Technical Meeting on Environmental Enteric Dysfunction, the Microbiome and Undernutrition

Nutritional and Health-Related Environmental Studies Section
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Book of Abstracts

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Intestinal barrier failure in Environmental Enteric Dysfunction: What do we know?

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In children living in the tropics where there is also a high burden of HIV, there are three major contributors to environmental enteric dysfunction (EED): environmental enteropathy which is the structural defect underlying EED and which leads to stunting, malnutrition enteropathy which is associated with wasting, and HIV enteropathy in children with HIV. In a series of three studies we have shown that epithelial breaches are a common pathophysiological feature which appears to permit high levels of translocation of bacterial molecules (lipopolysaccharide and DNA). These breaches can be identified in vivo using confocal endomicroscopy, and ex vivo using routine histology and using immunostaining for tight junction proteins. Zinc uptake into plasma was also inversely correlated with epithelial breaches. Epithelial breaches appear to represent a form of epithelial fragility which was correlated with low circulating glucagon-like peptide-2. We went on to analyse the transcriptome in a subset of biopsies collected from adults with minimal or severe enteropathy, and identified 23 differentially expressed genes in an independent agnostic process. Genes related to host defence (including antimicrobial peptides) and mucosal protection (including trefoil factor 3) were down-regulated. These data suggest that these three forms of tropical enteropathy share a common pathway of impaired mucosal protection and lead to breaches in the epithelium which then permit microbial translocation and lead to malabsorption. While there is much work to do to advance our understanding of these pathways, these data also suggest options for therapy.
Environmental enteric dysfunction (EED) is widespread, non-specific, upper intestinal inflammation characterized by villous atrophy and T-cell inflammation. This condition is thought to afflict hundreds of millions of children, primarily in south Asia and sub-Saharan. EED is associated with stunting, and thought to be a major cause thereof. A ‘gold standard’ biomarker or diagnostic test for EED has not yet been developed, but at present the dual sugar absorption test (lactulose:mannitol) serves as a bronze standard. In some populations the dual sugar absorption predicts subsequent linear growth. EED is associated with reduced absorption of macro- and micronutrients. Histology similar to EED is seen in malnourished children.

Interrogation of the host transcriptome in EED shows that a diversity of genes that function in the immune response are overexpressed. These include responses to viruses, bacteria and parasites; and are linked to the IgA response, phagocytosis and cell mediated immunity. KEGG pathways important to cell adhesion are disrupted in EED as well. In particular, certain enteroviruses are found in EED when compared to children with good gut health living in the same environment. EED is also associated with underexpression of mucin synthetic genes.

EED is reduced with deworming, high dose zinc and multiple micronutrient supplementation.

EED, because it is so widespread, is likely to be the end consequence of many insults. The insults range from inadequate dietary intake to overexposure to a large number of diverse microbes to exposure to specific microbes that target the small intestine. New tools to evaluate and ameliorate EED increase our ability in the near future to effectively reduce the burden of EED worldwide.
Intestinal infection and inflammation in severely malnourished children.

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In very undernourished children an additional complication ensues which compromises effective absorption of their limited nutritional intake. Intestinal barrier function becomes impaired and absorption pathways disrupted because of mucosal inflammation. Enteric infection initially causes transient breakdown of mucosal integrity and establishment of a proinflammatory environment within the intestinal mucosa. Epithelial damage induces recruitment from the bone marrow of innate immune cells (monocytes/macrophages and neutrophils) with potent inflammatory capacity, producing cytokines such as IL-1 and TNF-α. These contribute to antibacterial responses, but also impair epithelial tight junction integrity. Chronic mucosal production of proinflammatory cytokines may then occur because of non-specific ingress of dietary antigens and bacterial products of the microbiome through the disturbed epithelial barrier. Evidence for this process is given by the chronic elevation of inflammatory markers in the circulation (ESR, CRP etc) and the faeces (calprotectin, α-1 antitrypsin) – these are found in malnourished children at levels found in European children with active Crohn’s disease.

