



Aflatoxins and Maternal and Child Nutrition: Findings From Nepal, Mozambique, Uganda And Timor-Leste

Webinar Transcript

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Thank you for joining today's webinar to learn more about Aflatoxins and Maternal and Child Nutrition. My name is Hannah Koehn and I am a communication specialist at the Feed the Future Innovation Lab for Nutrition, and I will be your MC today. As more attendees are joining the webinar, I will begin to go over some of the housekeeping. I'll then hand it over to our moderator for today's webinar, Dr. Patrick Webb. I would like to direct all attendees to a few functions on the Zoom call. At the bottom of your screen, you should see a chat icon and a Q&A icon. Please use the chat feature to engage in relevant conversation with the other attendees. If you have a question for one of the panelists, please use the Q&A feature. Panelists will respond to questions and the Q&A as they are able throughout the webinar. We have allotted the final 20 minutes of this webinar for the Q&A as well, at which point the panelists will respond to any remaining questions from the audience. If you're experiencing any technical difficulties, please send a message to Dylan Curry, so that our technical support staff can work with you to resolve the issues. This webinar is being recorded and will be made available on the Innovation Lab for Nutrition website, as well as the USAID Advancing Nutrition website. You can also register for upcoming webinars and our previous recordings and slide decks on our website. I will repeat these technical housekeeping items in the chat throughout the webinar as people may be joining in at later times. Thank you again for joining us today. And now, I'd like to introduce the moderator for today's webinar, Dr. Patrick Webb. Dr. Webb is the Director of the Innovation Lab for Nutrition and is an

[Alexander Mc Farland Professor] of Nutrition at the Friedman School of Nutrition Science and Policy.
Dr. Webb, over to you.

Patrick Webb

Thank you Hannah, and welcome everyone. Great pleasure to be able to engage with you and share some recent findings from research in four different parts of the world: Uganda, Nepal, Mozambique, and Timor-Leste, all focused on aflatoxin issues relating to health and nutrition. So just a brief background. The Nutrition Innovation Lab of Feed the Future is one of the 25 more innovation labs that focus on various aspects of agriculture, multi-sector programming, and policy. These are ... what you see on the map are countries in which the Nutrition Innovation Lab has been working over the last 10 years not just on the aflatoxin issue but on a range of issues relating to agriculture to nutrition dimensions, policy, and governance issues, but also...

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... the mechanisms, biological mechanisms. To do all this type of work, we have a huge range of local and global partners. These logos capture some of those. None of this work could have been done just by us alone, and of course, great partnership with USAID in the whole activity.

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For these studies presented today, these are the primary, national, and international collaborators. And we thank them all for their participation in making this very rigorous science, and science to policy and practice agenda possible.

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So three major themes - I've already alluded to them that the Nutrition Innovation Lab has been focused on. One is on nutrition programming, one is on policy and governance dimensions. Today, we're focusing on better understanding biological mechanisms. What is it that is happening within the bodies of pregnant women or children in relation to their diets, their health, their access to clean water, that can enhance or impair nutritional outcomes? So what you're going to be hearing today is a range of different study designs from a range of different countries.

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Aflatoxins are just one of multiple mycotoxins. They're based on fungi that generate a sometimes visible, as in these pictures here...sometimes visible coating, a dusting of highly toxic metabolites known to cause cancer and a range of other growth impairments in humans, as well as in livestock and animals. When it's visible, like this, it's actually reasonable to discard or separate the infected ones, but it's not always visible. This makes it a very big challenge, both for public health and for agriculture interventions to try to improve this agriculture and trade.

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So, mycotoxins... there's a range of them. We're focusing here on aflatoxin B1, which is the main toxin, the most carcinogenic toxin. There are others and we are doing additional work to try and understand if these toxins work together in combinations or separately. They're often found together in a range of foods that are common across the tropics, particularly high humidity and high temperature seem to be conducive to the growth of this particular metabolite.

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There's been... there is a background... the role of mycotoxins is impairing livestock growth and actually mortality has been known for a hundred years or more. Effects on child nutrition are less well understood. There's been a lot of observational, mainly cross-sectional, studies and intervention trials trying to get at the links between aflatoxins and poor child growth. The evidence is mixed, which lead us to believe that as we focus on the role of agriculture and improved diets, to try to improve nutrition, we need to do better, we need to have prospective birth cohort evidence and other kinds of evidence to try and better tease out the effects of aflatoxins in the diet on linear growth, particularly moderated by birth outcomes.

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So what we have today is a stellar lineup of experts who've been leading a lot of this research. First, we're going to hear from Dr. Jackie Lauer, public health nutritionist. She's now a clinical assistant professor at Boston University. She did her work mainly in Uganda under the Innovation Lab focusing on environmental parameters as well as additional disease parameters like HIV, and how they affect environmental enteric dysfunction and aflatoxins in terms of outcomes on birth and child growth. Then, we have Kathy Heneveld who is an assistant researcher in the Nutrition Innovation Lab. She's got her

Master's from the Nutrition School at Tufts. She's been with us since 2016, and she's been coordinating playing a lead role in the Mozambique aflatoxin work. And then, Dr. Shibani Ghosh, research associate professor at the School. She's also the associate director for the Innovation Lab for Nutrition. She's got a wealth of experience from the Middle East to Africa and South Asia. PhD from UMass Amherst, and she's been playing a very important role overall. She'll be presenting on our work in Nepal and Timor-Leste. So without any more do, we will go straight to Jackie and launch into what is a fascinating world that links the microbiome to diets to health to nutrition. Thank you, Jackie.

Jacqueline Lauer

Thank you Patrick, and warmest welcomes to everybody. Today, I'm going to be presenting on two small but relatively interesting longitudinal studies that we conducted in Uganda, which will help us better understand the relationship between maternal aflatoxin exposure during pregnancy and various pregnancy outcomes.

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And in relation to this presentation, I have no disclosures. So, like many low and middle income countries, the food supply in Uganda is extremely vulnerable to mycotoxin contamination. This is both because of its climate, which includes things like heavy rains and sudden droughts, as well as high humidity and average temperature of 25 degrees Celsius, and because of a number of commonly practiced fungal promoting pre- and post-harvest activities, things like improper drying and storage practices. Additionally, aflatoxin exposure in Uganda is exacerbated by an overall lack of dietary diversity, because it is often the staple foods, such as maize, peanuts, and sorghum that are most aflatoxin-prone. As a wonderful resource, the PAC Report, that is the Partnership for Aflatoxin Control in Africa, is a great summary of the extent of aflatoxin contamination in various foods in Uganda. So, drawing from that report, I present this table here, which looks at aflatoxin levels in maize across the different agro-ecological zones and districts within Uganda. And it uses a range of the cut points that we have commonly used. As you can see on the table, more than 50 percent of analyzed samples exceeded the EU regulatory limit, which is four parts per billion, and in certain regions, 90 to 95 percent of maize samples exceeded this limit.

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And this is a similar table, but instead of maize samples, this is presenting on sorghum samples, where aflatoxins levels were shown to be even higher. So now, even using the FDA or WHO cut point of 20 parts per billion, three areas of Uganda had a 100 percent of sorghum samples exceeding the limit. In Gulu, where we conducted one of our studies, 85 of sorghum samples exceeded 20 parts per billion. So given this data, we would expect human exposure to aflatoxin be high among the Ugandan population. And we would expect this to be reflected in both serum levels, as well as in various health outcomes.

