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Environmental Enteric Dysfunction, WASH, and Nutritional Status of Women, Infants, and Young Children: Findings from Uganda, Sierra Leone, and Nepal

**Webinar transcript.**

**Yaritza Rodriguez**

Good morning, evening, afternoon to everyone. Thank you for joining today’s webinar to learn more about the Environmental Enteric Dysfunction, WASH, and Nutritional Status of Women, Infants, and Young Children: Findings from Uganda, Sierra Leone, and Nepal. My name is Yaritza Rodriguez, and I am a project coordinator at USAID Advancing Nutrition, and I will be your Emcee today. As more attendees are joining the webinar, I’ll begin by going over some of the housekeeping items and I’ll hand it over to Dr. Patrick Webb, Director of the Nutrition Lab for Nutrition, who will give a brief background on the lab and its research. And then, he will begin the webinar by introducing today’s moderator, Dr. Christopher Duggan.

I would like to direct the attendees to a few functions on this Zoom call, Zoom webinar. At the bottom of your screen, you should see a chat icon, and a Q&A icon.

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So, please, like I said before, use the chat function to engage in relevant conversation with other attendees and to introduce yourself. Panelists will respond to questions in the Q&A box, at which point, the panelists will answer your questions as they are able. We have allotted the final 20 minutes of this webinar for questions and answers, at which point the panelists will respond to any remaining questions from the audience.

If you’re experiencing technical difficulties, send a message in the chat box directly to panelists or a private message to AV support, so that our technical support staff can work with you to help resolve any technical issues. This webinar is being recorded and will be made available on the Innovation Lab for Nutrition and USAID Advancing Nutrition websites

You can also register for upcoming webinars and find the previous recordings and slide decks on our websites. I will repeat these technical housekeeping items in the chat throughout the webinar as people may be joining in at later times.

The moderator for today will be Dr. Christopher Duggan. Dr. Duggan is a pediatric gastroenterologist and a nutrition physician at Boston Children’s Hospital, a professor of pediatrics at the Harvard Medical School, and a professor at Harvard TH Chan School of Public Health. For the past 25 years, Christopher Duggan has been performing clinical trials in the fields of pediatric nutrition, gastroenterology, and global health. Dr. Duggan and colleagues have evaluated the efficacy of micronutrient supplementation in infants and young children born to women with or at-risk of HIV infection. But before handing it over to a moderator, we would like to introduce the Director of the Innovation Lab for Nutrition, Professor Patrick Webb, who will give a brief introduction on the Innovation Lab.

Dr. Webb, over to you

**Patrick Webb**

Thank you so much, and welcome to everyone to this webinar the fourth I believe, webinar series that’s for featuring, highlighting findings from the Feed the Future Innovation Lab for Nutrition’s research around the world.

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We have been conducting for the last 10 years, in collaboration with many, many partners, a series of studies of many different kinds and in many different countries, focused primarily in Sub-Saharan Africa and South Asia, all wrapped around the themes of trying to understand the ecological, economic, and human biological mechanisms that help translate agriculture through diets to improved health and nutrition. So, we’ve been looking at multi-sectoral programs, and the governance of policies and implementation issues, but also a range of biological mechanisms, human mechanisms, sometimes related to mycotoxins and food safety, such as foodborne diseases. But today, we’re dealing with what goes on in the gut.

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We do this work with a whole range of partners. Chris Duggan, who will chair this particular webinar comes to us from one of our core partners, from Harvard. We have John Hopkins, Tuskegee, and Purdue also as core partners, but as you can see many US, global, and national host country partners, doing many, many different things, but without whom we could not have engaged in the rigor and the scale of activities that we’ve been able to for this particular series of studies.

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We have a lot of specific collaborators for the studies that are going to be presented today. We are going to hear from findings relating to work in Nepal, Uganda, Sierra Leone, but also their global partners as well. These range from field workers collecting urine and feces, and trying to retain the cold chain all the way to labs, either in-country and outside the country. Trying to get an understanding of the importance of what is going on inside the gut in terms of permeability, the absorptive capacity for nutrients, to be able to translate what happens in a diet to what happens physiologically in terms of health and nutrition.

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I’m going to be… the research theme that we’re talking about here relates, as I said, to biological mechanisms.

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And we have a stellar line-up of really important findings relating to the three countries I mentioned, and Chris, as a moderator, could not be a better person. He has been involved with us from the beginning. He is a pediatric gastroenterologist. He leads the Center for Nutrition at Boston Children’s Hospital, but he’s also the medical director for the Center of Advanced Intestinal Rehabilitation. So, in terms of expertise, as well as being all round great guy, we are very honored to have Chris take on leadership of this webinar. So over to you, Chris.

**Christopher Duggan**

Thank you. Thank you Patrick and thank you Yaritza for this introduction. It was great, and it is my pleasure to moderate a really exciting session today, and I’m excited to see nearly 300 participants already, so I’m excited and hopefully more, as the seminar gets going. I would like now to introduce the panelists. Jacqueline Lauer, is a former Peace Corps volunteer, and a former post-doc with my program, who is now a public health nutritionist and clinical assistant professor at Boston University. Dr. Lauer’s research focuses on environmental contributors to poor growth and neural development among infants and young children in low resource settings, including environmental enteric dysfunction, which we’ll talk about extensively today, as well as aflatoxin exposure, which was the topic of our previous seminar. We’re also joined by Akriti Singh. In August, Dr. Singh successfully completed her thesis defense to receive her PhD from Tufts University’s Friedman School of Nutrition Science and Policy. Dr. Singh’s research focuses on determinants of maternal and child undernutrition in low and middle income countries, including diets, body composition, EED, gut microbiota, and water, sanitation, and hygiene, again the topics of her lectures today.

And finally, Dr. Shibani Ghosh, who is a research associate professor at the Friedman School of Nutrition Science and Policy, who is working closely with Patrick in her role as associate director of the Innovation Lab for Nutrition. She has extensive experience working in the Middle East, Sub-Saharan Africa, and South Asia. Dr. Ghosh’s research interest on understanding the role of agriculture in improving nutrition, while ensuring health, assessing the diet and non-diet determinations of nutritional status of infants and young children, and testing interventions aimed at improving maternal and infant nutrition and growth. So a stellar panel, and we’ll start with you Jackie, thank you.

**Jacqueline Lauer**

Great. Thank you Chris for that introduction, and warmest welcomes to everyone, and attendants for this topic which is primarily on environmental enteric dysfunction. Today, I’m going to be presenting findings from a few studies that we conducted in Uganda. During my time both as a PhD student at Tufts University, and during my post-doc at Boston Children’s Hospital, where I worked closely with Dr. Duggan, and because I am the first speaker in this webinar, I’ve planned to begin my talk by giving a brief background into environmental enteric dysfunction.

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And I have no disclosures in relation to this presentation.

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So, as highlighted by the 2013 Lancet Series, there were, and arguably continue to be, major problems in our approach to how we address stunting. The report found that if our ten best nutrition specific interventions, that is dietary interventions which are listed here on the slide, were to reach 90% of their target population, the problems of stunting would be reduced by only 20%. From these findings, many of us concluded that non-dietary contributors to stunting must have a much bigger role than we previously thought. And we ought to be looking a little more closely at the environment, at disease, and specifically at environmental enteric dysfunction as a driver for stunting.