Epithelial barrier function is compromised even in well-nourished children in resource poor countries (4-fold excess paracellular permeability compared to European children). Episodes of enteric infection transiently exacerbate this, inducing exaggerated mucosal inflammation. In order for this to settle effectively, a regulatory immune response dominated by IL-10 and TGF-β must occur. These cytokines both restore epithelial integrity and damp proinflammatory responses in nearby cells. Generation of mucosal regulatory cells depends on adequate zinc, vitamin A and D. Thus in advanced malnutrition a failed regulatory response occurs, leaving a mucosa dominated by Th1 and deficient in T regulatory responses. A chronically leaky intestinal barrier, with enterocytes showing impaired expression of disaccharidases and transporter function, is the consequence, establishing a vicious circle of further undernutrition. The mucosal immune response shifts further away from regulation, and dominance of IgG over IgA responses further inhibits defence against enteric infection. One additional consequence of this mucosal inflammatory response is loss of oral tolerance to dietary antigens – thus foods employed for nutritional rehabilitation themselves may induce enteropathy to exacerbate malabsorption. Thus paracellular permeability worsens during rehabilitation, while substitution of an isocaloric amino acid formula for lactose-free cow’s milk induced a substantially greater weight gain. Early work using the anti-inflammatory mesalazine has given encouraging early results.

Much more needs to be known about mucosal responses – remarkably it is unknown whether colonic inflammation occurs. However recognition of the chronic inflammatory state induced by the combination of undernutrition and repeated infection has been important. No malnourished European child with chronic loose stools, protein losing enteropathy and high inflammatory markers would simply be fed and denied appropriate investigation.

Overview of BMGF convening on environmental enteric dysfunction.

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In March 2015, The Bill & Melinda Gates Foundation (BMGF) hosted a convening on environmental enteric dysfunction (EED). The meeting focused on three fundamental questions related to EED: 1) what are the key questions that need to be considered when conducting interventional trials in children with, or at risk for, EED; 2) what factors need to be considered in developing a product use case and target product profile (TPP) for EED interventions; and 3) how would one design a clinical trial to prevent or treat EED? Participants from all over the world and from different disciplines met and small break out groups were utilized to discuss the finer points of intervention trials for EED. The uncertainties around the pathogenesis, definition and sequelae of EED, and need for good predictive biomarkers of EED were emphasized by participants and discussed at length, especially the linkage of EED to stunting and cognitive development. The consensus was that further evidence was needed to confirm those associations. Even with the complexities around case definition and pathogenesis of EED, there was a consensus to move forward with interventional trials and several possible trial designs were discussed, with important obstacles and considerations being identified and a refined target for an intervention trial emerging. Within the context of an interventional trial, there was discussion around whether to look at prevention or treatment and when to intervene. It was broadly decided that each of these would need to be separate approaches, each with their own TPP to use a metric of a successful intervention. A smaller group met for one more day to generate a broad draft TPP that could help evaluate what intervention(s) would be the best to test for EED. The TPP from that meeting will be further refined as more evidence on the pathogenesis, definition and sequelae of EED become available.
The human digestive tract is populated by a large number of microbes, whose cell number (10^{14}) has been estimated to exceed the number of body cells (10^{13}) by a factor of ten. The vast majority of these microorganisms are bacteria, while small proportions are archaea and yeasts. Interest in this microbial community has considerably increased owing to the realisation that gut microbes profoundly influence host functions and thereby contribute to health maintenance or disease development. Recognised functions of the gut microbiota include provision of a barrier against pathogens, also referred to as colonisation resistance, priming of both the innate and the acquired immune systems and extraction of energy from otherwise indigestible carbohydrates (dietary fiber). However, the intestinal microbiota may also contribute to the development of diseases such as ulcerative colitis and colorectal cancer. The application of metagenomics to the gut microbial ecosystem revealed truly remarkable correlations between certain diseases and the gut microbiome. It also led to the suggestion of the existence of a ‘core microbiome’ that encompasses key functions shared by each individual. However, the mechanisms underlying host-microbe interactions have not yet been unraveled.

The majority of microorganisms in the gut are considered commensals as they perform tasks that are beneficial for the host. However, even though this microbial community lives in harmony with its host most of the time it should be kept in mind that gut bacteria are not altruistic but take advantage of the constant temperature and the wide range of substrates available in the digestive tract. In return, by virtue of its immense metabolic potential the intestinal microbiota makes nutrients available to the host that otherwise cannot be utilized. For example, non-digestible carbohydrates, also referred to as dietary fiber, are fermented to short chain fatty acids, which can be utilized by the host. However, under certain circumstances the harmonic relationship between the host and its microbiota gets lost. Possible reasons include medication, a diseased state and/or unhealthy nutrition. Interestingly, various diseases are accompanied by alterations in the gut microbiota, often referred to as dysbiosis.
The intestinal microbiome in action – revealing the functions of individual microbiota members in health and disease by single cell stable isotope probing.