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So what are the health outcomes of aflatoxin exposure? Overall, human consumption of aflatoxin can have both acute and chronic health effects. Acutely high levels of exposure can lead to poisoning or what we call aflatoxicosis, and chronically, it can lead to an increased risk of liver cancer and potentially also to immunosuppression, low birth weight, and stunting in infants and young children.

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So in Uganda, aflatoxin contamination is estimated to cause approximately 3,700 new cases of aflatoxin-induced liver cancer per year. However, it's impact on birth outcomes and on stunting is a bit less clear. So, as I mentioned, we designed two different studies to look at maternal aflatoxin exposure and pregnancy outcomes in Uganda. The first one was a prospective study of HIV uninfected pregnant women in Mukono, Uganda. For those unfamiliar, Mukono is a large peri-urban district in the central region of the country, located about 20 kilometers outside of the capital city of Kampala. We conducted the study at Mukono health center. The objective was to examine the association between maternal aflatoxin exposure and infant birth outcomes, primarily interested in infant birth weight. And if you'd like to know more about this study, the details are published in *Maternal and Child Nutrition*.

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So briefly, this prospective study had a total of four visits. For the purposes of this particular analysis, the initial enrollment visit occurred between 9 and 27 weeks of gestation, and this is when a venous blood draw also occurred. Samples from this blood draw were later analyzed at the Wang Laboratory at the University of Georgia for levels of aflatoxin B1 using HPLC methods. Anthropometry at birth, which included weight, length, and head circumference was collected within 48 hours of delivery for a total of 220 live borne infants.

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So results from the laboratory analysis are shown here. All the maternal samples collected in the study had detectable levels of aflatoxin, which range from 0.71 to 95.6. The mean level was 8.55 and the median level was 5.71.

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Given the skewed distribution of aflatoxin levels, as evidenced in the previous slide, we log transformed AFBI levels prior to all analyses. So, this graph shows the correlation between log-transformed maternal AFBI levels located on the X-axis, and infant birth weight on the Y-axis. And as you can see, there is a small but significant negative correlation between the two.

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And likewise, there was an observed small, but significant, negative correlation between log transformed maternal aflatoxin BI levels and infant weight-for-age Z score at birth. And this was the table that was published in the manuscript. So here, we're still showing the relationship between log-transformed aflatoxin BI levels and birth outcomes, but now controlling for a number of other variables that are listed at the bottom of the table and that were collected using survey data. As you can see, there remains a significant relationship between the higher maternal aflatoxin BI levels and lower infant weight and weight for height Z score at birth. Furthermore, we found a significant association between higher maternal aflatoxin BI levels and smaller infant head circumference and head circumference for age Z score in both adjusted and unadjusted models.

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So building off this previous study, the goal of this second study was to examine the effect of maternal aflatoxin exposure during pregnancy and the rate of gestational weight gain in pregnant women, this time of mixed HIV status in this time in the north of Gulu, Uganda.

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For more information on this study, you can look to the Journal of Tropical Medicine and International Health. So data for this study was collected between the years of 2012 and 2013 as part of the Prenatal Nutrition and Psychosocial Health Outcomes Study, which was abbreviated PRENAPS and this was an observational longitudinal cohort study designed to explore relationships among food access, nutritional and psychosocial exposures, and several physical and mental health outcomes. This was a sample of a total of 403 HIV women, 133 of these were HIV-infected and 270 of these were HIV-uninfected. And if

you'd like more information on this parent trial, the PRENAPS study I've listed the clinical trials website here. So aflatoxin B1 levels were detected in 98.5 of samples in this study. And then, once again we analyzed them at the Wang Laboratory at the University of Georgia. This figure shows the distribution of aflatoxin B1 levels for HIV-uninfected women compared to HIV-infected women, and as you can see, aflatoxin B1 levels were significantly higher among HIV-infected women compared with HIV-uninfected women. The median for HIV-infected women was 4.8 and the median for HIV-uninfected women was 3.5. And notably, we observed 16 women that had aflatoxin levels over 100, and 15 of these 16 were in the HIV-infected group.

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So this shows unadjusted and adjusted models. Sorry that was cut off in the titles here, but we used linear mixed effect models to determine the unadjusted and HIV status adjusted differences in the rate of gestational weight gain per unit increase in baseline log transformed aflatoxin B1 level. So in summary, we found that a one log increase in aflatoxin levels was associated with a 16.2 grams per week lower rate of gestational weight gain and this was significant with a p. value of 0.028. So we also created separate models for HIV-infected women and uninfected women to examine whether the effect of aflatoxins on rate of gestational weight gain differed in the two study groups. And as you can see, the association between aflatoxin B1 and rate of gestational weight gain was stronger and significant only among HIV-infected women who were... are... were on ARTs. So for HIV-infected women, we observed a negative 2.57 grams per week per log aflatoxin B1. And for HIV-uninfected women, we observed a negative 7.5 grams per week per log aflatoxin B1. So, also in the study we wanted to rule out the possibility that HIV-infected women were perhaps geographically clustered and therefore shared a common food supply. Therefore, women's households were mapped using GIS according to HIV status, as well as serum aflatoxin B1 levels. We used GIS statistical tools to determine if there were any statistically significant hot spots, cold spots, or cluster outliers of HIV infection or aflatoxin levels using an inverse distance relationship. And in summary, we found no evidence of any geographic clustering within this study.

So in conclusion, maternal aflatoxin exposure during pregnancy appears to have a small but a significant effect on pregnancy and birth outcomes in Uganda, including gestational weight gain and infant WAZ/weight at birth. Maternal HIV-infection - even seemingly well controlled as it was in the study - appears to exacerbate these effects, although it's a bit unclear as to why. Perhaps there is a synergistic relationship between HIV and aflatoxin exposure with regard to immune suppression. Or perhaps, you have to take into account HIV's ability to impair liver function resulting in a decreased ability to detoxify

metabolites, including aflatoxins. This field would be advanced by future studies that include larger samples sizes, additional aflatoxins and fumonisins that are common in Uganda, besides aflatoxin B1, including aflatoxin B2, aflatoxin G1 and aflatoxin M1. And it would be interesting to not only continue research on HIV infection, but to collect data on viral load in order to be... in order to know if there is some sort of dose response relationship between HIV and viral load and aflatoxin levels. So, I look forward to continuing this discussion and answering any questions that you might have. And I will otherwise turn it over to my colleague. Thank you.

Patrick Webb

Thank you Jackie. Extremely interesting and useful, and you've sparked a lot of interest in the Q&A box and we'll come back to those questions later. Let me just highlight two really important things that will resonate into the subsequent presentations. One... that we're not just looking at a relatively simple relationship between aflatoxin level in the blood and stunting. What we are seeing is that aflatoxin in the blood, that's what Jackie just showed, of the mothers does seem to correlate with poor birth outcomes. And as we know, poor birth outcomes are the initial point at which stunting begins or it's already started in utero, but it's poor birth outcomes that lead to greater stunting down the road...impaired linear growth so think of it in that sense, as a process, not as a simple outcome of being stunted or not stunted. The second is that the interaction of aflatoxin with HIV and potentially other diseases that either compare immune function or aggravate inflammation in the body and make the impacts greater in terms of health and nutrition. So, we'll go straight to Kathy who is going to be talking about Mozambique cross-sectional study in the northern province of Nampula and a lot of interesting and complementary relationships. So over to you Kathy.

