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Assessment of aflatoxin exposure, growth faltering and the gut microbiome among children in rural Guatemala: protocol for an observational prospective cohort and bioreactor simulations

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ABSTRACT

Introduction Aflatoxin B1 (AFB1) is a carcinogen produced by *Aspergillus flavus* and *Aspergillus parasiticus* which grow on maize. Given the high prevalence of child stunting (ie, impaired growth) and other nutritional disorders in low-income and middle-income countries, where maize is consumed, the role of aflatoxin exposure may be significant. Observational reports have demonstrated associations between aflatoxin exposure and impaired child growth; however, most have been cross-sectional and have not assessed seasonal variations in aflatoxin, food preparation and dynamic changes in growth. Biological mechanistic data on how aflatoxin may exert an impact on child growth is missing. This study incorporates a prospective cohort of children from rural Guatemala to assess (1) temporal associations between aflatoxin exposure and child growth and (2) possible mediation of the gut microbiome among aflatoxin exposure, inflammation and child growth.

Methods and analysis We will prospectively evaluate aflatoxin exposure and height-for-age difference trajectories for 18 months in a cohort of 185 children aged 6–9 months at enrolment. We will assess aflatoxin exposure levels and biomarkers of gut and systemic inflammation. We will examine the faecal microbiome of each child and identify key species and metabolic pathways for differing AFB1 exposure levels and child growth trajectories. In parallel, we will use bioreactors, inoculated with faeces, to investigate the response of the gut microbiome to varying levels of AFB1 exposure. We will monitor key microbial metabolites and AFB1 biotransformation products to study nutrient metabolism and the impact of the gut microbiome on aflatoxin detoxification/metabolism. Finally, we will use path analysis to summarise the effect of aflatoxin exposure and the gut microbiome on child growth.

Ethics and dissemination Ethics approval was obtained from Arizona State University Institutional Review Board (IRB; STUDY00016799) and Wuqu' Kawoq/Maya Health Alliance IRB (WK-2022-003). Findings will be disseminated in scientific presentations and peer-reviewed publications.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Aflatoxin B1 exposure is potentially associated with impaired child growth.
⇒ Aflatoxin B1 exposure is associated with alterations in the gut microbiome.
⇒ Mechanistic work demonstrating how aflatoxin might lead to a growth phenotype is needed.

WHAT THIS STUDY ADDS

⇒ This study will reveal mechanistic explanations for hypothesised links among aflatoxin B1 exposure, the gut microbiome and growth.
⇒ This study will provide insights into the development of therapeutics for aflatoxin-related child stunting.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The outcome of this study will help support large-scale investments in aflatoxin remediation efforts on the part of public health organisations working to improve global child health.

INTRODUCTION

More than 40% of children under 5 years of age are at risk of not reaching their developmental potential, many due to the impact of stunting.[1] The rural Indigenous population in Guatemala has one of the highest rates of child stunting in the world. Interest in a possible role for aflatoxin in stunting has grown in recent years, made more compelling by the observation that many countries with high rates of stunting consume large amounts of maize and have documented aflatoxin in food sources.[2–7] However, most studies have been cross-sectional and have not assessed seasonal variations in aflatoxin, food preparation and dynamic changes in child growth. In addition, biological mechanistic data on

how aflatoxin may exert an impact on child growth are missing.

There have been many interventions to improve stunting in Guatemala, yet few have had tangible impact.[8] The gut microbiome has emerged as a key regulator of human health and nutrition, and a promising target for interventions.[9] Recently, our group demonstrated significant differences in children's gut microbiomes between those with high and low exposures to aflatoxins in Guatemala.[10] Our data also revealed potential shifts in diet-based aflatoxin exposure dependent on season and an association between diet-based aflatoxin exposure and child height-for-age.[11 12] To date, only a few animal studies have evaluated the impact of aflatoxin on the gut microbiome,[13–18] while direct investigation of the aflatoxin-exposed human gut microbiome is lacking. In addition to microbial changes induced by aflatoxin, gut microbiota can interact with aflatoxin through bioadsorption and biotransformation.[19–22] Aflatoxin can bind to extracellular structures on microorganisms (eg, *Lactobacillus* and *Saccharomyces*), which decreases its bioavailability.[19 22] Highly toxic aflatoxin such as aflatoxin B1 (AFB1) can also be metabolised by bacteria (eg, *Bacillus*, *Lactobacillus* and *Pseudomonas*) to less toxic or even non-toxic substances.[20] A close examination of aflatoxin degradation pathways and metabolites produced by human gut microbiota is also lacking.

