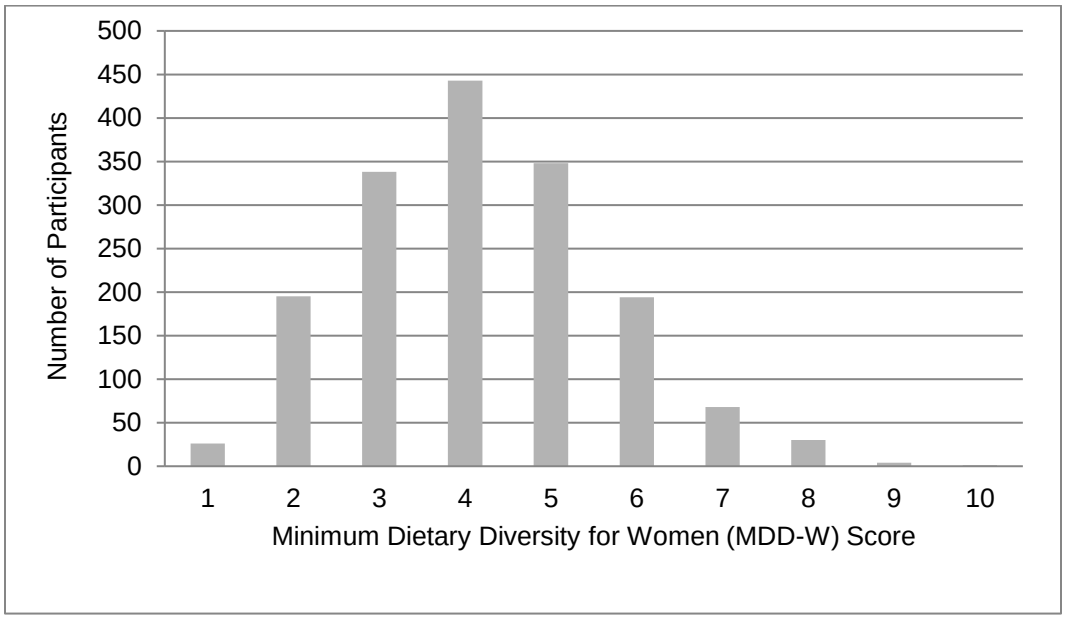
*n=1648; OLS, Ordinary Least Squares; Q, quantile, SES, socio-economic status; MUAC, mid-upper arm circumference. Standard errors in parentheses. *p<0.05; **p<0.01; ***p<0.001. [a] OLS regression; Model Adjusted R2 = 0.0639; [b] QR regression 10th percentile; Pseudo R2 = 0.0539 [c] QR regression 30th percentile; Pseudo R2 = 0.0698 [d] QR regression 50th percentile; Pseudo R2 = 0.0801 [e] QR regression 70th percentile; Pseudo R2 = 0.1010 [f] QR regression 90th percentile; Pseudo R2 = 0.1367. Model adjusted for location (Village Development Committee (VDC)).*

Figure 3. AFB1-lysine adducts in serum of pregnant women



AFB1, aflatoxin B1; Mean 3.2±8.3; Geometric mean 1.4 pg AFB1-lysine/mg albumin

Figure 4. Distribution of Minimum Dietary Diversity for Women (MDD-W) scores



CHAPTER 5: Are Good Agricultural Practices (GAP) Associated With Dietary Exposure to Aflatoxin Among Pregnant Women in Rural Nepal?

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Shortened version of title
Good agricultural practices and maternal aflatoxin

Acknowledgements

The authors express special gratitude to Dr. Maura Mack, Dr. Ahmed Kablan, the AflaCohort study team and the study participants, without whom this research would not have been possible.

Financial Support
Support for this research was provided by the Feed the Future Innovation Lab for Nutrition, which is funded by the United States Agency for International Development (USAID) under grant ID: AID-OAA-L-10-00006. The opinions expressed herein are solely those of the authors.

Authorship
JYAT contributed to the design of the study and was responsible for the analysis and writing of the manuscript. PW and SG were responsible for the overall design and planning of the study and contributed to the statistical analysis and writing of the paper. KB was responsible for the overall design and planning of the study. GS and BLR contributed to the statistical analysis of the study and its interpretation and contributed to the writing of the paper. DD and RS contributed to the design and implemented the study. AP supervised the fieldwork and coordinated acquisition of data. JW conducted the analysis of aflatoxin-exposure markers. All authors reviewed the manuscript for accuracy and approved the final manuscript.

Ethical Standards Disclosure: This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Nepal Health Research Council Ethical Review Board and the Tufts University Health Sciences Institutional Review Board. Written informed consent was obtained from all subjects/patients.

Number of tables: 6
Number of supplemental tables: 1
Number of figures: 3

Target journal: World Development
https://www.elsevier.com/wps/find/journaldescription.cws_home/386?generatepdf=true

Summary

Aflatoxins, naturally-occurring food-borne toxins, are linked to numerous adverse health effects. Exposure to such toxins is widespread in the semi-arid tropics where conditions for their proliferation are optimal, namely, high heat, humidity, many crop pests and diseases. Good Agricultural Practices (GAPs) by smallholder farmers and low-cost household-level food handling practices such as manual sorting can help prevent and control aflatoxin contamination. We used data from an ongoing USAID/Feed the Future-funded birth cohort study with 1675 mother-infant pairs in Banke, Nepal. In our assessment of GAPs we focused on households producing one or more of three widely consumed aflatoxin-susceptible food crops: maize (n=396), groundnuts (n=25) and chilies (n=37). The aims of this study were to: a) assess levels of aflatoxin knowledge and food sorting practices; b) identify GAPs applied in households producing aflatoxin-susceptible food crops such as maize, groundnuts or chilies; and c) determine if GAPs and/or sorting practices were correlated with lower aflatoxin levels among pregnant women living in farming households. Results from multivariate logistic regressions show limited associations between agricultural practices and maternal aflatoxin exposure. Efforts aimed at reducing aflatoxin in the diet are complicated by the fact that individuals are likely exposed through multiple commodities from multiple sources. Findings suggest that program implementers and policy makers should focus aflatoxin control attention broadly, at the food system level, rather than solely at the farm level, particularly in areas where off-farm food acquisition is common.

Keywords: aflatoxin, chili, drying, farming, good agricultural practices, groundnuts, home production, maize, markets, Nepal, pregnancy, post-harvest, storage

Introduction

Aflatoxins are highly toxic metabolites produced by several fungal species of the genus *Aspergillus* [41, 158, 159]. Aflatoxin B_1 (AFB_1) is the most common in foods and is considered carcinogenic [78]. Aflatoxin M_1 (AFM_1) is the major metabolite found in human urine, breast milk, and milk and meat when livestock have ingested contaminated feed [5]. *Aspergillus* fungi thrive in areas with hot and humid climates, such as the Terai (southern plain) region of Nepal where many staple crops are grown [12, 160]. In this climate, inappropriate field, harvest and post-harvest conditions can contribute to grain deterioration and aflatoxin contamination. In this paper, we assess agricultural practices used in households producing one or more of three widely consumed aflatoxin-susceptible food crops: maize (n=396), groundnuts (n=25) and chilies (n=37). We determine whether good agricultural practices (GAPs) and/or food handling practices during the 2015-2016 growing seasons were correlated with subsequently lower aflatoxin levels pregnant women living in those households.

Thus, the specific objectives of this study were to: a) assess levels of knowledge of aflatoxin contamination and handling practices of spoiled foods; b) identify GAPs that are being applied in households producing one or more of three aflatoxin-susceptible food crops - maize, groundnuts and chilies; and c) determine if GAPs and/or food handling practices were correlated with lower aflatoxin levels among pregnant women. Identifying how household drying and storage practices are associated with prenatal aflatoxin levels could have

significant implications for how best to prevent exposure to such toxins during a highly sensitive biological state.

A variety of agricultural commodities, such as grains, including maize, and groundnuts are susceptible to aflatoxin [161-163]. Consumption of low quality grains and nuts, either from home production or from the market, can increase dietary exposure [138, 163]. Often, the most at-risk populations are poor, have limited dietary variety, store foods for long periods, purchase low quality foodstuffs, and are reliant on highly susceptible foodstuffs such as maize and groundnuts [6, 8, 163]. Previous research shows that storage at temperatures below 18 degrees Celsius, plus reducing moisture level below 15% before storage, can stop the development of *Aspergillus flavus* [21, 82]. Moisture measuring devices help farmers determine if crops have been properly dried before storage to avoid mold growth [21].

Previous studies also have shown that prolonged post-harvest storage in the home, combined with high temperatures and humidity (resulting from improper drying and storage facilities) can increase aflatoxin contamination [79, 161]. Research by Joshi et al. in Nepal showed altitude bias, in which higher amounts of AFB_1 were produced by isolated *A. flavus* strains collected from the Terai region compared to the other two higher-altitude agro-ecological zones [13]. The onset of *A. flavus* production occurred during early storage in the Terai, and growth increased with time in storage; this contrasted with the other two zones where *A. flavus* growth was detected only after 9 months of storage.

Aflatoxin can proliferate at almost any point in a crop's life cycle – from pre-harvest (in stored seeds) to on-farm to post-harvest (storage). Codex Alimentarius recommends both Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP) for aflatoxin reduction [21]. GAPs are the primary line of defense for aflatoxin. GAPs are multifunctional; they not only improve food security and encourage environmental sustainability, they also contribute to improved food safety and reduce aflatoxin contamination in food [80]. In addition to helping prevent and control aflatoxin, GAPs can help farmers enter new markets and improve the health of farm workers [81]. At the production level, growth stress such as drought, insect damage and deficient irrigation can leave crops more vulnerable to *Aspergillus* fungi and aflatoxin contamination. The application of good drying and storage practices, applied at both the farm and value chain levels, help prevent aflatoxin production. Protecting the outer coat of grain or nuts during harvest and drying helps lower the likelihood of mold invasion [82]. Removal of damaged kernels (e.g. cracked, discolored, shriveled, moldy, musty, or with wormholes or rancid odor) has been shown to help maintain quality and reduce mycotoxin contamination [18, 21].

High temperatures, improper drying and inadequate storage conditions can exacerbate the problem by increasing the risk of *Aspergillus* mold growth [82, 158]. Long-term storage and inadequate storage infrastructure, whether at the farm, market or household level, are of special concern, especially in low-income areas characterized by climatic conditions conducive to aflatoxin development. Use of insecticides can indirectly reduce aflatoxin contamination by maintaining

the quality and integrity of the stored crop. Prolonged and improper storage can expose food crops to moisture, pests, rodents, and other problems that increase the food's susceptibility to aflatoxin. Contamination of crops with aflatoxin is an economic problem for producers as it can lead to lower yield, lower prices, and reduced livestock feed efficiency, reproduction and immunity [164].

Moreover, aflatoxin is also of great concern from a public health perspective. Its genotoxic, immunosuppressant, mutagenic, hepatotoxic nature is well documented [67, 68, 158]. Aflatoxin has been associated with impaired growth in animals and humans [165]. Owing to the threats aflatoxins pose to human health, authorities recommend exposure to aflatoxins be kept as low as possible [78, 163].

Previous studies in Nepal have indicated widespread contamination in both human food and animal feed [13, 166]. Koirala et al. [10] found a third of the food samples were contaminated with aflatoxins (30 ppb or more was used as the recommended maximum level in food). The human food with the highest proportion of samples showing any level of contamination was groundnut (peanut) butter (43%), followed by groundnuts (34%), maize grits/flour (32%), and maize (corn) flakes (31.5%). Manufactured foods such as maize (corn) flakes (26%) and wheat flour (25%) were the two foods with the highest proportion contaminated above 30 ppb. These data suggest widespread contamination in Nepalese markets.

Another study involving Nepalese women showed potential for hand-sorting visibly diseased maize kernels [18]. Women were able to decrease both

fumonisins and DON (two other types of mycotoxins) to acceptable levels (<1000 ng/g); however, the women's ability to discriminate and efficiently remove diseased kernels varied by their level of training and experience.

A recent analysis of aflatoxin levels in the Aflatoxin Birth Cohort Study in Banke showed 94% of women were exposed during pregnancy (**Chapter 4/forthcoming manuscript**). Use of GAPs may reduce aflatoxin levels in family members including pregnant women residing in households implementing such practices.

Methods

Data for this study were from an ongoing birth cohort study following 1,675 maternal-infant dyads living in 17 Village Development Committees (VDCs)[9] of Banke, a district in the tropical mid-western southern plains (Terai) region of Nepal. The study commenced enrollment and prenatal data collection in July 2015, was forced to halt for 3 months due to nation-wide strikes, and resumed in December 2015. The longitudinal study involves several time points. For this paper, we used data from the prenatal interview (July 2015-July 2016).

Healthy pregnant women aged 16-49 less than 30 weeks into their pregnancy were recruited using a rolling recruitment strategy. Women were excluded if they did not plan to deliver in the study area, had a mid-upper arm circumference

[9] In 2017, the Ministry of Federal Affairs and Local Development dissolved VDCs and replaced them with rural municipalities (The Himalayan Times, March 2017). Since this research was conducted prior to the disbanding of VDCs, the old structures were used throughout the analyses.

(MUAC) less than 17.5 cm, were severely anemic (less than 7 g/dL) [100] or hypertensive (more than or equal to 140/90 mm Hg) [167]. Women meeting any of the biological exclusion criteria were counseled and referred to the closest health facility for appropriate health care. Venous blood samples and the data listed below were concurrently collected from pregnant women at enrollment.

Household Survey

Trained research enumerators administered a tablet-based electronically programmed survey in each enrolled participant's home. Data collected included: 1) household characteristics (e.g. infrastructure, assets, size and composition, water, sanitation and hygiene); 2) maternal characteristics such as education, diet/food consumption frequencies in the past week, recent illness, birthing history; and 3) annual household farming practices and food purchases. Survey questions were designed to capture information on current adoption and use of pre-harvest, harvest, and post-harvest GAPs (Figure 5). Specifically, women were asked about materials used to dry and sort crops and use of moisture measuring devices. Women were also asked about storage conditions, such as storage length, use of insecticides or fungicides, and quality checks of infrastructure used to store food crops. GAP and serum aflatoxin (Figure 6) data shown are for households reporting eating only home-produced maize (n=396), groundnuts (n=25), or chilies (n=37).

Anthropometry

After administering surveys, research enumerators collected maternal anthropometry data in the woman's home. Seca 874 digital scales, ShorrBoard® Stadiometers, and adult (65 cm) MUAC measuring tapes were used to collect maternal weight, height and MUAC. Anthropometric measurements were recorded to the nearest 0.1 kg or 0.1 cm.

Blood Sample Collection and Analysis

After respondents completed the household questionnaires with the data collector, a nurse visited each pregnant woman in her household to collect finger-prick blood for anemia testing (using HemoCue® Hb 301 Systems) and 3-5 ml of blood from the antecubital vein for aflatoxin testing. To ensure sample integrity, samples were stored in cool boxes filled with wet ice and transported to the laboratory for processing within 5 hours of collection. Blood was allowed to clot for half an hour at room temperature and subsequently centrifuged at less than 5000 RPM for 10 minutes. Serum was frozen at -20 degrees Celsius or lower and within a week air-shipped to a -80 degrees Celsius freezer located at the Patan Academy for Health Sciences in Kathmandu.

A 400-microliter aliquot for each woman was air-shipped on dry ice to the University of Georgia for aflatoxin B_1-albumin adducts analysis. A high-performance liquid chromatography (HPLC) with fluorescence detection method was used to assay serum samples for the presence of aflatoxin-albumin conjugate [93-95]. Samples were analyzed for aflatoxin covalently bound to blood

albumin via lysine. Aflatoxin results were adjusted for serum albumin concentration. This aflatoxin B1 measurement reflects exposure during the previous 2-3 months [96]. In accordance with standard practice, values under the lower limit of detection (LOD) of <0.4 pg aflatoxin-lysine/mg albumin were substituted with a constant value of half the LOD [97]. There is no established safe level of exposure [98]; therefore, for these analyses any detected level of aflatoxin is considered potentially harmful.

Variable Computations

Each woman was assigned to a wealth index quintile, a proxy of household wealth created using Principal Component Analysis (PCA) applied to a list of household possessions [16, 106-108, 112]. Literature on PCA and the Nepal Demographic Health Survey guidelines were used to construct the wealth index [106-108, 112]. Household characteristics (type of roof, floor, walls, toilet, cooking fuel, piped water, number of household members) and asset ownership (livestock, radio, TV, mobile phone, bicycle, motorcycle, and electric fan) were used to construct a household wealth index. Variables found in <1% of the sample were not included in the analysis. Variables (with the exception of number of household members) were dichotomized for the PCA.

Twenty-four hour food frequency data were used to calculate dietary diversity (DD) scores, a commonly used proxy for diet quality [104]. Minimum diversity measurements were calculated using FAO Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [104]. For the FAO Minimum Dietary

Diversity Score, food items from the seven-day dietary intake recall were categorized into one of the 10 FAO MDD-W food groups. Minimum dietary diversity was defined as consuming at least 5 of the 10 food groups in the previous 24 hours.

Data were available for all seasons except autumn. Autumn is characterized by wet, cool weather. Winter months tend to be dry, and temperatures are coldest from December-February. The coldest months have average daily temperatures ranging from a low of 6 to a high of 30 degrees Celsius [92]. Temperatures are warmest in April - June with a maximum daily temperature of 35-40 degrees Celsius, and rainfall and humidity are highest during the rainy (monsoon) season, July - August [92]. Monsoon is the cropping season for rice and maize.

Statistical Analysis

The aflatoxin albumin adducts data (the outcome variable) were not normally distributed and therefore were natural log-transformed for all statistical analyses. All statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX). A P value of less than 0.05 indicates the estimated coefficient is different from zero in a two-tailed test of statistical significance.

Bivariate analyses were conducted using Student’s t-tests and ANOVA for continuous variables and chi-squared tests for categorical variables. Covariate-adjusted parameter estimates with 95% confidence intervals were computed using ordinary least squares (OLS) regression. We calculated variance inflation

factors (VIF) in order to diagnose multicollinearity in the model. The association between the maize-producing household's post-harvest and food handling practices and serum aflatoxin levels was studied in the context of other risk factors, such as seasonal variations, dietary diversity, socioeconomic status, and maternal education, among others. Covariates in the full model were selected based on their potential for confounding the relationships of interest in this study. More specifically, the aflatoxin levels in pregnant women from maize producing households was modeled as a function of various GAPs:

$$Log(A) = \beta_0 + \beta_1 (P) + \beta_2 (M) + \beta_3 (D) + \beta_4 (R) + \beta_5 (SP) + \beta_6 (C) + u$$

where Log(A) is the log value of serum aflatoxin levels in pregnant women, *P* is use of plastic sheets/tarps for drying the crop*, M* months of storage*, D* method for determining dryness*, R* rodent attacks during storage, and *SP* storage problems. To control for a series of potential confounding factors that may be correlated with prenatal aflatoxin levels and GAPs, we included *C*, a series of maternal and household dietary and sociodemographic characteristics (recent intake of maize and groundnuts, season, location, age, education and wealth index). **Table 8** provides summary statistics for the variables included as confounders in the model.

Results

Almost all pregnant women in the study (94%) tested positive for aflatoxin B1-lysine adducts (mean ± SD maternal aflatoxin of 3.2± 8.3 pg/m albumin) (**Table 8**). The average age of the women was 23.6 years, and mean maternal aflatoxin levels were significantly higher in women ages 16-19. Levels of serum aflatoxin were significantly higher for women visited in the winter months.

More than a third of the women (37%) reported having no education. Forty-one percent of the women were classified as anemic during pregnancy with hemoglobin levels of less than 11 g/dL. Fifteen percent of the women were of very short stature (≤145 cm [103]), and 33% had a low MUAC (≤23 cm). Thirty-nine percent of the respondents had achieved a minimum dietary diversity (defined as ≥ 5 of the 10 food groups in the previous 24 hours). Aflatoxin levels were not significantly associated with the aforementioned parameters.

Maternal aflatoxin knowledge and household removal of spoiled food

Respondents were asked (in local languages) if they had previously heard of aflatoxin. Respondent awareness was extremely low (1%) (**Table 9**). When asked what households do with spoiled food, almost 10% reported discarding the food, and 66% reported culling spoiled portions of food before consumption. Almost 15% of households reported feeding spoiled food to animals, making alcohol, mixing moldy grain with non-moldy grain, or drying. Nine percent of women reported consuming the spoiled food. Neither having knowledge of

aflatoxins nor discarding or removing spoiled foods was significantly associated with lower levels of serum aflatoxin in pregnant women.