Katherine Heneveld

Thanks Patrick, and thank you very much for joining us today. First, next slide please, so first to provide a little background on why we conducted the study. Primarily, it was because there is very little known about infant and young child exposure to aflatoxins in Mozambique and how such exposure is related to child growth. Studies have been conducted to assess aflatoxin levels in crops and soil samples, as well as in ground nuts sold in markets in Maputu, with both showing high levels of aflatoxin contamination, specifically in the Nampula province, where we conducted the study, over half of the children under five

are stunted, and the population in Nampula is highly dependent on crops that are commonly contaminated with aflatoxin, including maize, groundnuts, and cassava.

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So, as I mentioned before, the study was conducted in Nampula province in the ten Feed the Future zones of influence districts in that province highlighted in green on the map. The study was a cross-sectional study conducted in November and December of 2018 and focused on two groups of children: both 6 to 23 months of age and 24-59 months of age. With the assistance from the National Institute of Statistics, we were able to use the 2017 census enumeration areas as our sampling clusters and using the probability proportional to size, we randomly selected urban and rural clusters in each district. And then we randomly selected household from the census list in each selected cluster. So because of this sampling strategy, our results from the study are representative of children 6 to 59 months of age in the 10 districts. For data collection, there were three main elements. First was the household questionnaire. This was conducted at the household and was split into two questionnaires: one for the female caregiver of the selected child, and the other for the head of the household. Among other things, the caregiver questionnaire gathered information on child and caregiver diets, child morbidity, WASH, and IYCF practices. And the household's head questionnaire focused on agricultural practices and socioeconomic indicators. Then, at the nearest health clinic, anthropometrists measured the child's weight, height or length, MUAC, head circumference, and knee-heel length. Phlebotomists then conducted a finger prick to test for anemia and malaria. If the child did not have severe acute malnutrition or severe anemia, the phlebotomist then took a venous blood draw to test for aflatoxin B1 exposure. Although we are not focusing on anemia and malaria in this presentation today, we found an anemia prevalence of 76 and 67 of children had malaria. So the first two objectives of this study were to assess the mean aflatoxin levels of each age group and to examine the difference in mean levels between the age groups. As we hypothesized we found that children in the older age group had higher levels of aflatoxin than the younger age group, and this was statistically significant. Overall, we found that 90 percent of children in the study had a detectable level of aflatoxin in their serum sample. The mean aflatoxin levels and rate of detectability found in the study were similar to results from other Innovation Lab studies conducted in Uganda and Nepal.

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So the third objective of the study was to enumerate the association between aflatoxin and linear growth or stunting. Similar to rates reported in the 2011 DHS survey, we found that 45 percent of

children were stunted. Disaggregated, this was 32 percent of children 6 to 23 months of age, and 52 percent of children 24 to 59 months of age. In the entire sample, we found a significant relationship between stunting and aflatoxin where a child was 60 percent more likely to be stunted with each unit increase in aflatoxin level. The units of aflatoxin used in these models is logged transformed aflatoxin, which is standard and standardized by child's body weight in kilograms. We standardized aflatoxin levels by body weight because of their complex relationship in children. Even after adjusting for age, we found a relationship between aflatoxin and body weight, and hypothesized that a larger body weight allows for more accumulation of aflatoxin. Linear regressions also showed a significant and negative relationship between height or length for WHZ scores and aflatoxin, indicating that a higher aflatoxin level was associated with lower HAZ. Both logistic and linear regression models were also run on individual age groups, resulting in similar and significant findings for children 24 to 59 months of age. However, models for the younger children were not found to be significant.

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So now, we will look into some of the factors associated with aflatoxin levels. First is the diet. As I mentioned at the beginning of the presentation, the population in Nampula is highly dependent on maize, groundnuts, and cassava. The bar graph on the screen shows the percent of children in the study who consumed these foods in the past 24 hours. So, you can see that the consumption of cassava is much more prominent in our study sample than maize or groundnuts. I do want to note however that these patterns varied by district. And some districts relied more on maize than cassava. In relation to aflatoxin exposure, we found that overall consumption of groundnuts and cassava were significantly associated with increased aflatoxin levels. Only groundnut consumption by the older children was significantly associated with increased aflatoxin levels when our models were conducted on separate age groups. And we did not find any significant relationship between aflatoxin and maize consumption.

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So, next we looked at agricultural practices. Post-harvest practices around grain drying and storage are known to impact grain quality and therefore potential mold development. Here we will focus on two aspects of grain drying, the location of drying and the type of method used. I also want to note that we collected a substantial amount of information on storage practices. However, we did not see any significant relationship with any of these practices in child aflatoxin exposure. Regarding location, farmers dry their grain in the field after harvest after bringing it from the field or both. An FAO technical brief on seed and grain storage systems emphasizes that grain must be dried rapidly taking care to cover

it if it rains and to avoid overexposure to the sun. We hypothesized that drying grain only in the field does not meet these criteria and therefore leads to greater risk for aflatoxin contamination. Concerning methods, there are many common methods used to dry crop and we categorize them into improved and unimproved groups. Methods in the improved category include using fans, placing grain on platforms or plastic sheets or hanging grain under the rood or in the kitchen. These methods dry crops quickly, reduce the risk of infestation and moisture damage, and do not have high labor requirements. Unimproved methods include drying grain only in the field, spreading grain directly on dirt, cement, or brick floors or drying on the roof. The bar graph here describes these drying practices for maize and groundnut producers in the study. Overall, over half of study households produced maize and/or groundnuts. A little over a third of maize producers dried their crop out of the field, while two-thirds of groundnut producers dried their crop out of the field. Only around 15 percent of maize producers used improved drying practices. Unfortunately, we were not able to collect agricultural practices related to cassava production due to overall questionnaire length and the desire to limit participant burden.

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So, how did these drying practices relate to aflatoxin levels? Linear regression found that children in maize-producing households had lower aflatoxin levels if the household dried the maize out of the field compared to only drying in the field. A relationship of similar magnitude was found with drying methods where children in households using improved drying practices had significantly lower aflatoxin levels than those in households that did not use improved drying practices. In addition to other covariates, these models were adjusted for intercropping as it is known to reduce the risk of brain damage due to pest infestation.

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Similarly to maize, we found a significant relationship between aflatoxin levels and improved groundnut drying practices. Children in groundnut producing households that used improved drying practices had lower aflatoxin levels compared to children in households that did not use improved drying practices, but we did not find a significant relationship between aflatoxin levels and groundnut drying location. So, in conclusion, we found ubiquitous exposure to aflatoxin in the study sample as well as high prevalences of stunting, anemia, and malaria. We found that children were more likely to be stunted if they had higher aflatoxin levels, and that relationship was stronger in the older age group. Factors significantly associated with increased aflatoxin levels in children included groundnut and cassava consumption, as well as poor maize and groundnut drying practices.