We aim to assess temporal changes in diet, aflatoxin exposure and linear growth faltering in a prospective cohort of children from rural Guatemala, a country that has one of the highest rates of child stunting and aflatoxin exposure in the world. We will prospectively evaluate the association among AFB1 exposure, height-for-age growth trajectories, and the gut microbiome over 18 months for 185 children aged 6–9 months at enrolment. In addition, we will use bioreactors inoculated with faecal samples to evaluate the response of the gut microbiome to varying levels of AFB1 exposure, and the impact of the gut microbiome on aflatoxin detoxification/metabolism. We hypothesise that (1) aflatoxin consumption impacts child linear growth by altering the composition of the gut microbiome and inciting a systemic inflammatory response; (2) aflatoxin exposure alters luminal nutrient metabolism by the gut microbiome and (3) certain gut microorganisms metabolise aflatoxin and may be protective against aflatoxin exposure.

METHODS

Study setting and design

In this project, we will work in rural Guatemala in collaboration with Maya Health Alliance, the lead local institution, which facilitates primary care and research in service of the local Indigenous Maya population. Maya Health Alliance works alongside Indigenous communities to improve access to healthcare as well as leading clinical trial and observational studies on complementary feeding, stunting, dietary quality and early child development.[23–25]

This study includes field-based and lab-based components (figure 1). For the field-based component, children 6–9 months of age, only one child per household, will be enrolled in the study and followed for 18 months through 24–27 months of age. During the 18 months when children are enrolled in the study, households will be visited for data collection three times, at 9-month increments. Household visits will consist of surveys (eg, dietary intake), anthropometric measurements, sampling of household maize stores and foods, and collection of venous blood specimens and faecal samples.

Blood samples will be used to measure serum AFB1-lysine (AFB1-lys) adduct levels, C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Faecal samples will be used to analyse faecal calprotectin, gut microbiome composition and microbial metabolites such as short-chain fatty acids (SCFAs). Faecal samples will also be used in the lab to seed bioreactors which will then be dosed with varying levels of AFB1 (see the 'Bioreactor setup and sampling' section). Microbial metabolites (eg, SCFAs), AFB1 and its degradation products, microbiome structures (DNA) and functions (RNA) will be analysed.

Outcomes

The primary growth outcome will be height-for-age difference (HAD) scores, calculated as the difference between measured height-for-age and the median

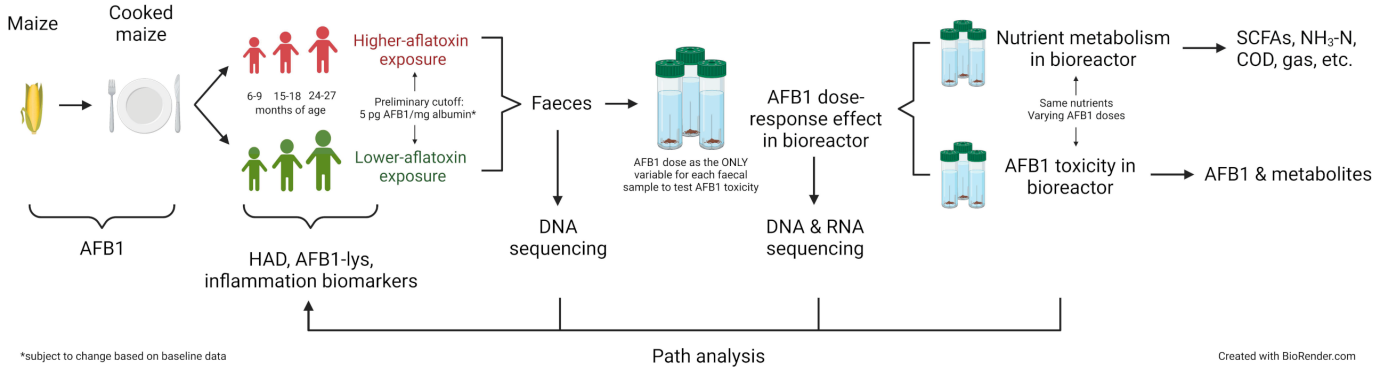

Figure 1 Overall project design and path analysis integration. AFB1, aflatoxin B1; HAD, height-for-age difference; AFB1-lys, aflatoxin B1-lysine; SCFAs, short-chain fatty acids; NH3-N, ammoniacal nitrogen; COD, chemical oxygen demand.