Livestock ownership

More than half of the women (57%) lived in households that owned livestock (**Table 9**). Of these, 36% reported noticing mold in their animal feed in the past year. Of those who identified mold on the animal feed, 23% reported feeding the contaminated grain to the animal and 10% reported fully discarding the contaminated feed. The majority of participants (67%) reported removing the mold-contaminated portions or mixing the contaminated feed with clean animal feed. Women from households reporting feeding contaminated feed to animals did not show significantly higher aflatoxin levels than those from households that discarded the feed.

Sources of maize, groundnuts and chilies

Table 10 shows the sources of maize, groundnuts and chilies for study participants. **Figure 7** shows aflatoxin levels by source of aflatoxin-prone food. Seventeen percent of the participants reported no consumption of maize in the past year. Off-farm food acquisition was common in this cohort. Over a third (35%) of the households reported having received all of the maize they consumed as a gift or in exchange for another good or service. Another 24% of the participants reported obtaining the maize solely from the market, while 24% reported consuming only home-produced maize. Less than 1% of participants

reported both purchasing maize in the market and producing their own maize. Levels of serum aflatoxin were significantly higher in women eating only home-produced maize ($p<0.01$) and women eating only market-sourced maize ($p<0.01$) than they were in the group who were gifted or who had traded the maize.

Virtually all participants reported having consumed groundnuts (97%) and chilies (99.9%) in the past year. Of those who reported consuming groundnuts in the past year, 91% consumed product purchased from the market. Almost 4% reported having received the groundnuts in-kind (as a payment, gift, or bartered), <2% reported eating home-produced groundnuts only, and less than 1% reported consuming groundnuts from both home production and the market. Participants who had only consumed home-produced groundnuts had lower levels of aflatoxin than those who received the groundnuts as gifts or in exchange for another good or service ($p<0.1$).

Most participating households (93%) reported acquiring their chilies from the market. A little over 1% of participants reported having received all chilies in-kind, while 2% reported having home-produced all the chilies they consumed. Another 4% of participants reported having consumed a mixture of home-produced and market-sourced chilies. Participants who had self-produced or purchased their chilies in the market had lower levels of aflatoxin than those who received the chilies in-kind ($p<0.1$).

Farmers' maize, groundnut and chili drying practices

Table 11 shows maize, groundnut and chili farmers' use of drying practices. Eighty percent of maize farmers reported harvesting their crop at maturity, whereas 11% reported harvesting after the crop had passed the stage of maturity (some breakage detected). About half (48%) of maize farmers reported leaving maize in the field for one or more days after harvest. Fourteen percent of maize farmers reported not using any additional drying methods once the maize was brought home from the field. Those who dried maize after bringing the harvest in from the field reported doing so by spreading the grain directly on the bare ground (33%), on plastic sheets/tarps (24%), or on platforms (13%). Prenatal aflatoxin levels were higher in women from households using platforms to dry their maize than those in households not using platforms but the relationship was not significant ($p=0.081$). Levels were lower in women from households reporting spreading the maize on plastic sheets/tarps ($p<0.05$).

Among maize farmers, visual observation was the most common method for determining dryness (61%) followed by visual observation and sound (either cracking between teeth or shaking) (24%) or sound only (13%). A very small portion (3%) of maize farmers reported using moisture detection devices to measure grain dryness precisely. Women in households that used auditory methods as the sole measure for maize dryness had the highest levels of serum aflatoxin ($p<0.01$). Use of screening/gravity tables designed to remove heavily mold-contaminated or damaged kernels was nearly nonexistent (<1%) among maize farmers.

Most groundnut farmers (72%) reported harvesting their nuts at maturity; however, 24% harvested after nuts reached maturity. Sixty-four percent of groundnut producers reported leaving groundnuts standing in the field for more than a day after harvest. Virtually all (96%) reported additional drying after bringing the groundnuts home from the field. The most commonly reported method of drying the groundnuts was on plastic sheets/tarps (40%), followed by spreading on bare ground (36%) or drying on platforms (20%). One-half of groundnut farmers (48%) reported using a mixture of visual observation and sound to determine dryness. The remainder reported using either visual observation (24%) or auditory (28%) methods for determining groundnut dryness.

None of the groundnut producers reported using a moisture device to determine dryness of their food crop, nor did they report using screening/gravity tables to remove moldy/damages nuts. Aflatoxin exposure was lower in women in households using visual and auditory methods to determine dryness compared to women from households using visual methods only; however, this relationship was not significant ($p=0.093$). This study did not detect any other significant relationships between GAPs related to drying and serum aflatoxin levels for the participants in groundnut-producing households, possibly because of the small sample size.

Most chili producers (70%) reported harvesting chilies at maturity, while 11% reported harvesting after maturity. All chili producers reported leaving their chili crop standing in the field for more than a day after harvest. Chili producers reported drying on plastic sheets/tarps (67%), directly on bare ground (50%) or

on platforms (14%). Some chili-producing households also reported hanging chilies to dry (11%) and women in these households had significantly higher aflatoxin levels than those who did not hang chilies to dry ($p<0.05$). The main method for determining chili dryness was visual observation (92%), while 8% of chili producers reported using a moisture device to determine dryness. No other significant relationships were detected between post-harvest practices and serum aflatoxin levels for chili producers, possibly due to the small sample size.

Farmers' maize, groundnut and chili storage practices

Storage practices of maize, groundnut and chili farmers are shown in **Table 12**. More than half (56%) of maize farmers stored maize on cobs in the husk, while the rest stored shelled maize. Very few maize farmers (<2%) reported using chemical insecticides in their storage unit, and none reported having used fungicides in the storage units. Maize was usually stored for more than 6 months. Longer periods of storage were positively correlated with aflatoxin levels. Aflatoxin levels were significantly higher in women who reported maize storage of 1-3, 4-6 and 7-9 months compared to those in households not storing home-grown maize ($p<0.05$). Although a quarter (28%) of maize producers reported storing maize for longer than 10 months, these women did not show significantly higher aflatoxin levels than women from households not storing their maize.

A large percentage of maize farmers took time to prepare their storage units before filling them with new maize. For example, 69% of maize producers reported checking storage units for leaks, and over half (55%) reported checking

units for infestation. A third of farmers reported checking the storage unit for rips/tears or leftover spoiled grain from the previous harvest, and checking for moisture.

Fourteen percent of maize farmers reported problems with insects in the past year, while 16% reported having rodents in their storage units in the past year. Women from households where rodents had attacked stored maize had significantly higher levels of aflatoxin in blood than those who did not report a rodent attack ($p<0.01$). The most important problem reported in the past year was rodents (7%), followed by insects (3%) and mold (<1%). Only 2% of maize farmers reported having been trained in proper storage techniques.

Insecticide and fungicide use during storage was nonexistent for groundnut producers. Almost half of groundnut producers reported long durations of storage, with more than half storing for longer than 6 months. A large percentage (84%) of groundnut farmers reported checking storage units for infestation, leaks (76%) and rips/tears (48%). A smaller percentage of groundnut farmers reported checking storage units for leftover spoiled grain (20%) or moisture (28%) before storage. Very few groundnut producers reported having their food crop attacked by pests (8%), and 16% reported rodent attacks. The two groundnut producers (8%) who reported having received training in proper storage techniques had higher levels of aflatoxins than those who had not received training ($p<0.05$).

None of the chili producers in this study reported applying insecticides or fungicides during storage. A third (35%) reported storing their chilies for more than a year, while 22% reported not storing chilies at all. Most chili farmers

reported checking the storage unit for leaks (78%), infestations (49%) and moisture (43%). Women in households that checked for infestation had lower levels of aflatoxin than those who did not check for infestation ($p<0.01$). A smaller percentage reported checking containers for rips/tears (30%) and leftover spoiled grain (24%). Very few chili farmers reported pests or rodent attacks on stored chilies (3% and 8%, respectively). Only a few farmers (9%) reported having received training on proper storage practices.

Multivariate analysis of maize farming households' post-harvest GAPs and maternal serum aflatoxin levels

Table 13 shows the regression results using crude and adjusted ordinary least squares (OLS) regression approaches. When controlling for other measured factors, such as seasonality and consumption of aflatoxin-prone foods such as maize and groundnuts, the OLS regression models showed only use of plastic sheets/tarps for drying the maize to be associated with lower maternal aflatoxin levels ($\beta=-0.30$, $p<0.1$). Having had the maize attacked by rodents ($\beta=0.36$, $p<0.1$) as well as more frequent consumption of maize in the previous week ($\beta=0.11$, $p<0.05$) were positively associated with aflatoxin levels. Given the small number of groundnut and chili-producing households in this cohort, no multivariate regression analyses examining GAPs and maternal aflatoxin levels were conducted for these households.

Discussion

Despite farming households' frequent use of certain well-known GAPs, including proper drying and removal of contaminated/moldy grains and nuts, almost all participants in this study, regardless of their household's involvement or reliance on farming, showed evidence of aflatoxin exposure, albeit at lower levels than have been observed in some other parts of the world. Aflatoxin awareness was equally low for pregnant women from farming and non-farming households. The pathway of exposure is complicated by the multiple food sources that can be contaminated and is not restricted to home-produced foodstuffs. Instead, contaminated foods are likely to come from a variety of sources, including foods produced at home, received in-kind (as a payment, gift or bartered), or purchased in markets. Levels of aflatoxin exposure observed in this study likely reflect consumption of various foods susceptible to aflatoxin from multiple sources.

This study showed both low awareness of aflatoxin contamination (1%) and widespread prevalence (94%) of aflatoxin B1-albumin adducts in pregnant women in the Banke district of Nepal. Detectable levels of aflatoxin were found in 94% of the serum samples, with a mean serum AFB_1 concentration of 3.2 (range: undetectable to 147) pg/mg-albumin and a geometric mean concentration of 1.37 (CI: 1.30-1.44) pg/mg-albumin. Although the widespread aflatoxin exposure observed in these pregnant women is consistent with previous studies, measured levels in these women were much lower than those observed in other studies [4, 6, 7, 24, 32, 168]. Rice, the cornerstone staple grain in the Nepalese diet [160],

typically harbors low levels of aflatoxin contamination [136]. It is possible that the overall lower levels of aflatoxin may be due to high rice consumption.

We examined culling of damaged kernels among all households enrolled in the birth cohort study following previous literature that shows removal of damaged kernels can help reduce levels of aflatoxin contamination [78]. While 66% of households reported sorting and discarding spoiled portions of food, another 9% reported discarding all the food. Nine percent of the respondents reported eating the spoiled food. Our results are in line with a similar study in Ghana [5] with 140 adults that showed that 85% of the respondents reported discarding spoiled grains; 8% reported feeding them to animals; and 7% reported eating the spoiled grains [5]. The Ghana study likewise showed that even with such practices, 100% of the adults tested positive for aflatoxin albumin adducts. While sorting was commonly practiced, no significant correlation was found between this practice and lower aflatoxin levels.

One of the major challenges with solely relying on household-level sorting as an aflatoxin reduction method is that while the mold is visible, the toxin itself cannot be detected visually. Removing visibly moldy kernels may not entirely remove the toxin. Previous literature examining the impact of culling damaged grains on aflatoxin reduction shows that successful sorting and removal of contaminated grains depends largely on training, personal judgment, and the household’s economic situation [169].

Efforts aimed at reducing aflatoxin in the diet are further complicated by the fact that individuals are likely exposed through multiple commodities from

multiple sources. In this study, the primary source for most of the maize, groundnuts and chilies consumed by pregnant women was either the market or a gift or traded item. Receiving in-kind food and a high reliance on market-purchased food limits consumers' information on the quality and safety of the food consumed. It is likely that even when a household avoided damaged maize, individuals within the households continued to consume aflatoxin via other susceptible foods, such as groundnuts or chilies, many of which were not self-produced [1, 10, 170]. Hence, efforts to improve crop quality and overall food safety cannot be restricted to the household. There is a need for a better understanding of market sources of contamination. Research on commonly contaminated foods at each step of the value chain is warranted to provide a richer understanding of seed-to-table interventions for improved quality and crop safety.

Previous findings from the AflaCohort Study showed higher levels of aflatoxin in women having recently consumed maize and/or groundnuts (**Chapter 4 in dissertation/forthcoming manuscript**). Those results suggest these are important contributors to aflatoxin in the diet, even among populations who consume relatively low amounts of maize and groundnuts. Chilies warrant further study as another important potential source of aflatoxin. Chilies are highly susceptible to aflatoxin and are common in Nepalese diets [170-172]. To reduce aflatoxin exposure from foods, both the commonly contaminated staple foods, such as maize, and ancillary sources like groundnuts and chilies should be treated as potential dietary sources of aflatoxin exposure. Further studies

examining quality of foods that are exchanged or sold in markets are indispensable for understanding potential contaminants. Studies should quantify the levels consumed, via detailed dietary recalls and quantification of aflatoxin in the foods consumed. Such studies can help establish a dose response of the compounded levels from all foods translating into serum aflatoxin levels.

An additional potential source for dietary exposure to aflatoxin includes consumption of contaminated animal products such as milk, eggs or meat. This study examined the relationship between livestock ownership, animal feed handling practices, and serum aflatoxin levels; women in households that fed contaminated feed to animals did not have statistically significant differences in aflatoxin levels compared with those from households that discarded the feed. This lack of association in this sample could reflect the low number who fed contaminated feed to animals, low consumption of animal products in general, or lack of variation in diets among participants, combined with the fact that aflatoxin contamination occurs in the wide range of foods from multiple sources that are commonly consumed in the study area.

High temperatures and humidity levels characterize the Terai region, especially during the summer and monsoon seasons. Therefore, carefully drying crops before storage is an important step for maintaining quality and diminishing mold growth. Women from farming households reported using several drying practices that can reduce mold growth contamination [173]. A total of 24%, 40% and 66% of maize, groundnut and chili producers, respectively, reported spreading crops to dry on plastic sheets/tarps or on platforms, away from the

bare ground. Most reported examining dryness of the crop via visual or auditory examination. Very few farmers reported using moisture measurement devices to test levels of humidity. Although farmers reported drying crops and examining them for dryness before storage, the relationship between the aforementioned GAPs did not translate into detectable or consistently lower serum aflatoxin levels in the multivariate analyses.

Participants reported long storage periods, with much of the maize, groundnuts, and chilies stored for more than 6 months at a time. There was a tendency of serum aflatoxin levels to be higher in women from maize-producing households that stored their maize versus those who did not store maize for longer than a month after harvest. Women from households that had stored maize for 1-3 months or 4-6 months had the highest mean levels of aflatoxin. These levels were significantly higher than women from households where maize was not stored for longer than 30 days ($p<0.1$). Aflatoxin levels for women in households where maize was stored for 7-9 months was significantly higher than for women in in households that did not store maize ($p<0.05$).

Although women in households where maize was stored for more than 10 months also showed higher mean levels of aflatoxin than those who did not store their maize, this difference was not significant. This could partly be because most participants, regardless of their farming practices, are consuming a combination of contaminated foods from home production and the market. It may also be because the maize in these households is destined for sale rather than personal

consumption. While not significant, a greater percentage of participants storing their maize for more than 7 or 10 months reported selling their maize (**Table 14**).

Analyses from this study revealed low levels of formal training on proper storage techniques and low usage and/or access to technologies, such as fungicides, among producers of aflatoxin-prone foodstuffs. Previous research in Nepal showed potential for *A. flavus* growth suppression through application of certain agents such as lemon grass oil, *Mentha arvensis* oil, *Zanthoxylum alatum Hemsl* (Timur) oil, citric acid, lactic acid, and tartaric acid; however, sodium chloride and neem leaves did not show a protective effect [13]. Information on non-synthetic chemical fungicide use was not collected as part of this study. Farmers may have been applying local (or traditional or non-synthetic) remedies to reduce fungal growth that we did not document.

This study had a few limitations. Statistical tests and definitive conclusions for groundnut and chili-producing households were limited by the unexpected small sample size. Many of the coefficients were small and only statistically significant at the 10% level. Mindful of these caveats, our findings highlight a need for further exploration of impacts of good agricultural practices on human aflatoxin exposure. Results from this study reinforce the growing number of programs and research studies examining reduction methods at the market and value chain levels [10, 174-176].

Aflatoxins are not found in any single food, nor do they proliferate during one single season or because of a single poor post-harvest practice. Instead, aflatoxins are found at many points in the food system and, when conditions are

favorable, can proliferate in nearly any segment of the food supply chain, including household production, processing and storage; market transport, wholesaling and retailing; and in-kind acquisition. Therefore, a farm or household-level approach to reducing dietary exposure to aflatoxin is not likely to suffice in Banke or similar settings where most households, regardless of their participation in agriculture, rely on markets supplied by multiple local, national and international sources.

More research is needed to identify the most cost-effective points of intervention. A validated bundle of low-cost, culturally appropriate household and market-level GAPs could help reduce aflatoxin levels in addition to improving the overall quality of food. Successful interventions are likely to incorporate a mixture of strategies, ranging from commonly used household practices such as sorting shriveled, moldy, discolored or undersized pieces and the use of hermetically sealed storage structures to market-level interventions including government monitoring of distributors.

Tables and Figures

Table 8. Sample descriptives

	n	%	Mean AFB1	SD	Geo mean AFB1 (95% CI)		
Aflatoxin B_1 exposure							
Undetectable/unexposed (LOD<0.4 pg/mg)	95	5.8	-	-	-	-	
Exposed	1553	94.2	-	-	-	-	
Age Category							
<20 (REF)	347	21.1	4.4	11.2	1.6	(1.4-1.9)	
21-24	627	38.1	3.3	7.6	1.4	(1.3-1.5)	*
25-29	470	28.5	2.4	4.4	1.2	(1.1-1.4)	***
30-34	135	8.2	3.0	12.9	1.2	(1.0-1.4)	**
35+	69	4.2	2.2	2.6	1.3	(1.0-1.6)	†
Schooling							
None (REF)	606	36.8	2.5	5.0	1.3	(1.2-1.4)	
Some primary (1-5)	321	19.5	3.0	5.6	1.4	(1.3-1.6)	
Some secondary (≥6)	721	43.8	3.8	11.0	1.4	(1.3-1.5)	
Wealth Index [a]							
Poorest (REF)	328	19.9	3.3	9.0	1.4	(1.3-1.6)	
Poor	330	20.0	3.0	7.2	1.2	(1.1-1.4)	†
Middle	330	20.0	2.8	6.3	1.3	(1.2-1.5)	
Rich	329	20.0	3.9	9.1	1.5	(1.4-1.7)	
Richest	331	20.1	3.0	9.3	1.3	(1.2-1.5)	
Religion							
Hindu (REF)	1257	76.2	3.2	8.5	1.3	(1.3-1.4)	
Buddhist	5	0.3	3.4	5.3	1.2	(0.0-9.3)	
Muslim	363	22.0	3.1	7.2	1.4	(1.2-1.6)	
Christian	23	1.4	5.4	11.2	1.8	(1.0-3.2)	
Ethnicity							
Brahmin (REF)	77	4.7	3.5	9.1	1.5	(1.2-2.0)	
Chettri	299	18.1	3.7	9.2	1.4	(1.3-1.6)	
Tharu	168	10.2	2.6	7.1	1.0	(0.8-1.2)	**
Muslim	360	21.8	3.0	7.1	1.4	(1.3-1.6)	
Dalit	380	23.1	2.9	8.6	1.3	(1.2-1.5)	
Other	364	22.1	3.5	8.5	1.5	(1.4-1.7)	
Anemia (<11 g/dL)							
No (REF)	977	59.3	3.5	9.3	1.4	(1.3-1.5)	
Yes	671	40.7	2.7	6.4	1.3	(1.2-1.4)	†
Maternal Stature							
Short/Average (>145 cm) (REF)	1422	86.4	3.2	8.3	1.4	(1.3-1.4)	
Very Short (≤145 cm)	224	13.6	3.2	8.3	1.5	(1.3-1.7)	
MUAC							
Average (>23 cm) (REF)	1099	66.7	3.2	8.5	1.4	(1.3-1.4)	
Low (≤23 cm)	549	33.3	3.3	7.9	1.4	(1.3-1.5)	

Minimum Dietary Diversity (FAO MDD-W)							
No (REF)	1003	60.9	3.0	7.3	1.3	(1.2-1.4)	
Yes	645	39.1	3.6	9.6	1.4	(1.3-1.6)	
Season of serum sample collection							
Non winter (REF)	937	56.9	1.8	4.2	1.0	(1.0-1.1)	
Winter	711	43.1	5.0	11.4	2.0	(1.9-2.2)	***

AFB1 (pg/mg) values were log-transformed before analysis. Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001. Geo, geometric; LOD, lower limit of detection; REF, reference; MUAC, mid-upper arm circumference; FAO, Food and Agriculture Organization of the United Nations. Maternal mean MUAC borderline significantly negative association with maternal AFB1. Mean hemoglobin of 11.2±1.2; mean mid-upper arm circumference of 24.1±2.5; mean dietary diversity score of 4.2±1.5, minimum dietary diversity defined as ≥ 5 of the 10 food groups in the previous 24 hours (day or night). [a] The wealth index quintiles were constructed using Principal Component Analysis (PCA) of a set of common asset indicators. See text for full description of the variables used in the PCA. Numbers do not always add up due to missing responses.