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So looking at the UNICEF Malnutrition Framework, which depicts pathways to poor nutrition and mortality for young children, the role of inadequate dietary intake has arguably been a disproportionate focus for the nutrition community, and conversely, the disease pathway has been defined too narrowly. We previously thought to include mostly diarrheal diseases and other common childhood illnesses. I would propose to reframe this as [morbid] inflammation, immune suppression and growth hormone suppression pathway. And there is growing evidence on the drivers of this pathway, which means that not only environmental enteric dysfunction, but also exposure to toxins, including aflatoxins exposure, which was touched on in last week’s webinar, and exposure to other environmental determinants, including air pollution and pesticides.

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So, the following are the questions I intend to focus on in my research, and the ones I will touch on for the remainder of my presentation today. So, to begin with, how should EED be assessed, in other words, what biomarker(s) should we be using? Also, what are the underlying contributors to EED? What is the relationship between infants/child EDD and poor growth outcomes and micronutrient deficiencies? And what is the relationship between maternal EED during pregnancy and poor birth outcomes?

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So again, what is EED and how is it assessed is how I’ll begin my talk.

Problematically, EED still has no universal case definition or diagnostic criteria, instead, we characterize the condition by changes to both the structure and the function of the small intestine. And these changes include things likes blunting of the villi, reduced epithelial surface area and absorptive capacity, altered mucosal barrier integrity, and intestinal and systemic inflammation.

So you can see two intestine biopsies depicted here on the slide. One is showing a healthy intestine on the top, and one with EED on the bottom, where you can clearly see the blunted and inflamed villi, and a naturally reduced absorptive capacity. It is believed that these changes to the small intestine developed throughout infancy, as a result of exposure to chronic… as a result of chronic exposure to enteropathogens due to living conditions of poor water, and sanitation and hygiene, or what we refer to as WASH. So in fact, it is believed that EED is almost practically ubiquitous among infants and children living in such conditions.

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So this schematic depicts the two primary pathways by which EED can lead to stunting in children or perhaps too low birth weight in the case of maternal EED. In infancy, young children living in conditions of poor WASH, frequently ingest enteropathogens, which can affect the structure and the function of their small intestine, leading to intestinal inflammation and villi’s atrophy. This, in it of itself, can reduce the absorptive capacity of the small intestine, and ultimately result in poor linear growth. Additionally, it can lead to what we call microbial translocation or the passage of bacteria across the intestine, which ultimately leads to systemic inflammation. Therefore, it is believed that these children with EED are in a state of constant low grade immune activation. So not only does the body have to divert nutrients towards fighting this inflammation, but it is also thought that systemic inflammation can suppress the growth hormone, in some [] and in some growth factors banning protein access, which is essential for normal linear growth.

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So EED was actually first recognized in the 1960s and the 1970s in a series of studies of which I am specifically mentioning to. The Lindenbaum study was the first to identify the condition in returned Peace Corps volunteers, and later it was discovered that the structure and function of the intestine returned to normal after moving to a sanitary environment, the conclusion being that EED is reversible. The Chacko study was a necropsy study, which added to the literature by confirming that infants are not born with EED, and therefore it is an acquired condition, likely from the environment.

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Arguably, however EED did not become noteworthy until the 1991 study from the Gambia. The objective of this study was to explore the relationship between intestinal disease and poor growth in Gambian children who were 2 to 15 months old. The study found that up to 43 percent of observed growth faltering could be explained by EED. Notably, however, this 43 per cent has not been replicated, but a number of studies since, including around, have also linked EED to poor growth outcomes in children.

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The Lin study assessed EED using the lactulose mannitol or the L:M test. In an ideal world, intestinal biopsies would provide the best method for assessing EED. However these are unpractical and often unethical in field-based settings. In biopsies, historically, the most commonly used proxy biomarker has been the L:M test. All that has been changing more recently.

From the L:M test… for the L:M test, participants are asked to drink a solution containing two different sugars: one is a small monosaccharide or the mannitol, and the other is a larger disaccharide or lactulose. Urine is collected over the course of approximately 4 to 5 hours. In a healthy gut, the mannitol would be readily absorbed and the lactulose would not, as it is too big. In EED, however, mannitol is not so readily absorbed because of the blunted villi, and the lactulose would be [] because of the increased inability of the gut. Therefore a high L:M ratio is thought to be indicative of the condition of EED.

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So the L:M test, despite being considered somewhat of a silver standard for assessing EED, is [frought] with limitations. First, it is time-consuming, in that the test can take up to 5 hours or even longer if we have a stubborn child. It is both burdensome on both participants and on research staff. It’s expensive to administer and to analyze. It often has a high rate of test failure due to things like spilled urine or contamination of stool. It lacks formal evaluation studies. It fails to capture a number of the key domains of EED, especially the inflammation component of the condition that we think is so critical. And it has proven to be inconsistently correlated with EED symptoms and growth outcomes in young children.

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So, as a result of the limitations of the L:M test, numerous biomarkers have been proposed across a range of biological specimens, especially various [] markers, many of which are listed here on the slide. These proposed markers assess the various domains of EED previously mentioned, including things like intestinal permeability, microbial translocation and inflammation. Notably, the ones that Chris and I have used a lot are microbial translocation markers, specifically the anti-flagellin and anti-lipopolysaccharide or LPS, and immunoglobulins.

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One thing to note however is that correlation studies looking at panels of these biomarkers have shown little relation among them, particularly across different domains of EED. And overall, this is considered to be a big limitation of most EED research at the moment, as it is believed that infants and young children can experience EED differently, especially with regards to the severity that each child experiences in terms of the different domains of the condition.

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So now that we’ve looked at how EED is assessed, I want to turn to what are the underlying drivers of EED. As we mentioned, it is believed that EED results from the chronic ingestion of enteropathogens and toxins due to living and poor WASH conditions.

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However, with regard to the actual evidence, this is actually still considered somewhat of a hypothesis, primarily because very few studies have attempted to actually link EED to poor WASH. This slide shows more or less the summation of these studies, which so far have linked EED to things like [] of the soil, to animal exposure, to care giver hygiene. However, mostly all of these are using small observational studies. Ours, which is also a small observational study, is the first to link EED to poor household water quality.

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So we performed a cross-sectional study which was embedded within the context of a larger longitudinal birth cohort study or the Ugandan Birth Cohort Study, abbreviated UBCS. We assessed EED at the age of 12 to 16 months using the 4 to 5 hours lactulose mannitol test on a population of 385 infants from south-western Uganda. Anthropometry and covariate data were abstracted from the birth cohort, in which visits were conducted every 3 months. Water quality in this study was assessed at 6 months, using a portable water quality test. In this case, we used the Aquagenix Compartment Bag Test which detects and quantifies E.coli contamination in 100mL samples of water.

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So this table shows the primary water source for each of the households in our sub-study, and they are disaggregated by safe and unsafe depending on the results of the Compartment Bag Test, because there is no safe threshold for E. coli contamination in water. The presence of any detectable E. coli in the Compartment Bag Test was considered to be unsafe water. Overall, there is not a statistically significant difference in the presence of E. coli contamination between water sources that are traditionally considered improved, and those that are traditionally considered unimproved. With regard to individual water sources, there were some differences in safety. For example, surface water was much more likely to be unsafe, but rainwater in fact was much more likely to be safe. Overall, these results make us question how we measure WASH variables in our studies and emphasize the need to actually measure contamination levels, rather than using sources of proxy indicators for this.