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So from this study, a few future research areas have been identified. First, there are several initiatives targeting aflatoxin contamination in different crops in Mozambique. Second, the association between cassava and aflatoxin levels was not fully examined in the study. We found a relationship with drying... with dietary consumption of cassava, but the link between cassava, agricultural practices and aflatoxin levels warrants further investigation. A better understanding of the exposure to aflatoxin in urban areas is needed as our study focused primarily on rural areas. This includes examining the source of dietary exposure in the way of home, production, or market purchases. Finally, now that we found a significant relationship between stunting and aflatoxin in this cross-sectional study, the next step is to examine how aflatoxin affects linear growth through longitudinal studies. Thank you for your time.

Patrick Webb

Thank you Kathy. Really interesting again and so you started bringing in some the nuances about what is actually done on the farm or not done, the technologies available for understanding moisture content and storage, and whether or not foods are procured from the market and which foods, including cassava, not just peanuts and maize. So, the challenge here is to understand that this isn't just a farm-based problem. It's a market-based problem and a food system-wide problem, and therefore, while a lot of the questions that have been posed are about testing on the farm and interventions pre-harvest, we have to try and understand that this is a food system-wide problem that requires attention from multiple sectors and institutions. And Shibani Ghosh is going to share some findings from Nepal and Timor, and bring in some more of those dimensions. So over to you, Shibani.

Shibani Ghosh

Thank you Patrick and greeting to everybody from across the world for joining us. I'm pivoting from sub-Saharan Africa to talk about our work on serum aflatoxins and markers of growth in Nepal and Timor-Leste, and I'd like to add that a lot of this work has been done by Johanna Andrews Trevino, who is our postdoc at Tufts University. Unfortunately, she isn't able to... she's on the panel but she isn't able to actually be on the presentation. So, yes, thank you, thank you Grace. So I think one of the things we find in sub-Saharan Africa is around household production and linked to maize and groundnut

consumption. And as we've seen with Kathy's work, cassava as well. What was very interesting in 2012-2013, when we started working on aflatoxins, was very little literature emerging from Asia, despite the fact that maize and groundnut are consumed in South Asia as well. And particularly, I'm just going to give a little bit of background on Nepal and Timor last. We do know that the stunting rates in these countries are high, though in Nepal, there's been a substantial reduction in stunting as many of you on this webinar are familiar and aware. But there have been studies, a few studies on aflatoxin contamination in food products, and not just in commodities and agricultural commodities, but across the board in oil, in formula, in corn flakes, so in any kind of processed food product, aflatoxin contamination has been found. And this one particular study and it's from 2005. And the John Groopman and colleagues from Johns Hopkins had assessed aflatoxin in pregnancy and actually in the placenta at birth and in offspring of Nepali women in the Terai district of Salahi, and they found that these were correlated, that these women having aflatoxin at birth were likely to have kids who had aflatoxin at two years of age. So that led us down to the path of sort of saying, we really need to understand what's happening from the pregnancy through post-birth through early life with respect to aflatoxin and different outcomes, particularly given the literature around stunting and aflatoxins, we thought that that would be important to look at. That being said, since we started the study in Nepal, we did... there is a paper that was published on the [PLoS](#) network by Felicia Wu and her colleagues, where they did not see a relationship between aflatoxin and stunting in children 36 months of age.

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Now when you look at Timor-Leste, one of the reasons why we went to Timor-Leste is because we were invited actually, it was Patrick Webb who led the work in supporting the Ministry of Health in Timor-Leste, and UNICEF, and the University of Indonesia in adding an aflatoxin survey to the national survey of Timor-Leste. And in addition to that aflatoxin data that was collected, we found one study that has just been published last year on aflatoxin contamination in maize and groundnuts. And what they found was that about 11 percent of the maize and 12 percent of the groundnut samples had aflatoxin levels higher than the EU cutoffs, but in pretty much... the detectable level of aflatoxin was 80 to 90 percent of the samples. And they did look at the aflatoxin data from the Timor-Leste survey, which we are also going to be presenting, and reported an 80 percent detection rate in the samples.

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So let me just go into Nepal. A substantial part of this presentation is going to be on Nepal because we've conducted a longitudinal prospective... longitudinal observational birth cohort study in the Banke

District of Nepal, and now this is the latest, I think, I hope I got the correct map of Nepal with its new provinces. And what you have here, you see Banke is actually in what is called the Terai Plains of Nepal, and it is bordering India. And we've worked in about what are called 17 Village Development Communities or VDCs, which I don't believe exist now with the new structure, but it was pretty much about a third of Banke districts that was within our catchment area for the study. And we recruited 1,675 women in pregnancy and we following them and their infants up to two years of age.

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Please, so this is just to give you the timeline of the... of what we call the AflaCohort Study. It began in 2015 and we finished our last data collection in March or April last year. We initially had just one phase to the study which was to start with prenatal recruiting women on a rolling basis in pregnancy, and if possible in the first trimester, but we ended up rolling a lot of women were being identified only in the second and the third trimester. And we measured them either once or twice during pregnancy, and then we followed them up at birth, 3 months, 6 months, 9 months of age of the child and 12 months. That was the Phase I, and then we were fortunate to have additional funding that allowed us to at least follow up on half of the cohort, which is about 700 kids we were able to follow up at 18 to 22 months of age, where in addition to aflatoxin measurements, we were also able to add other mycotoxins as well as do an assessment of EED. Now for each of these time points, we collected a venous sample on the mother as well, and when the child was born, post-birth from 3 months onwards, we collected a venous sample on the child to measure serum aflatoxin levels on the mother in pregnancy and the child starting at 3 months of age.

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So let me just... this is basically the serum aflatoxin B1 concentration and from... that was detected in pregnancy at the child in the 3 months, 6, 12, and 18 to 22 months. And as you can see, we have just about 700 kids by the time we're reaching that last time point. And the second column that you see in this table is the detectable range which is about 95 percent of women had detectable levels of aflatoxin and we're presenting, you see, the mean and the standard deviation and the geometric mean. In terms of presenting data on aflatoxin, we almost always have to use a geometric mean because of the fact that the distribution of aflatoxin is extremely skewed, which sort is the reason why all our analyses also include logged transformations.

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Now, in terms of the outcome of interest, the primary outcome of interest in follow-ups was looking at change in length... length-for-age Z score, and then the prevalence of stunting. So what you're seeing here is a graph that we plotted in the LAZ distribution over time. So you go from birth to 18 to 22 months, and what you are seeing is the blue line ... the dashed blue line is the children, the subsample at 18 to 22 months, and there is a shift towards the left, which indicates an increase in the number of kids who are moving ... either becoming stunted or who are moving in the direction of being stunted. And you can see that in the box, 15 percent of the infants at birth were classified below two standard deviations, length for age and at 12 months it was 27 percent of the kids, and by 18 and 24 months it's about 40 percent of the kids. So you are seeing that progression, which has been documented by many others, that stunting in the first two years of live, you see this rapid shift in the direction of being stunted.