Table 9. Knowledge of aflatoxins, food handling behavior and livestock ownership

	n	%	AFB1±SD (pg/mg)[a]
Knowledge of aflatoxins			
No	1630	98.9	3.1±7.5
Yes	18	1.1	9.4±34.4
General behavior after identifying contaminated / moldy food			
Consume food (REF)	155	9.4	2.9±5.2
Make alcohol, feed to animal, mix with good grain, dry	242	14.7	2.6±5.0
Sort out contaminated/moldy portions	1089	66.2	4.5±9.5
Discard food	158	9.6	2.2±3.1
Household livestock ownership in the past year			
Do not own	712	43.2	3.2±8.3
Own	936	56.8	3.2±8.3
Mold identified on animal feed in the past year			
No	598	64.3	3.5±9.7
Yes	332	35.7	2.6±4.9
Action taken after identifying contaminated / moldy animal feed in the past year			
Fed contaminated feed to animal (REF)	75	22.7	3.2±6.9
Discarded moldy portions of animal feed or mixed with clean animal feed	221	67.0	2.4±4.2
Discarded animal feed	34	10.3	2.6±3.5

AFB1, aflatoxin B1; SD, standard deviation; REF, reference. [a] Values are mean ± SDs. Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001. AFB1 values were log-transformed before analysis. None of the comparisons were significant. Numbers do not always add up due to missing responses.

Table 10. OLS regression results for the relationship between maternal serum aflatoxin levels and sources of maize, groundnuts and chilies

	n	%	AFB1±SD (pg/mg)[a]	Crude OLS			Adjusted OLS			R^2
MAIZE										0.17
Did not consume	280	17.0	3.2±7.4	-			-			
In-kind (gifted or traded) only	568	34.6	2.4±5.4	REF			REF			
Home production only	396	24.1	4.1±11.2	0.31	(0.072)	***	0.24	(0.075)	**	
Market only	389	23.7	3.5±8.8	0.21	(0.072)	**	0.22	(0.076)	**	
Home production and market	11	0.7		0.08	(0.334)		0.10	(0.367)		
GROUNDNUTS										0.15
Did not consume	45	2.7	2.3±4.0	-			-			
In-kind (gifted or traded) only	59	3.6	2.5±4.6	REF			REF			
Home production only	25	1.5	1.6±3.3	-0.49	(0.264)	†	-0.35	(0.193)	†	
Market only	1505	91.4	3.3±8.6	0.0006	(0.147)		0.03	(0.130)		
Home production and market	12	0.7	2.3±2.1	0.05	(0.350)		0.13	(0.311)		
CHILIES										0.15
Did not consume	4	0.2	0.7±0.3	-			-			
In-kind (gifted or traded) only	23	1.4	6.9±16.3	REF			REF			
Home production only	37	2.3	3.2±6.4	-0.37	(0.293)		-0.59	(0.316)	†	
Market only	1524	92.6	3.1±7.3	-0.45	(0.232)	†	-0.50	(0.278)	†	
Home production and market	58	3.5	5.7±19.9	-0.24	(0.272)		-0.42	(0.316)		

Standard errors reported in parentheses; Coefficients on the control variables (maize consumption in the past week, groundnut consumption in the past week, season, location, age, schooling and wealth) omitted to preserve space; Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001; OLS, ordinary least squares; AFB1, aflatoxin B1; SD, standard deviation; pg, picograms; mg, milligrams; REF, reference; [a] Values are mean ± standard deviations; Tests for statistical significance apply only to those who reported consuming the crop in the past year. In-kind group is the reference group; AFB1 values were log-transformed before analysis; Numbers do not always add up due to missing responses; Maize model n=1364, Groundnuts model n=1601, Chilies model n=1642

Table 11. Harvest and post-harvest drying practices among sample farmers, by crop

	CROP PRODUCED									
	MAIZE n=396			GROUNDNUT n=25			CHILI n=37			
	n	%	AFB1±SD (pg/mg)[a]		n	%	AFB1±SD (pg/mg)[a]		n	%	AFB1±SD (pg/mg)[a]	
Time of harvest												
Before maturity	33	8.4	2.0±1.9		1	4.0	0.2±n/a		7	18.9	2.3±0.5	
At maturity	316	80.4	4.0±11.2		18	72.0	1.0±0.5		26	70.3	3.9±7.5	
After maturity (some breakage)	44	11.2	6.5±14.4		6	24.0	3.4±6.8		4	10.8	1.5±1.1	
Days drying in the field after harvest												
0 days	203	51.3	4.3±13.3		9	36.0	2.7±5.5		0	0	-	
≥ 1 day	193	48.4	4.0±8.3		16	64.0	0.9±0.5		37	100	3.2±6.4	
Drying method after harvest [+]												
Did not dry (REF)	54	13.6	5.2±20.0		1	4.0	1.3±n/a		0	0.0	-	
Field	23	5.8	3.4±4.4		1	4.0	0.5±n/a		35	97.2	3.4±6.5	
Fan	0	0.0	-		0	0.0	-		0	0.0	-	
Platform	53	13.4	5.4±11.1	†	5	20.0	1.7±3.7		5	13.9	5.8±7.3	
Bare ground	131	33.1	3.7±6.6		9	36.0	0.7±0.4		18	50.0	1.9±2.6	
Plastic sheets/tarps	97	24.3	3.4±11.0	*	10	40.0	2.5±5.2		24	66.7	3.0±4.1	
Drying floor	23	5.8	2.1±2.5		0	0.0	-		2	5.6	1.9±0.6	
Mechanically	0	0.0	-		0	0.0	-		0	0.0	-	
Hanging	21	5.3	5.6±13.0		0	0.0	-		4	11.1	12.0±16.0	*
Other	8	2.0	1.9±1.9		2	8.0	0.9±0.5		6	16.7	1.3±0.9	
Method for determining dryness												
Visual observation (REF)	237	60.5	3.4±8.0		6	24.0	3.9±6.6		33	91.7	3.5±6.7	
Sound (teeth or shaking)	50	12.8	5.9±11.1	**	7	28.0	0.7±0.4		0	0.0	-	
Visual examination and sound (teeth or shaking)	93	23.7	3.6±8.7		12	48.0	0.8±0.4	†	0	0.0	-	
Moisture measuring device	12	3.1	2.2±2.0		0	0.0	-		3	8.3	1.1±0.7	

Use of screening table									
No	394	99.5	4.1±11.2	25	100.0	1.6±3.3	37	100.0	3.2±6.4
Yes	2	0.5	1.3±0.7	0	0.0	-	0	0.0	-

AFB1 (pg/mg) values were log-transformed before analysis. + Categories are not mutually exclusive. AFB1, aflatoxin B1; SD, standard deviation; REF, reference. [a] Values are mean ± SDs. Two-tailed tests, [†]p<0.1; *p<0.05; **p<0.01; ***p<0.001. Numbers do not always add up due to missing responses.

Table 12. Post-harvest storage practices among sample farmers, by crop

	CROP PRODUCED								
	MAIZE n=396			GROUNDNUT n=25			CHILI n=37		
	n	%	AFB1±SD (pg/mg)[a]	n	%	AFB1±SD (pg/mg)[a]	n	%	AFB1±SD (pg/mg)[a]
Stripped maize from cob before storage									
As grain	172	43.9	4.0±9.6	-	-	-	-	-	-
With husk	220	56.1	4.2±12.3	-	-	-	-	-	-
Treat storage unit with chemical insecticides									
No	390	98.5	4.2±11.3	25	100.0	1.6±3.3	36	100.0	3.2±6.4
Yes	6	1.5	1.1±0.7	0	0.0	-	0	0.0	-
Treat storage unit with chemical fungicides									
No	396	100.0	4.1±11.1	25	100.0	1.6±3.3	36	100.0	3.2±6.4
Yes	0	0.0	-	0	0.0	-	0	0.0	-
Duration of storage									
Did not store (REF)	45	11.4	2.1±3.3	3	12.0	6.6±9.3	8	22.2	3.4±5.8
1-3 months	83	21.0	5.2±16.6 †	4	16.0	0.8±0.7 *	4	11.1	9.6±17.0
4-6 months	102	25.8	4.9±12.2 †	6	24.0	1.0±0.6 †	6	16.7	2.8±3.1
7-9 months	57	14.4	4.3±10.3 *	6	24.0	0.8±0.4 *	5	13.9	2.5±2.6
10+ months	109	27.5	3.4±6.7	6	24.0	0.9±0.4 †	13	36.1	1.8±2.7
Storage unit preparation [+]									
Check for rips/tears	143	36.1	4.9±15.0	12	48.0	0.8±0.5	11	29.7	5.2±10.3
Check for leftover spoiled grain	1180	29.8	4.5±14.6	5	20.0	4.3±7.3	9	24.3	3.1±5.4
Check for moisture	150	37.9	5.1±15.3	7	28.0	3.1±6.3	16	43.2	3.1±4.5
Check for leaks	272	68.7	4.3±12.2	19	76.0	1.8±3.8	29	78.4	2.7±4.7

Check for infestation (e.g. vermin, bugs)	218	55.1	3.9±11.6		21	84.0	1.6±3.6		18	48.7	1.9±3.9
Cleaned (e.g. dirt, spider webs)	22	5.6	2.8±3.4		0	0.0	-		0	7.4	-
Nothing	2	0.5	1.6±1.6		0	0.0	-		1	2.7	0.6±n/a
Pests attacked stored food/seed											
No	339	85.6	4.1±11.6		23	92.0	1.6±3.4		36	97.3	3.3±6.4
Yes	57	14.4	4.1±7.9		2	8.0	0.6±0.1		1	2.7	0.6±n/a
Rodents attacked stored food/seed											
No	331	83.6	3.9±11.5		21	84.0	0.9±0.5		34	91.9	2.9±6.2
Yes	65	16.4	5.4±9.1	**	4	16.0	4.7±8.3		3	8.1	6.7±9.2
Most important storage problem											
No storage problem (REF)	355	89.7	4.2±11.7		24	-	0.9±0.5		32	86.5	3.0±6.3
Insects	13	3.3	1.8±1.6		0	-	-		0	0.0	-
Rodents	26	6.6	3.3±4.3		1	100.0	1.6±3.3		2	10.8	5.7±7.7
Mold	2	0.5	13.3±5.6	*	0	-	-		1	2.7	1.0±n/a
Received training on proper storage techniques											
No	385	97.7	4.2±11.3		23	92.0	0.9±0.5		34	91.9	2.9±6.2
Yes	9	2.3	3.3±5.3		2	8.0	8.8±11.9	*	3	8.1	6.4±9.4

AFB1 (pg/mg) values were log-transformed before analysis. [+]Categories are not mutually exclusive. AFB1, aflatoxin B1; SD, standard deviation; REF, reference. [a] Values are mean ± SDs. Two-tailed tests, [†]p<0.1; *p<0.05; **p<0.01; ***p<0.001. Numbers do not always add up due to missing responses.

Table 13. OLS regression results for the relationship between serum aflatoxin levels and post-harvest practices in maize farming households

Dependent variable: Maternal AFB1-lysine adducts	Crude OLS			Adjusted OLS		
Dry maize using plastic sheets/tarp						
No	(REF)			(REF)		
Yes	-0.34	(0.135)	*	-0.30	(0.153)	†
Storage duration						
Did not store	(REF)			(REF)		
1-3 months	0.41	(0.215)	†	0.23	(0.216)	
4-6 months	0.35	(0.208)	†	0.23	(0.216)	
7-9 months	0.47	(0.232)	*	0.20	(0.255)	
10+ months	0.29	(0.206)		0.30	(0.213)	
Method for determining dryness						
Visual observation	(REF)			(REF)		
Sound (teeth or shaking)	0.47	(0.177)	**	0.22	(0.203)	
Visual examination and sound (teeth or shaking)	0.01	(0.139)		0.07	(0.163)	
Moisture measuring device	0.08	(0.336)		-0.02	(0.344)	
Rodents attacked stored maize						
No	(REF)			(REF)		
Yes	0.42	(0.157)	**	0.36	(0.191)	†
Most important maize storage problem						
No storage problem	(REF)			(REF)		
Insects	-0.13	(0.327)		-0.11	(0.336)	
Rodents	0.17	(0.235)		-0.09	(0.304)	
Mold	2.09	(0.820)	*	1.53	(0.819)	
Frequency of maize consumption in the past week	0.15	(-0.051)	**	0.11	(-0.050)	*
Frequency of groundnut consumption in the past week	0.08	(0.038)	*	-0.01	(0.041)	
R^2					0.17	
Adjusted R^2					0.10	

OLS, ordinary least squares; REF, reference; Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001; Coefficients on the control variables (location, age, schooling, season and wealth) omitted to preserve space; AFB1 values were log-transformed before analysis; Standard errors reported in parentheses; n=392.

Figure 5. Good agricultural practices (GAP) for aflatoxin prevention and control

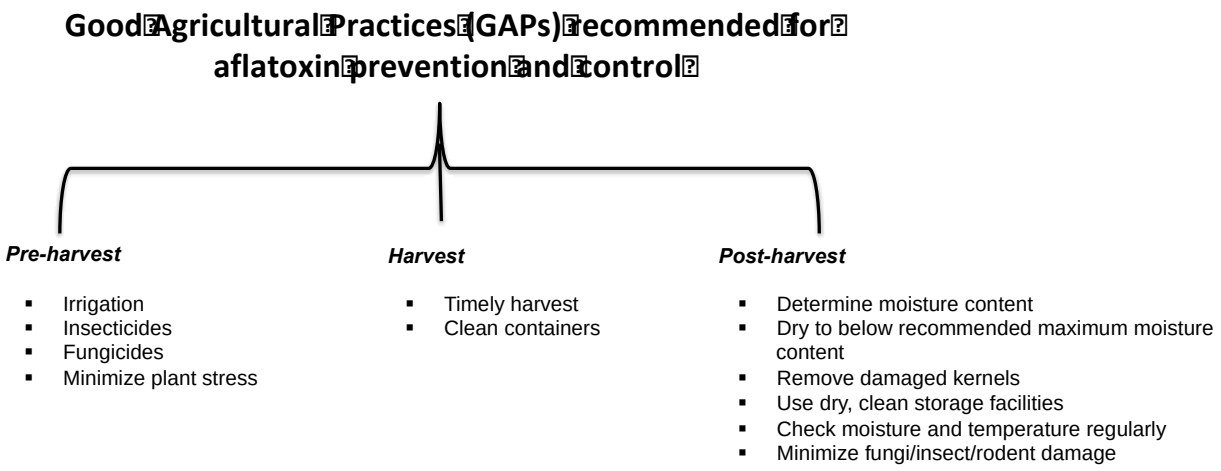


Figure 6. Maternal serum aflatoxin levels for pregnant women in maize, groundnut or chili-producing households

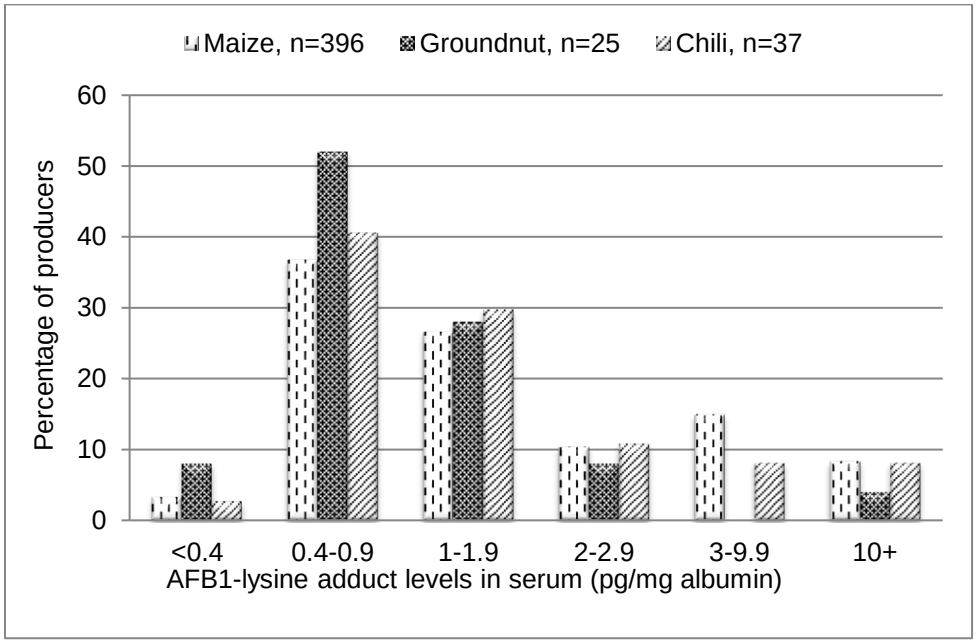
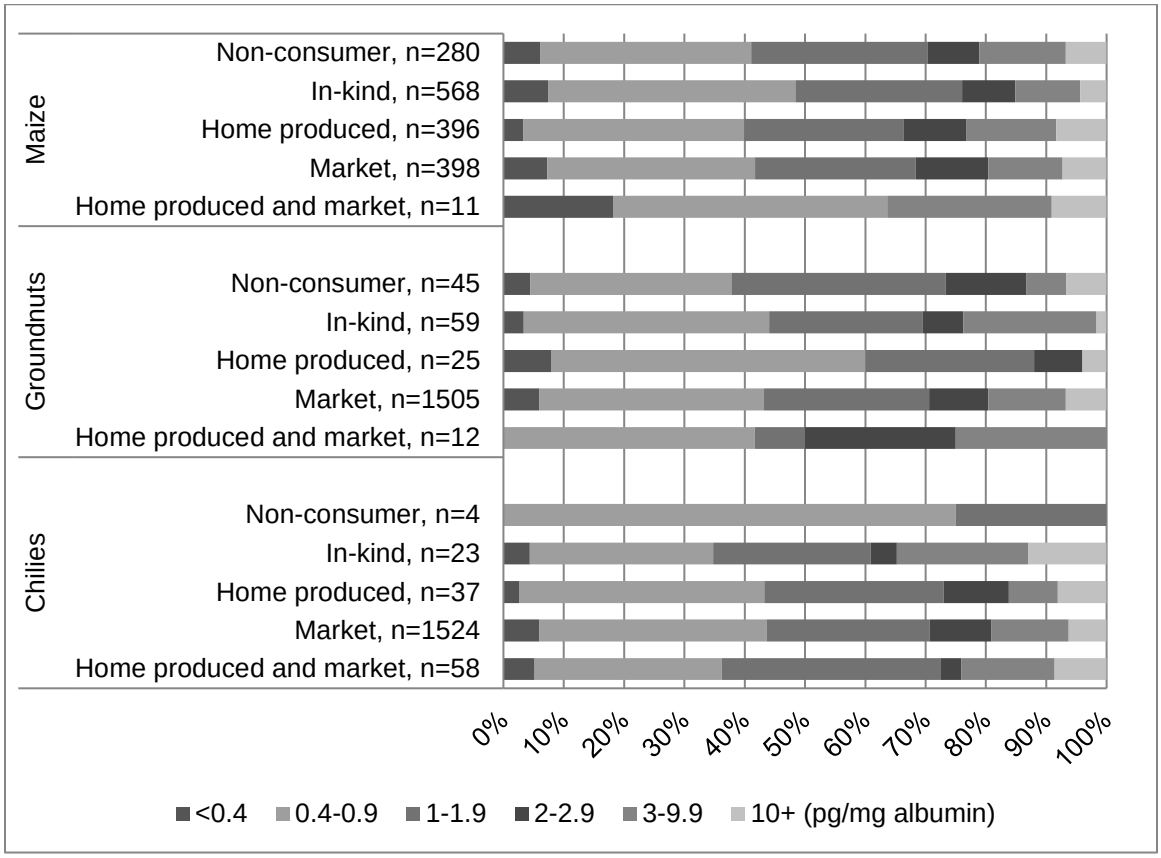


Figure 7. Distribution of maternal aflatoxin levels by sources of maize, groundnuts and chilies



Supplementary material

Table 14. Maize sales and storage duration

	n	%	AFB1±SD (pg/mg)[a]		% selling maize
Duration of storage					
Did not store (REF)	45	11.4	2.1±3.3		15.9
1-3 months	83	21.0	5.2±16.6	†	15.7
4-6 months	102	25.8	4.9±12.2	†	12.8
7-9 months	57	14.4	4.3±10.3	*	28.1
10+ months	109	27.5	3.4±6.7		23.2

AFB1 (pg/mg) values were log-transformed before analysis. AFB1, aflatoxin B1; SD, standard deviation; REF, reference. [a] Values are mean ± SDs. Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001.