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It is common knowledge that nutrition has an immense impact on human and animal health, but the role of common intestinal microorganisms in mediating the beneficial or detrimental effects of diet is less appreciated. Genomic and postgenomic studies have revealed exciting insights into the composition and metabolic potential of the intestinal microbiota and intriguing correlative associations with human/animal nutrition and disease. Large microbiome sequencing projects, such as the US human microbiome project or the European Meta-HIT initiative, have greatly raised scientific and public awareness of the importance of our microbial residents. However, while these predominately “big sequence data”-driven programmes can generate new hypotheses about the potential role of the complex microbiota, the true physiological roles of individual microbiota members and their contributions to host ecosystem function remain largely undetermined. It is thus important to focus on human and animal microbiome function in host nutrition and disease to provide both significant advances in scientific understanding and targeted dietary interventions. In vivo applications of diets or dietary compounds labelled with harmless stable isotopes in nutritional studies represent an attractive option to reveal host and microbiota physiologies and quantify nutrient fluxes. Here, I will focus on recent methodological advances\textsuperscript{1,2,3} that enable studying the identity and metabolic role of single microbial cells directly in the intestinal environment. These technologies will allow us to make an important step towards revealing the complex trophic interplay between individual populations of microbial cells and how they influence nutrient and energy flow to the human or animal host.


Enteropathy in severe acute malnutrition.

Environmental enteropathy (EE) strictly refers to an asymptomatic condition, but well known association of severe malnutrition and malabsorption suggests a more severe lesion than in EE. We carried out a study of enteropathy in 34 children with severe acute malnutrition and persistent diarrhea in the malnutrition ward in the University Teaching Hospital, Lusaka. All biopsies showed severe villus blunting and epithelial damage. Lactase deficiency was observed in 40%. Levels of bacterial translocation were very high, and inversely correlated with the growth hormone IGF-1. Intestinal permeability also very high, both when measured by lactulose/rhamnose testing and by leakage of serum proteins into gut secretions. The most severe intestinal damage was associated with autoantibodies which usually characterise coeliac disease even though these children were not taking gluten in the diet, suggesting that the severity of the enteropathy can permit food-related and auto-reactive dysfunctional immune responses. Studies of zinc uptake were complicated by the zinc supplements prescribed according to WHO recommendations, indicating that isotopic studies will be required to understand micronutrient balance in these severely ill children.
EED is very common among children in many low- and middle-income countries, if defined by an abnormal lactulose:mannitol test. Many have no symptoms, not all are stunted but it is likely that most have some degree of impaired growth and thereby a catch-up potential. Children with EED have been shown to have a reduced carbohydrate and protein absorption and the gastrointestinal inflammation might also reduce appetite. It is therefore plausible that a diet with energy and macronutrients composition as recommended for children with MAM will be appropriate for children with EED. To secure catch-up growth, the diet should contain 10E% from high quality protein, and 25-35% from fat to ensure adequate energy density. A higher protein intake may cause metabolic stress. Furthermore, specific nutrients seem to be able to influence some of the components of EED, e.g. to reduce intestinal inflammation, improve the intestinal barrier and support repair and growth of the intestinal mucosa. Zinc has been shown to be effective but only few studies have tested the effects of macronutrients. Animal studies, especially studies of piglets with intestinal injury due to early weaning, have shown positive effects of specific amino acids. E.g. glutamine and glutamate are important for mucosal repair. Intervention studies have shown effects of alanyl-glutamine supplementation on weight gain and barrier function in children from Brazil and improved intestinal permeability in adult HIV patients. n-3 fatty acids have been suggested to reduce gastrointestinal inflammation. However, an intervention study among 3-9 months old children from The Gambia did not show effects on growth or intestinal integrity.

It is possible that supplements with specific nutrients, e.g. amino acids or fatty acids, can improve intestinal repair and function and thereby reduce the severity of EED. However, more studies are needed to prove this. Better biomarkers of gut function are needed to evaluate the effects of such interventions in children and animal models, e.g. malnourished piglets, can be used to study the effects of such intervention on the gut mucosa in detail.
Environmental Enteric Dysfunction and stunting - making the links.