height-for-age from the WHO's Child Growth Standards reference population. The primary exposure outcome will be serum AFB1-lys, which will be used to estimate aflatoxin exposure. Children will be divided into 'high' and 'low' AFB1-lys groups using a potential cut-off of 5 pg AFB1/mg albumin (pending exposure data in the baseline assessments).[4 26–29] Secondary exposure outcomes will be markers of systemic and intestinal inflammation, including ESR, CRP, SCFAs and faecal calprotectin. These outcome measurements will be collected at three time points beginning at 6–9 months of age and will be collected every 9 months until the final time point at 24–27 months of age. In addition to these outcomes, we will evaluate the faecal microbiome as a mediator between exposures and outcomes. Other outcomes include the results from the bioreactor experiments, including AFB1-induced microbiome composition and function changes, microbial metabolite profiles and AFB1 degradation products.

Eligibility criteria

Participants will include children from Maya Health Alliance catchment areas located in the Departments of Chimaltenango, Sololá, Sacatepéquez and Suchitepéquez. Maya Health Alliance community health clinics and health centres will be the primary source for identifying potential children. In addition, the project will be promoted through community centres, churches, schools and community leader meetings.

Inclusion criteria are as follows:

- Infants who are 6–9 months of age at baseline.
- At least one caregiver willing to provide written informed consent and participate in study activities.
- Permanent residents of the communities or planned residence in the study area at least for the 24 months following enrolment.
- Singleton birth.

Exclusion criteria are as follows:

- Infants with moderate to severe acute malnutrition (weight-for-length z-score ≤−2).
- Infants with a chronic medical condition that affects growth and/or requires special care, present at baseline or diagnosed subsequently during the study observation period, such as:
  - Congenital heart disease.
  - Genetic conditions.
  - Kidney disease.
  - Neurological deficits.
  - Problems of cleft lip or palate.
- Infants whose caregivers have cognitive or other impairments that prevent them from providing informed consent or reliable information.
- Concurrent participation in any other clinical trial.

Sample size

Our planned sample size is 185 children. This is based on assumptions of an SD for HAD of 3.5 cm and an intrasubject correlation coefficient of 0.8 for repeated measures, based on recent Guatemalan Demographic Health Survey Data[30] and Maya Health Alliance observational data.[31–33] With these assumptions, a sample of 154 children will allow us to detect a minimum difference in HAD slopes of 1.0 cm between high and low AFB1-lys groups (above or below 5 pg AFB1/mg albumin) with 80% power, at an alpha level of 0.05. The total sample of 185 participants includes an increase of 20% to account for possible dropouts or lost to follow-up and will also allow for detection of the same 1 cm difference in slopes if our SDs are smaller than expected (as low as 2.5).

Recruitment

For the recruitment process, study staff will identify potential participants and inform caregivers of the study using a recruitment script, and directly answer questions or concerns about the study. This will either be conducted by phone, in routine healthcare settings or via home visits. Interested caregivers will be screened for eligibility via a rapid screening including general information on the child's demographic characteristics and inclusion criteria. Caregivers of children that meet the inclusion criteria after the rapid screening will review the informed consent form the same day or in a rescheduled home visit. Staff conducting recruitment and informed consent activities will be bilingual (Spanish and Kaqchikel, or other Mayan languages, as appropriate), and will provide information in the caregiver's preferred language.