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So the first paper that we have published, and this is under Johanna's name as the lead author in the Journal of Nutrition, was to assess gestational serum aflatoxin levels and birth outcomes. And what you see here is our adjusted models and we're just presenting the estimates in the form of an odds-ratio for the relationship of aflatoxin in pregnancy and low birth weight, small-for-gestational age, stunting at birth, and preterm birth. And the only birth outcome that we found significant in the case of the Nepali women was small-for-gestational age. And I'd like to add that the prevalence of low birth weight was about 20 percent and that small-for-gestational-age was around in the realm of 30 to 35 percent, so this is a population which has a significant percentage of low birth weight and SGA infants.

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In terms of looking at growth over time, this is the latest analysis that has been done and this has been submitted. So this is a draft manuscript that has been submitted and is under review, so not yet published data, but what we are presenting here are the beta estimates for length, and LAZ, and the odds-ratio for stunting for two different variables: one where the aflatoxin is an absolute value, a logged transformed aflatoxin value, and the other one is where we have standardized it by body weight of the child, as we found that there was a significant correlation between levels of aflatoxin and increasing body weight, and that was irrespective of age. So what we find is that length ... the weight was using just absolute value or standardized by body weight, was significantly lower as aflatoxin levels increased. And the same goes from length-for-age Z score and the stunting odds-ratio. And just to add that these are

fixed effects models where we have included only the data for the children from 3 to 22 months of age, and these are what are called as contemporaneous analyses. We also ran a lagged analysis, which unfortunately I realize we don't have the slide here for and what we found was only length... change in length was affected by the prior aflatoxin level in that child. Again, these were fixed effects models. The other thing that we also found that is not here in this slide is knee-heel length, which is a measure of long bone growth, was significantly lower in kids with higher levels of aflatoxin, and that relationship with knee-heel length existed in a contemporary model or if you will, a pooled cross-sectional model, as well as in the lagged model. And that's something that is very interesting to look at in terms of a measure of anthropometry that is knee-heel length.

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We also looked at weight-for-age and weight-for-length Z scores and what we did find was a negative association with weight-for-age, but we did not find a relationship with weight-for-length Z score.

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Now the question that we had was now that we've looked at the exposure levels, we've looked at what it translates to with respect to a major outcome such as linear growth or stunting over time... do we have a sense of where ... what is the source of this contamination, particularly within these communities in Nepal, which are close to urban areas, but which are also agricultural. So one of the analyses that was done with data that was collected in pregnancy was to look at the diets of pregnant women and the levels of aflatoxin B1 in pregnancy. And what you see is... you do not see a relationship with maize, but you do see a relationship with groundnut consumption. And there is a huge seasonality effect for aflatoxin levels in pregnant women with women having significantly higher levels in winter compared to the spring or summer months. And we looked at the relationship of diet and aflatoxin using both OLS model, a simple linear regression, as well as a quantile regression model, just to ascertain if there were differences in relationships as you went along that aflatoxin distribution. And you do see that milk consumption, while it does not appear as significant ... as being significantly correlated in the OLS, it does seem to become significant at certain quintiles, but there isn't really a pattern here that we are observing.

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The other question then was: what was the source of these aflatoxin prone foods. It seems to me that groundnuts were clearly a major issue within the diets of these women. So we had collected data on the

source of the food that was being used in the households, and we looked at maize, groundnuts, and chilies, as it turns out chilies are also quite highly contaminated with aflatoxin, particularly in South Asia. And what you see here is essentially... it didn't seem ... it seems to be that home production and market both were significant sources of contaminated foods. This is when we compared to those people who had in-kind consumption, and the same thing applies for groundnuts as well as for chilies. And in fact with chilies, what you see, and with groundnuts, what you see is that the market dependence of groundnuts and chilies is very high in this catchment area. And if you go to the next slide, you can see that.., and I think Grace could you click one more time because I think there is an animation there, I apologize, one more please, sorry, thank you, that's it. So what you see here maize or corn was not really consumed by this population, it's not surprising in the Terai, not many people consume corn, but they were consuming peanuts and chilies, and most of them were acquiring the peanuts and chilies from the market.

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So then the next question we had was we have to understand where the children... what the source of aflatoxin is for children besides AFB1 and we do know that AFM1 does appear in breast milk, it's a metabolite of AFB1, so we wanted to assess what is the level of AFM1 in breast milk. Is it correlated with the growth of these kids and what are the sources of AFM1? And this is a paper that is in draft form that has been led by Ashish Pokharei who's been working on the Aflacohort study since 2016-2017, and has done some fabulous work on this analysis. And what you see here is 80 percent of the samples had detectable levels for AFM1. They are low, they are not high, they are within the cutoffs that are allowed for levels in milk. But what you do find is that women who have consumed yogurt, milk, oil, or ripe pumpkins, had higher levels of aflatoxin M1, whereas those who had consumed legumes in the past 24 hours had lower levels of aflatoxin, and once again, the seasonal element does remain. AFM1 seems to be higher in those women who were measured in winter compared to the spring months. We did not find a relationship of AFM1 with directly with linear growth.

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Then, we also looked at the dietary determinants of serum AFB1 in the children themselves, and this was looked at 12 months of age because our assumption was that the kids, while not everybody was exclusively breastfed, that most of them would be and that they would start introducing foods at around 6 months. So we looked at diet at 9 months, and AFB1 at 12 months, as well as diet at 12 months and

AFBI at 12 months. And what you see, these are models that were run by the lead author, Ashish Lamichhane, who is also one of our coordinators for the Nutrition Innovation Lab work in Nepal. And he looked... he used both OLS and quantile regression models and on the left side, what you're seeing is the lagged relationship which is diet at 9 months in cedar maple toxin at 12 months. And he found a positive association with kids who had been given large fish, who had consumed infant formula, would consumed groundnuts and for some reason cauliflower, had significantly higher levels of serum aflatoxin. The interesting thing is he did find a negative association with maize consumption, but only 3 percent of the kids had actually received any maize in their diet, which is why we're not entirely sure whether that's a very relevant finding. In terms of the sort of cross-sectional analyses or cross-sectional relationships, again the large fish and groundnuts were significantly associated with higher levels of aflatoxin and we found a negative association with kids who had been provided bananas or mangoes. We didn't see anything with diet diversity at all, and I have to say that we didn't see that in the women as well. And I'm not quite sure whether it's because it's not able to pick up the nuances that are needed for this analysis. I don't know why we wouldn't see diet diversity relationships, but I think the banana and the mango were a reflection of improved... a good quality diet

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In addition, as I mentioned in our Phase 2, we have had the opportunity to assess other mycotoxins because within the interim of this project being undertaken, there have been papers from Tanzania where fumonosins were found to be associated with stunting. So we were able to add in one more measure of AFBI in the children 18-22 months of age. We kind of had graduated them out and then we were able to re-consent them and do another blood draw. You know we've had some very fabulous folks on the ground who have been working on the Aflacohort study thanks to HKI in Nepal, and you know and of course the Aflacohort households themselves. And we do see, and I have to say that I'm presenting only the descriptive statistics, because at this moment Johanna is working on assessing what the relationships are of these different aflatoxin mycotoxins to each other, to EED as well as to the linear growth of these kids at 22 months of age. But in a sense, what I'd like to point out that pretty much all of them had detectable levels of ochratoxins and fumonosins. There was a 100 percent detection rate and about 85 to 87 percent had AFBI and DON.