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Stunting – low height or length for age – is frequently referred to as either as a component of the clinical syndrome of Environmental Enteric Dysfunction (EED) or closely associated with it. 165 million children worldwide suffer from stunting, with prevalence especially high in sub-Saharan Africa and in South and Southeast Asia. Once established, stunting has known consequences for immediate mortality risk, long-term neurological development and, in adult life, risk of non-communicable disease. 200 million children annually do not reach their developmental potential due to stunting. Although a proportion of babies are born stunted, stunting prevalence further increases after birth, and predominantly during the first year of life. This – plus the observation that geographical distribution of stunting is heterogeneous both between and within countries – suggests that environment is a powerful determinant of stunting risk. Existing efforts – mainly nutritional – to prevent or reverse stunting have had limited effect.

Linear growth failure has been observed in association with markers of EED in infants, children and even some adults in The Gambia, Malawi, Bangladesh, Zimbabwe, Nepal and Peru. Other studies have demonstrated associations between stunting and EED’s putative risk factors: including specific enteric infections, pathways of general faeco-oral contamination, and socio-demographic factors. Ongoing interventional and observational studies with EED and stunting as outcomes of interest will help improve our understanding of the multifactorial nature of stunting, and the role that EED plays in its pathogenesis.
Pathogenesis of stunting in malnourished children.

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Stunting in malnourished children reflects the combined effects of intrauterine growth restriction (IUGR), postnatal growth failure, and blunted catch-up growth.

IUGR results from maternal malnutrition and placental insufficiency, with deficits in fetal organ growth from IGF-1 and insulin deficiencies, IGF resistance, and glucocorticoid excess. IUGR limits organ growth during a critical developmental window and thereby predisposes to postnatal stunting.

Growth failure and lack of catch-up growth during postnatal life are exacerbated by small bowel inflammation, infection, and persistent/recurrent bouts of malnutrition. These cause variable food intake, macro- and micro-nutrient deficiencies, cytokine excess, T3 deficiency, GH resistance, IGF-1 deficiency, and IGF-1 resistance. Leptin deficiency may contribute.

Nutrient delivery and rapid catch-up growth are associated with visceral fat deposition and insulin resistance, predisposing to metabolic syndrome and type 2 diabetes. Cognitive deficits in stunted children result from the effects of macro- and micro-nutrient and IGF deficiencies on brain development in early life.

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The impact of infection and inflammation on intrauterine growth restriction.

Environmental enteric dysfunction (EED) is associated with linear growth failure in childhood. This condition has been suggested to be caused by deranged bacterial composition and function in the gut, leading to malabsorption of nutrients, translocation of bacterial components through a damaged intestinal wall, and a systemic inflammation that restricts growth by interfering with its hormonal control. According to this model, aberrations in the intestinal bacterial composition – largely caused by repeated ingestion of bacteria - are key determinants of linear failure. If this were the case, growth stunting could be prevented by interventions that targeted food, water and sanitation hygiene (WASH), possibly combined with a dietary supplementation.

In several studies and trials in Malawi, our group has documented an asymmetric fetal growth failure, manifested as a low mean length but normal mean weight-for-length and head-circumference at birth. In a cohort of 1391 pregnant women, we have studied the predictors of intrauterine growth restriction, using multivariate regression models and pathways analyses with structural equation models (SEM). Neonatal weight, length, and head-circumference were all predicted by the duration of pregnancy, but otherwise the measures of body size were differentially predicted. Chronic maternal infections, such as HIV, malaria and deep dental infections explained especially linear growth failure, maternal nutritional status was important for ponderal growth, and head growth was least predicted by external factors.

The results indicate the importance of chronic maternal infections in causing linear growth failure in fetal period, probably through their interference with hormonally driven elongation of long bones. Similar mechanisms are likely to mediate growth failure also in infancy and early childhood. Therefore, in areas where chronic or frequent infections are prevalent among mothers or children, WASH interventions, with or without nutritional support, may not prove very successful in stunting prevention.
Stable isotopes and gut function.