CHAPTER 6: *In utero* serum aflatoxin levels are not associated with adverse birth and anthropometric outcomes in Nepal: Results from a prospective birth cohort study

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Running title: In utero aflatoxin and birth outcomes Nepal

Acknowledgements

The authors express special gratitude to Dr. Maura Mack, Dr. Ahmed Kablan, AflaCohort study team and the study participants, without whom this research would not have been possible. The author's responsibilities were as follows – JYAT: contributed to the design of the study and was responsible for the analysis and writing of the manuscript with feedback from BR, GS, PW and SG. PW, SG and KB were responsible for the overall design and planning of the study. JYAT conducted the statistical analyses with feedback from BR, GS, PW and SG. DD and RS contributed to the design and implemented the study. AP supervised the fieldwork and coordinated acquisition of data. KP contributed to the design of the study. JW measured aflatoxin-exposure markers. JYAT had primary responsibility for the final content. All authors: reviewed the manuscript for accuracy and approved the final manuscript.

Footnotes

Support for this research was provided by the Feed the Future Innovation Lab for Nutrition, which is funded by the United States Agency for International Development (USAID) under grant ID: AID-OAA-L-10-00006. The opinions expressed herein are solely those of the authors.

Number of tables: 4
Number of supplemental tables: 2
Number of figures: 3

Intended for submission to the Journal of Nutrition, IP 4.145
https://academic.oup.com/jn/pages/General_Instructions#General Information

Abstract

Background: Exposure to aflatoxin has recently garnered increased attention as a possible contributor to adverse birth outcomes and poor linear growth. Objective: Investigate the role of aflatoxin exposure during pregnancy on adverse birth outcomes such as low birth weight, small-for-gestational-age, stunting at birth and preterm birth.

Methods: This study used maternal and newborn data from an ongoing USAID-supported birth cohort study in Banke, Nepal (n=1621 mother-infant pairs). Maternal aflatoxin B1 (AFB1)-lysine adducts were determined using high performance liquid chromatography. Data on aflatoxin B1, sociodemographic and health characteristics, and anthropometry were collected during the prenatal visit. Infant height and weight were collected at birth. Logistic regression analyses were used to determine if *in utero* aflatoxin exposure was associated with adverse birth outcomes, as well as to identify other potential risk factors associated with low birth weight (LBW, < 2.5 kg), small-for-gestational-age (SGA, <10th percentile for gestational age and gender), stunting at birth (length-for-age z-score≤-2) and preterm birth (born before 37 weeks of gestation). Infants measured within 72 hours of birth were included in the anthropometric regression analyses.

Results: The geometric mean AFB1-lysine adducts was a relatively low 1.37 pg/mg albumin (CI: 1.30-1.44, range: undetectable-147 pg/mg albumin). Almost 20% of infants were of LBW, and more than half (51.5%) of infants were SGA. Sixteen percent of infants were stunted at birth. Moreover, 13% of infants were born preterm (before 37 weeks of gestation). After adjusting for potential confounders, prenatal AFB1 exposure was not a statistically independent predictor of adverse birth outcomes.

Conclusions: Findings from this study did not show an association between *in utero* aflatoxin exposure and selected adverse birth outcomes in Nepal. Results suggest either a lack of an association or an as yet unidentified threshold health effect that could not be detected due to the relatively modest aflatoxin levels in this sample.

Keywords: aflatoxin, birth outcomes, length, weight, *in utero*, Nepal, preterm, small-for-gestational-age

Introduction

Two important public health concerns in Nepal are the alarming rates of low birth weight (LBW) (11-16%) and high rates of stunting in children below age five (36%) [16, 87-89, 177, 178]. A critical window for interventions to promote appropriate physical and cognitive growth, often referred to as the “first 1000 days”, extends from conception through the child’s second birthday. Growth faltering may begin during the period of gestation and continue for at least the first 2 years of life [28]. Small birth size is a crucial determinant of an infant’s health and survival later in life [179, 180]. Being born small increases both mortality and morbidity, and can increase the likelihood of stunting later in childhood [181, 182]. Given enormous health and development burdens associated with low birth weight and stunting in children, it is important to identify potential causes and solutions. Exposure to aflatoxin, a type of mycotoxin, has recently garnered increased attention as a possible contributor to adverse birth outcomes and poor linear growth.

Several human studies in Africa substantiate significant associations between high levels of serum aflatoxin B1-lysine adduct levels during the first 1000 days and growth faltering in infants and young children [1-4, 7, 64, 66, 165, 183]. Aflatoxin is a naturally occurring, carcinogenic environmental toxin that can damage human health, particularly during pregnancy when a rapidly developing fetus is susceptible to adverse conditions [59]. Aflatoxin crosses placental barriers and exposure during pregnancy could impair fetal growth [7, 23]. Previous research has established a potential link between aflatoxin exposure

during pregnancy and adverse anthropometric birth outcomes such as low birth weight [23, 24, 32, 184]. Furthermore, an extensive body of animal health research has documented that a high level of aflatoxin in foods presents a threat to an animal's overall health, growth and reproductive outcome [43-47, 49, 51, 53, 55-57]. For example, aflatoxin at high levels has been shown to cause cancer in laboratory animals. Piglets exposed to maternal aflatoxicosis exhibited growth impedance, thymic shrinkage, impaired peripheral immune efficiency and defects related to zinc intestinal absorption [54].

Several human studies in South Asia have shown widespread exposure to aflatoxin. A study using archived (1999-2001) blood samples collected from pregnant women in the Sarlahi district of Nepal showed 94% of women were exposed to aflatoxin during pregnancy [14]. The same study detected aflatoxin B1-lysine adduct/mg albumin adducts in Bangladeshi human cord blood samples, suggesting a fetus can convert aflatoxin into toxicologically active compounds. A second, more recent study in Bhaktapur, Nepal showed that childhood exposure to aflatoxin was pervasive; however, this study did not find a link between chronic exposure to aflatoxin and postnatal child growth [147].

Although aflatoxin is known to be harmful to animals and humans, much remains unknown about the birth outcome risks associated with *in utero* aflatoxin exposure. Furthermore, there is a lack of consensus on what constitutes critical levels of exposure for adverse health outcomes other than hepatocellular carcinoma. Findings from animal experiments and cross-sectional human studies suggest a need for additional research regarding the *in utero* effects of aflatoxin

on newborns, especially given the unfavorable linkages cited between human aflatoxin exposure and low birth weight and subsequent poor linear growth. Thus, one of the main objectives of this prospective birth cohort study was to investigate the effect of aflatoxin exposure during pregnancy on risk for adverse birth outcomes. Outcomes of interest include low birth weight, small-for-gestational-age, stunting at birth and preterm birth.

Methods

Study design and participants

A power of 80% was used to identify the necessary sample size to detect a difference of 225 grams or larger in birth weight in infants exposed to aflatoxin *in utero* compared to those who were not exposed. Calculations showed a minimum of 56 infants were necessary to answer our main research question on birth weight.

The sample size for our study was 1621. Participants were enrolled in the AflaCohort Study, a prospective birth cohort study that recruited 1675 pregnant women from 17 Village Developments Committees (VDCs)[10] in the Banke district of Nepal. Eligible participants were healthy pregnant women 16-49 years of age, <30 weeks gestation at the time of enrollment, intending to deliver and stay in the study area. Of 1675 women and infant pairs enrolled in the AflaCohort Study, a

[10] In 2017, the Ministry of Federal Affairs and Local Development dissolved VDCs and replaced them with rural municipalities (The Himalayan Times, March 2017). Since the research was conducted prior to the disbanding of VDCs, the old structures were used throughout the analyses.

total of 1648 maternal blood samples were tested for aflatoxin and a majority of them (n=1634) were visited within 72 hours of birth between August 2015-March 2017. A total of 1621 had both serum aflatoxin values and birth anthropometric measurements within 72 hours of birth. The study used prenatal and post-birth survey data from the AflaCohort birth cohort study.

Written informed consent was obtained in accordance with the Nepal Health Research Council Ethical Review Board (NHRC ERB) and the Tufts Health Sciences Institutional Review Board (HS IRB) prior to enrollment.

Trained research staff administered in-person structured electronic surveys to participants in their homes. Data collected during the prenatal survey included mother's diet, anthropometry, nutritional knowledge, education, gestational age, hemoglobin, and serum aflatoxin levels. Information was also collected on household water, sanitation and hygiene (WASH), socioeconomic status (SES) and food insecurity. Data collected in the post-birth visit included prenatal care, birth outcomes and child anthropometrics. Participants with missing data, multiple births, or visited more than 72 hours after birth were excluded from this analysis.

Maternal blood collection and assessment of AFB1

After the prenatal survey was complete, a study nurse visited participants in their homes to collect 3-5 ml of blood from the antecubital vein using 5ml BD Vacutainer® blood collection tubes. In the field, samples were stored on wet ice in cool boxes to maintain sample integrity. Samples were transported

within 5 hours of collection to a laboratory in Kohalpur, Banke. At the lab, blood was left to clot for 30 minutes at room temperature and subsequently centrifuged at less than 5000 RPM for 10 minutes. The serum was separated and frozen at -20 degrees Celsius or lower. Within a week of collection, samples were air-shipped to a -80 degrees Celsius freezer at the Patan Academy for Health Sciences in Kathmandu.

Once prenatal data collection was complete, and maternal serum samples were ready for aflatoxin testing, a 400-microliter aliquot from each participating woman was air-shipped on dry ice to the University of Georgia for aflatoxin B_1-albumin adducts analysis. Serum samples were assayed for the presence of aflatoxin-albumin conjugate using a validated high-performance liquid chromatography (HPLC) with fluorescence detection method [93-95]. This method measures aflatoxin covalently bound to blood albumin via lysine and reflects AFB_1 exposure during the previous 2-3 months [96]. Aflatoxin results were adjusted for serum albumin concentration, and exposure was presented as picograms of aflatoxin B_1 lysine adducts per mg of albumin. Samples were analyzed in accordance with standard practice with a lower limit of detection (LOD) of <0.4 pg aflatoxin-lysine/mg albumin, and a constant value of half the LOD was used for values under the LOD [97]. Neither onset for exposure, exposure duration, nor critical nor specific serum aflatoxin threshold dose for immunotoxic effects have been established to date, making it difficult to draw definitive conclusions regarding safe levels in humans. Therefore, for this

analysis any aflatoxin detected in maternal serum samples is considered potentially harmful.

Study outcomes

Study outcomes included were birth weight (kilograms), low birth weight (<2.5 kg), small-for-gestational-age (SGA) (<10th percentile for gestational age and gender), weight-for-age (WAZ), birth length (centimeters), length-for-age (LAZ) z-scores, and stunting at birth (LAZ≤-2**)**. Additional birth outcomes included preterm birth (PTB) (born before 37 weeks of gestation), stillbirth (born with no signs of life at or after 28 weeks gestation) and miscarriage (fetal loss before 28 weeks gestation).

Low birth weight infants are defined as weighing less than 2.5 kilograms at birth, regardless of gestational age or gender. Infants may be born with a LBW as a result of being born early (before 37 weeks of gestation), because of intrauterine growth restriction (IUGR), or both [180, 185]. SGA was defined as less than 10th percentile for gestational age and gender relative to the World Health Organization's (WHO) multinational fetal growth population standard [121, 186][11]. Using small-for-gestational age, in addition to low birth weight, allowed for

[11] The INTERGROWTH-21st Project fetal growth were also applied to estimate SGA rates in this sample. The primary purpose of applying both sex-specific growth charts to the data was to identify to what extent the choice of cut-offs influences the rates of SGA and the associations between aflatoxin and odds of SGA. Rates of SGA varied depending on the reference population used (30% with INTERGROWTH and 50% using the WHO charts). However, choice of reference population did not affect the final bivariate or multivariate results. While both reference populations are recommended for countries that have not

a distinction between being born too small because of prematurity or being born small at term; it also allowed for weight differences between male and female infants [187]. Because birth weight is not the only measure indicating *in utero* growth restriction, birth length was also examined. Stunting at birth was defined as less than or equal to -2 standard deviations below the WHO-reference length for age (i.e. <1 month) median standardized z-scores [118].

Seca 874 digital scales and Infant/Child/Adult ShorrBoard® Stadiometers were used to measure infant (tared) weight and length. Infant weights and lengths were measured to the nearest 0.1 kg and 0.1 cm, respectively. The 2006 WHO Growth Standards [115] were used to calculate z-scores of WAZ, WLZ and LAZ. Z-scores were calculated using Stata WHO Child Growth Standard macros [116, 117]. Infants with z-scores either below or above six standard deviations from the WHO reference median were excluded prior to analysis [118]. Gestational age at delivery was determined by estimating the duration between the first day of the last menstrual period (LMP) and the birth date. Study team members were trained to use a standard protocol when prompting and recording information on LMP.

In utero aflatoxin exposure

The geometric mean aflatoxin level of 1.37 (CI: 1.30-1.44) in this cohort was relatively low compared to earlier studies [7, 14, 24, 92]. Prenatal aflatoxin levels

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developed their own charts, we present the SGA prevalence based on the WHO multinational fetal growth charts.

were treated as continuous variables to avoid loss of information. Levels of exposure were also included in analyses as ordinal variables. For example, levels were categorized into tertiles and quartiles based on the observed distribution of data. However, the lower quantiles had very low variability due to the right-skewed nature of the aflatoxin data (**Figure 8)**. Therefore, this type of categorization was not used in the final analyses. Instead, all analyses examined separately the relationship between prenatal aflatoxin exposure and birth outcomes with both a continuous predictor variable and a dichotomous aflatoxin exposure indicator. The aflatoxin albumin adducts data were log transformed during analyses to address the non-normal distribution of the data, and the binary variable was defined to indicate levels either below or above the geometric mean of 1.37 pg/mg albumin adducts.

Additional variables

Data collected from the mother at the prenatal visit included age, ethnicity, education, smoking habits, nutritional knowledge, dietary diversity, antenatal care (ANC) visits, and last menstrual period. Information for computing socioeconomic status was also collected during the prenatal visit. Principal Component Analysis (PCA) methodology was used to calculate socioeconomic status [16, 106-108, 112]. Dietary data were assessed using a food frequency questionnaire (FFQ). The FFQ captured information on frequency of consumption of certain foods in the past 7 days and 24 hours (**Supplemental Table 19**). The FAO Minimum Dietary Diversity for Women of Reproductive Age (MDD-W) guidelines [104]

were used to calculate minimum diversity measurements. Foods consumed in the past seven days were categorized into one of the 10 FAO MDD-W food groups: (1) grains/roots/tubers, (2) pulses, (3) nuts and seeds, (4) dairy, (5) meats, (6) eggs, (7) dark green leafy vegetables, (8) other vitamin A sources, (9) other vegetables, (10) other fruits. Responses were coded to compute a minimum dietary diversity score [188]. Minimum dietary diversity was defined as having consumed ≥ 5 of the 10 food groups in the previous 24 hours (day or night) [104].

Additionally, hemoglobin levels were measured using HemoCue® Hb 301 Systems. As per the WHO recommendations [100], anemia during pregnancy was defined as hemoglobin levels of less than 11 g/dL. Maternal height, weight and mid-upper arm circumference (MUAC) were measured using Seca scales, ShorrBoard® Stadiometers and adult (65 cm) MUAC measuring tapes, respectively. Low MUAC during pregnancy was defined as ≤23 cm [101]. Anthropometric measurements for women were also recorded to the nearest 0.1 kg or 0.1 cm. Very short maternal stature was defined as ≤145 cm [90, 103, 113].

Statistical analyses

Anthropometric analyses were restricted to the sample of women with serum aflatoxin data who had singleton births with no congenital malformations between 26-45 weeks of gestation and measured within 72 hours of childbirth (**Figure 9**). Key analytical covariates were based on evidence from previous literature [189]. Other factors that could potentially influence birth outcomes included age, diet,

education, wealth, anemia, short stature, smoking, antenatal visits, and infant birth month [90, 113, 190, 191]. Infant birth month data are shown in **Supplemental Table 20**.

Variables were examined for distributional properties and, when appropriate, were divided into categories based on sample size or biological significance (i.e. anemia, low MUAC). Exploratory analyses were conducted using Student t-tests for continuous maternal aflatoxin levels and chi-squared tests for categorical variables.

Logistic regression models were used to identify factors associated with low birth weight (LBW), small-for-gestational-age (SGA), stunting at birth, and preterm birth. The association of each covariate was first assessed individually using a binary logistic regression model to estimate crude odds ratios. Next, multivariate logistic regression models including key covariates were used to estimate adjusted odds ratios. Covariates considered in the final models included infant's sex, household socioeconomic status, and maternal characteristics such as schooling, hemoglobin levels, height, parity, MUAC, dietary diversity, and smoking during pregnancy. All models were adjusted for location (VDC) and birth month [125, 126]. A P value of less than 0.05 indicates the estimated coefficient is different from zero in a two-tailed test of statistical significance. Statistical analyses were conducted using Stata Version 14.2 (StataCorp LP, College Station, TX).

Results

Maternal aflatoxin exposure

Aflatoxin albumin adducts were detected in 94% of maternal blood samples. The distribution was skewed to the right, with levels varying from undetectable (<0.4) to 147 pg/mg albumin adducts. The mean ± SD of maternal aflatoxin B1-lysine adducts for women in this cohort was 3.2±8.3 pg/mg of albumin (geometric mean 1.37 (CI: 1.30-1.44), range undetectable-147 pg/mg). Mean levels in this cohort were relatively low compared to levels found in earlier studies [7, 14, 24, 92] with pregnant women and children.

Maternal sociodemographic and health characteristics

Table 15 shows maternal characteristics and infants' birth month. The mean maternal age was 23.6±4.7 years. Almost 37% of the women reported no education, while 23% had completed a primary education, 32% a secondary education, and 9% more than secondary. Thirty-four percent of the women were primigravida. Almost 41% of the pregnant women were anemic. Almost 14% were of very short maternal stature. One-third of women (33%) had a low MUAC during pregnancy, and almost 40% did not achieve minimum dietary diversity.

Maternal serum aflatoxin levels and birth outcomes

Table 16 shows descriptive infant data by gender. Almost 51% of the infants were female. The average gestational age at birth was 39.5±2.4 weeks for both male and female infants. The mean birth weights for male and female infants

were 2.9±0.5 kilograms (kg) and 2.8±0.4 kg, respectively ($p<0.001$). The mean WAZ scores were -1.00±1.03 for both male and female infants. Almost 20% of infants were born LBW (weighing less than 2.5 kg). Significantly more female infants (22.5%) were LBW than male infants (17.3%) ($p<0.05$). More than half (51.5%) of infants were small-for-gestational-age (SGA). A larger percentage (53.1%) of the female infants were SGA than the male infants (50.3%), but this difference was not significant at the $p<0.05$ level. One noteworthy caveat when considering the high rates of SGA and preterm births is the use of LMP to estimate gestational age at birth. LMP-based assessment of gestational age at birth is not as accurate as ultrasound estimates; it might therefore imprecisely estimate gestational age [192].

The mean birth lengths for male and female infants were 48.5±2.2 centimeters (cm) and 47.8±2.1 cm, respectively ($p<0.001$). The mean LAZ scores for male infants were -0.88±1.17 and for female infants. Sixteen percent of infants were stunted at birth. More male infants (17.7%) were stunted at birth than female infants (14.4%), but this difference was not significant at the $p<0.05$ level. Moreover, 13% of infants were born preterm (before 37 weeks of gestation). There was no significant difference in preterm birth rates between male and female infants.