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So, I'm going to move on to the Timor-Leste aflatoxin survey and as I mentioned, this aflatoxin data comes from a national survey that we had an opportunity... we were very fortunate enough through

Patrick's interactions with Timor-Leste and UNICEF and USAID to support that data collection. This was a quantitative national level survey that was conducted in 2014 and there was a sub-sample of about 600... 520 to 600 mothers and children who were measured in a study of biomarkers, which includes both micronutrients, as well as serum aflatoxin. And similar to the way other data were handled, we looked at anthropometric measurements, converted them to Z-scores using the WHO standards and we log transformed AFB1 data for analysis.

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So what you see here is there were about just over 500 children that we had data on for cedar maple toxin, and we had about 83 percent detectable rate with a mean value of 1.4. Look at the geometric, it's about 0.6.

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Looking at the aflatoxin adduction log transformed LAZ and stunting, what we do find is there is a significant relationship, a negative one for LAZ and a positive one for stunting. So the risk of stunting goes up with every unit increase in the lysine adduct aflatoxin

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So, I think I just want to conclude on these two major studies that we have conducted and we still have ongoing analyses in Nepal and I'm going to talk about that in a second. But I just want to sort of point out to some key conclusions compared to what levels Jackie and Kathy have presented in Mozambique and Uganda, the levels of aflatoxin in the Nepali and the Timor-Leste populations are low. But what we are seeing in the longitudinal work that from three month of age, you are detecting aflatoxin in the infants, and it is going higher as they get older and they are being exposed to a diet that is contaminated. The other thing that I didn't get time to spend on in the results section is that the exposure is ubiquitous. So we looked at levels by socioeconomic status, education, location. We thought if any if this could modify... you know... is there any difference between the groups and we find that no... in fact there is no difference. We did, I mentioned, made a comment about the existing weight status and the correlation with aflatoxin and we are not entirely sure why that's the case, because animal studies show weight gain is impeded in animals that are provided contaminated feeds. So I think this might have... this is unique to what we are seeing, and we need to understand better about the role of body composition, lean versus fat mass. We do see low levels of exposure in pregnancy and that increases the risk of small-for-gestational age babies and there is a longitudinal relationship between early life exposure to aflatoxin,

linear growth, and stunting. And what we are seeing, particularly in the case of Nepal, that consumption... the exposure reflects consumption of different aflatoxin-prone foods coming from multiple sources. So that's a critical point that I think we started this ... Patrick started this off before I started talking about Nepal and Timor-Leste. The sources can be from different locations and it can be across the food system.

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So just we thought that these would be from a context of the Asian countries that we've been working in but we need to have some future research, we do need to think about interventions, and these will have to be context-specific interventions. And it has to be a food system approach given the sort of multiple sources of contamination. We are working to understand the co-exposure with other mycotoxins and child growth, and testing the associations with inflammation and environmental enteric dysfunction. So that's really actually ongoing right now, as well as a relationship between aflatoxin exposure and cognitive function. What's really critical I think is we need to understand what are the long-term effects of low-level exposure... low level of chronic exposure because what we are seeing in this observational longitudinal work is that the levels in Nepal are low, but they are consistently present in this population. There is no shift in the level of detection, if you will by putting it very simply, despite the fact that the levels are actually low.

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So yes, this is just to sort of reiterate some of the ongoing work and I just want to highlight the last two bullets here. We are also looking at aflatoxin in food. We had a sub-study that we were fortunate to conduct with the Feed the Future Innovation Lab for reduction of post-harvest loss, and we are working with them in assessing for the role of aflatoxin in different food products and its relationship with the blood markers and the growth outcomes, as well as looking at agricultural practices and its relationship with food after toxin exposure. This is specifically for the Nepal Banke study.

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I think I have two more slides which then will open. I apologize, this is a little bit of a long session but I just want to sort of highlight a couple of things which I've also mentioned in the conclusions: it is that we do see chronic exposure in pregnancy and early life of aflatoxins. There are public health implications to that. And for those of you who work in the realm of post-harvest agriculture, interventions, or policy-making, I think targeting aflatoxin might be an important issue to consider within the context of the

health effects. Again, I sort of want to emphasize we are seeing chronic exposure but low level exposure. We don't know what that means because most of the regulatory cutoffs that you find ... the research around the regulatory cutoffs is around acute exposure, which is obviously a metric for setting those cutoffs.

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And I think what we are seeing is different foods besides maize and groundnuts are... seem to also be sources of contamination, like cassava and chilies, and there are different sources and the role of markets and addressing gaps in ensuring a clean, safe, and nutritious food supply is going to be critical. So I think we have to go beyond aflatoxin mitigation of maize and groundnuts on-farm to off-farm and beyond. At the end, I'd like to say that this really emphasizes what we're seeing is it really emphasizes the need for enhancing multi-sectoral collaborations and using a food systems approach for aflatoxin mitigation. Thank you so much for listening and I think we should be open for questions, yes.

[Patrick Webb](#)

Thank you Shibani, and thank you all three presenters for very clear, concise, and yet compelling presentations that have stimulated a huge amount of interesting questions. And we fortunately have plenty of time to deliver into those. So, I'm going to start with Jackie, and let's address some of the more physiological questions first, perhaps before then going to broader questions about: well what can we do about this? So Jackie from Ahmed, there's a question specifically about the HIV link with the expression of the aflatoxin outcomes and he's wondering if it's possible that HIV infection is somehow over-expressing or increasing the stability of whatever it is that converts aflatoxin into its most dangerous form... its carcinogenic form or is it, as you said, immune suppression and/or a cumulative effect of inflammation. Just say a little bit more about that relationship if you could.

[Jacqueline Lauer](#)

Well, the fact is we really don't know. I mean I would say that there's only maybe one or two studies that have... human studies I should say... that have provided data on this. And if anybody is interested, there is a researcher [Jolly et al] had a paper out as well from the University of Alabama that looks at

this question too. And then of course our paper. And I don't believe there is too much more in literature. But the hypotheses from animal models and from the scant human literature that's out there suggests exactly what you said. I mean it could be HIV is just increasing people's... suppressing immune systems, creating more infections, which is creating higher aflatoxin levels. I think it's worth looking at the liver, in particular. We know that HIV has effects on liver and ARTs do as well. The interesting thing about this population from our standpoint was that we had every reason to believe, because this study was done after Uganda kind of implemented kind of their very strong program on controlling HIV, that these were very well controlled HIV infection, and it's likely that viral loads in this population are extremely low, leading to the question of perhaps it's not the HIV infection, even if it could be related to the ARTs that they're on, which we also known to have effects on the liver, and potentially the liver's ability to metabolize aflatoxins. So I think those are two pathways that have a lot of potential, but need further exploration.

Patrick Webb

Thank you yes, still a lot to be done. So Kate, and potentially both Shibani and Jackie as well, can you explain to several people asking why aflatoxin level was logged and that there was standardization by child weight, and so that relationship between logging the aflatoxin in the analysis, and the standardization against the children's weight.