Field data and sample collection

The study will involve three household visits at 9-month intervals. During these visits, the team will conduct surveys and anthropometric measurements, collect samples of maize, and collect venous blood specimens from the children (less than 2 mL/kg body weight). In addition, the team will place a sterile diaper on the child at the start of each visit and collect faeces on defecation. The maize samples will be tested for AFB1 concentrations, and a probable daily intake (PDI) score will be estimated via cooking practices, maize consumption recall and child body weight. The team will conduct parallel non-consecutive 24-hour dietary recalls using a locally validated method. Breastfeeding practices will be recorded, although previous data suggest that breast milk is not a clinically significant source of aflatoxin exposure in the population being studied and similar settings.[2] Blood samples will be collected by a trained nurse phlebotomist. Faecal samples will be collected in raw form and in glycerol and stored at −80°C until further processing. In addition to the planned testing, blood and faecal samples will be archived for future testing related to areas of specific interest to the study aims of this research project.

Bioreactor setup and sampling

We will use bioreactors to investigate the AFB1-gut microbiome interactions and use these results to elucidate microbiome-related observations in the cohort study.[34–36] We will select 20 children from the lower-aflatoxin

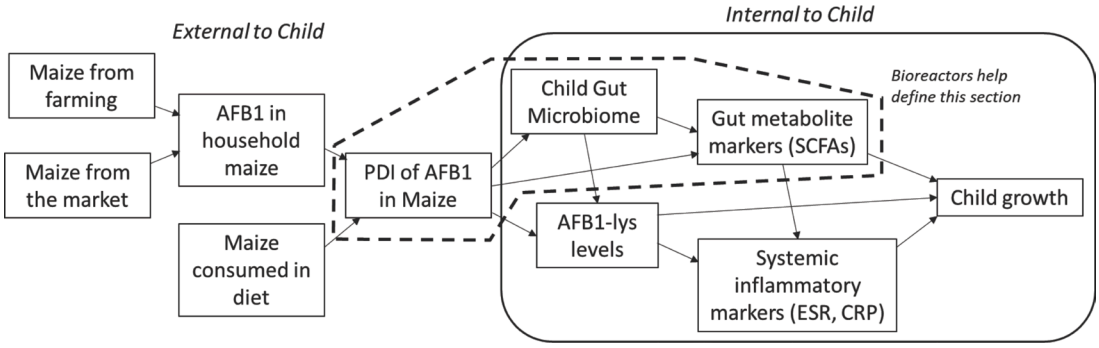

Figure 2 Diagram of hypothesised pathways in aflatoxin-child growth model. AFB1, aflatoxin B1; PDI, probable daily intake; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; SCFAs, short-chain fatty acids.

exposure group and 20 from the higher-exposure group based on their AFB1-lys levels, HAD scores and faecal microbiome composition (figure 1). We will collect their faeces in glycerol at two time points and combine those from the same age and same group as the inocula for bioreactors (ie, four inocula in total). We will feed the bioreactors with maize starch (the primary carbohydrate source in the maize-based diet and the matrix that AFB1 is associated with), other necessary nutrients and three doses of AFB1 that represent the maximum, minimum and average amount of AFB1 ingested by the children. We will operate the reactors in a fill-and-draw mode to simulate transit and retention time in the colon. We will collect liquid samples periodically for metabolite analyses (eg, SCFAs, AFB1 degradation products), and microorganisms for microbiome composition (DNA) and function (RNA) analyses.

Statistical methods

We will use the statistically appropriate correlation-based methods to evaluate associations between the hypothesised pathways (figure 2). We will assess growth trajectory differences between higher and lower aflatoxin exposure groups (as defined above) using a longitudinal mixed model.[37] We will control for the effect of a set of potential confounding factors and covariates (eg, diet, age, sex) collected in the household survey. Furthermore, to describe the mediating, moderating, direct and indirect effects among the microbiome, aflatoxin exposure, child growth outcomes and other factors, we will use path analysis and latent growth models.[38 39]

Data management and confidentiality

Study personnel will be trained on standard operating procedures for recruitment, enrolment and data collection tasks. Data quality will be ensured using native data field definition functions in digital data capture software and ongoing quality control measures such as database review and random audits of in-field operations. Each subject will be assigned a unique study ID number and this number will be the only link between their name and research data. Identifiable data will be retained by Maya Health Alliance for at least 3 years from the date of study completion or primary outcomes are published, whichever is later. Deidentified datasets will be transferred to Arizona State University and may also be deposited in public data repositories at the time of publication. No identifiable data will be released publicly.