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Stable, non-radioactive, isotopes provide a methodology to trace dynamic metabolic processes in a non-invasive manner in humans. Stable isotope breath tests utilise a $^{13}$C labelled tracer which is metabolised by a rate-limiting process of interest and the excretion of $^{13}$CO$_2$ in breath can be measured serially and kinetic parameters derived from the $^{13}$C excretion data. Stable isotope breath tests are particularly well suited to children and pregnant women. In environmental enteric dysfunction (EED), stable isotope breath tests have found some clinical use. The classical clinical $^{13}$C breath test is the diagnosis and monitoring of Helicobacter pylori (HP) infection, an enteropathogen associated with peptic ulcers, gastritis and upper GI cancer. The HP breath test, to this day, represents one of the best examples of the diagnostic value of $^{13}$C breath tests because of the clear and unambiguous nature of the diagnostic test. In EED, several $^{13}$C labelled substrates have been used to assess bacterial colonisation/infection in the small intestine and are largely carbohydrate based. $^{13}$C xylose, $^{13}$C sucrose and $^{13}$C sorbitol have been used but generally suffer from poor specificity and sensitivity. The kinetics of $^{13}$C appearance in $^{13}$CO$_2$ from these tests is also complicated by variations in intestinal transit time which may interfere with a diagnosis of small intestinal bacterial overgrowth. Further work is needed to ascertain if combining breath tests with other clinical outcomes measures such as permeability or inflammation may increase specificity and sensitivity of a diagnostic test for EED. Other multi-isotope approaches are possible which allow assessment of bioavailability of labelled metabolites from mammalian digestive and absorptive processes (integrating gut function) in comparison with metabolites that are produced by the resident microbiome. The “Holy Grail” in EED diagnosis is a point-of-care biomarker with high specificity and sensitivity that allows unambiguous clinical decision making. Greater insight into EED microbiome function may yield opportunities for organism specific targeting with isotopic tracers. Combined breath tests which allow both microbiome function and transit to be measure simultaneously may also improve the specificity and sensitivity of existing tests.
New combination non-invasive tests for mucosal dysfunction, inflammation and gastrointestinal microbiome composition and activity.

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Multifactorial elements contribute to optimal gut function including motility and regional residency time differences. In order to minimise perturbation of the gastrointestinal ecosystem, comprising the different regions of the gut, the design of new non-invasive tests to interrogate gut function in free living individuals, at different levels, is an unmet need. These constitute detecting luminal parameters including resident bacteria presence and activity, assessment of small intestinal mucosal damage and permeability and indicators of regional residency time. In order to develop “fit for purpose” functional tests to better understand gut absorptive capacity in childhood we have used a stable isotope breath test and other breath tests to detect and monitor progression of enteropathy in different populations in Australia. This began with independent tests such as the $^{13}$C sucrose breath test for small intestinal damage; the dual sugar permeability test for detecting changes in barrier function; categorising the fermentative signature and detecting the presence of small bowel bacterial overgrowth (SBBO). Data from populations of indigenous children in the Northern Territory vs. children in Metropolitan Adelaide with and without Environmental Enteropathy (EE) respectively will be presented. The progression to the use of combination non-invasive tests in these settings with discussion on the advantages and disadvantages of this approach will be described. The use of combination tests in intervention studies and the ability to define subsets based on their extent of absorptive dysfunction will be presented. Additionally, the potential for these to enhance the interpretation and clarify the efficacy of interpretation of outcomes will be highlighted. The impact of the gut microbiome patterns and activity and its influence on the extent and severity of the host’s stressed mucosa and their putative role in conferring susceptibility to impaired growth and defining candidate strategies for management of EE will be discussed.
Stable isotope techniques to investigate oral-cecal transit time, lactose malabsorption and starch digestion/fermentation.

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Application of stable isotope techniques can give insight in various biological processes. To assess the oral-cecal transit time lactose-$^{13}$Cureide is administered together with the meal. Lactose-$^{13}$Cureide resists enzymatic degradation in the small intestine but is cleaved by colonic bacteria. As result of the bacterial fermentation of labelled urea, $^{13}$CO$_2$ is released and can be measured in breath. A rise of $^{13}$CO$_2$ thus indicates the arrival of the head of the meal in the cecum.

Lactose maldigestion can be diagnosed by means of orally administering a solution of $^{13}$C-labeled lactose. The addition of a tracer amount of the reference sugar $^2$H-glucose enhances the discriminative power, as it enables correction of the inter-individual variations of the insulin effect and detection of general sugar malabsorption. In plasma $^{13}$C- and $^2$H-labeled glucose is measured and the $^{13}$C/$^2$H glucose ratio calculated. This ratio increases postprandial to around 1 in lactose digesters but only up to ca. 0.46 in lactose maldigesters.