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Furthermore, in our study we found that children from households with safe water had significantly better mannitol lactulose ratios and growth outcomes compared to children from households with unsafe water.

Also, in this study, we retrospectively linked L:M ratios measured at 12-16 months to poor growth at 6 and at 9 months. But a limitation of the study was not having prospective growth data from the time the L:M test was performed. The results of this study have been published in the American Journal of Tropical Medicine and Hygiene if you would like more information.

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So the next topic is: What is the relationship between EED and linear growth in infants and young children in Uganda?

So this is a map of the UNCEF 2019 State of the World Children’s Report. Overall, this map shows that stunting remains an enormous public health problem, which most of us know. Globally, about one in five children under 5 years of age is considered stunted. However the rate is much higher in South Asia and Sub-Saharan Africa, where [], where it’s about one in three children, it’s about 29% in Uganda where these studies were conducted. While the rate has slowly declined over the last few decades in sub-Saharan Africa, the absolute number of children who are stunted is actually increasing, mostly due to population growth, although of course we now have to worry about COVID’s impact on these rates as well.

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So just by way of a reminder, this schematic summarizes the two main routes by which we believe EED may contribute to malnutrition, specifically poor absorption route and the systemic inflammation route. On the systemic inflammation route, we at Boston Children’s Hospital have been focused on microbial translocation markers, specifically anti-flagellin and anti-LPS IgA and IgG.

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So we performed an additional cross-sectional study of 548 six-month old infants who were enrolled in the same Ugandan birth cohort study. We measured anti-flagellin and anti-LPS at the Gewirtz Laboratory at Georgia State University. Also in this study, we measured systemic inflammation markers, specifically AGP and CRP, as well as markers of iron status, including hemoglobin, soluble transferrin receptor, and ferritin. We assessed the associations between these markers and infant’s growth, using adjusted linear regression analysis.

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So overall in adjusted models, which included adjustments for systemic inflammation, we found a significant association between higher anti-flagellin and anti-LPS IGs, and reduced growth at 6 months of age, specifically length-for-age Z scores in our sample of 548 infants.

We also found an association between higher systemic inflammation markers, both AGP and CRP, and reduced length-for-age Z scores. We did not however find an association between any of these markers in either weight-for-age Z scores or weight-for-length Z scores at 6 months of age.

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We also found a relationship between higher anti-flagellin and anti-LPS markers and reduced iron status, specifically lower immunoglobulins levels and also higher sTfR. This significant relationship between EED biomarkers and higher ferritin levels was odd, but we believe this may have to do with incomplete adjustment for inflammation.

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In this graph, we show the correlation between the different anti-flagellin and anti-LPS IGs, which are present on the X axis, and the immunogobulin levels, which are present on the Y axis. In all cases, you can see the significant negative association between the two.

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So for more information on this study, it can be found in the report which was published recently in the Journal of Nutrition.

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And the last study that I want to mention is the one looking at maternal EED and birth outcomes. So, the objective of this study was to assess the relationship between maternal EED during pregnancy and adverse birth outcomes, primarily birth length. We performed a prospective cohort study in the town of Mokono, Uganda, located 25 miles outside of the capital city of Kampala, and we measured EED in this case using both the urinary L:M test as well as serum anti-flagellin and anti-LPS IGs. And we assessed relationships in 258 pregnant women using unadjusted and adjusted linear regression models.

So this study consisted of four distinct visits. There was first an enrollment visit conducted at Mokono Health Center IV, where venous blood draw occurred. Samples from the blood were sent to the laboratory for analysis, for anti-flagellin and anti-LPS. Also, at this visit, we conducted an ultrasound to have a more accurate measurement of the gestational age. Roughly a week after the enrollment visit, a lactulose and mannitol test was performed on women. And at delivery, infant anthropometry, including length, weight, and head circumference, was collected within 48 hours of delivery.

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So this graph show the correlation between maternal anti-LPS IgG specifically, which is on the X axis, and gestational age at birth on the Y axis. Again, we consider the biggest strength of the study to have ultrasound, and therefore more accurate gestational age data, and as you can see, we found a significant negative relationship between the two, suggesting that microbial translocation in particular could be associated with pre-term birth.

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And finally, in both unadjusted and adjusted analysis, we found that higher concentrations of anti-flagellin IgG and anti-LPS IgG in particular, were significantly associated with shorter length of infant gestational age at birth, lower length at birth, and lower LAZ at birth.

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And so more information on the study can be found in the manuscript which is published in the American Journal of Clinical Nutrition.

So by way of wrapping up, some conclusions.

Within the field of EED, a number of key challenges still persist, including a lack of an agreed-upon case definition and diagnostic criteria for EED. Given that EED has the multiple domains that we discussed, potentially a multi-plex panel of biomarkers, may be a promising path forward to establish an agreed-upon way of assessing EED in the field. The connection between WASH and EED is still hypothesized, but it does have strong biological plausibility. Notably, WASH interventions often do not provide sufficient protection from environmental contamination to prevent or to ameliorate EED and to improve growth outcomes. This was essentially the takeaway messages from the WASH benefits in the [Shine] trials. There is however mounting evidence that EED impairs linear growth in infants and young children in low and middle income countries. However, more research is needed on the role of EDD maternally, when it comes to birth outcomes, in infants, when it comes to micronutrient deficiencies and other forms of undernutrition.

So that’s the slide deck to Akriti’s presentation, and I will wrap up before I return to your questions. Otherwise, we’ll turn it over to Akriti.

Thank you very much.

**Akriti Singh**

Thank you Jackie. So the title of my presentation is Environmental Enteric Dysfunction during Moderate Acute Malnutrition in Sierra Leone.

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I’d like to take a few minutes to talk about why we studied EED during moderate acute malnutrition. So globally, it is estimated that there are about 50 million children with acute malnutrition or wasting, and two-thirds of them have moderate acute malnutrition, which is defined as having a weight-for-height or length between minus 2 or minus 3, or mid-upper arm circumference between 11.5 and 12.5 centimeters. MAM is a public health concern, because children with MAM are 3 times more likely to die than children without MAM, and these children are also at risk of progressing to SAM, or severe acute malnutrition, which has a much higher associated risk of mortality. In food insecure contexts, MAM children receive supplemental food as treatment. However, not all children who receive treatment recover, and program success rates have been reported to be between 50% and 100%. A number of risk factors could be, you know, allotted for to contributing to these low treatment success rates, one of which could be environmental enteric dysfunction or EED. And while EED has not been studied among children with MAM, studies like Campbell and Farras have reported that EED is present among children with SAM. And in addition to looking at the role of EED in treatment outcomes for children with MAM, it is also worthwhile to see if there is an association between EED and linear growth among children with MAM, because the majority of children with MAM are stunted, and they continue to grow poorly, even after receiving treatment.

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So we heard from Jackie’s presentation that there are a number of different biomarkers of EED, such as the L:M markers and also the [several] markers that she used in her studies. In addition to these, there are also non-invasive fecal markers. An example are fecal proteins and fecal mRNA transcripts, common fecal protein markers are also antitrypsin or AAT, which is a lack of permeability, neopterin (NEO), myeloperoxidase (MPO), which are markers of inflammation, and with the mRNA transcripts, we’re not just talking about two or three markers, but we can assess a whole range of markers that can then be used to construct scores, and these can then tell us, most possibly, about a more wide variety of characteristics of EED or domains of EED, and what is happening in the small intestine during this condition. So, some of the markers that we looked at in Sierra Leone were the gut inflammation score or GI score, a marker of inflammation, the gut permeability or the gut structure score that is a marker of permeability, and the gut defense score, which is a marker of anti-microbial gut defense.