Unadjusted correlations between mean maternal aflatoxin levels and birth outcomes are shown in **Table 17**. The table also shows characteristics of infants whose mother's aflatoxin levels during pregnancy were above the median or not. Mean maternal aflatoxin levels were higher in mothers of preterm, stillborn,

stunted, LBW, and SGA infants, though this difference was not significant at the $p<0.05$ level. Of the 182 preterm infants, almost 39% were stunted, 41% were low birth weight, and 20% were SGA. Mean AFB1 levels were higher in the stunted, LBW and SGA preterm infants, but in models that included the full set of covariates listed previously, these differences were not significant. Of the full term infants, almost 13% were stunted, 17% LBW and 56% SGA. Levels of maternal aflatoxin were not higher in full term infants who showed signs of *in utero* growth retardation.

Multivariate logistic regression results for association between *in utero* aflatoxin exposure and adverse birth outcomes

Table 18 shows results of the multivariate logistic regression analyses for four adverse birth outcomes: low birth weight, small-for-gestational-age, preterm and stillbirth. After adjusting for covariates: gestational age, location (VDC), birth month, education, household wealth, hemoglobin levels, stature, parity, MUAC, dietary diversity, number of ANC visits, and smoking, maternal aflatoxin albumin adducts were not significantly correlated with any of the four birth outcomes.

Among the covariates, low maternal stature (OR=1.95, 95% CI: 1.33-2.84, $p<0.01$) and average MUAC (OR=0.91, 95% CI: 0.86-0.97, $p<0.01$) were significantly associated with LBW. Primigravida women had significantly higher odds of having a LBW infant than those who had been pregnant before. Higher dietary diversity scores (OR=0.87, 95% CI: 0.78-0.97, $p<0.05$) and increased

number of ANC visits (OR=0.76, 95% CI: 0.64-0.89, p<0.01) were significantly related to lower odds of having a low birth weight infant.

Women who had serum aflatoxin levels above the geometric mean had 18% higher odds of having a small-for-gestational-age infant than those with aflatoxin levels below the geometric mean (OR=1.18, 95% CI: 0.93-1.50) in the adjusted model; however, this relationship was not statistically significant. Moreover, women in the richest wealth category had lower likelihood of having a SGA infant than women in the poorest category (OR=0.64, 95% CI: 0.44-0.94, p<0.05). Higher hemoglobin levels during pregnancy were significantly associated with 10% higher probability of SGA (OR=1.10, 95% CI: 1.00-1.21, p<0.05). Women of short stature showed double the likelihood of having a SGA infant (OR=2.17, 95% CI: 1.54-3.06, p<0.001). Women in their first pregnancy had significantly higher odds of having a SGA baby than those who had been pregnant before.

Maternal serum aflatoxin levels were not significantly associated with odds of stunting at birth. For women of short stature, the likelihood of having a stunted infant at birth was 2.34 times larger than the likelihood for a woman of average height (OR=2.34, 95% CI: 1.59-3.45, p<0.001). Additional covariates significantly negatively associated with stunting included having had one previous pregnancy (OR=0.50, 95% CI: 0.34-0.74, p<0.01), two previous pregnancies (OR=0.38, 95% CI: 0.24-0.60, p<0.001), three or more previous pregnancies (OR=0.37, 95% CI: 0.23-0.59, p<0.001) and number of ANC visits (OR=0.74, 95% CI: 0.62-0.88, p<0.01). The likelihood of male infants being stunted at birth were 1.46 times higher than that of female infants (OR=1.46, 95% CI: 1.07-1.97, p<0.05).

In utero aflatoxin levels were not associated with preterm deliveries. Every additional year of schooling was associated with lower odds of having a preterm birth (OR=0.94, 95% CI: 0.89-0.99, p<0.05). Odds of having a preterm birth were lower for every centimeter of MUAC during pregnancy (OR=0.92, 95% CI: 0.85-0.99, p<0.05). Odds of experiencing a preterm delivery decreased with every additional ANC visit (OR=0.59, 95% CI: 0.49-0.71, p<0.001).

Discussion

This study used longitudinal birth cohort data to examine the relationship between *in utero* aflatoxin exposure and adverse birth outcomes, such as low birth weight, small-for-gestational-age, stunting and preterm delivery. One in five infants was born low birth weight. As expected, preterm infants were more likely to be LBW than full term infants. On the other hand, a greater proportion of full term infants (56%) were SGA than preterm infants (22%). The high rates of LBW, SGA and stunting (16%) suggest intrauterine growth restriction was common [180].

Findings from this study did not show an association between *in utero* aflatoxin exposure and selected adverse birth outcomes. The lack of correlations between aflatoxin exposure during pregnancy and birth outcomes suggests either a) an as yet unidentified threshold health effect; b) a relationship confounded by other mycotoxins that often coexist with aflatoxin; or c) a lack of an association within the context of this specific population. The relationship may be complicated by other mycotoxins that often coexist with aflatoxin, such as

fumonisin, which may be confounding this relationship. Furthermore, variations in genetics and age can influence toxicity and excretion of aflatoxin, complicating the potential link between aflatoxin and birth outcomes [84, 193].

The high prevalence (94%) of prenatal aflatoxin exposure is comparable to previous studies in Africa [66] and South Asia [14]. However, the geometric mean maternal aflatoxin level of 1.37 (CI: 1.30-1.44) in this cohort was much lower than average levels found in pregnant women and children in previous studies [7, 14, 24, 92]. A previous study in Sarlahi, Nepal [14] showed a geometric mean of 25.3 pg/mg in pregnant women (n=141, range: 0.45 to 2939.30 pg aflatoxin B1-lysine adduct/mg albumin). Turner et al. [7] reported geometric mean levels of 40.4 pg/mg in maternal blood (almost 30 times the geometric mean observed in the current study) while Shuaib et al. [24] reported average levels of 10.9 in pregnant women (more than three times the average of this current study). Another recent study in Nigerian children [9] showed a geometric mean level of 2.6 pg/mg. The higher blood aflatoxin levels in previous studies may reflect different diets. Other populations may have a higher reliance on dietary staples such as maize and groundnuts, two food crops with a high susceptibility to aflatoxin contamination. Rice, a staple in this area of Nepal [160], tends to harbor low levels of aflatoxin [136].

This study showed a positive association between maternal aflatoxin levels and low birth weight that was not statistically significant after adjusting for other relevant factors. This finding concurs with non-significant results from Maxwell et al. [25] in Nigeria and Turner et al. [7] in The Gambia. Results from Turner et al.

[7] showed strong relationships between *in utero* aflatoxin exposure and children's poor linear post-natal growth (lower weight-for-age and weight-for-height z-scores). However, their study did not detect a relationship between *in utero* aflatoxin exposure and weight or length at birth.

Birth weight results from our study differ from prior studies conducted in Ghana [24], the United Arab Emirates (UAE) [32] and Kenya [23], which did detect an association between aflatoxin exposure during pregnancy and low birth weight. A study by Shuaib et al. in Ghana [24] used cross-sectional data to investigate the association between various birth outcomes and serum aflatoxin B1-lysine adduct levels in pregnant women. After controlling for socio-demographic variables and potential confounders such as malaria parasitemia, anemia and worm infections, that study found that mothers in the very high aflatoxin quartile (>11.34 pg/mg albumin) were more likely to have low birth weight infants (OR, 2.09; 95% CI, 1.19–3.68). Infants from those mothers showed a trend of increasing risk for LBW compared to respondents in the lowest exposure quartile. Moreover, a study in the UAE showed high *in utero* aflatoxin exposure and a significant negative correlation between birth weight and serum aflatoxin B1-lysine adduct levels ($p<0.001$) [32]. An earlier study in Kenya showed exposure present among half of the pregnant women tested and found mean birth weights of females born to aflatoxin-positive mothers were significantly lower than those born to mothers free of aflatoxin [23].

Results reported here from Nepal did not show a statistically significant association between aflatoxin exposure during pregnancy and small-for-

gestational age. These conclusions are similar to those from Shuaib et al. [24]. The discordance between the two studies' results regarding *in utero* aflatoxin exposure and reduced birth weight may be a function of the low mean aflatoxin levels in our sample. A threshold level of exposure may be needed to produce detectable adverse birth outcomes. That level remains to be determined for human subjects. The low levels of aflatoxin in this sample prevent further exploration to determine a possible cut-off value.

This study did not detect a relationship between aflatoxin exposure and stunting at birth. Thirteen percent of the children were stunted at birth. Such evidence confirms that early-life linear growth failure occurs frequently in this part of Nepal. Child stunting is a public health concern, not only because of the known linkage to increased morbidity and mortality in children, but also because of the link between stunting and reduced cognitive development and thus economic growth [29]. These aflatoxin-stunting at birth results corroborate findings from a study by Turner et al. [7], which showed no relationship between *in utero* aflatoxin and birth length. While that study did not show a relationship with length at birth, it did show a negative association between aflatoxin exposure and postnatal length.

Additionally, while animal studies have shown a relationship between aflatoxin and decreased live births [52, 194], this study did not find a relationship between either preterm births or stillbirths and the levels of *in utero* aflatoxin exposure. Such results corroborate the findings from previous human studies [23, 74, 91] showing no relationship between aflatoxin exposure and such

adverse birth outcomes. Note that neither this study nor any of the previous studies was designed to assess a causal relationship between aflatoxin exposure during pregnancy and preterm or stillbirth.

This study has strengths and weaknesses. The longitudinal nature of this study allows for simultaneous adjustment of statistical models for important confounding factors such as household wealth, diet and health. Data have been collected periodically on infants enrolled in this study, and future analyses will help further elucidate the relationship between maternal aflatoxin exposure, birth outcomes, infant aflatoxin exposure (through breast milk and food) and linear growth.

Second, the study has a much larger sample than previous studies examining these associations. Moreover, this study uses a validated quantitative biomarker to measure exposure to aflatoxin over a period of two to three months [96]. Also, infants' supine length, a measurement often overlooked in field research due to challenges with accuracy [123], was measured within 72 hours of birth for most study participants. Lastly, because this study uses a community-based rather than a clinically-based recruitment strategy, results can be generalized to similar populations in the area and possibly to other populations in Nepal and nearby nations with similar diets.

Both the relatively low mean levels of *in utero* aflatoxin exposure and the use of LMP to estimate gestational age are possible explanations for a failure to determine any evidence of association. The generally modest levels of aflatoxin in this sample could have limited our ability to detect health effects if such

adverse outcomes only result above a certain threshold. Dating last menstrual period has drawbacks, mainly poor recall accuracy and inaccuracy when menstruation cycles are irregular. LMP estimates are often off by a couple of weeks, and studies have shown ultrasound to be the most accurate form of dating gestational age [114, 192].

However, in settings where ultrasounds are not feasible, LMP serves as a low-cost, practical, though less precise alternative for measuring gestational age. Moreover, the high prevalence of SGA should be interpreted with caution. Previous literature has shown that the reference population chosen can strongly affect SGA prevalence [120]. This study used the WHO's recently published Multinational Fetal Growth Charts [121], which are recommended for countries that have not developed their own charts.

Results from this study support literature showing strong intergenerational effects on birth outcomes. Short maternal stature was significantly associated with LBW, SGA and stunting (after adjusting for current socioeconomic status, parity, and other factors), all parameters of *in utero* growth failure that often coexist. Women who are stunted tend to have stunted offspring [123, 195]. Such evidence warrants the scaling up of evidence-based interventions to reduce the intergenerational cycle of poor growth. The higher likelihood that primigravida women will have stunted infants or infants of lower average weight suggests a need for additional targeting of first time and adolescent mothers who may, both for biological and social reasons, be at a higher risk of experiencing adverse birth

outcomes. Improving a woman's nutritional status and health before pregnancy can help improve the chances of her children having a healthy start to life.

Although these findings from Banke, Nepal do not confirm the previously suggested association between *in utero* aflatoxin exposure and adverse birth outcomes, future work should both re-examine existing relationships between prenatal aflatoxin exposure and birth outcomes and explore whether the lack of a relationship in this particular study is due to the lower levels observed thereby suggesting a need to examine multi-country data to elucidate a threshold level of *in utero* aflatoxin exposure beyond which there is a likelihood of a deleterious effect on birth weight. Furthermore, additional studies are needed to understand how different diets and individual detoxification pathways influence excretion and toxicity of *in utero* aflatoxin. Last, other mycotoxins that often coexist with aflatoxin (e.g. fumonisin) may be implicated, prompting further research into this issue of co-exposure to multiple toxins.

Tables and Figures

Table 15. Sociodemographic and health characteristics of Nepalese women and infants enrolled in the AflaCohort Study

	n	%	AFB1 Mean ± SD	p-value Log AFB1
Age Category				0.0081
16-20	340	21.0	4.3±11.2	
21-24	617	38.1	3.2±7.7	
25-29	466	28.8	2.4±4.4	
30-34	133	8.2	3.0±13.0	
35+	65	4.0	2.1±2.6	
Schooling				
None	595	36.7	2.5±5.0	0.4773
Some primary (1-5)	317	19.6	3.0±5.5	
Some secondary (6-10)	567	35.0	4.2±12.1	
More than secondary (10+)	142	8.8	2.6±5.2	
Wealth Index Quintile				0.0951
Poorest	316	19.5	3.5±9.5	
Poor	327	20.2	2.8±6.5	
Middle	326	20.1	2.6±6.4	
Rich	323	19.9	4.0±9.4	
Richest	329	20.3	3.0±9.2	
Parity				0.0448
Primigravida	547	33.7	4.0±10.5	
1 previous pregnancy	426	26.3	2.8±6.0	
2 previous pregnancies	303	18.7	3.0±9.8	
3+ previous pregnancies	345	21.3	2.4±4.2	
Anemia (<11 g/dL)				0.0559
No	964	59.5	3.5±9.4	
Yes	655	40.5	2.7±6.3	
Stature				0.4547
Short/Average (>145 cm)	1400	86.7	3.2±8.3	
Very Short (≤145 cm)	539	13.5	3.1±8.4	
MUAC				0.4575
Average (>23 cm)	1082	66.8	3.1±8.5	
Low (≤23 cm)	539	33.3	3.3±7.9	
Minimum Dietary Diversity (FAO MDD-W) ≥ 5 food groups [a]				0.1002
No	984	60.7	3.0±7.3	
Yes	637	39.3	3.6±9.7	
Smoking during pregnancy				0.9072
No	1600	98.7	3.2±8.3	
Yes	21	1.3	2.6±3.5	
Maternal serum sampling season				0.0000
Spring (Basanta)	504	31.1	2.2±5.0	
Summer (Grishma)	386	23.8	1.3±2.8	

Rainy (Barsha)	32	2.0	1.2±1.3
Autumn (Sharad)	0	0	*ND*
Prewinter (Hemanta)	237	14.62	6.6±14.1
Winter (Shishir)	462	28.5	4.2±9.6

n, sample; AFB1, aflatoxin B1; g, grams; dL, deciliter; cm, centimeter; MUAC, mid-upper arm circumference; FAO, Food and Agriculture Organization of the United Nations; MDD-W, Minimum Dietary Diversity for Women; ND, No data. [a] MDD-W defined as ≥ 5 of the 10 food groups in the previous day or night. Numbers may not always add up due to missing responses; n=1621.

Table 16. Birth outcomes, by gender

Characteristic	n (%) or mean ± SD	Q1	Median	Q3	p-value
Infant sex					
Male	718 (49.4)	-	-	-	
Female	736 (50.6)	-	-	-	
Gestational age at birth (weeks) [a]					
Male	39.5±2.4	38.3	39.7	41.0	0.8470
Female	39.5±2.4	38.4	39.6	41.0	
Birth weight (kg)					
Male	2.9±0.5	2.6	2.9	3.2	0.0000
Female	2.8±0.4	2.5	2.8	3.0	
WAZ					
Male	-1.00±1.03	-1.7	-0.9	-0.3	0.9858
Female	-1.00±1.03	-1.6	-1.0	-0.4	
LBW (<2.5 kg)					
Male	124 (17.3)	-	-	-	0.0130
Female	165 (22.5)	-	-	-	
SGA [b]					
Male	360 (50.3)	-	-	-	0.3010
Female	389 (53.1)	-	-	-	
Birth length (cm)					
Male	48.5±2.2	47.2	48.6	50.0	0.0000
Female	47.8±2.1	46.6	48.0	49.2	
LAZ					
Male	-0.88±1.17	-1.6	-0.8	-0.1	0.8501
Female	-0.87±1.07	-1.5	-0.8	-0.2	
Stunted at birth (LAZ≤-2)					
Male	127 (17.7)	-	-	-	0.0800
Female	105 (14.4)	-	-	-	
Preterm birth [c]					
Male	87 (12.1)	-	-	-	0.2075
Female	95 (12.9)	-	-	-	
Stillbirth [d]					
Male	23 (2.9)				0.9260
Female	15 (1.9)				
Miscarriage [e]	27 (1.6)	-	-	-	

n, sample; SD, standard deviation; Q1, first quartile; Q3, third quartile; kg, kilograms; WAZ, weight-for-age z-score; LBW, low birth weight; kg, kilograms; SGA, small-for-gestational-age; cm, centimeter; LAZ, length-for-age z-score; [a] based on last menstrual period (LMP); [b] <10th percentile for the weight for gestational age and gender relative to standard population; [c] born before 37 weeks of gestation; [d] baby born with no signs of life at or after 28 weeks gestation; [e] pregnancy in which the fetus did not survive or in which the fetus was born before the 28th week of pregnancy, information on child gender not available. Numbers may not always add up due to missing responses. Indicators involving anthropometric measurements exclude infants visited within 72 hours after birth.

Table 17. *In utero* aflatoxin levels and birth outcomes

	n (%)	AFB1 Mean ± SD	p-value log AFB1	n (%) AFB1 ≥ geo mean	p-value AFB1 ≥ geo mean
Preterm birth [a]					
No	1272 (87.5)	3.0±7.6	0.0690	520 (41.0)	0.1030
Yes	182 (12.5)	4.2±11.8		86 (47.3)	
Stillbirth [b]					
No	1610 (97.7)	3.2±8.1	0.9541	637 (28.3)	0.9820
Yes	38 (2.3)	4.9±13.6		14 (26.9)	
Low birth weight (<2.5 kg) [c]					
ABW	1159 (80.0)	3.1±8.2	0.0585	472 (28.9)	0.1270
LBW	289 (20.0)	3.4±8.5		132 (31.4)	
Birth weight for gestational age					
AGA	705 (48.5)	3.1±9.4	0.1478	282 (28.6)	0.2080
SGA [d]	749 (51.5)	3.2±6.9		324 (30.2)	
Stunted at birth (LAZ≤-2) [c]					
No	1215 (84.0)	3.1±8.0	0.9345	497 (29.0)	0.1760
Yes	232 (16.0)	3.3±9.3		106 (31.4)	
PRETERM INFANTS					
Low birth weight (<2.5 kg)					
ABW	107 (58.8)	3.5±11.1	0.1061	47 (43.9)	0.2830
LBW	75 (41.2)	5.1±12.7		39 (52.0)	
Birth weight for gestational age					
AGA	146 (80.2)	3.9±12.7	0.0824	68 (46.6)	0.7120
SGA [d]	36 (19.8)	4.8±7.4		18 (50.0)	
Stunted at birth (LAZ≤-2)					
No	113 (61.5)	3.9±11.4	0.4483	49 (43.4)	0.1790
Yes	69 (38.5)	4.6±12.5		37 (53.6)	

FULL TERM INFANTS					
Low birth weight (<2.5 kg)					
ABW	1052 (83.1)	3.0±7.8	0.3669	425 (40.4)	0.4070
LBW	214 (16.9)	2.8±6.3		93 (43.5)	
Birth weight for gestational age					
AGA	559 (43.9)	2.8±8.4	0.1371	214 (38.3)	0.0950
SGA [d]	713 (56.1)	3.1±6.9		306 (42.9)	
Stunted at birth (LAZ≤-2)					
No	1102 (87.1)	3.0±7.6	0.304	448 (40.7)	0.6840
Yes	163 (12.9)	2.8±7.6		69 (42.3)	

n, sample; SD, standard deviation; AFB1, aflatoxin B1; ABW, average birth weight; LBW, low birth weight; kg, kilograms; AGA, appropriate-for-gestational-age; SGA, small-for-gestational-age; LAZ, length-for-age z-score; Geometric mean=1.37 pg/mg aflatoxin albumin adducts; [a] born before 37 weeks of gestation; [b] baby born with no signs of life at or after 28 weeks gestation; [c] Does not account for gestational age of child (preterm or full term); [d] <10th percentile for the weight for gestational age and gender relative to standard population. Numbers may not always add up due to missing responses. Indicators involving anthropometric measurements exclude infants visited >72 hours after birth.