Data monitoring, harms and auditing

The rate of subject accrual and compliance with inclusion/exclusion criteria will be monitored monthly during the recruitment phase. The study may be stopped early if there is regional or national political instability or difficulty with recruitment or retention that significantly impacts the ability to evaluate the study endpoints. The study has minimal risk to subjects. The anticipated risks include lost productivity or interference with domestic routines for the caregivers of enrolled children, risk of psychological stress or stigma for caregivers discussing possible delays in child development, risk of accidental disclosure of personal or confidential data, and risk of pain or infection associated with blood draws. To address these risks, standard operating procedures have been developed, including a specific operating procedure governing behavioural distraction techniques and limited phlebotomy attempts for children. The study is not expected to have any significant adverse events, but any perceived adverse events or complaints from participating communities or caregivers will be reported to the institutional review board (IRB) and granting authorities. Staff will be trained to report adverse events following established protocol. Staff will provide counselling to caregivers when laboratory results are returned and assist with linkages to clinical care when indicated.

Patient and public involvement

Patients or the public were not involved in the design of our research protocol. However, prior to initiating field work, community meetings will be held to solicit feedback on planned research activities, and results obtained from the study will be communicated back to the community in regular community meetings.

Informed consent

Study staff members will explain the study and obtain written informed consent from the caregiver/legal guardian of the child participating in the study. Informed

consent will be administered in Spanish or the Mayan language of the caregiver's choosing. Children participating in the study are less than 24 months old and are not capable of providing assent. Once signed informed consent is given, the study staff member will provide the caregiver will be provided with a signed copy of the informed consent (online supplemental file 1).

Dissemination policy

Laboratory results will be returned to participating caregivers by study staff. Results will be explained in detail and linkages to clinical care facilitated when indicated. We will educate the public and the scientific community by publishing in peer-reviewed scientific journals, presenting our findings at microbiome-related, toxicology-related and environmental engineering-related conferences and webinars, and by conducting town-hall style meetings with participating communities.

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Contributors All authors contributed to the study design, sample size calculations and writing of the protocol for this study. QC, HG and LEV-G prepared the first draft of the paper. All authors contributed to revisions of the manuscript and contributed to the revision of the final manuscript. All authors have read and approved the final manuscript.

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Competing interests No, there are no competing interests.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The study protocol has been approved by the Maya Health Alliance IRB (WK-2022-003) in Guatemala and the Arizona State University IRB (STUDY00016799) in the USA.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement No data are available.

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Wuqu' Kawoq

Consent to be a Research Participant

Key Information for Aflatoxin Exposure, Growth Faltering, and the Gut Microbiome among Children in Rural Guatemala

You are being invited to take part in a research study about maize consumption and child growth.

WHAT IS THE PURPOSE, PROCEDURES, AND DURATION OF THE STUDY?

By conducting this study, we hope to learn more about the quality of maize we consume and the relationship to the health and development of our children. In particularly we will be looking for a type of fungus sometimes present in maize. In large enough quantities, this fungus has the potential to be harmful. Your participation in this research will last about one and a half years. We will conduct visits during four separate timepoints spaced about six months apart. At each of the four timepoints, we will visit you three times, one primary visit in-person and two shorter visits (potentially by phone or in-person). In the primary visit, we will ask you about the health of your child, we will take anthropometric measurements and collect information on his (her) diet. We also take one sample of poop and blood on each visit. Additionally, we will ask you to provide small samples of the maize you have at home. In the two shorter visits we will collect additional information on your child's diet.

WHAT ARE THE KEY REASONS YOU MIGHT CHOOSE TO VOLUNTEER FOR THIS STUDY?

By participating in this study, you will help us figure out ways to better support growth and development in children like those in your community. Also, if we find harmful fungus in the maize, we hope to learn and guide families in better practices for maintaining healthy maize in storage and cooking.. Your child, if enrolled in the study, will get laboratory and clinical exams to see if he/she has some problems (i.e. infections, anemia), and access to a physician that can explain the results. Additionally, your child will be monitored for growth and if needed your child will be referred to the nutrition program from Wuqu´ Kawoq for follow-up.