Katherine Heneveld

Sure. So first for just the nature of aflatoxin level is that it is highly skewed. So we did need to take a log transformation at the beginning just to be able to have accurate regression results. And then, when we're looking at its relationship with stunting or linear growth, there seems to be some relationship in children between body weight and aflatoxin level that is... that still remains when we adjust for age. So we aren't quite sure exactly what this relationship is, but we just wanted to make sure to standardize by weight, so we aren't attributing any of the relationship of aflatoxin and stunting to the child's weight.

Patrick Webb

Shibani, do you want to elaborate perhaps a little bit more on the weight issue?

Shibani Ghosh

I just realized I was on mute, I think Kathy said is very, very well. We started... we saw this... it's very interesting... we saw this in the longitudinal data in Nepal, where we were seeing this, you know, increase in age, even after you controlled for age, there was a relationship of weight and aflatoxin. And I think that sort of laid down this path of looking at: is there potentially some issue linked to body composition? There isn't very much out there in terms of whether differing body composition of lean versus fat mass could be the reason why you're seeing more accumulation of aflatoxin, but that's one direction that one has to go and investigate. We don't have actually the answers. We do know that we see... we saw this in the longitudinal data in Nepal, so essentially the same kids overtime. We have seen this in Mozambique, and as well as the additional data that we have been looking at in Uganda, we're seeing that. So it seems to be something across the different groups that we have been studying.

Patrick Webb

So sticking with you Shibani. So two queries here. One from [] about can we just be explicit, are we seeing aflatoxin levels positive, specifically associated with linear growth of the young child?

Shibani Ghosh

Yes I believe that that is yes.

Patrick Webb

So we are still working on those analyses and have followed children from birth in some of the countries as they grow. And we're looking explicitly at that, then controlling for the birth weight and the birth outcome and small-for-gestational age. Shibani, did you want to say more?

Shibani Ghosh

Yes, I just wanted to sort of say that the paper that has just been submitted was looking at the longitudinal relationship of aflatoxin and length, aflatoxin and length-for-age Z score, and aflatoxin and stunting. And across the board, we are seeing the relationship between aflatoxin and growth.

Patrick Webb

Right. So a final one, sticking with you Shibani. Biomarkers, what can we say... there's been an explicit question about: is there a relationship between aflatoxin and anemia, but I know we've also been doing other micronutrient biomarkers and can you say something about that?

Shibani Ghosh

Yes, and I might ask Katie to jump in because we did look at aflatoxin and anemia in Mozambique, and I think we didn't really find a relationship that was significant. Katie is that...? So we did because anemia and in the case of sub-Saharan Africa, malaria is very common and we were thinking that we really need to understand, particularly with the Ugandan findings around HIV, that do infectious diseases increase the risk of accumulation of aflatoxin and we didn't see that correlation with malaria ok as well or did we see that? □ So there was a relationship, but it wasn't significant. It also has to be kept in mind that anemia and malaria prevalences in the Mozambican children were very high, so almost half or more of the kids had either anemia and malaria, and so there's a different discussion altogether. In terms of micronutrients and its relation and aflatoxin we are looking at our data in Uganda right now. Unfortunately, we're not at that point where we can present everything, but we are looking at micronutrient markers, inflammation markers, and aflatoxin individually with their relationship with growth, but then their interaction with each other and we're not actually seeing very much there. We're talking about a sample size of around 16,200 kids, but we're not seeing anything with micronutrients and aflatoxin, at least at this moment.

Patrick Webb

At this moment. And I just want to come back to one of the really important points you made earlier, that even if the prevalence rates of the mean levels in Asia, and Nepal, and the Timor samples are relatively low, remember that there is no absolute cutoff where some low level exposure level is okay. There should be no exposure, we should find none in the blood. But even though the levels are higher in Africa than we're seeing in Asia, chronic long-term exposure even at low levels may well be having significant effects on health and growth through the lifetime. Right so it's not just 'oh it's okay to have low levels' if those low levels are consumed every day from birth onwards, then there's... especially for small children... there's a danger of rapidly rising above toxic levels in the blood and we have to bear that in mind. So the diet, the seasonality, all matter. And Katie, on diet, Barbara Best was curious about the result on large fish and were fish broken down into large and small fish? The answer is yes, but do you want to just explain a little bit more how we assessed the whole diet in all of these countries, but particularly in Bangladesh?

Katherine Heneveld

Well, Bangladesh is a different study, I'm sorry.

Patrick Webb

I'm sorry, Mozambique.

Katherine Heneveld

We did assess the full diet in Mozambique. We haven't really looked at other dietary factors yet, related to aflatoxin levels. We focused on the foods that we know to be commonly contaminated with aflatoxin. However Nampula does border or does have, you know, quite a bit of coastline, so we do know that the population there is reliant on fish. And so, that is something that we should look into.

Patrick Webb

Jackie, Uganda is one of the countries in Africa with very high levels... Tom Mayhem is asking: 'Well why lower levels in Asia than sub-Saharan Africa?' Do you want to talk about that, but also: 'In your experience of having lived in Uganda, how aware are people, whether farmers or policy-makers, of this problem, and what are people thinking in terms of answers?'

Jacqueline Lauer

So I can only speak to the Uganda context and then maybe somebody can chime in about patterns they see working in south-East Asia. But of course I think a lot of it has to do in Uganda, with a heavy reliance on staple foods that make up the majority of their diet. So of course, maize, groundnuts, and cassava being large staples, and also the three foods that we see with very high levels, going back to the data I presented about kind of crop levels. And then you know, a lot of kind of the harvesting and storage practices that you see a lot as well. Like I said, the climate kind of being very aflatoxin-prone. So, between the climate and the harvesting and storage practices, as well as the lack of dietary diversity, I think kind of creates perfect storm in Uganda as well as several other East-African countries. I don't know Shibani how that contrasts to maybe Southeast Asia, and why there might be lower levels there. And also I mean the HIV infection you have to take into account if we do believe that HIV increases exposure levels of aflatoxins you know, there is a large population in East African that is HIV-infected.

Shibani Ghosh

Yes, I think I can speak for Nepal... the population in Nepal is very reliant on rice, so the majority of the staple food it's not so ... is rice and there is some addition of groundnuts and some additional needs to the diet, and then the chilies that are contaminated may not be consumed... they are... I mean people do eat spicy food but you need to probably... there must be a certain amount you need to heat on a daily basis and I think that that's the only thing I can think of that the amount of maize, groundnuts, and cassava that are consumed in sub-Saharan African, because those are the main meals whereas the case of Asia particularly in this context in Nepal, rice is the main meal. The key difference between the two areas is... at least where we were working on between Uganda and Nepal, is the difference in

contamination from household production versus from the market. I think that there is a lot more dependence in Asia on the markets compared to rural areas... that's sort of... yes.

Patrick Webb

That speaks to what I was alluding to earlier about a food system approach... a multi-sectoral approach. This kind of problem has to be addressed not just from the Ministry of Agriculture's perspective. It's not just about production. It's clearly about cleaning up the food system at large. It's clearly about national policies and regulations and setting standards and testing regimes to manage international cross-border trade, as well as market food safety concerns in a broader sense. Right, so the public health dimension, the trade dimension, the agriculture dimension need to work together to be able to resolve this problem... in the longer run... that doesn't mean it's too complex to address. It just means we need the right evidence to convince policy-makers that this is the right thing to do, and by generating evidence that's we're doing, that there is a clear rigorous effect on birth outcomes and child growth, then this just adds one more piece of evidence to the jigsaw puzzle relating to child development and growth. Shibani: breastmilk. Felicia Wu was really interested in the finding that we didn't see on AFMI breastmilk correlated with the child anthropometry: Was that a surprise to you?