Table 18. Multivariate logistic regression analyses of maternal aflatoxin levels and birth outcomes

	LBW [1] n=1445	SGA [2] n=1451	Stunted at birth [3] n=1444	Preterm birth [4] n=1451
	Adjusted OR (95% CI)			
Maternal aflatoxin levels [a]				
Below geometric mean	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
At/above geometric mean	1.13 (0.84-1.53)	1.18 (0.93-1.50)	1.25 (0.90-1.72)	0.91 (0.64-1.30)
Schooling (years)	0.95 (0.91-1.00) *	0.97 (0.94-1.00) †	0.95 (0.91-1.00) †	0.94 (0.89-0.99) *
Wealth index quintile				
Poorest	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Poor	0.62 (0.40-0.96) *	0.99 (0.70-1.41)	0.90 (0.56-1.44)	1.16 (0.68-1.97)
Middle	0.82 (0.53-1.26)	0.85 (0.60-1.21)	0.84 (0.52-1.34)	1.31 (0.78-2.23)
Rich	0.67 (0.43-1.07) †	0.98 (0.69-1.40)	0.92 (0.57-1.48)	0.97 (0.54-1.72)
Richest	0.72 (0.44-1.18)	0.64 (0.44-0.94) *	0.81 (0.48-1.37) *	1.50 (0.81-2.62)
Hemoglobin (g/dL)	0.98 (0.87-1.11)	1.10 (1.00-1.21) *	0.95 (0.84-1.07) *	0.87 (0.76-1.00) †
Maternal stature				
Average (>145 cm)	1.0 (REF)	1.0 (REF)	1.0 (REF)	1.0 (REF)
Short (≤145 cm)	1.95 (1.33-2.84) **	2.17 (1.54-3.06) ** *	2.34 (1.59-3.45) ** *	1.42 (0.90-2.23)
Parity				
Primigravida	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
1 previous pregnancy	0.31 (0.21-0.47) ** *	0.50 (0.38-0.67) ** *	0.50 (0.34-0.74) **	0.82 (0.52-1.28)

2 previous pregnancies	0.26 (0.16-0.40)	** *	0.36 (0.26-0.51)	** *	0.38 (0.24-0.60)	** *	0.76 (0.46-1.26)	
3+ previous pregnancies	0.36 (0.23-0.55)	** *	0.49 (0.35-0.69)	** *	0.37 (0.23-0.59)	** *	0.68 (0.42-1.13)	
MUAC (cm)	0.91 (0.86-0.97)	**	0.96 (0.91-1.01)		0.95 (0.89-1.02)		0.92 (0.86-1.00)	*
FAO MDD-W score	0.87 (0.78-0.97)	*	0.98 (0.90-1.06)	†	0.97 (0.87-1.08)		0.94 (0.83-1.07)	
Infant sex								
Male	0.76 (0.57-1.01)	†	0.93 (0.74-1.15)		1.46 (1.07-1.97)	*	1.03 (0.74-1.45)	
Female	1.00 (REF)		1.00 (REF)		1.00 (REF)		1.00 (REF)	
ANC visits	0.76 (0.64-0.89)	**	1.04 (0.93-1.17)		0.74 (0.62-0.88)	**	0.60 (0.50-0.72)	** *
Smoking during pregnancy								
No	1.00 (REF)		1.00 (REF)		1.00 (REF)		1.00 (REF)	
Yes	1.98 (0.68-5.81)		1.65 (0.64-4.30)		0.73 (0.15-3.44)		0.87 (0.18-4.12)	

Adjusted for gestational age, VDC, trimester and birth month. [a] Geometric mean=1.37 pg/mg aflatoxin albumin adducts;. Two-tailed tests, †p<0.1; *p<0.05; **p<0.01; ***p<0.001. LBW, low birth weight; SGA, small-for-gestational-age; OR, odds ratio; CI, confidence interval; g, grams; dL, deciliter; cm, centimeter; MUAC, mid-upper arm circumference; FAO, Food and Agriculture Organization of the United Nations; MDD-W, Minimum Dietary Diversity for Women; ANC, antenatal care. The relationships between maternal aflatoxin levels and adverse birth outcomes were also explored using Ordinary Least Squares models, and restricted to full-term infants only. Results did not differ and are therefore not presented.

[1] Logistic regression; Model Pseudo R^2 = 0.14

[2] Logistic regression; Model Pseudo R^2 = 0.07

[3] Logistic regression; Model Pseudo R^2 = 0.11

[4] Logistic regression; Model Pseudo R^2 = 0.12

Supplemental material

Table 19. Women's Food Frequency Questionnaire (FFQ)

	Food Item(s)	How many times have you eaten _____ in the past 7 days?	How many times have you eaten _____ in the past 24 hours?
3.2.1	Ricebhat/riceroti		
3.2.2	Corn dhido/bhat/roti		
3.2.3	Wheat/buckwheat dhido/roti		
3.2.4	Millet dhido/roti		
3.2.5	Daal (any)		
3.2.6	Maseura		
3.2.7	Other legumes (including chickpeas, dried peas, lima beans and soybeans)		
3.2.8	Groundnuts		
3.2.9	Milk		
3.2.10	Curds/whey		
3.2.11	Milk tea		
3.2.12	Vegetable oil (any)		
3.2.13	Ghee		
3.2.14	Hydrogenated oil (Banaspati Ghee)		
3.2.15	Eggs (any)		
3.2.16	Chicken/duck		
3.2.17	Goat		
3.2.18	Buff		
3.2.19	Pork		
3.2.20	Large fish (fresh)		
3.2.21	Small fish (fresh)		
3.2.22	Dried fish		
3.2.23	Snails		
3.2.24	Dark green leafy vegetables		
3.2.25	Carrots		
3.2.26	Gundruk		
3.2.27	Ripe pumpkin		
3.2.28	Green beans (Bodi, simi)		
3.2.29	Green peas (mutterkosa)		
3.2.30	Gourd (lauka, ghiraula, bitter, titekarela, jhimni)		
3.2.31	Okra/Ladies finger/Bhindi		

3.2.32	Eggplant		
3.2.33	Potatoes		
3.2.34	Tomato		
3.2.35	Cauliflower		
3.2.36	Cabbage		
3.2.37	Drumstick (sajCHan)		
3.2.38	Green jackfruit		
3.2.39	Ripe mango		
3.2.40	Jackfruit (ripe)		
3.2.41	Guava		
3.2.42	Orange/tangerine		
3.2.43	Ripe papaya		
3.2.44	Apple		
3.2.45	Pineapple		
3.2.46	Banana		
3.2.47	Jaard/Rakshi		
3.2.48	Instant Noodles (packet, e.g. Wai Wai)		
3.2.49	Snacks (beaten rice, puffed rice, sweets, biscuits, dalmot, popcorn)		

Table 20. Infant birth month

Infant birth month [a]	n	%
Poush (December-January)	52	3.2
Magh (January-February)	34	2.1
Falgun (February-March)	60	3.7
Chaitra (March-April)	83	5.1
Baisakh (April-May)	94	5.8
Jestha (May-June)	125	7.7
Ashar (June-July)	165	10.2
Shrawan (July-August)	240	14.8
Bhadra (August-September)	243	15.0
Ashwin (September-October)	244	15.1
Kartik (October-November)	171	10.6
Mangsir (November-December)	110	6.8

[a] http://calendar-nepali.com/about/Nepali-Calendar-Months.html

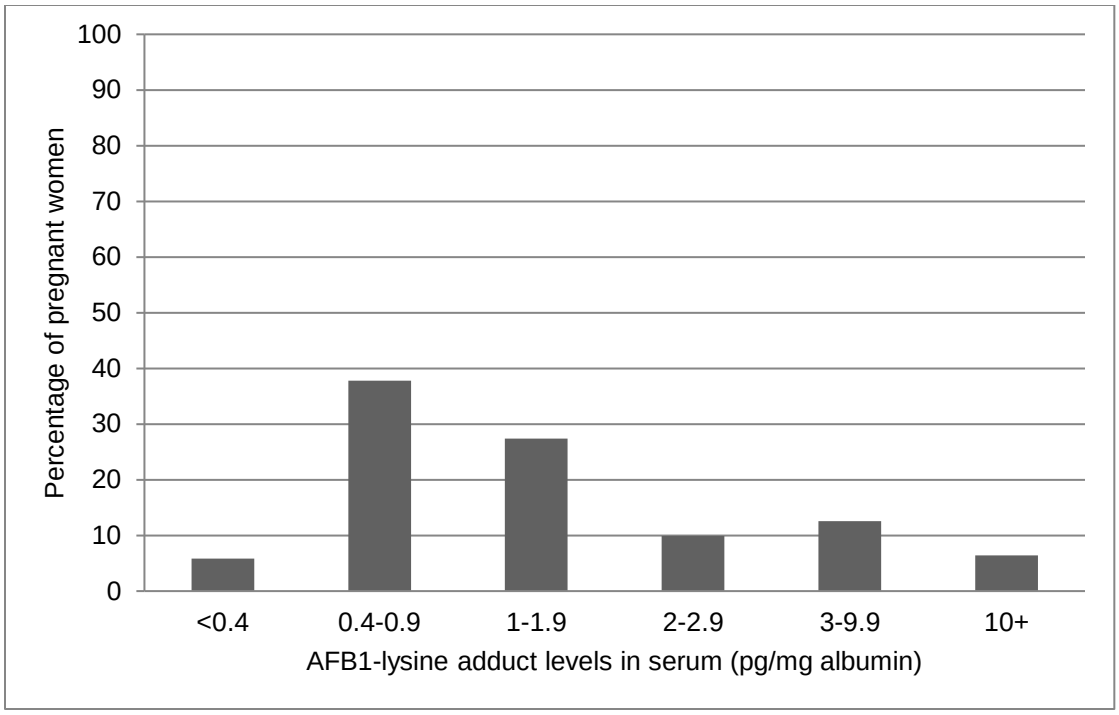

Figure 8. Distribution of prenatal serum aflatoxin levels

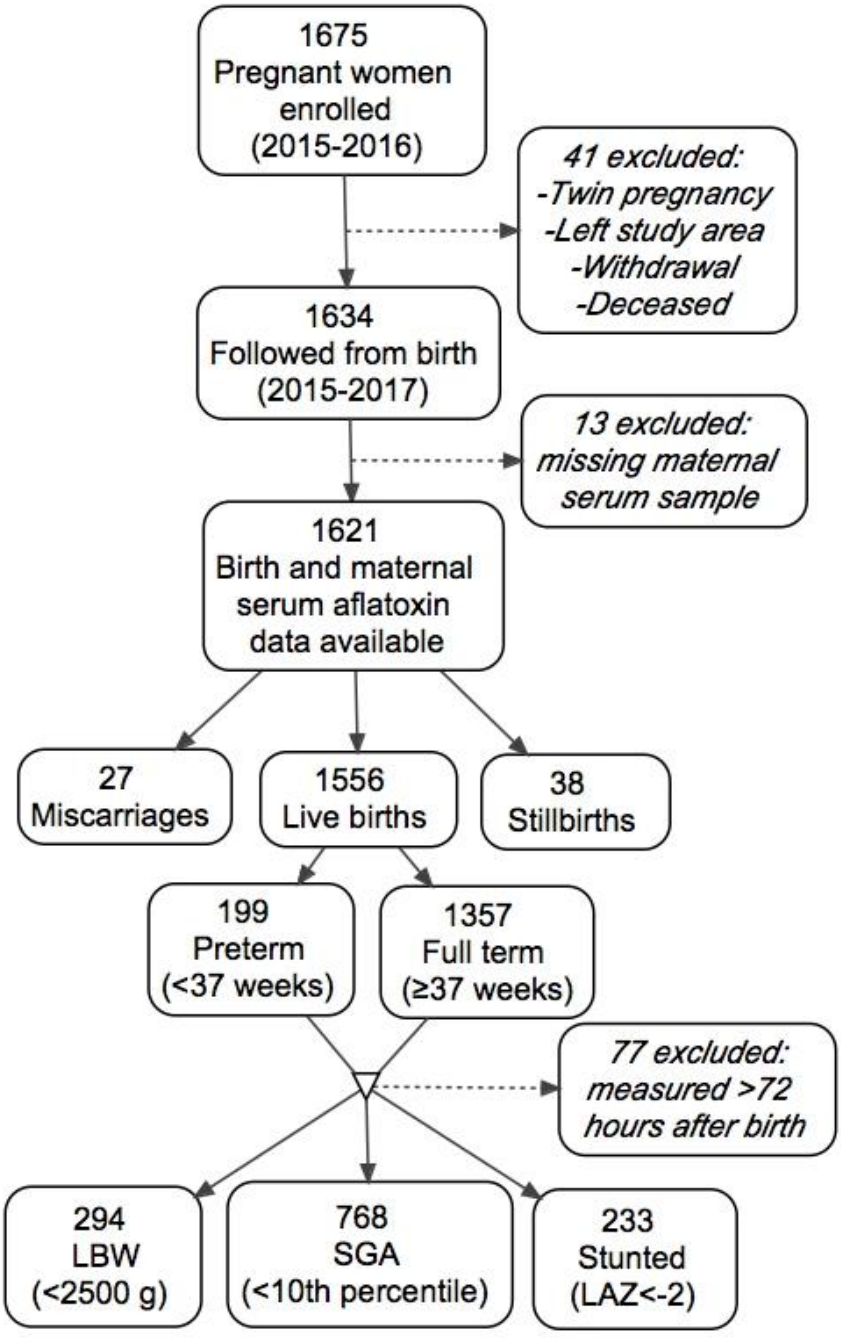

Figure 9. Flow of AflaCohort enrollment and birth outcomes

CHAPTER 7. SUMMARY AND DISCUSSION

While recent studies have suggested a strong association between aflatoxin exposure and stunting during the first 1000 days [196], much remains unknown about factors contributing to aflatoxin exposure and its effects on birth outcomes. A birth cohort study, implemented in the Banke district of Nepal, with 1675 mother-infant pairs gave us the opportunity to explore potential dietary and agricultural pathways of exposure and to address unanswered questions regarding the link between aflatoxin exposure during gestation and subsequent birth outcomes. The topics explored in this dissertation are important in light of the documented widespread presence of aflatoxin in developing countries, the recognized association between aflatoxin and poor linear growth in children, and the limited research that had taken place in South Asia, even though aflatoxin contamination seems to be widespread in the region [10, 14, 15, 134, 149]. An improved understanding of the factors associated with aflatoxin exposure, particularly during the biologically sensitive period of gestation, will help inform appropriate interventions for the region.

Summary of Findings

Chapters 4 and 5 of this dissertation presented evidence of multiple dietary, seasonal, and behavioral practices that increase aflatoxin exposure of pregnant women. Chapter 6 presented the results from this large birth cohort study that found no relationship between prenatal aflatoxin levels and adverse birth outcomes. While the three studies in this dissertation concentrated on the first

270 days (gestation), upcoming analyses from the same birth cohort will measure aflatoxin exposure throughout the rest of the 1000 days and its potential link to postnatal linear growth.

Chapter 4 examined the relationship between dietary diversity and aflatoxin levels during pregnancy. It also identified which of the food items studied were correlated with higher aflatoxin levels during this biologically sensitive state. Findings showed that even though some women were consuming relatively small quantities of maize and modest amounts of groundnuts, consumption of such foods was strongly correlated with higher aflatoxin levels. In Banke, maize and groundnuts are typically harvested before the winter months. In this population, women tested for aflatoxin in the winter months, following the maize and groundnut harvests, showed the highest levels of serum aflatoxin. Given fluctuations in supply throughout the year, consumption also appears to be seasonal. Women interviewed in the winter months had higher average frequencies of maize and groundnut consumption than women visited in non-winter months. Furthermore, in the population of pregnant women studied, greater dietary diversity was not associated with lower aflatoxin levels.

The research presented in Chapter 4 aimed to understand from where households habitually sourced aflatoxin-prone foods (i.e. maize, groundnuts and chilies). A very small percentage of households relied on self-production of these foods. Only 25% grew maize, and less than 1% produced groundnuts or chilies. Instead, maize, groundnuts, and chilies were often purchased in the market, and a large percentage was received in-kind. Results showed low aflatoxin

awareness among pregnant women living in farming households, regardless of which crops the household produced.

Furthermore, in Chapter 5 we used structured questionnaires to identify farmers' practices and determine if Good Agricultural Practices (GAPs) are currently being applied by farmers in a region of Nepal in which the population is heavily engaged in agriculture. We tested the potential link between GAP use and aflatoxin levels in sera of pregnant women. Generally, the use of one or more GAP was low (30%) in this population. The practices that were applied by some included harvesting the crops at maturity, drying kernels and chilies before storage, and checking storage units for leaks or pest infestations. Use of either insecticides or fungicides was rare. Many farmers reported long storage times (more than 6 months). The relationship to GAPs was complicated by the fact that households consume multiple aflatoxin-prone foods that come from multiple sources (i.e. home-produced, market, in-kind).

Chapter 6 examined four selected birth outcomes – low birth weight, small-for-gestational-age, stunting at birth and preterm birth in relationship to aflatoxin exposure during pregnancy. Rates of LBW, SGA, stunting at birth and preterm birth were 20%, 52%, 16% and 13% in this sample. Findings reinforce the conclusions from Maxwell et al. [25] and Turner et al. [7, 171] who showed no significant relationship between prenatal aflatoxin exposure and anthropometric birth outcomes. This relationship is complicated by multiple factors. As suggested by Mitchell et al. [147], the impacts of prolonged aflatoxin exposure on child growth might depend on the levels of exposure, levels that in many settings are

much higher than we found. Also, complex biological pathways and genetic differences may influence the toxicity and excretion of aflatoxin [9, 66, 193]. Moreover, the aflatoxin- fetal growth relationship is further complicated by other mycotoxins that often coexist with aflatoxin (e.g. fumonisin, deoxynivalenol) [141, 197].

Strengths and Limitations

A major strength of these studies was the large sample size, which was powered to detect differences in birth weight and postnatal stunting in women with high versus low aflatoxin levels. The longitudinal nature of the study allowed us to control for multiple confounding factors that could affect the results. We used a community recruitment strategy, thereby making the findings generalizable to the area and possibly to similar regions in Nepal, and perhaps neighboring India. Since seasonal variations influence the concentration of aflatoxins, maternal serum samples were collected during five out of the six seasons. Furthermore, standardized measuring equipment was used to measure infant anthropometry, and most infants were measured within a 72-hour window after birth. LMP-based gestational age was available for most infants and was applied to all anthropometric birth outcomes analyses. Accounting for gestational age allowed us to discern potential differences between preterm and full-term infants.

Although we worked with a large sample, the purposive recruitment strategy may have led to an underestimation of aflatoxin exposure levels among pregnant women since women who decided to enroll in the study may have wanted to

know more about their health than those who chose not to participate. Also, given the overall low levels of aflatoxin in this population, it is possible that we did not have sufficient women in the high aflatoxin level group to detect significant differences in birth outcomes. It is possible that the limited evidence detected of a relationship between GAPs and exposure to this toxin may be an artifact of the sample size, which was not powered to detect differences among the small numbers of self-sufficient farmers on this specific aflatoxin biomarker.

Further larger studies with farmers are required to confirm these results. Another potential limitation is that measuring AFB1 levels once during the pregnancy may not provide a full picture on aflatoxin exposure throughout the 9-month gestational period, especially given suspected seasonal variations. However, a recent study in Nepal [14] showed fairly constant aflatoxin exposure levels during pregnancy. Therefore, we believe this measurement provides exposure information over a significant portion of the *in utero* period and can be considered a valid reflection of general exposure during pregnancy [64]. Moreover, LMP used to estimate gestational age at birth is not as accurate as using ultrasound measurements [192].

Suggestions for Future Research

In addition to studying dietary recall data and serum aflatoxin levels, as was done for this dissertation, future studies should quantify aflatoxin levels in the foods consumed by pregnant women and their children. Such quantification will allow for a better understanding of which foods are most often contaminated with

aflatoxin, and also specify how the levels of contamination vary by type of food consumed and season, as well as how that contamination translates into serum levels during the first 1000 days. A study by Gong et al. [2] showed exposure levels increased significantly when children are weaned and begin to eat solid foods, some prone to aflatoxin contamination. By identifying the most commonly contaminated complementary foods and quantifying aflatoxin levels in them, programmers and policy-makers can more efficiently target and reduce aflatoxin levels in children during the critical first two years of their lives.