WHAT ARE THE KEY REASONS YOU MIGHT NOT CHOOSE TO VOLUNTEER FOR THIS STUDY?

If you participate in the study, you will need to participate in visits conducted during four timepoints spaced every six months and facilitated by a study nurse. At each of the four timepoints, we will visit you three times, a primary visit and two shorter, secondary visits. During the primary visit you will be asked to provide samples of your child's blood, feces (poop), and some household maize samples. Therefore, the study requires your time and willingness to provide the samples.

DO YOU HAVE TO TAKE PART IN THE STUDY?

If you decide to take part in the study, it should be because you really want to volunteer. You will not lose any services, benefits or rights you would normally have if you choose not to volunteer.

WHAT IF YOU HAVE QUESTIONS, SUGGESTIONS OR CONCERNS?

The person in charge of this study is Dr. Peter Rohloff at Wuqu' Kawoq. If you have questions, suggestions, or concerns regarding this study or you want to withdraw from the study, his contact information is 502-5000-5833.

Detailed Consent

Title: Aflatoxin Exposure, Growth Faltering, and the Gut Microbiome among Children in Rural Guatemala

Principal Investigator: Peter Rohloff

Sponsor: National Institutes of Health (NIH)

Introduction

You are being asked to participate in a medical research study. We are going to tell you more about the study.

After hearing about the study, you can decide to participate or not. It is your decision. If you decide to participate, and then do not want to continue, you can leave the study.

The decision not to participate will not affect your relationship with Wuqu' Kawoq in any way. In other words, you will not be penalized for not participating.

For example, you will not lose the right to receive Wuqu' Kawoq medical care.

Before deciding:

- Please read or listen to this document
- Please listen as one of our workers explains everything to you
- Please ask any questions you have

You can take a copy of this document to keep at home. You can take the time you need to decide to participate, up to one week. For example, you may want to talk with your family or friends before deciding.

Do not agree to participate unless your questions have been answered. When you agree to participate, you are not giving up your legal rights.

What are we going to do?

From this study, we are trying to learn more about the quality of maize we consume and the relationship to the health and development of our children (including intestinal function). In particular, we will be looking for a type of fungus sometimes present in maize. In large enough quantities, this fungus has the potential to be harmful. In this study, we will work with around 200 children between 6 and 24 months of age in your community and other similar communities.

Once you decide to participate, your participation in this study will consist of the following activities: At each of the four primary visits we will ask you about 1) household characteristics, your child's health and diet, 2) conduct measurements of your child's length, weight, head circumference, and arm circumference, 3) collect a blood sample from your child, 4) collect a fecal sample from the diaper we will provide you for your child, and 5) collect a sample of maize from your current cooking supply. In total, we estimate this to take about 90 minutes.

We will also conduct two shorter, secondary visits with you at each of the four timepoints, which can be done by phone or in-person. These visits will be to collect additional information on your child's daily food consumption and will take about 15-30 minutes.

If you decide to participate in the study, you will receive a series of visits at four time points spaced six months apart during the project from a nurse from Wuqu' Kawoq. During each of the four timepoints, we will visit you three times, one primary visit and two, shorter, secondary visits for a total of 12 visits over the whole study. If possible, we will aim to conduct one primary visit that is in-person to ask questions and collect samples and then two follow-up visits via phone to ask a shorter list of questions. However, it may be necessary to conduct two in-person visits if all samples cannot be collected in the primary visit. The results of the growth and counseling will be given the same day. We will share the results of the clinical tests (blood, poop) and explain them to you one or two weeks after collection. In each home visit we will do exactly the same procedure. We will provide a guide for correct feeding practices according to the child's age. Each visit will happen six months apart, until your child is between 24-27 months of age.

During visits from the study, if there are any procedures or questions that make you uncomfortable, you can tell the researcher that you do not want to complete them. You can also decide where in your home is the best place to conduct the visits. We plan to conduct all of these visits in the comfort of your home, unless you think there is another option that suits you better, for example coming to the clinic at Wuqu' Kawoq.