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So this diagram walks you through the environmental exposures that likely lead to EED, that then likely leads to poor growth. But one area in this pathway that has not been studied as extensively are the changes in the microbiota profile that occur as a result of environmental contaminants. When I talk about the microbiota, I’m talking about microbes that colonize the gut. These could be bacteria, they could be viruses, protozoa, etc. And you may have also heard of this term being referred as the microbiome, both terms can be used interchangeably.

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Two recent studies have shown that the gut microbiota is altered among children with EED. One study was from Bangladesh, another from Malawi, that then also showed that these alterations are associated with poor linear growth. Among children with moderate acute malnutrition, there are also changes to the gut microbiota, and furthermore, what some studies from Malawi have shown was that there are specific foods, if provided to children with MAM, they can lead to growth of a more beneficial microbiota, so more beneficial microbes in the gut microbiota. And while we know that the gut microbiota is altered during EED and during MAM, what remains to be seen is whether these changes are universal across geographic locations.

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Now for the objectives of the study in Sierra Leone, we examined associations between different biomarkers of EED and growth during and recovery from MAM. It was also to compare the microbiota profile of children with and without MAM and of MAM children with different levels of EED. And then finally, to examine the relationship among WASH conditions, EED, and microbiota profile of children with MAM.

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So this study was conducted in collaboration with the Food Aid Quality Review, Four Foods MAM Treatment Study conducted in Pujehum district of Sierra Leone. The children were 6 to 59 months of age and were identified as having MAM based on the mid-upper-arm circumference state, and they did not have any complications. They were given one of four foods as treatment, however EED was only assessed at enrollment into the program. And we collected urine and fecal samples for this purpose, and fecal samples were also collected from a small number of children without MAM for the microbiota analysis.

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So, what did we find with the first objective?

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Well, first of all, we found a very high burden of EED among children with MAM. This is one of the first studies to report this using the L:M test or the fecal protein markers, for which we have more well established cutoffs.

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And then, when we looked at the association between the EED biomarkers and recovery from MAM, which we define as achieving a mid-upper arm circumference greater than or equal to 12.5 centimeters within 12 weeks of supplementation, what we found was that children with higher GD score, gut defense score, were more likely to recover from MAM, and children with lower intestinal permeability measured using AAT, were also more likely to recover from MAM.

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Then, when we looked at the association between these biomarkers and growth during MAM, what we found was that children with high inflammation, measured using the GI score had lower length-for-age Z score, and lower weight-for-length Z score, and that children with high intestinal permeability, measured using the GS score also had lower weight-for-length Z score. So in this objective, the results showed that certain biomarkers of EED are associated with recovery from MAM and growth during them.

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In the second objective, we focused on the microbiota, and what we found was that…

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… that the gut microbiota of children with MAM, shown in red, was enriched in inflammogenic taxa, or inflammation-inducing microbes, such as gamma proteobacteria whereas the microbiota of children without MAM were enriched in the beneficial taxa such as the lactobacillus ruminis. I talk about taxa because with this analysis, we don’t always have information at the species level. So it could be at any of the higher taxonomic level such as []

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And when we looked at the overall microbiota structure of children with EED, measured using the GI score, what we found was that children with higher … sorry lower GI score had less inflammation, had more microbial diversity, that is shown in the figure to your left, than children with high GI score. Similarly, the microbial communities between children with low and high GI score was also different, as shown in the figure to your right.

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And what we also found was that the gut microbiota of children with high GI score was enriched in inflammogenic taxa, such a Streptococcaceae, shown in red, where the microbiota of children with low GI score was enriched in the beneficial taxa, such as Rosebuna, and B. faecis.

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So basically, with the second objective, what we showed was that the microbiota is altered during MAM and EED. And with the third objective, we were looking at the association between WASH, EED, and microbiota, and what we found was that…

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When we looked at the association between the EED biomarkers and household drinking water sources, we first created a binary variable where drinking water source was defined as improved or unimproved based on the UNICEF/WHO Joint Monitoring Program definitions. So improved drinking water source would be public tap or a tube borehole and unimproved would be surface water or unprotected wells. And what we found here was that lower intestinal permeability was associated with improved drinking water source at the household, using the L:M exclusion ratio, the [T Parker] as well as the GS score.

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And when we looked at the association between the biomarkers and household sanitation facility, this was again defined as improved or unimproved based on the Joint Monitoring Program definitions, so improved would be ventilated [] and unimproved would be open defecation or open pit, what we found here was that low intestinal inflammation measured using the [] was associated with improved sanitation facility.

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And when we looked at WASH practices by the household WASH conditions, what we found was that more children living in households with unimproved sanitation facility were observed putting soiled or animal feces in their mouths. And that’s the only practice that was statistically significant.

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And in terms of the gut microbiota, what we found was children living in households with improved sanitation facilities were enriched in beneficial taxa such as Roseburia, Colincella, and [bifidal] bacterial species.

So, to summarize, we saw that certain biomarkers of EED are associated with recovery from MAM, as well as growth during MAM, and that the gut microbiota of children with MAM were enriched in inflammogenic taxa. And that children with MAM living in households with improved WASH conditions had lower risk of EED and had beneficial microbes. And finally, that the alterations of the gut microbiota are associated with MAM, as well as gut inflammation during MAM, which raises the possibility of microbiota-directed or targeting the microbiota with interventions, such as nutrition and WASH to treat children with MAM or EED.

Thank you. And now I would like to hand it over to Shibani for her section to the presentation.

**Shibani Ghosh**

Thank you Akriti, and good morning, afternoon, evening everybody. I’m very privileged to be speaking after Jackie and Akriti, who have done such phenomenal work with their desktop research, and I’ve been fortunate to be part of Akriti’s committee as well as being Jackie speech teacher. So it’s a moment of being very, very proud of both of them. And I’m sort of speaking on behalf of another person, she’s on the panel but she’s unable to be online right now, [Johanna Andrew Cimino], who has been the third PhD that has been working with the Nutrition Innovation Lab on issues linked to such themes as biological mechanisms, and last week we talked extensively about micro-toxins, particularly aflatoxin and our work in Nepal around the AFLACOHORT study. And so I am sort of extending… Johanna and I have extended some of the analysis that has been part of the AFLACOHORT study to look at EED markers, water, hygiene, sanitation, but also looking at EED markers and aflatoxin levels in young children. So that is really the focus of this last presentation.

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So I don’t need to go through the background that Jackie and Akriti have laid out so very nicely. And I realize that I have an error in my first bullet, but I’ve already spoken about Nepal [] but I think all of you who are joining from Nepal are aware of the rates of stunting. And that also that the environmental enteric dysfunction, as Jackie particularly laid out, has been implicated in the development of the stunting syndrome, if you will. And we’ve seen that poor WASH practices are associated with EED. There’s also this postulation that toxins such as aflatoxin may induce EED, and that might be the mechanism by which it increases… causes poor gut health, and possibly poor absorptive capacity in children.