$^{13}$C-labelled starchy products are used to measure the rate and degree of starch digestion. For correction of the insulin effect (glucose disposal) a tracer amount of $^2$H-glucose is constantly infused. With this dual isotope technique the underlying processes which determine postprandial glucose concentrations can be estimated: the rate of appearance of starch-derived glucose, the rate of appearance of glucose produced in the liver and the rate of glucose disposal into tissues. $^{13}$CO$_2$ in breath gives information about the portion of digested carbohydrates that is oxidized. However, in case of a meal containing indigestible carbohydrates, both small intestinal digestion and colonic fermentation are contributing to breath-$^{13}$CO$_2$ as soon as those carbohydrates enter the cecum. Then it is necessary to measure also hydrogen in the breath and apply a curve fitting method to be able to distinguish $^{13}$CO$_2$ produced by colonic fermentation from that produced by glucose oxidation.
Assessment of zinc metabolism in EE using stable isotopes.

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Zinc (Zn) homeostasis and metabolism may be impacted by EED via several processes. Under normal conditions, Zn absorption is consistent with a saturation response model, which predicts that absorption from increasing doses of ingested Zn will approach an asymptote, with progressively smaller gains in the amount absorbed for the amount ingested. In adults, the 2 strongest predictors of the amount of Zn absorbed are the amount ingested and the amount of dietary phytate. Recent modelling of Zn absorption based on multiple studies using stable isotopes in young children indicate that age is positively associated with Zn absorption, but dietary phytate did not have an effect. Results of studies in children with high risk of EED have shown mixed effects on absorption, ranging from impaired to higher than expected. In several studies using supplements or fortification, the total daily Zn absorbed has met estimated physiologic requirements, but this benchmark has been achieved only with intakes well above those of infants and young children in westernized settings. Another critical aspect of homeostasis is excretion of endogenously secreted Zn. The inflammation and dysfunction of the gastrointestinal tract in EED may lead to excessive secretion, impaired reabsorption, or both; either would lead to excessive faecal losses. Some studies have supported a relationship between endogenous Zn losses and markers of gut function, but data are extremely limited. We have also examined the size of the exchangeable Zn pools (EZP) in young children in austere settings, as a putative index of Zn status. The results have generally been consistent with absorption data: larger EZP associated with higher daily Zn absorption. Future studies are needed to better estimate the “patho-physiologic”requirement for children with EED and the daily intakes needed to achieve them, while considering factors governing normal Zn homeostasis as well as those that may effectively increase daily requirements. Such investigations are urgently needed to refine interventions.
Lessons from iron fortification trials in Ivory Coast, Kenya and Pakistan with a focus on the gut microbiome.

Iron deficiency anaemia (IDA) remains a major contributor to the global burden of disease and most of this burden is in tropical regions where the etiology of IDA is due to poor diets and infections. However, providing iron in these settings may also increase risk for infections. Low absorption of iron fortificants results in >90% of the iron passing unabsorbed into the colon. Most iron in the human body is tightly bound to various proteins that limit iron supply to potential pathogens, but there is no similar system for the sequestration of dietary iron in the gut lumen. Iron is a growth-limiting nutrient for many gut bacteria. Iron is essential for the growth and virulence of many pathogenic enterobacteria, whereas beneficial barrier bacteria, such as lactobacilli, do not require iron. In a randomized controlled trial (RCT) in Ivorian children fed iron-fortified biscuits, there was a significant increase in the number of enterobacteria (P < 0.005) and a decrease in lactobacilli (P < 0.0001) in the iron group after 6 months. In the iron group, there was an increase in the mean fecal calprotectin concentration (P < 0.01). In contrast, an RCT of high dose iron supplements in low-to-middle-income South African children showed no major effects on the gut microbiota or inflammation. We have also examined the safety of in-home iron fortification for infants; this strategy is recommended in developing countries to control anaemia but low absorption typically results in >80% of the iron passing into the colon. Iron is essential for growth and virulence of many pathogenic enterobacteria. We determined the effect of high- and low-dose (12.5 mg iron vs. 2.5 mg iron) in-home iron fortification on the infant gut microbiome and gut inflammation in two RCTs in 6-month-old Kenyan infants consuming home-fortified maize porridge daily for four months. The primary outcome was gut microbiome composition analysed by 16S pyrosequencing and qPCR. At baseline, 63% of the total microbial 16S rRNA could be assigned to Bifidobacteriaceae but there were high prevalences of pathogens. Using pyrosequencing, an increase of enterobacteria, particularly Escherichia/Shigella, the enterobacteria/bifidobacteria ratio, and Clostridium by +FeMNP was detected. Most of these effects were confirmed using qPCR; e.g., +FeMNP increased pathogenic E. coli strains. Further, +FeMNP increased faecal calprotectin. In this setting, provision of iron fortification to weaning infants adversely affects the gut microbiome, increasing pathogen abundance and causing gut inflammation.
Environmental enteropathy, oral vaccine failure and growth faltering in infants in Bangladesh

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Background: Environmental enteropathy (EE) is a subclinical enteric condition found in low-income countries that is characterized by intestinal inflammation, reduced intestinal absorption, and gut barrier dysfunction. We aimed to assess if EE impairs the success of oral polio and rotavirus vaccines in infants in Bangladesh.