The risks for you and your child

If you agree to participate in the study, you will receive a visit from a study nurse. The main risk from the study is that it will take up some of your time to participate. You may also experience stress or emotional discomfort from answering study-related questions. You can skip any question that makes you uncomfortable. There is a risk of stress as well caused by potentially abnormal findings, we will coordinate with a physician to provide clinical evaluations, counseling and aftercare if this is the case.

The laboratory exams that we will take are of minimal risk. However, we will take a blood sample on each visit. This can cause some stress to you and your child. The procedure is safe, and we will use aseptic techniques and the best practices for blood collection in small children. Also, the nurse in charge of this procedure has extensive experience in collecting blood from small children. The quantity of blood we will take is ~15 mL (about 1 tablespoon), which is within the parameters of safeness for small children. There is a small risk of infection from the collection, however, if infection occurs, treatment from Wuqu' Kawoq will be provided.

Collecting the fecal (poop) sample from your child's diaper will be done by our staff, and it may take some time, depending on how long it takes your child to poop. We will make every effort to schedule the visit at a time that is convenient for you, and to minimize the time we are in your home, but you may still have some emotional stress if the visit takes some time.

Sometimes young children can get sick. This is probably not due to the study, but you can still inform the team. If we find a child with acute malnutrition or another very serious health problem, we will treat him or her or refer for treatment accordingly.

In addition, there may be cases of COVID-19 in our region or your community and there is a risk of getting COVID-19. Our team will use very strict protective equipment and disinfection procedures to keep this risk low.

We will collect information about your child's health. Because of this, there is a small chance that your information may be seen occasionally by someone other than your doctor, nurse or other trusted person. We will work to prevent this from happening.

Sometimes families make decisions about participating in research projects together. If there is another family member that you feel needs to help you make the decision to participate, then we can talk to that person before you make your decision.

Will you benefit from taking part in this study?

Test results from the study will be available to you as caregivers, and a member of our team will explain to you what they mean. Additionally, if a child is diagnosed with abnormal findings, a physician will interpret and provide a reference to a specialist (if necessary). Any care that is required from a hospital or medical specialist will will not be covered by the project however.

If a participant child has anemia, or acute malnutrition, that requires center-based care, we will arrange the reference of the child for further evaluation, depending on the urgency, to either the Wuqu´ Kawoq nutritional program or to the Health Center from the Ministry of Health of Guatemala, according to each individual case.

What will it cost you to participate?

There will be no costs for you to participate. You will not be charged for any of the study activities.

Payment

You will not be given any money for your participation. You will receive a gift (a basket of household items and/or foods) with a value of around 50.00 quetzales that will be delivered during the principal visits as compensation for your time.

Security

Wuqu' Kawoq and the Arizona State University, are responsible for conducting the study. All private study records will be maintained in accordance with the laws and study records will be maintained for at least three (3) years from the date of study completion or primary outcomes are published, whichever is later.

In the study we will record personal information about your child. When possible, we will use a number or code, instead of your child's name, to protect them. Your names or other important information will not be included in any study report. However, when we publish reports about the study, we may share an anonymous version of information collected during the study.

In a very special situation, we would have to share your name if we are ordered to do so by a court or a judge.

Can you choose to withdraw from the study early?

You can choose to leave the study at any time. You and your child will not be treated differently if you decide to stop taking part in the study.

If you choose to leave the study early, data collected until that point can be removed by contacting Dr. Peter Rohloff at the phone number (+502) 5000-5833.

You may be removed from the study if:

- Ø You or your child are not able to follow the directions.
- Ø The study team finds that your child's participation in the study is more risky than beneficial to them.
- Ø The agency paying for the study chooses to stop the study early for a number of scientific reasons.

Are you participating, or can you participate in another research study at the same time as participating in this one?

You may not **be able** take part in this study if you are currently involved in another research study. It is important to let the investigator know if you are in another research study. They will evaluate if the research affects your participation in this study.

What happens if your child gets hurt or sick during this study?

If your child gets sick or injured by being in the study, Wuqu' Kawoq would help you get medical treatment. However, Wuqu' Kawoq and the sponsor will not pay you for this medical treatment. The only exception to this rule is if it is proven that your injury or illness is caused directly by the negligence of an employee of Wuqu' Kawoq.