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And that’s just… I’m sort of using what is Akriti’s slide on looking at what is EED, which is a process and a syndrome. On the top, where you see environmental contaminants, I’ve highlighted the fact that in addition to microbes that are introduced through poor WASH practices or through poor WASH itself, you have also aflatoxins and other micro-toxins. And of course this is a postulation, this is a hypothesis, and we’re trying to see whether this actually does translate into poor nutritional status.

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For those of you who were not on our webinar last week, I do suggest please go and listen to the webinar where we have presented extensively about the AFLACOHORT study for those of you who… one day last week… The AFLACOHORT study is an observational but cohort study that was conducted in the Banke District of Nepal, and we followed 1,675 mother-infant dyads starting from the second trimester of pregnancy in the women through almost 24 months age of the child.

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So here’s just to give you the timeline of the AFLACOHORT study, which began in 2015 and finalized its last data collection point in March 2019, which was last year. The launch was, as I said, started with the prenatal in the second trimester of the women, and then we followed the women at birth, 3 months of age for the child, 6 months, and so on. Originally, the AFLACOHORT study which you see on the slide is Phase one, was meant to go through the infants only 12 months of age, and we were fortunate to receive additional funding that would allow us to do follow-ups on the child between 18 months of age and 24 months of age. So what I have highlighted here is the time point where we were able to collect additional serum samples to assess micro-toxins beyond aflatoxin. But also we were able to administer the L:M test on the infants thanks to our research manager [Ashish Boukra] who was extremely valuable in doing this work. And today, what I’m going to be presenting is some of the preliminary analysis that is emerging from that data.

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So, I’m focusing only on that specific time point, so you have an understanding of the sort of demographics and the descriptives of this population. The child at 18 to 22 months of age in our AFLACOHORT study, about half of them were girls, 20% of them were low birth weight, and about 7% of them had diarrhea in the past two weeks. A lot of them had achieved minimum dietary diversity, but you can see that stunting, underweight and wasting problems are quite high in the Banke district, as was low head circumference for HZ score.

And the last two points I wanted to make was the two metrics that are often used in programming and in policy around WASH, which is improved water source, and improved toilet facility. And what we see is that 96% of the households were reporting an improved water source, and about 64% were reporting an improved toilet facility.

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So this is just to give you the descriptives on the EED markers, and like Jackie and Akriti, we had the opportunity only to collect a urine sample, and assess the L:M ratio in these kids. We do have serum samples that are… that could be analyzed for inflammatory markers, as well as for microbial translocation markers, but we are in a sort of the COVID lockdown right now, we are not expected doing any work between the US and Nepal. So what you see here, is the L:M ratio and the percentage of lactulose excreted as well as the percentage of mannitol excreted. You do see that this level of this L:M ratio is very similar to what Akriti and Jackie were reporting and is considered on the higher end.

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And then, this is again the assessed mycotoxin exposure in these kids. The first one is aflatoxin and you do see that it’s pretty much the detection rate, and I want to highlight the second column here which the most important one for the purpose of this discussion is that we had about 85 % of the kids had detectable levels of aflatoxin. For ochratoxins and fumonisins, there were 100% detection rate and for DON we had 87%. And what you do see across the aflatoxins is that these are extremely skewed distributions from very large numbers of kids on the lower end of the distribution, and a few kids who are only in the upper end of the distribution.

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So we ran quite a few [] regression models and what we are presenting here is the relationship of the EED markers, in this case, we’re using the lactulose mannitol excretion ratio, percent lactulose and percent mannitol, and its relationship with WASH markers and mycotoxins, in the case of the improved water source, because there was so little availability, so there was really nothing we could run in terms of regression model. In terms of the improved water source, what you see here is that there is a significant relationship between the household reporting and improved water source, and the child EED marker LMER ratio. And as a negative relationship which indicates that if the child was in a house with an improved water source … toilet source, excuse, they had lower LMER ratios. What we did look at was mycotoxins and fumonisins, and we find there is no correlation between mycotoxins and fumonisins and EED markers LMER percent L and percent M. And all these models have been adjusted for the age of the child, the number of household members, the mother’s education level and the geographic location, which could be a significant confounder.

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So in conclusion, we do find a significant association of markers of intestinal permeability and absorption and access to an improved toilet facility and as I mentioned, we don’t see it with the improved water source. At least with our data report we have right now, we don’t see any relationship of the LM markers with either aflatoxin or fumonisin. If we are able to assess other inflammation markers, as well as microbiota translocation markers, then that would be something we would like to revisit. But at this moment, we are analyzing the associations with growth and development along with EED and aflatoxin markers.

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I just wanted to bring in this particular slide together all the three different presentations that have been done, and what this means with respect to research and policy implications. And I think Jackie and Akriti have laid out very nicely what the research implications are. And what we find in the Nutrition Innovation Lab is that our work has allowed us to assess the linkages between WASH, the microbiota, infection, health, and nutritional status. And using both stunting and moderate acute malnutrition, what we’re hoping is that this sort of advances in thinking in nutrition science and applied nutrition and its intersection with environmental contamination. But what is very, very clear from Jackie’s work and Akriti’s work is that we need to have longitudinal studies and randomized studies that address the specific issues that have been highlighted. Particularly, and I think there was a little chat on the Q&A about what the intervention was… or what interventions are being used for targeting WASH and that we really need to understand the source, the extent and the intensity of contamination when we are planning responses. The one last thing I would say is what we have done in these studies has really been focusing on using very innovative biomarkers, and obviously that is something that can be done in the research round. But in the programming round, I think the idea would be, as I readily, to start looking at water quality, not just the source of the water, and not just use proxy indicators, such as water source and improved facilities, because there could be so many things between that set up of the water improvement resource and actually the water consumed that could be the reason why you see such high levels of contamination.

So on that, I’m going to hand it back over to Chris Duggan who is going to take the next steps of this webinar. Thank you.

**Christopher Duggan**

Thanks so much Shibani, and Akriti and Jackie for a really excellent presentations. As you can imagine, the chat group has been very active during the presentations, and I think it would be great to kind of answer some of the questions that were either left unanswered or can expand on the ones that were in part answered by the panelists and the moderators. So, there were several questions that were answered in part, but I guess it would be nice to know if we could review them in more detail. I’ll pose the first one to Jackie, I know you’ve started answering this question. A number of our attendees were asking about the role of exclusive breastfeeding in the cohort that you described. Would you like to comment a little bit on how that was assessed in the field as well as in the statistically in the analysis.

**Jacqueline Lauer**

Yes, definitely. Because we were looking at infants at 6 months of age for the studies linking EED and infant and child growth, we didn’t have much variability within that variable to kind of be able to control for it. I think about 98 percent of women reported that they were breastfeeding to some extent at that time, and because it was right at that six months mark. It was a time when we weren’t expecting some of them to be transitioning away from exclusive breastfeeding, so we weren’t going to kind of penalize them for that and make a marker of whether or not they were exclusive breastfeeding or not, and the marker whether they are breast feeder or not did not have much variability. So unfortunately, because we were kind of limited by using data from the birth cohort study for recovery, and not kind of collecting our own more granular data on breastfeeding, we weren’t able to do much with that variable. But I appreciate the question. I also am interested in that as a research question as well, I would just think that you would have to kind of work that into the studies that you are designing in order to collect kind of much more specific breastfeeding, amounts, and labels, and etcetera.