Shibani Ghosh

Well actually to be honest, I think because there is at least one other paper that I know of in Egypt, where they have reported AFMI association, I don't know whether it was with growth, or whether it was with linear growth, or whether it was with weight gain, and so we were expecting that there would be some relationship, and the only thing we can think of is that while it's there, it's in very low levels. That's the only explanation we have... yes sorry. I know and we actually looked at it because the AFMI is a metabolite so we can't actually... there can't be any conversion back to AFBI, at least that's my understanding. And so we were a little stunned because we couldn't quite sort of say 'we're seeing it, it's in the milk which is being provided to those kids', but we're not seeing that relationship. It could also be that there are so many factors that could be affecting the child in addition to the presence of AFBI and the food that they might be given, as well as poor WASH, sanitation, hygiene. There might be so many other things going on, but this in itself is not the most important factor.

Patrick Webb

Right. So Jackie, maybe you could talk too a little bit about confounders in these analyses, not just for Mozambique but others... some people are asking well but have we looked at other factors? You've already mentioned 'Yes, we looked at diet, and also income, wealth, education. What other factors do we have to control for to be able to tease out the relationship between aflatoxin and child growth?

Jacqueline Lauer

So, I believe in our Uganda study, we controlled for all the variables that we collected in our survey that were also correlated with birth weight. So we did control for things like maternal age, and weight, and blood pressure, and education... education levels. And I think they were similar to what we controlled for in the Uganda birth cohort study, which the data is not published. In this particular study we again only included HIV-uninfected women and we did not include women with severe anemia or multiple pregnancy. So thankfully we had in that case ultrasounds used to better assess gestational age so we could get at that... yes, does that answer your question?

Patrick Webb

Yes. So I think it's quite important to get the message across, which is why I was using the term 'we need to clean up the entire food system'. It's the one thing that really struck me. We haven't presented the prevalence rates of exposure very strongly in this particular webinar, but in most of each of these countries, we're looking at these samples, 80, 85, 90 per cent of all the people, all the women in the samples, and the children having some detectable level of aflatoxin in the blood, right. So that's really important to understand. In Timor-Leste it was very clear that it's didn't matter if you were in the top income quintile or the bottom income quintile, or highest wealth, or lowest education, highest education because a lot of people purchase food from the market, as well as own production. This problem gets everywhere, which means you're not protected necessarily by education and income unless you're going to trusted sources of the food. So we need to pay much more attention to not just the characteristics of the households and the fields in the farms, but the characteristics of the market

and the way that governments are regulating food safety issues and understanding those, because that's a really important dimension. That said, different geographies in countries can have different rates, obviously because of the temperature and moisture issues, and Shibani there was one question about: Why the study was done in Banke, even though... because the aflatoxin rates were relatively low compared potentially to the hills, where we know that there's more maize grown-up in the regular diet. So why was it Banke?

Shibani Ghosh

Yes, that's a very good question. But before I go into that, I just want to say one of the risk factors that we needed to control for with respect to the Nepal analysis of birth outcomes was maternal height, because that is one of the major predictors of particularly length at birth in infants in South Asia. And so that is another one and I think there are some sort of ecological analyses that have been done about the role of maternal height in determining the risk of being born stunted and risk of being stunted and low height for weight Z score. In terms of the location, location, location, for those of you who are from Nepal will be familiar with this issue of being able to reach communities, so that you can actually recruit people, do the data collection, but also get those samples... those biological samples out of those communities to central locations. So our initial plan was to do one of the Terai districts, which is where stunting and wasting is very high, that was Banke, and a hill district where we know that there are lower levels, but there are also more maize and groundnut consumption. Unfortunately, logistically it would have been extremely impossible for us to reach those communities in the hills and maintain a cold chain. So that was one of the reasons. We also do know that in Banke, groundnut consumption, particularly in that belt and the Terai belt, while maize isn't very high, people do consume groundnuts. And that would be another reason, but I have to say honestly, that in the hills it would have been practically impossible to do any venous blood draws, because we wouldn't be able to guarantee the integrity of the sample.

Patrick Webb

Thanks Shibani. Several people have been asking how easy it is to test. And so let me just make the point that it is actually the test kits are quite easy, and cheap, and reliable for testing... testing mycotoxin content of foods themselves. Some of them can be done actually in the field on the farm, others are lab-based, but requiring cold chain and then doing the assays of blood serum samples is very,

very complex, and actually very, very expensive. And not every country in the world has the capacity of doing that, be it the lab analysis or the cold chain. So this work represents quite an investment, not just of people's time and intellectual capital, but real money and resources and logistics to make it possible. Those of you who, like Felicia Wu and others here who've been doing these kinds of studies, know very well how challenging it can be to maintain cold chain. Finally, let's just have a quick look you know what can we do Shibani... a few final words. Can we reduce contamination? Are there simple ways, technological or policy that we can apply to try and reduce people's exposure to this problem? What else do we need to do?

Shibani Ghosh

So I think I'm not the agriculturalist and I know that might be people on this who are better equipped to answer this question, but there are simple methods, whether there are improved drying and storage, methods that need to be scaled up, particularly within the African context you're seeing that seems to be very critical. I do also know that there is folks at IITA are working on trying to get an on-farm biological tool which is afla safe, which is applied to the soil and that sort of really drastically reduces the levels of aflatoxin, even in the post-harvest storage period. There are also studies that have been conducted by Purdue University around the use of hermetic storage bags, and I know that Bill Masters is a lot more familiar on this as well as folks at Purdue like Jerry Shively and Jake, who is the Director of one of the Innovation Lab whose last name I cannot remember... sorry Jack but they have published a really, really nice RCT on use of hermetic storage bags in Uganda actually which is a very, very, very relevant publication. That's if anybody is interested in looking at solutions around storage, around drying as well as the IITA work around afla-safe which I think has been scaled up in some countries. So that is basically... those are the tools of the intervention points if you are looking at on-farm, post-harvest, on-farm pre- and post-harvest, but the question really... the big critical question is what do you do in a system like in the case of Nepal where you have open borders, where you have a market reliance is incredible for basic needs and there is sort of this back and forth of different of different food products. I think that particular kind of food system requires a lot more thought in terms of how do we create some kind of a framework where you address household production, but also market contamination. So I think I don't have an answer but I think we do need to think about it differently, depending upon the context.

Patrick Webb

Yes, thank you Shibani. That was an excellent summary. We have to address this from the field perspective, from the market perspective, from the regulatory perspective. This evidence here presented today by these three amazing speakers confirms that there's a public health dimension to this relationship as well. We will be posting the presentations and the recording on our website, which you can see there. Thank you for an excellent engagement, so many questions, and so many answers. This is just one webinar in a long series. You can find the whole schedule of webinars on the website as well. Look out for them on social media and we look forward to engaging with you again on additional topics through the FDF Nutrition Innovation Lab. Thank you all for your participation and be safe and well.

Shibani Ghosh

Thanks everybody. Thank you.



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