Methods: We conducted a prospective observational study of 700 infants from an urban slum of Dhaka, Bangladesh from May 2011 to November 2014. Infants were enrolled in the first week of life and followed to age one year through biweekly home visits with EPI vaccines administered and growth monitored. EE was operationally defined as enteric inflammation measured by any one of the faecal biomarkers reg1B, alpha-1-antitrypsin, MPO, calprotectin, or neopterin. This study is registered with ClinicalTrials.gov, number NCT01375647.
Biomarkers of EED including the kynurenine tryptophan ratio.

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As EED is defined by the altered physiology of the gut in a manner that compromises child growth, the identification of individual subjects with EED at a point of time is made difficult by the multifactorial origins of linear growth failure in children. Biomarkers must be developed with the goal of identifying children for which enteric dysfunction is a principal contributor to this linear growth failure for selective inclusion in clinical trials of therapies for EED. Current biomarkers of importance include permeability tests with carbohydrates (lactulose, mannitol, rhamnose), fecal markers of permeability (claudin-3), bacterial translocation (EndoCab, sCD14, bDNA), fecal markers of intestinal inflammation (myeloperoxidase, calproctectin, neopterin, alpha-antitrypsin) and tissue repair (REG-1B), and markers of systemic immune activation (alpha-1-glycoprotein, hsCRP, IL-6). Recently, kynurenine and tryptophan have been evaluated as additional markers of EED and predict linear growth failure and vaccine non-responsiveness in children in Peru and Tanzania. This suggests immunotolerance may be an important factor to consider in the evaluation of EE and evokes possible links between host metabolome and immune response to fecally contaminated environments.
Use of faecal host transcripts as biomarkers of EED.

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Background: Although widely used as a surrogate for small bowel permeability and absorptive capacity, urine lactulose to mannitol (L:M) ratio has several limitations as a marker of EED, including cumbersome sample collection and a lack of standardization of methods and normal values. We sought to identify a stool-based mRNA biomarker of EED that would have the advantage of simplifying sample collection and might reflect other processes that contribute to the pathophysiology of EED.

Methods: Stool samples collected from children in rural Malawi at risk for EED with accompanying urine L:M data were analysed. Total nucleic acids were extracted using a magnetic silica particle-based method, and target mRNA sequences were quantified using digital droplet PCR and normalized to a constitutively expressed gene. Human transcriptome array (HTA) was performed on nucleic acids extracted from stool to better characterize differences in gene expression between healthy children and those with EED. Several computational models were employed to test the use of a combination of biomarkers to predict L:M and EED severity.

Results: The mRNA extraction and quantification methods used reliably detected mRNA transcripts of interest in stool. Of the targets tested, regenerating islet-derived protein 4, REG4, best differentiated between children with normal and significantly elevated urine L:M ratios. A Random Forest model using 11 biomarkers was better able to predict L:M and EED than any single marker alone. Transcriptome analysis revealed differential gene expression when comparing children with and without EED, and identified 13 specific transcripts associated with EED including those of chemokines, immunoglobulin Fc fragments, and activators of inflammatory cells.

Conclusions: We have developed a sensitive and reliable method for extracting and quantifying mRNA in stool and demonstrated differential patterns of gene expression in children with EED compared to healthy controls. With further investigation, these methods may prove useful for identifying an alternative to the L:M ratio as a marker for EED.
Biomarkers of EED in the MAL-ED Study.