**Christopher Duggan**

Excellent, thank you Jackie. Akriti, there were several questions about your presentation as well. Some of the examples of foods that lead to growth beneficial gut biota.

**Akriti Singh**

Sure, that’s a really interesting paper. I hope many of you will read it. Some of the foods were chick pea flour, soy flour and banana that were able to lead to beneficial microbes among children with MAM.

**Christopher Duggan**

That’s great, thanks Akriti. [Clemence Marley] had a question about the role of EED in defining… in the definitions that you used for moderate acute malnutrition specifically. You use MUAC as the inclusion criteria. If you used weight-for-height Z scores in the inclusion criteria, would those results have changed much?

**Akriti Singh**

Hmm. We don’t know. The program in which this study was embedded used MUAC as their indicator to enroll children in the program, which is what was used in the analysis.

**Christopher Duggan**

Excellent, thank you. Shibani, one of the questions that was posed was the question about minimal dietary diversity, which was seen in 70% of the study that you presented, and that was thought be you know quite high. Is that atypical for the population under study, do you think that could have affected some of the results?

**Shibani Ghosh**

I just realized I was on mute. Yes, it is something that… I saw that question as well. It is a little higher than what one would see in the Terai of Nepal there. One of the biggest issues has been that the minimum acceptable diet and the minimum acceptable diversity for infants and young children does not seem to move higher than 30 or 40 % and I could be wrong. So I think Johanna and I need to really look at why these kids, and it may be that these particular kids were part of the study, so they were getting exposed to messages that led them to have a better diet diversity. But the relationship that were are seeing is after we control for minimum dietary diversity, so I mean the fact would not seem to really have an effect. Is that… is that… does that answer the question?

**Christopher Duggan**

Yes. I think so. To all three panelists, [Manuela Mazuro] who is in Guatemala asked about any experiences in mycotoxins or information markers in breastmilk. Could that provide some clues regarding stunting before six months? We’re expecting some thoughts on that very interesting comment.

**Shibani Ghosh**

Chris I’m sorry. I just kind of missed that. Could you…

**Christopher Duggan**

Sure, we’ve talked extensively today about serological markers and stunt markers, but what about breastmilk markers? Inflammation or pathogens or components of pathogens that might trigger this? Any knowledge there?

**Shibani Ghosh**

Yes I think… Manuela thank you, I saw your question. We did … we haven’t assessed breastmilk for inflammation markers. I think that is really an importantly interesting question. We have assessed it for aflatoxin M1, which is a metabolite of aflatoxin B1. We found that pretty much more sort of samples did have aflatoxin M1 in very low levels. So it below what we consider as [EU] cutoff for what is acceptable aflatoxin M1 in milk. So, yes, I think that’s definitely a very good point, and something we should pursue if we have more examples.

**Christopher Duggan**

Excellent, thank you. [Ellen Milner] is commenting. She says: It’s disappointing that most of the studies using WASH indicators are not aligned with post MDG, sustainable development goals, and improve water source is insufficiently aligned with current evidence, improved sanitation is also insufficient and does not incorporate the negative externalities of poor sanitation from neighbors. Is there a reason that basic water service based on coverage and basic handwashing? Are there any plans to [] WASH variables that incorporate these service ladders? Any comments from our panelists on that interesting comment?

**Jacqueline Lauer**

I can comment on that. I used the GMP criteria as well and incorporated things like distance to water source and was not kind of seeing an association with what we found in the compartment bag test. Either I think that the GMP is noble, and they should be emphasizing a lot more actually testing the water. They do to some extent, but probably don’t go far enough considering I do think that seems to be the actual only way to kind of get at how clean the water source is. I think an interesting finding we also saw that may kind of explain why some things like improved versus unimproved or distance to water source are not doing a great job kind of explaining contamination levels. It seems that a lot of the contamination is happening kind of post-collection, perhaps based on kind of the utensils that are being been used, whether or not the water is being stored with a cap or not, and things like that. I think they’ve probably kind of under emphasized it also in the GMP. So, it’s a good start moving away from just the dichotomy on improved and unimproved but it’s still not aligning with what we are seeing when we are measuring the kind of actual contamination levels.

**Christopher Duggan**

Thanks Jackie.

**Shibani Ghosh**

Chris, could I add…
You wanted to add something about your WASH work… observational work that you did on WASH, if you saw anything?

**Akriti Singh**

Sure, so we did look in the analysis about using the dichotomous variables, especially for drinking water. The issue that we ran into was that some of those groups were not balanced, so we had a lot of participants who got their water from tube wells or burble, and very few who got them from unprotected springs for example. It was much more balanced when you looked at improved versus unimproved. But I do recognize the limitations for that analysis. I think Shibani’s question was “Did we find any differences with the WASH observation data?”
So when we looked at household drinking water quality, using a slightly… you know a proxy measure because we didn’t actually test the drinking water quality, we didn’t find differences in how the drinking water was stored among households that sourced their water among improved and unimproved sources, but when looking at the dichotomous variables, we are finding these differences. I think the next step for this study would be to then go and test the water to see ‘Was there contamination in drinking water that was sourced from unimproved or improved sources, and was there a difference? But unfortunately, we did not do that.

**Christopher Duggan**

Great. Thanks Akriti, thanks Shibani. Thanks Jackie. Several other interesting questions have come up. Two have the same theme of animal feces. We have a question on ‘Have you addressed exposure to chicken and how about exposure to animal excreta and EED. Another question at the same time came through on the same point. So, let me ask our panelists about exposure to chicken and other feces.

**Shibani Ghosh**

Is Jackie on? I have not seen her.

**Jacqueline Lauer**

Yes, I’m one. Again, I think… we did measure this very carefully actually in my first study I presented on water quality. We did actually collect good data on exposure to animals, exactly kind of ‘Which animals in particular’ as well as ‘How far they were from the household?”, and things like that. Unfortunately, [] I don’t think there was enough variability. It seems like children were kind of saturated by animals feces and being around the animals, most households kept animals in their homes. So while we didn’t see much, that might be just simply because it was not a variable with a lot of variability unlike the unsafe and safe water, which was put very evenly among households, very nicely, and so we got the variability while we couldn’t exactly quantify how much children were exposed to bacteria from feces, it was clear that all children were hanging around animals most of the time. So that could be why. But of course, the study that I presented on the slide was showing the studies that had been done linking poor sanitation to EED. There is a study there that I would refer you to, linking exposure to animals and EED. So there is some evidence for it, we just do not see it in this particular case.

**Christopher Duggan**

Thank you Jackie. Shibani?

**Shibani Ghosh**

Could I add one point? I think one of the issues one faces when you are in an environment, and I think this might also address what [Ellen] pointed out to that you are in an environment where there’s contamination across the board, and the sort of this across household contamination that happens, and when you’re doing a study, even if you have the most rigorous methods of data collection, if you don’t see a variation, then you are going to not be able to… if you’ve done that regression, you’re going to be like… well there’s no relationship, but in fact, what it is there is very little variation in that population and for you to see any difference. And I think that’s why we often run into what Jackie has been indicating. And someone asked that question about improved water source for Nepal, where 96% reported improved water source, and I bet you it’s the same issue that everybody has some form of water source that they perceive as improved. And we have the list of what is improved and what is unimproved. But I think it’s not an individual, it’s an environmental and a community-level issue that does not allow us to differentiate between households in this kind of analysis. Sorry, Chris I was just…

**Christopher Duggan**

No, excellent that’s great. I have a couple more methodological questions that I’ll bring to the panelists, but I do want to reach a way to have time to develop to think about the policy implications of our work, because these are fascinating analytic approaches. But let’s ask one more kind of global question, which is one of the attendees asked about: Did any of the studies attempt to assess levels and types of mother-child interactions? Did improved maternal stimulation make a difference in helping children with moderate acute malnutrition improve neural development as well as growth? So, Akriti, I’m pointing to you since this is a question about MAM.