Our study, like previous similar studies [84, 146, 151], identified seasonality as a strong predictor of prenatal aflatoxin exposure; however, none of these studies carefully examined exactly how seasonal variations increase exposure to aflatoxin. Future studies should be designed to both collect a wider range of aflatoxin-prone food samples (maize, groundnuts, rice, wheat, chilies as well as other foods) throughout the year and to examine how contamination levels vary by season. Seasonality may also affect food source. When market prices vary, consumers may change the amounts consumed, choose different foods, or opt to purchase lower quality foods that are affordable. Identifying when and what foods are most contaminated will improve targeted efforts at aflatoxin reduction.

On a broad level, GAPs help address a complex set of pest and storage challenges to optimize food safety and in some cases profitability. While there is evidence that GAPs can reduce aflatoxin contamination in food [21] more research is needed to understand how application of good practices translates into levels of aflatoxin in human sera. More research is also needed to identify

barriers and facilitators for farmers' ability to implement GAPs. GAPs must be of value to farmers and pertinent to the agro-ecological setting in which the aflatoxin-prone food is being grown.

Future studies may be able to identify a bundle of practices most relevant for the specific setting based on cropping seasons, *Aspergillus* strains, weather fluctuations, concurrent pest and storage challenges, monetary cost, and economic return to farmers, and time use. Proper training and access to information and inputs are key to effectively apply GAPs. While the GAPs examined in this study were selected based on their potential contribution to aflatoxin prevention, ultimately implementation of such GAPs should lead to overall food safety and crop quality and profitability.

Another related and important area for future research is exploring feasible market-level interventions for the reduction of aflatoxin. Future studies cannot focus only at the farm level and overlook the importance and complexity of the market that we found was the principal source of the aflatoxin-prone foods identified in this region. Off-farm food acquisition was common in our study and is expected to become more relevant as rapid urbanization continues in Nepal [198], where some towns are growing 5-7% a year. Even if aflatoxin-reduction efforts include promotion of farm level GAPs, an increasingly large portion of the population will still be exposed to aflatoxin through markets. Therefore, in addition to targeting households, markets need to be monitored and made responsible for meeting food safety standards. Which foods and points of entry in

the value chain would be most useful to improve food safety are yet to be identified for this production environment.

More research on the effects of prenatal aflatoxin exposure on birth outcomes is needed. Literature on this relationship is limited and reports mixed results. Additional research is needed to determine a threshold of exposure during gestation for adverse birth outcomes to manifest. Given the literature on interindividual variation in the bioactivation and detoxification of aflatoxin [199], future studies should be designed to detect such physiological differences (e.g. metabolizing enzymes) that may alter the influence aflatoxin has on birth outcomes.

Future studies need to determine if the timing of aflatoxin exposure during gestation affects fetal outcomes. Negative effects of aflatoxin on birth outcomes may not only depend on exposure levels, the fetus' response to the toxin may also vary depending on the stage of gestation in which a fetus is exposed. Studies in the future should consider measuring aflatoxin exposure repeatedly, starting in early pregnancy. The availability of continual biological samples from the same women would allow researchers to assess temporal variability in aflatoxin exposure and its possible role in adverse birth outcomes. It will also allow for a better understanding of seasonal variations in exposure.

Furthermore, to understand effects on anthropometric birth outcomes, a good measure of gestational age must be available. Accurate gestational age measurements help elucidate how much of the reduced weight or height are attributable to IUGR, early birth, or both.

Policy Implications

This research suggests improving the quality of maize and groundnuts, especially immediately following harvest when consumption levels increase, presents opportunities for reducing aflatoxin exposure. This may be even more relevant in parts of Nepal where these foods are consumed in larger quantities than in Banke [200]. Also, given the high amount of serum aflatoxin levels not explained by maize and groundnut consumption and studies showing contamination is common in chilies in other South Asian nations [149, 171], and our qualitative observations in field visits; we recommend that chilies be considered a known source of aflatoxin contamination. Rice, which can harbor low levels of aflatoxin, and is a fundamental component of the Nepali diet [134, 136, 155, 156], cannot be discarded as a conceivable source of low doses of aflatoxin in this population.

The research presented in this dissertation underscores the importance of a holistic approach to aflatoxin reduction, where interventions are implemented at every step of the food system. GAPs, which are multifunctional, not only help prevent and control aflatoxin; they also ensure safety and overall quality of food products throughout the food chain. Because we found use of universally recommended GAPs to be relatively low in the area, it is reasonable to assume that increased training on and use of context specific GAPs may reduce aflatoxin contamination in the diet and food system. Given limited resources, programs will need first to identify the most cost-effective points of entry, including crop production and management. From an agricultural perspective, this seems

feasible, given that many of the GAPs recommended for aflatoxin prevention and control provide multiple benefits to farmers.

Further, given the high reliance on markets in the area, multifaceted, integrated market-level interventions to manage aflatoxin risk throughout the supply chain need to be considered. Continued surveillance of contaminants may help directly target aflatoxin-ridden foods and ensure timely prevention and control. In addition, surveillance and early market intervention can ensure food imported into the country meets minimum food safety standards. Seasonality, which plays a big role in contamination, should be prioritized when designing surveillance programs and designing market-based reduction strategies. Women were exposed all year long; however, the highest levels were detected after harvest.

Investing early in the health of a child helps lay a strong foundation for lifelong prosperity and wellbeing. Our study on birth outcomes provides evidence there are high rates of *in utero* growth faltering in this region of Nepal. These findings corroborate those from previous studies showing the pronounced role of intergenerational nutrition and health effects on linear growth. Therefore, effective strategies to improve fetal outcomes must include provisions to improve adolescent and women's nutrition before pregnancy occurs.

Our results, together with the mixed results from previous studies, underscore how inconclusive the evidence is at this point of the relationship between aflatoxin and birth outcomes. They also highlight the importance of scaling up research efforts to elucidate this relationship. Key research priorities are to

understand how aflatoxin is metabolized differently depending on weight, age and other physiological differences during pregnancy; how timing of exposure plays a role in the relationship; and what the threshold values are for fetal outcomes to be adversely affected.

When put in the context of health and overall food safety, aflatoxin exposure is widespread in this region of Nepal and poses a health risk to pregnant women. However, with the current mixed evidence on the aflatoxin-fetal growth relationship, and the many other major public health challenges facing Nepal, it is too early to recommend aflatoxin reduction be prioritized in programs and interventions designed to improve birth outcomes.

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APPENDICES

Appendix 1: Principle Component Analysis for wealth index creation

Table 21. Results from Principal Component Analysis (PCA) for wealth index creation

	Mean	SD	Factor Score
Household members per room	16.94	1.64	0.2436
Finished Wall	0.63	0.48	0.2303
Finished Roof	0.71	0.45	0.2069
Cement Floor	0.26	0.44	0.3228
Electricity	0.88	0.33	0.3407
Improved Cooking Fuel	0.28	0.45	0.2692
Own Home	0.94	0.24	-0.0988
Piped Water	0.25	0.44	-0.0255
Treat Drinking Water	0.03	0.16	0.0866
Improved Toilet	0.66	0.47	0.3406
Do Not Share Toilet	0.50	0.50	0.2872
Own Livestock	0.57	0.50	-0.0426
Radio	0.14	0.34	0.1066
TV	0.58	0.49	0.3195
Mobile	0.93	0.25	0.1921
Bicycle	0.84	0.37	0.0017
Motorcycle	0.18	0.38	0.2142
Electric Fan	0.80	0.40	0.3709

The household members per room values are reversed for PCA application purposes. Households with less members/room have the highest scores.

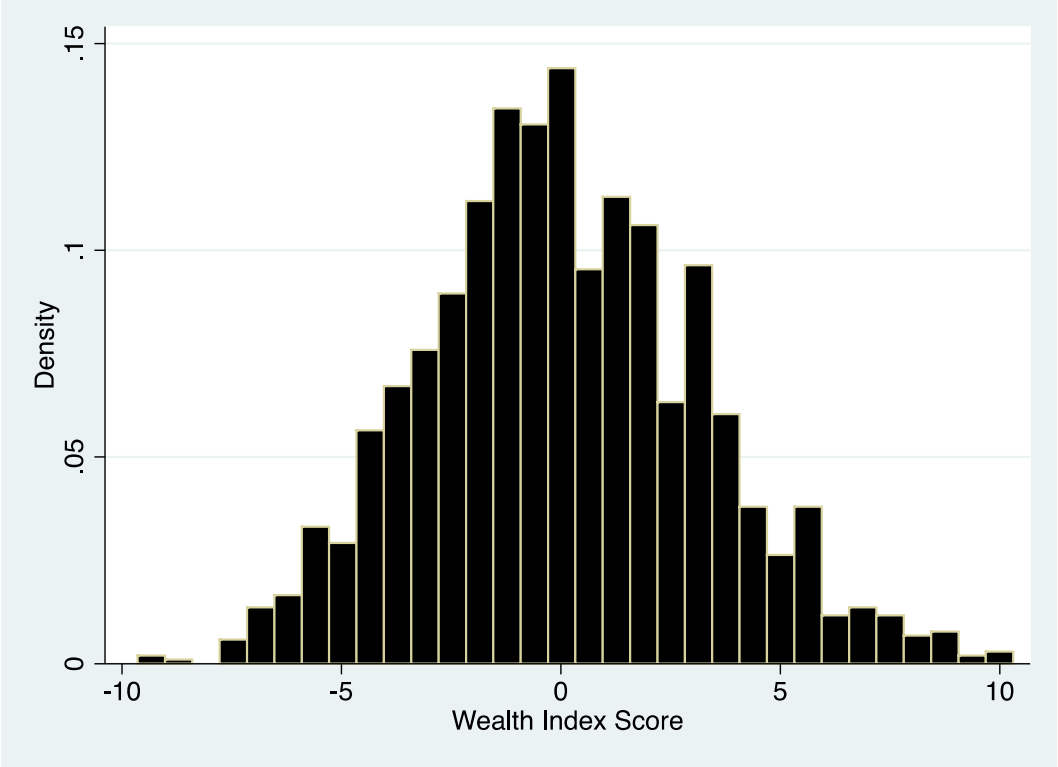

Figure 10. Distribution of wealth index score

Appendix 2: Dietary and Agricultural Modules from the Prenatal Questionnaire (Chapters 4-6)

Module 3 Section 2: Women's Food Frequency
Read Aloud: Now I am going to ask you about what you ate yesterday (or the day before if yesterday was unusual), from the time you woke up to the time you slept. Please only mention foods and drinks that you consumed yourself. I will now ask you some questions about your diet in the past 7 days.

		How many times have you eaten _____ in the past 7 days?	How many times have you eaten _____ in the past 24 hours?
		00=None 01-98=No of times 99=DK	00=None 01-12=No of times 99=DK
3.2.1	Ricebhat/riceroti	RICEWK/RICEWK_TIME	RICEDY/RICEDY_TIME
3.2.2	Corn dhido/bhat/roti	CORNWK/CORNWK_TIME	CORNDY/CORNDY_TIME
3.2.3	Wheat/buckwheat dhido/roti	WHETWK/WHETWK_TIME	WHETDY/WHETDY_TIME
3.2.4	Millet dhido/roti	MILTWK/MILTWK_TIME	MILTDY/MILTDY_TIME
3.2.5	Daal (any)	DAALWK/DAALWK_TIME	DAALDY/DAALDY_TIME
3.2.6	Maseura	MASEWK/MASEWK_TIME	MASEDY/MASEDY_TIME
3.2.7	Other legumes (including chickpeas, dried peas, lima beans and soybeans)	LEGUWK/LEGUWK_TIME	LEGUDY/LEGUDY_TIME
3.2.8	Groundnuts	PEANWK/PEANWK_TIME	PEANDY/PEANDY_TIME
3.2.9	Milk	MILKWK/MILKWK_TIME	MILKDY/MILKDY_TIME
3.2.10	Curds/whey	CURDWK/CURDWK_TIME	CURDDY/CURDDY_TIME
3.2.11	Milk tea	MTEAWK/MTEAWK_TIME	MTEADY/MTEADY_TIME
3.2.12	Vegetable oil (any)	VEGOWK/VEGOWK_TIME	VEGODY/VEGODY_TIME
3.2.13	Ghee	GHEEWK/GHEEWK_TIME	GHEEDY/GHEEDY_TIME
3.2.14	Hydrogenated oil (Banaspati Ghee)	HYDOWK/HYDOWK_TIME	HYDODY/HYDODY_TIME
3.2.15	Eggs (any)	EGGSWK/EGGSWK_TIME	EGGSDY/EGGSDY_TIME
3.2.16	Chicken/duck	CHIKWK/CHIKWK_TIME	CHIKDY/CHIKDY_TIME
3.2.17	Goat	GOATWK/GOATWK_TIME	GOATDY/GOATDY_TIME
3.2.18	Buff	BUFFWK/BUFFWK_TIME	BUFFDY/BUFFDY_TIME
3.2.19	Pork	PORKWK/PORKWK_TIME	PORKDY/PORKDY_TIME
3.2.20	Large fish (fresh)	LFISWK/LFISWK_TIME	LFISDY/LFISDY_TIME

3.2.21	Small fish (fresh)	SFISWK/SFISWK_TIME	SFISDY/SFISDY_TIME
3.2.22	Dried fish	DFISWK/DFISWK_TIME	DFISDY/DFISDY_TIME
3.2.23	Snails	SNALWK/SNALWK_TIME	SNALDY/SNALDY_TIME
3.2.24	Dark green leafy vegetables	DGLYWK/DGLYWK_TIME	DGLYDY/DGLYDY_TIME
3.2.25	Carrots	CARRWK/CARRWK_TIME	CARRDY/CARRDY_TIME
3.2.26	Gundruk	GUNDWK/GUNDWK_TIME	GUNDDY/GUNDDY_TIME
3.2.27	Ripe pumpkin	PUMPWK/PUMPWK_TIME	PUMPDY/PUMPDY_TIME
3.2.28	Green beans (Bodi, simi)	GBEAWK/GBEAWK_TIME	GBEADY/GBEADY_TIME
3.2.29	Green peas (mutterkosa)	GPEAWK/GPEAWK_TIME	GPEADY/GPEADY_TIME
3.2.30	Gourd (lauka, ghiraula, bitter, titekarela, jhimni)	GOUDWK/GOUDWK_TIME	GOUDDY/GOUDDY_TIME
3.2.31	Okra/Ladies finger/Bhindi	OKRAWK/OKRAWK_TIME	OKRADY/OKRADY_TIME
3.2.32	Eggplant	EGPTWK/EGPTWK_TIME	EGPTDY/EGPTDY_TIME
3.2.33	Potatoes	POTAWK/POTAWK_TIME	POTADY/POTADY_TIME
3.2.34	Tomato	TOMAWK/TOMAWK_TIME	TOMADY/TOMADY_TIME
3.2.35	Cauliflower	CAULWK/CAULWK_TIME	CAULDY/CAULDY_TIME
3.2.36	Cabbage	CABBWK/CABBWK_TIME	CABBDY/CABBDY_TIME
3.2.37	Drumstick (sajCHan)	DRUMWK/DRUMWK_TIME	DRUMDY/DRUMDY_TIME
3.2.38	Green jackfruit	GJACKWK/GJACKWK_TIME	GJACKDY/GJACKDY_TIME
3.2.39	Ripe mango	MANGWK/MANGWK_TIME	MANGDY/MANGDY_TIME
3.2.40	Jackfruit (ripe)	JACKWK/JACKWK_TIME	JACKDY/JACKDY_TIME
3.2.41	Guava	GUAVWK/GUAVWK_TIME	GUAVDY/GUAVDY_TIME
3.2.42	Orange/tangerine	ORANWK/ORANWK_TIME	ORANDY/ORANDY_TIME
3.2.43	Ripe papaya	PAPAWK/PAPAWK_TIME	PAPADY/PAPADY_TIME
3.2.44	Apple	APPLWK/APPLWK_TIME	APPLDY/APPLDY_TIME
3.2.45	Pineapple	PINEWK/PINEWK_TIME	PINEDY/PINEDY_TIME
3.2.46	Banana	BANAWK/BANAWK_TIME	BANADY/BANADY_TIME
3.2.47	Jaard/Rakshi	ALCOWK/ALCOWK_TIME	ALCODY/ALCODY_TIME

3.2.48	Instant Noodles (packet, e.g. Wai Wai)	NOODWK/NOODWK_TIME	NOODDY/NOODDY_TIME
3.2.49	Snacks (beatenrice, puffedrice, sweets, biscuits, dalmot, popcorn)	SNACWK/SNACWK_TIME	SNACDY/SNACDY_TIME

Module 11 Section 2: Aflatoxin Knowledge and Practices

Read aloud: Now I am going to ask you questions about crops you produce.

		11.2.a Rice RICE	11.2.b Wheat WHEAT	11.2.c Corn CORN	11.2.d Sorghum/ Millet SORMIL	11.2.e Pulses PULS	11.2.f Groundnuts (e.g. Peanuts) GROUDNUT	11.2.g Chilies & Spices CHILI
11.2	In the past year did you produce _____? 1=yes 0=no *If no, move to next food item* CROPSITEMLIST/CROPSITEMYESNO							
Pre-harvest								
11.2.1	…. Where did you get your seeds? (check all that apply) ☐ 1=Kept from own production *(if unchecked, skip to 11.2.3)* ☐ 2=Neighbor/friend ☐ 3=Purchased ☐ 4=Program recipient, specify program (_____) ☐ 5=Other ☐ 98=DK SEED/SEEDSPE							
11.2.2	…..If kept from own production, how were the seeds stored? (check all that apply) * ☐1=Bery/Bhakari ☐2=Muja-koBhakari ☐3=Suliout ☐4=Thungki ☐ 5=Thangro ☐6=Dhansar ☐7=Kath-koBhakari ☐8=Gundari-koBhakari ☐9=Chitra/Choya-koBhakari ☐10=Kotho ☐11=Doko ☐12=Dalo/Bamboo basket ☐13=Dehari and Kothi ☐ 14=Gagro and Ghyampo ☐15=Dhukuti ☐16=Eaves of the houses ☐ 17=Sealed container (plastic, metal or glass) ☐18= Plastic bag/sack ☐ 19=Burlap(jute) bag/sack ☐20=Other (specify _____) ☐98=DK SEEDSTORE/SEEDSTORESPEC							
11.2.3	Where was the storage unit? ☐1=Field ☐2=In the house ☐3=In the courtyard ☐4=I did not store my seeds ☐98=DK STOUNIT							
11.2.4	…Did you apply any fertilizer to your field? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.6* FERT							

11.2.5	…. If yes, what kind of fertilizer did you use? (check all that apply) ☐ 1=Chemical fertilizer (Urea, DAP, Potash) ☐ 2=Farmyard Manure (FYM) (Animal waste matter) ☐ 98=DK FERTKIND							
11.2.6	…. Did you use any insecticides in your crop standing field? ☐ 1=Yes ☐ 0=No ☐ 98=DK INSECT							
11.2.7	…. Did you use any fungicides in your crop standing field? ☐ 1=Yes ☐ 0=No ☐ 98=DK FUNGI							
11.2.8	…. How do you control weeds? ☐ 1=Manually ☐ 2=With herbicides ☐ 3=Both ☐ 98=DK WEED							
11.2.9	…. Did you treat the seeds with insecticides before planting? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.11* SEEDTRT							
11.2.10	If yes, what insecticides did you treat the seed with? ☐1=Aldrin ☐2=Lindane ☐3=Chlordane ☐4=DDT ☐5=Endosulfan ☐6=Dementon-s-methyl ☐7=Dichlorvos ☐8=Dimethoate ☐ 9=Fenitrothion ☐10=Malathion ☐11=Monocrotophos ☐12=Parathion methyl ☐13=Phosphamidon ☐14=Carbayl ☐15=Carbofuran ☐ 16=Methomyl ☐17=Propoxur ☐18=Cypermethrin ☐19=Deltamethrin ☐20=Fenvalerate ☐21=other ☐98=DK SEEDTRTSPEC							
11.2.11	…. Did you treat the seeds with fungicides before planting? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.13* SEEDFUNGI							
11.2.12	If yes, what fungicides did you treat the seed with? ☐ 1=Carbendazim ☐ 2=Carboxin ☐ 3=Copper oxychloride ☐ 4=Edifenphos ☐ 5=Mancozeb ☐ 6=Thiram ☐ 7=Tridemorph ☐ 8=other ☐ 98=DK SEEDFUNGISPEC							
11.2.13	…. Did you use irrigation? ☐ 1=Yes ☐ 0=No ☐ 98=DK IRRI							
11.2.14	…. Did you plant other crops (intercrop) in the same field? ☐ 1=Yes ☐ 0=No ☐ 98=DK INTER							