"Negligence" means that standard medical care was not followed.

If you believe you have become ill or have been injured in this study, you should contact Dr. Peter Rohloff at the telephone number +502 50005833.

What if new information is learned during the study that might affect your decision to participate?

You will be informed if the investigators learn new information that could change your mind about having your child stay in the study. You may be asked to sign a new informed consent form if the information is provided to you after your child has joined the study.

Will you be given individual results from the research laboratory tests?

Although the gathered information is for research purposes and is not meant to provide individual clinical diagnosis or information, you will receive personal results from the blood test and poop once we have the laboratory analysis results. These results will not be shared with

anyone other than you. If further diagnosis is needed, a physician from Wuqu´Kawoq will explain the results if abnormal values are found.

Future use of your protected health information or specimen(s).

Any information we collect from you will be stored securely and separately from your name or other personal information. This collected information will only be shared with other people collaborating with the research at Brigham and Women's Hospital, Harvard Medical School, ASU, CDC and CIENSA, but it will not have your name or personal information. Finally, we will store samples we collect from you (blood and feces) so that in the future, if better tests become available that help us better understand gut function or child development, we can look at your sample. We will not use your samples for any other reason.

Contact information

Contact Dr. Peter Rohloff at +502 50005833:

- If you have any questions about this study or your participation in it,
- If you feel you have had an injury related to the study, or
- If you have questions, concerns or complaints about the study

Contact the Institutional Review Board of Wuqu' Kawoq at +502 50005833.

- If you have questions about your rights as a research participant.
- If you have questions, concerns or complaints about the investigation.

INFORMED CONSENT SIGNATURE PAGE

You are participating or are authorized to act on behalf of the participant. This consent includes the following:

· Key Information Page

· Detailed Consent

You will receive a copy of this consent form after it has been signed.

I have read or the information above has been read to me and I have received all clarifications and answers to any question I had. I voluntarily consent for my child to participate in this study.

Agrees to participate: _____ Yes _____ No

_____ _____

Printed name of research subject's legal representative **Date**

**Research subject's legal representative*

_____

Signature or printed thumb from research subject´s legal representative

_____

Printed name of child participant

_____ _____

Printed name of person obtaining informed consent

Place and date

_____

Signature of [authorized] person obtaining informed consent

Wuqu' Kawoq

Frequently Asked Questions (FAQs) for Potential Research Participants

FAQs for Aflatoxin Exposure, Growth Faltering, and the Gut Microbiome among Children in Rural Guatemala

Below is a list of frequently asked questions (FAQs) that potential research participants may have when being described the research background.

What is the fungus you are testing for in my maize?

The scientific name of the fungus is called *Aspergillus flavus* and *Aspergillus parasitcus*. The toxin that this produces is called Aflatoxinas, perhaps you have heard of it, or even notice it. When it is really bad, the maize can look like this picture. However, sometimes it is present and we don´t know.

Why is this fungus an issue?

This fungus can create a toxin, called aflatoxin, that in large quantities, or when is frequently consumed, can negatively affect the health of some animals and humans.

How do aflatoxins negatively affect me or my family?

In large quantities, or when is consumed regularly for extended periods of time, studies show that aflatoxin may cause issues in the intestines as well as it may affect the long-term health

the liver. That is why we want to learn more about it and how it potentially have other effects in the health.

What does this fungus come from?

This fungus is in the environment, however, during the growing season of maize, the fungus is attracted to the cob of the maize plant. If the maize is moist/wet, this can encourage the fungus to grow, therefore, upon harvesting, drying and processing practices of the maize are important.

Is this fungus present in my maize?

We don't know. This fungus has been found all over the world (in the USA, Europe, Sub-Saharan Africa, etc.). Previous research in Guatemala has found this fungus present here, however, it is more prevalent in humid climates, such as the coast. Sometimes it depends of where the maize comes from and the way of dry and storage, so even in cold weather like here can be present. That is why we will examine the corn that you or your family is consuming, and from other people of the community.

Can this toxin be prevented?

Yes. With good practices of maize production, storage and cooking. The important prevention is to reduce the humidity and not consume the maize that has already visible fungus, and this can be achieved in different forms.