**Akriti Singh**

Sure. In my study, we did not. But in [FEQR], there is another study on neurocognition, they didn’t look at whether stimulation led to improvements in neurocognition, but what that study did find was that after supplementation, there were improvements in neurocognition

**Christopher Duggan**

Great. And similarly we’ve done some work in Tanzania that’s linked by markers of EED with different aspects of the Bayley scales of infant development, but we haven’t formally measured maternal stimulation. So that’s a great question. Ok, so, why don’t we open the door a little bit up to kind of given the science, given the state of the science, what are the policy implications? We’re obviously doing this not just to earn about science, but to have a positive impact and develop more broadly-based policy for improving growth in young children. Let me push that up to the panelists and say, knowing what we know now, knowing what we reviewed so well in the past few minutes, what are the next steps in terms of advising the people that make health policy in low and middle income countries? Are the data strong enough to say: Hey, EED is a major problem in the countries we reviewed? Or do we need more data to define it better, collated with important outcomes, and perhaps to do more interventions studies that would suggest not only we do know how to measure it, but we know how to treat it. It’s a tall order, I’ll start with Shibani since []

**Shibani Ghosh**

I was hoping Jackie turned off her mic.

**Jacqueline Lauer**

I could go, I could also go.

**Christopher Duggan**

That’s your lead, start off Jackie.

**Jacqueline Lauer**

I’ll start off. Just a few points to make about that. You know I think we’re all kind of coming off in this community like having done a serial of post-mortem on WASH benefits and on [Shine] you know WASH is not you know the most kind of popular thing to think about or to fund right now. But, you know, I would say… I would say there is a lot of good evidence from observational studies that point to a strong link between WASH and EED, including kind of what Akriti and I have been saying. So, to not give up on WASH but to not think about WASH in such rudimentary terms, that we need to be thinking about WASH, both like Akriti said more at a community level, and also comprehensively, kind of resurges we should kind of expect, you know from ourselves and better from kind of the communities that where’re working with in terms of what they deserve in terms of WASH, and simply having an improved latrine does not mean they have sanitation, and simply having access to a borehole doesn’t mean they’re drinking clean water. So to keep that in mind and to kind of keep WASH, I think, at the forefront would be good. And then, in terms of EED, certainly as researchers, we need to kind of set our own case definition and set a line of how we’re thinking about measuring this. And there is a lot of work going on, though admittedly every country has sort of passed their own markers that are daunting []. But I think, as Chris mentioned, it’s also probably time to start rolling out better randomized controlled interventions studies. Chris and I did one looking at zinc and found that zinc supplementation did not have an effect on improving markers of EED. [] So that was a disappointing result. It kind of wakened us to the idea where we were working on that manuscript that there just was not a lot of good regress interventions studies for EED and we should probably start again some kind of work on diet as well.

**Christopher Duggan**

Thanks Jackie.

**Shibani Ghosh**

Sorry, I was looking at Akriti, but I think I can jump in. I think on the research end, we still have a lot to unpackage, if you will, around EED. This is just a starting point. But what we are seeing is that EED is out there, it is prevalent, it could be correlated in some cases to certain health outcomes, but maybe not adverse. That does not take away the fact that it is a condition that is pretty debilitating when you’re talking about gut health, and particularly, increasing risk of inflammation. We can talk about whether that causes poor absorption of nutrients or not and to what extent it causes. For the fact of the matter, it is increasing the risk of poor health in young children and in mothers who are… adolescent woman who are going to be new mothers. So, I think that’s one point, and I think Jackie has nicely articulated the research needs here. On the policy end, I would say that it’s very hard when you are a policy maker or a programmer to think of how to bring these very biological elements of our research into policy and programming. And I think that the first place I would think about starting is just sort of say… and I know that a lot of people have already worked on thinking about the WASH indicators, and that just having improved versus unimproved sources, which is something that is highlighted even in our presentations when we are looking at it dichotomously, isn’t sufficient to indicate that that community actually is free of pathogens and is free of contamination. On the food end, we are already talking about the food systems approach, and that does bring in food safety. And I feel like we need to start talking more about environmental safety as well. And not matter how we make such studies, we can go back and forth about this, if there’s no variation of population, we won’t see the relationship. But that doesn’t mean that those issues are not important. So I would say that thinking about … taking food systems and food safety to another level of bringing in environmental contamination and environmental safety around WASH. The other thing that I want to point out and I think that was one of the questions on neurocognition and maternal stimulation… so a lot of the work that we’ve been doing … we have very focused research questions that need to be answered, but I’m hoping, as we’re doing these webinars, that we are putting the puzzle together with these different pieces. So we have research that has looked at the role of animal source foods, that has looked at head circumference-for-age Z scores, which could be possibly a proxy of development, particularly in young pediatric populations. I think there’s a lot out there that needs to be brought together, and I’m hoping, as we go towards the conclusion of these webinar series, that our charge as the Nutrition Innovation Lab will be to bring all these pieces together for folks out there. I really appreciate all the questions because that’s making us think a little more deeply on what we want to bring together as… here’s what the output of the Nutrition Innovation Lab is. So that’s… on that note, I want to pass it back to you Chris.

**Christopher Duggan**

Excellent. Thanks Jackie and Akriti.

**Akriti Singh**

I do have a comment. I think that my work in Sierra Leone has shown is that it really added to the evidence base, especially around thinking about not just, you know, what impact do these interventions on nutritional WASH have on anthropometric outcomes, but also pushing us to think more about the gut microbiota, because perhaps, this is where we need to be focusing on using the gut microbiota and different taxa as a targets for these interventions. And you know, for program implementers, the science is not there yet, where we can say: You do need to promote consumption of banana or soy flour, as one study showed. So, this is where the science is heading, and I hope that in the near future we will be able to make those recommendations.

**Christopher Duggan**

Excellent. Thank you. I’m reminded of course in terms of … I hope everyone that added to the chat group was able to view last month’s really interesting paper in the New England Journal, where [Jeff Gordon, Tommy Dockner] and colleagues did some fantastic work about how the gut microbiota was influential on growth of young children. And I do know that the Gates Foundation has taken up with a moderate enthusiasm this idea of microbiome-directed complementary feeding. So I believe that will be very high on the research agenda over the next months to years.

Patrick Webb, you’ve been awfully quiet. I wonder if you have any comments on the policy implications of the work we’ve heard about today. I know you’re handicapped a little bit by IT support today.