11.2.15	…. Did you use proper plant spacing? ☐ 1=Yes ☐ 0=No ☐ 98=DK SPACE							
11.2.16	…. Did you clean harvesting equipment (e.g. mattock, pick axe, sickle) prior to harvest? ☐ 1=Yes ☐ 0=No ☐ 98=DK EQCLEAN							
11.2.17	…. Did you clean out handling (e.g. carrying basket) and drying equipment prior to use? ☐ 1=Yes ☐ 0=No ☐ 98=DK HANDCLEAN							
Harvest								
11.2.18	….. How did you harvest/ thresh?….. ☐ 1=Manually ☐ 2=Mechanically ☐ 3=Both ☐ 98=DK HOWHARV							
11.2.19	….. When did you harvest/ thresh?….. ☐ 1=before ___ reached maturity ☐ 2=immediately when ____ reached maturity ☐ 3=sometime after ____ reached maturity (some seed breakage) ☐ 98=DK WHENHAR							
Postharvest								
11.2.20	…. After harvest/ threshing, how many days was the __ dried in the field? ☐ 1=_____ days ☐ 999=DK DRYDAYS/DRYDAYS_DAY							
11.2.21	…. After harvest, did you…. ☐ 1=hold ___ in the field for more than 6 hours ☐ 2=transfer ___ to a drying/storage location in less than 6 hours after harvest ☐ 98=DK HOLD							
11.2.22	…. Did you dry the _____ immediately after harvest/ threshing? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.24* DRYAFTER							
11.2.23	If yes, how did you dry ____? (do not read responses/check all that apply) ☐ 1=In the field ☐ 2=With fans ☐ 3=On platforms ☐ 4=Spread directly the dirt ☐ 5=Spread on plastic sheets on the ground ☐ 6=Spread on drying floor (cement or brick) ☐ 7=Mechanically ☐ 8=Tied with rope for hanging around the house under the roofs for drying ☐ 9=Other ☐ 98=DK DRYMETHOD							

11.2.24	…. Before storage, did you strip the cobs or leave the husk on? CORNCOB ☐ 1=strip the cobs ☐ 2=left husk on ☐ 98=DK							
11.2.25	…. Did you dry the pulses/beans in the pod or did you remove the beans from the pod? PULSEPOD ☐ 1=dried with pod attached ☐ 2=separate the bean from the pod ☐ 98=DK							
11.2.26	…. Did you use screening or gravity tables to clean the _____? ☐ 1=Yes ☐ 0=No ☐ 98=DK SCREEN							
11.2.27	…How did you know the seed or grain was well dried? (do not read responses/check all that apply) ☐ 1=Sound produced while breaking seed with teeth ☐ 2=Sound produced wile shaking seeds ☐ 3= Visual observation ☐ 4= Seed moisture measuring device ☐ 98=DK DRYKNOW							
11.2.28	…. After drying ___ did you immediately store it, consume or sell it? (check all that apply) ☐ 1=stored ☐ 2=consumed ☐ 3= sold ☐ 98=DK *If consumed only, sold only or don't know, go to 11.2.30* AFTERDRY							
11.2.28a	How long did you store the ____? ☐1=Up to 1 month ☐2=2 months ☐3=3 months ☐4=4 months ☐ 5=5 months ☐6=6 months ☐7=7 months ☐8=8 months ☐9=9 months ☐10=10 months ☐11=11 months ☐12=12 months MPROSTO							
11.2.29	Where did you store the _____? (check all that apply) * ☐1=Bery/Bhakari ☐2=Muja-koBhakari ☐3=Suliout ☐4=Thungki ☐ 5=Thangro ☐6=Dhansar ☐7=Kath-koBhakari ☐8=Gundari-koBhakari ☐9=Chitra/Choya-koBhakari ☐10=Kotho ☐11=Doko ☐12=Dalo/Bamboo basket ☐13=Dehari and Kothi ☐ 14=Gagro and Ghyampo ☐15=Dhukuti ☐16=Eaves of the houses ☐ 17=Sealed container (plastic, metal or glass) ☐18= Plastic bag/sack ☐ 19=Burlap(jute) bag/sack ☐20=Other (specify _____) ☐98=DK STORELOC/STORELOCSPEC							
11.2.30	…. Did you clean out storage equipment (e.g. bins, sacks) prior to storage? ☐ 1=Yes ☐ 0=No ☐ 98=DK STORECLEAN							

11.2.31	…. Did you mow around storage bins to discourage insect/rodent activity? ☐ 1=Yes ☐ 0=No ☐ 98=DK STOREMOW							
11.2.32	…. Was the storage area dry and moisture free? ☐ 1=Yes ☐ 0=No ☐ 98=DK STOREDRY							
11.2.33	…Did insects attack your stored food and/or seed? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.35* INSATTACK							
11.2.34	If yes, what did you do with that ___? ☐1=threw it away ☐2=sorted out the contaminated parts ☐3=mixed it with the products for sale ☐4=fed it to the animals ☐5=nothing ☐ 98=DK ATTACKPRACTICE							
11.2.35	…Did rodents attack your stored food and/or seed? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.37* RODENT							
11.2.36	If yes, what did you do with that ___? ☐1=threw it away ☐2=sorted out the contaminated parts ☐3=mixed it with the products for sale ☐4=fed it to the animals ☐5=nothing ☐ 98=DK RODENTPRAC							
11.2.37	…. Did you scalp _____before storage (remove trash, cracks and shriveled kernels)? ☐ 1=Yes ☐ 0=No ☐ 98=DK SCALP							
11.2.38	…. Did you use insecticides or any other insect repellent around and/or inside the storage area? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.41* REPELLENT							
11.2.39	If yes, when did you apply insecticides? (check all that apply) ☐ 1= before the appearance of insect infestation in storage ☐ 2=right after the appearance of insect infestation in storage ☐ 3=at the later stage after severe infestation ☐98 = DK REPELLENTSTAGE							

11.2.40	What insecticides did you treat the seed with? ☐ 1=Aldrin ☐ 2=Lindane ☐ 3=Chlordane ☐ 4=DDT ☐ 5=Endosulfan ☐ 6=Dementon-s-methyl ☐ 7=Dichlorvos ☐ 8=Dimethoate ☐ 9=Fenitrothion ☐ 10=Malathion ☐ 11=Monocrotophos ☐ 12=Parathion methyl ☐ 13=Phosphamidon ☐ 14=Carbayl ☐ 15=Carbofuran ☐ 16=Methomy ☐ 17=Propoxur ☐ 18=Cypermethrin ☐ 19=Deltamethrin ☐ 20=Fenvalerate ☐ 21=other ☐ 98=DK SEEDWHCINS							
11.2.41	…. Did you use fungicide around and/or inside the storage area? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.44* FUNGIUSE							
11.2.42	If yes, when did you apply fungicides? ☐ 1= before the appearance of molds in storage ☐ 2=right after the appearance of molds in storage ☐ 3=at the later stage after severe infestation ☐ 98 = DK FUNGIWHEN							
11.2.43	What fungicides did you use to treat ___ ? ☐ 1=Carbendazim ☐ 2=Carboxin ☐ 3=Copper oxychloride ☐ 4=Edifenphos ☐ 5=Mancozeb ☐ 6=Thiram ☐ 7=Tridemorph ☐ 8=other ☐ 98=DK WHCFUNGI							
11.2.44	…. How often did you check the stored _____for molds or damage (e.g. for insect activity, high temperatures, mold growth or sprouting at the top of the grain)? ☐ 0=never ☐ 1=once every few months ☐ 2=once a month ☐ 3=twice a month ☐ 4=more than twice a month ☐ 98=DK CHECK							
11.2.45	…In which month did you dry the ____(check all that apply) ☐ 1=January ☐ 2=February ☐ 3=March ☐ 4=April ☐ 5=May ☐ 6=June ☐ 7=July ☐ 8=August ☐ 9=September ☐ 10=October ☐ 11=November ☐ 12=December ☐ 13=I didn't dry it *(go to 11.2.47)* ☐ 98=DK DRYMONTH							
11.2.46	…How often did you sun dry the ____? ☐ 1=Once a month ☐ 2=Every 2 to 3 months ☐ 3=Every 4 to 5 months ☐ 4=Every 6 + months ☐ 5=Never ☐ 98=DK SUNDRY							

11.2.47	Did the ____ get wet after harvest/threshing? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.50* *WET*							
11.2.48	If yes, how many times did it get wet since harvest? ☐ 1=____times ☐ 98=DK WETTIME/WETTIME_NUM							
11.2.49	What did you do with the wet ___? ☐ 1=Dry it again ☐ 2=Nothing ☐ 98=DK WETPRAC							
11.2.50	….Have you received any training on proper storage techniques? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go skip to 11.2.52* TRAINING							
11.2.51	If yes, from which organization? ☐ 1= Specify (_____) ☐ 98 = DK ORG/ORGSPEC							
11.2.52	…How long did you store the ____ before consuming it? ☐ 0 = I did not consume/store it *(skip to 11.2.55*) ☐ 1=less than one month ☐ 2=1-2 months ☐ 3=3-5 months ☐ 4=6-7 months ☐ 5=8-10 months ☐ 6=11-12 months ☐ 7=more than 12 months (1 year) ☐ 98=DK STOREMTH							
11.2.53	….Did you have storage problems? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.55* STOREPROB							
11.2.54	If yes, which storage problem was the most important? ☐ 1=Insects ☐ 2=Rodents ☐ 3=Birds ☐ 4=Mold ☐ 5=Other, specify _____ ☐ 98=DK PROBIMP/PROBIMPSPE							
Sales								
11.2.55	Do you sell the _____? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.60* SALE							
11.2.56	…. Did you have any trouble selling ____ because of mold/discoloration/damage? ☐ 1=Yes ☐ 0=No ☐ 98=DK *If no, go to 11.2.58* SALEPROB							

11.2.57	If yes, what did you do with the unsold ____? (check all that apply) ☐ 1=destroy the moldy parts ☐ 2=destroy it all ☐ 3=feed it to animals ☐ 4=mix with good grain ☐ 5=nothing, consume it ☐ 6=other ☐ 7=make alcohol ☐98 = DK SALEPRAC							
11.2.58	…. Did you check and mark (name, date of harvest, location, etc.) the batches of _____ before selling? ☐ 1=Yes ☐ 0=No ☐ 98=DK CHECKM							
11.2.59	…. Did you check or test the ___ for mold before selling? ☐ 1=Yes ☐ 0=No ☐ 98=DK SELLCHECK							
GENERAL PRACTICES								
11.2.63	When you plant, what do you do to prepare the seeds before planting? (do not read responses/check all that apply) SEEDPREP SEEDPREPSPEC	☐ 1=check for/remove broken seeds ☐ 2=check for/remove moldy seeds ☐ 3=check for/remove dirt or foreign material ☐ 4=dip in a solution ☐ 5=mix with animal dung ☐ 6=other, specify (____) ☐ 7=nothing ☐ 98=DK							
11.2.64	What do you do to prepare your storage units (e.g. bins, sacks) for storing after harvest? (do not read responses/check all that apply) STORUNITPREP STORUNITPREPSPEC	☐ 1= Check sacks for rips or tears ☐ 2= Check sacks for leftover spoiled grain ☐ 3= Check storage units for moisture ☐ 4= Check storage units for leaks ☐ 5= Check storage units for infestation (vermin, bugs) ☐ 6= Other, specify(____) ☐ 7=nothing ☐ 98=DK							
11.2.65	Why do you dry the crops after harvest? (do not read responses/check all that apply) HARVAFTDRY HARVAFTDRYSPEC	☐1=To avoid mold ☐2= To avoid insects ☐3= Maintain quality ☐4= Avoid spoilage ☐5=Other, specify (____) ☐6=I do not dry my crops ☐98=DK							
11.2.66	What is the longest time you held your crops before drying them? TIMECROP	☐ 1=1-4 hours ☐ 2=4-6 hours ☐ 3=7-24 hours ☐ 4=more than one day ☐ 98=DK							
11.2.67	Do you do anything if the storage area becomes too hot? STORAGEHOT *If no, go to 11.2.69*	☐ 1=Yes ☐ 0=No ☐ 98=DK							
11.2.68	If yes, what do you do? STORAGEHOTSPEC							
11.2.69	Do you do anything if the storage area becomes very cold? STORAGECOLD *If no, go to 11.2.71*	☐ 1=Yes ☐ 0=No ☐ 98=DK						

11.2.70	If yes, what do you do? STORAGECOLDSPEC	
11.2.71	Have recent floods or other natural disasters affected your crop storage in the last year? STORFLOOD *If no, go to 11.2.73*	☐ 1=Yes ☐ 0=No ☐ 98=DK
11.2.72	If yes, specify (check all that apply) STORFLOODSPEC	☐ 1=flood ☐ 2=drought ☐ 3=frost ☐ 4=earthquake ☐ 5=pestilence ☐ 6=other ☐ 98=DK
11.2.73	Do you examine crops for mold? MOLDEXAMINE	☐ 1=Yes ☐ 0=No ☐ 98=DK
11.2.74	If you see and/or detect mold/fungus on your food, what do you do with it? (do not read responses/check all that apply) MOLDEXAMINEWH MOLDEXAMINEWHSPEC	☐ 1=destroy the moldy parts ☐ 2=destroy it all ☐ 3=feed it to animals ☐ 4=mix with good grain ☐ 5=nothing, consume it ☐ 6=other, specify (_____) ☐ 7=make alcohol ☐ 98 = DK
11.2.75	Why are molds potentially a problem? (do not read responses/check all that apply) MOLDKNOWLEDGE MOLDKNOWLEDGESPEC	☐ 1=dangerous ☐ 2=taste bad ☐ 3=bad for health ☐ 4=other (specify_____) ☐ 98=DK
11.2.76	Have you heard of aflatoxins? AFLAKNOWLEDGE *If no, skip to 11.3.1*	☐ 1=Yes ☐ 0=No ☐ 98=DK
11.2.77	Have you ever heard about testing for aflatoxins? AFLAHEARD *If no, go to 11.3.1*	☐ 1=Yes ☐ 0=No ☐ 98=DK
11.2.78	Do you test your products for aflatoxins? AFLATEST *If no, skip to 11.3.1*	☐ 1=Yes ☐ 0=No ☐ 98=DK
11.2.79	If yes, when do you test for aflatoxins? (do not read responses/check all that apply) AFLATESTWHEN AFLATESTWHENSPEC	☐ 1=before storage ☐ 2=during storage ☐ 3=before sale ☐ 4=after sales ☐ 5=before feeding to animals ☐ 6 =right before eating ☐ 7=other (specify_____) ☐ 98=DK
11.2.80	How do you test for aflatoxins? (check all that apply) AFLATESTHOW AFLATESTHOWSPEC	☐ 1= visual observations ☐ 2=black light ☐ 3=chemical analysis ☐ 4=other (specify_____) ☐ 98=DK

Appendix 3: Birth Questionnaire (Chapter 6)

Module 1: Household Information & Characteristics
Section 1: Interview Information

Number	Question	Response	Variable name
1.1.1	Data Time Point (1-6 or one year visit)	☐	HDTIMEPT
1.1.2	Date of interview (dd/mm/yyyy)	☐☐/☐☐/☐☐☐☐	DAY/MONTH/YEAR
1.1.3	VDC	*(Droplist: Basudevpur, Bageswari,*	HVDC
1.1.4	Ward	*(Droplist 1-9)*	HWARD
1.1.5	Household ID	☐☐☐☐	HHID
1.1.6	Index Woman ID	☐☐☐☐☐	HHWID
1.1.7	Index Child ID	☐☐☐☐☐	HHCID
1.1.8	Village/Community	*(text)*	HVILL
1.1.9	GPS	"Get GPS Coordinates"	LATITUDE/LONGITUDE/ALTITUDE/ACCURACY
1.1.10	Interviewer/Nurse ID 1	☐☐☐	HID1/HID1N

Module 5: Birth Event
Section 1: Birth Event
I am now going to ask you questions about your recent delivery.

5.1.1	What was the birth outcome?	☐1=Live birth ☐ 2=Stillbirth *(skip 5.1.12, 5.1.13)* ☐3=Spontaneous abortion/ miscarriage *(skip 5.1.2a/b & 5.1.7-5.1.13)* ☐4=Other _____ (specify) ☐98=DK	MPREGOUT MPREGOUTSPEC
5.1.2	On what date did you give birth?	(dd/mm/yyyy)	DAYB/MONTHB/YEARB
5.1.2a	Child name		CNAME
5.1.2b	Child gender	0 = male 1 = female 2 = other	CGENDER
5.1.3	How many antenatal care visits occurred during your pregnancy (total)?	☐1=_____*(0-20)* Visits ☐98=DK	MNOANCT/MNOANCT_NUM
5.1.4	During this pregnancy, were you given or did you buy any iron tablets?	☐ 1=Yes ☐ 0=No ☐ 98=DK *If no/dk, go to 5.1.6*	MANCIRONT
5.1.5	If yes, during this whole pregnancy, for how many days did you take the tablets?	☐1=____ *(1-280)* days ☐98=DK	MIRONDAYST/MIRONDAYST_NUM

5.1.6	During this pregnancy, did you receive any drug for intestinal worms?	☐ 1=Yes ☐ 0=No ☐ 98=DK	MTREATWORMT
5.1.7	Were there any complications to the birth? (select all that apply)	☐1=Prolonged labor (> 24 hrs) ☐2=Congenital anomalies (physical deformities) ☐3=Multiple births ☐4=Infection/Fever ☐5=Caesarean birth (operation) ☐6=Instrumental delivery ☐7=Breech ☐8=Other, specify (____) ☐9=None ☐98=DK	MCOMPLBIR/MCOMPLBIR_SPE
5.1.8	Where did you deliver?	☐1=Own home alone ☐2=Own home with SBA ☐3=Other's home ☐4=Sub health post ☐5=Health post ☐6=Primary health care ☐7=Public Hospital ☐8=Private Hospital ☐ 9=Other_____(specify) ☐98=DK	MDELIVER MDELIVSPE
5.1.9	Did a FCHV visit you after you gave birth?	☐ 1=Yes ☐ 0=No ☐ 98=DK	MDELIVFCHV
5.1.10	Do you know the child's birth weight?	☐1=Yes, confirmed with child health card ☐2=Yes, remembers ☐3=Doesn't remember *to 5.1.12)* ☐98=DK *(Go to 5.1.12)*	CBIRWTKN
5.1.11	If yes (1 or 2), What was the child's birth weight?	☐.☐ kg (0.2-7 kg)	CBIRTHWT
5.1.12	Is the child still alive?	☐ 1=Yes ☐ 0=No *If yes, go to module 13*	CALIVE
5.1.13	If no, what was the age of the child at the time of death?	☐1=_____ *(0-20)* days ☐98 = DK *End interview*	CALVDAYS/CALVDAYS_NUM

Module 13: Laboratory Tests and Anthropometric Measurements
Section 2: Child Anthropometry
Enumerator/Nurse: Skxip if outcome of pregnancy (5.1.1) was a stillbirth or spontaneous abortion/miscarriage

	Question	**Index child**
13.2.4a	How many days has it been since the child was born?	☐☐ days (range 0-31) DAYBIR
13.2.5	Birth Weight 1	☐☐.☐ kg (range: 0.2-7 kg) ACBW1
13.2.6	Birth Weight 2	☐☐.☐ kg (range: 0.2-7 kg) ACBW2
13.2.7	Birth Weight 3	☐☐.☐ kg (range: 0.2-7 kg) ACBW3
13.2.8	Birth Length 1	☐☐.☐ cm (range: 10-60 cm) ACBL1
13.2.9	Birth Length 2	☐☐.☐ cm (range: 10-60 cm) ACBL2
13.2.10	Birth Length 3	☐☐.☐ cm (range: 10-60 cm) ACBL3
13.2.26a	Did you measure the index child's anthropometry?	☐ 1=Yes ☐2= yes, but incomplete ☐ 0=No ACCOMP
13.2.27	Referral?	☐ 1=Yes ☐ 0=No ACREF1

1.1.11	Outcome of Interview	1. completed 2. incomplete 3. absent 4. refused 5. could not locate	HINTOUT