**Patrick Webb**

Definitely handicapped. Alright, well thanks Chris. I think it’s important what Akriti just said. These studies have been focused on pushing forward our understanding through the science of links, let’s say… how various metrics… how environmental enteric dysfunction can be measured to begin with? How that links to diet and water quality and how each of those link to various kinds of outcomes relating to nutrition, not just wasting but linear growth and micronutrient status, obviously, what we’ve presented is just the tip of the iceberg. Now what we were not doing, was evaluating WASH interventions. I think that needs to be spelled out. And I think we need to do a lot more of both. We need to continue to push the frontiers of our understanding of what EED means for these children, physiologically and in terms of growth and nutrition, and future health, and where are the entry points for interventions that can make a difference, and if in fact, and I saw [Felicia Wun] was asking in one of the questions, if appropriate sanitation and clear water sources are not sufficient, then what are going to be the additional activities that governments should be promoting, that development partners should be focused on? And communications, obviously better practices, better understanding of what exclusive breastfeeding means and not feeding contaminated water to infants. There are lots of other dimensions that come under the rubric of policy. But we need to speak clearly to the policy makers and the governments in these countries to communicate where our understanding is going and what kinds of actions are feasible, and then test them in the field. I think all of those things need to be done. That’s where we’re heading and I think that’s what these kinds of studies contribute to.

**Christopher Duggan**

Excellent, thank you. Thank you Patrick. Scoring through the questions, I see many have been answered not by the panelists but by the participants, which is terrific. And a question has been raised about… Akriti, I’ll ask you to come perhaps on the WASH interventions, because one of the attendees was wondering whether the WASH interventions didn’t include chlorine and how could that perhaps affect the absence of effect on cryptosporidium. And I’m sorry, maybe that was Jackie that reviewed some of the lack of efficacy of the WASH interventions. So either Jackie or Akriti. Any thoughts on that?

**Akriti Singh**

My study did *not* look at … was not focusing on the WASH interventions.

**Christopher Duggan**

Right, and Jackie, any thoughts on your review of the literature for WASH and whether cryptosporidium is an important factor in EED? And while you’re thinking about that, I guess I will address another comment that came up in the chat, which is this idea that of course the antibodies that your work has often used are indeed antibodies to gram-negative cells walls in components, and therefore leave open the possibility that they don’t really explain any of the exposures to other organisms, including viral organisms, including gram-positive bacterial organisms. And furthermore, you can argue that the presence of these gram-negative antibodies, gram-negative components that are found in the gut stream, we assume that they are related to gastro-intestinal exposure of gram-negative bacteria but there are mucous membranes that indeed these antibodies could be absorbed from as well as the skin. So, I do think that a lot of caution is warranted, for when we think if these things are truly EED biomarkers. So Jackie, any thoughts on cryptosporidium as a possible cause of EED?

**Jacqueline Lauer**

Yes. I think that was more measured… I think in kind of WASH benefits, I’ll probably direct you there. What we did is because probably E. coli is probably like considered most common in water contaminants, we’ve kind of used it as like a proxy marker for perhaps how contaminated the water with both [] and parasites, which unfortunately was just because we did not get kind of the water tested for that. Yes, but I think going forward [] and not just look at E. coli and actually exactly what the water is contaminated with, including cryptosporidium.

**Christopher Duggan**

Excellent, thank you.

One question was posed about the role of probiotics, we think of EED as an entity where bacterial enteropathogens are present in the gut and causing damage. But what about the role of healthy bacteria, one of the panelists can answer on that.

**Akriti Singh**

Sure, I can talk about the one study that looked at whether giving lactulose GG to children 3 to 5 years in Malawi had an impact on EED and they found that it did not. My response to this would be that perhaps the age group was slightly older, and we need to look at younger age groups because we know that EED picks around 12 months, and perhaps we also need to be looking at other probiotics in addition to lactulose GG. And this is where when focusing on what are the beneficial bacteria that are present in children without EED might be helpful.

**Christopher Duggan**

Excellent, thanks Akriti. Jackie?

**Jacqueline Lauer**

The probiotics study was negative and our results on kind of zinc, RCT, antibodies have been as well. There have been EED therapeutic trials, mostly kind of small, but I don’t think there have been any ones that have shown any positive impacts on EED, which is kind of why I attempt to go back to kind of the WASH question. And not only is it a convention, but I think in terms of therapeutics that we have tried, and I’m not sure there are any positive results, we perhaps need to kind of go back and start thinking about prevention and WASH, while keeping open you know for the therapeutic trials, but just kind of to point out that we have not really seen much success in the ones that have been tried thus far.

**Christopher Duggan**

Excellent, so I’m going to… keeping my eye on the clock, I’m going to ask our three panelists for a little bit of a wrap-up comments on both their presentations and the discussion today. We’ll go on the same order if that’s ok, so Jackie any wrap-up thoughts?

**Jacqueline Lauer**

I’ve loved the discussion. It’s always great to come and talk about what we’re measuring, how we’re measuring, if we’re actually measuring what we’re talking about. And I think there are very important things to keep in mind. Chris and I have a lot in the pipeline when it comes to EED, and we’re very excited to work with paths to develop maybe a multi-plex assay that’s looking at the various domains of EED. And I know Akriti has exciting work on [] which is also I think a kind of future direction as field is headed. So there’s tons of exciting work within EED if you’re thinking about kind of getting involved in it. It’s a super interesting field that still needs a lot of people kind of tackling it from all different angles. So, I’m looking forward to any future collaborations with anybody out there who may be interested.

**Christopher Duggan**

Excellent. Akriti, any comments?

**Akriti Singh**

Sure, we’ve studied EED and we’ve used a number of different biomarkers and we found that a lot of studies… you know we tested interventions, WASH, nutrition, and we did not find that it improved EED. And my response to that would be: let’s think about how we are studying EED, which biomarkers we’re using. I think the mRNA transcripts might be a comprehensive way to study this condition. And in addition to using the biomarkers, let’s think about the age groups of the kids, some have looked younger, some have looked older, but I think when we measure EED what’s going to be important, how we measure it, the study design. So I hope studies that continue to work on EED and looking at the association with growth, and looking at which interventions can improve EED, think about how EED is measured, and the age of the study participants in the design of the study.

**Christopher Duggan**

Excellent, Shibani?

**Shibani Ghosh**

Thank you, and I think what I’m really excited about … from this webinar and the previous ones is that we’ve generated a lot of discussion, and we’ve got such fabulous attendees here, who should all actually… some of them, I wish we could have them speak to us with such great ideas and talks moving forward. In terms of the… from my perspective as applied researcher, I think what I’m really excited about is that we will be able to take nutrition science down to applied nutrition, but we’re also moving it towards folks who are working in programming and policy. That, I think, has been a very entrusting journey for me within the context of this work that has been conducted around EED, and the biomarkers of EED, and WASH, and nutritional status. I do think that we are moving forward in different studies to look at interventions which are more sort of programmatic interventions that could then be measured using innovative markers to see what within the WASH, what within the realm of improving practices in the household animal livestock interactions could maybe shift the marker for EED and for nutritional status of those populations. So there’s is a lot more going into more of the interventions as we move forward. For me, this is a starting point, it’s been an exciting journey, and I’d like to continue with all of you on this. Thanks so much.

**Christopher Duggan**

Thank you Shibani, and Akriti and Jackie for your really excellent presentations. Thank you for the Nutrition Innovation Lab for supporting this seminar as well as our colleagues at USAID. I’ve learned a lot. We need more tools to measure EED impacts, as Shibani noted, for therapy. So, let’s keep the conversation going, please visit our website, keep us up on your progress, and we’ll do the same. So thanks very much to all who participated today. And see you soon.

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