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The MAL-ED study is a multi-site birth cohort study designed to improve the understanding of interactions between undernutrition and enteric infections and measure the consequences of these adverse events in early life on child growth and development. The cohort enrolled 2145 children in 8 countries: Brazil, Peru, Tanzania, South Africa, India, Bangladesh, Nepal and Pakistan. The project is now in its 7th year and follow-up to five years of age is planned, with the first children already having completed 5 years of follow-up. The project has yielded important findings on the etiology of enteric infections in diverse settings, the permeability of the intestine as measured by the lactulose mannitol test in the first two years of life, contributed to the evaluation of non-invasive biomarkers for EED, and evaluated multiple analytic frameworks for the analysis of causality in EED.
Child stunting and anaemia are intractable public health problems in developing countries that have profound short- and long-term consequences. The Sanitation Hygiene Infant Nutrition Efficacy (SHINE) trial is a proof-of-concept, 2x2 factorial, cluster-randomized, community-based trial in two rural districts of Zimbabwe that will test the independent and combined effects of protecting babies from faecal ingestion (Factor 1, operationalised through a WASH intervention) and optimising nutritional adequacy of infant diet [Factor 2, operationalised through an infant and young child feeding (IYCF) intervention] on length and haemoglobin at 18 months of age. SHINE is motivated by the hypotheses that, (1) environmental enteric dysfunction (EED) is a major underlying cause of both stunting and anaemia, (2) chronic inflammation is a central characteristic of EED mediating these conditions, (3) EED is primarily caused by faecal ingestion in conditions of poor water, sanitation and hygiene (WASH), (4) interrupting faeco-oral transmission through baby-targeted WASH interventions will reduce the onset of EED, and (5) WASH and infant feeding interventions will have at least additive effects on reducing stunting and anaemia. Within SHINE we are measuring two causal pathways. The biomedical pathway comprises the infant biologic responses to the WASH and IYCF interventions that ultimately result in attained stature and haemoglobin concentration at 18 months of age; it will be elucidated by measuring biomarkers of intestinal structure and function (inflammation, regeneration, absorption and permeability); microbial translocation; systemic inflammation; and hormonal determinants of growth and anaemia among a subgroup of infants enrolled in an EED sub-study. The programme impact pathway seeks to elucidate the mediating and modifying determinants of intervention impact; it will be modelled through measures of fidelity of implementation of interventions, uptake of promoted behaviours and practices, and household and caregiver characteristics. This presentation focuses on the rationale, design and methods underlying the SHINE trial in Zimbabwe.

[The SHINE trial is registered at http://Clinicaltrials.gov as protocol NCT01824940.]
THE WASH Benefit Trial: an evaluation of the impact of water, sanitation, hygiene and nutritional interventions on child growth, development and environmental enteric dysfunction in Kenya and Bangladesh.

Environmental enteric dysfunction (EED) is common among children living in poor environments, where water quality, hygiene, and sanitation (WASH) facilities are inadequate or of poor quality and where food insecurity is common. However, data on EED from intervention trials designed to improve these conditions are lacking and many WASH interventions have suffered from low rates of uptake. The WASH Benefits trial is an ongoing two country cluster randomized trial of improved water quality, sanitation, hygiene, and nutrition interventions with sites in rural Kenya and Bangladesh. Interventions in each domain were selected based on extensive formative research on hardware, nutrient supplements and behaviour change methods that might facilitate the greatest uptake in the target populations and lead to reductions in diarrheal disease or improved growth. Households with pregnant women were enrolled at baseline and are being followed for two years to monitor growth, diarrheal disease, and EED biomarkers among the children born. A total of 5551 and 8248 households have been enrolled and two-year follow-up visits will be completed in March and June 2016, in Bangladesh and Kenya, respectively. In a subsample of approximately 1100 children per country, urine, blood and stool samples are being collected and biomarkers of EED, including the urinary lactulose:mannitol test and faecal markers of neopterin, alpha-1 antitrypsin, and myeloperoxidase will be measured. This session will present an overview and rationale for the study design, study progress, and data on uptake and adherence for both countries.
Influence of pro/pre-biotics and other interventions on gut health.

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Probiotic organisms are live microorganisms which are believed to actively enhance health of consumers by improving the balance of microflora in the gut when ingested live in sufficient numbers. Commonly used probiotics organisms include *Lactobacillus* spp., *Bifidobacterium* spp. and *L. casei*. The strains *L. rhamnosus* GG, *Saccharomyces cerevisiae* Boulardii, *L. casei* Shirota, and *B. animalis* Bb-12 are the most investigated probiotic organisms.

To maintain the effectiveness of probiotic organisms or probiotic products, prebiotics including fructo-oligosaccharides (FOS), lactose derivatives such as lactulose, lactitol, galacto-oligosaccharides (GOS), and soyabean oligosaccharides are added. The combinations of pre- and probiotics have synergistic effects in promoting growth of probiotic, particularly *Bifidobacterium*, in the colon. Foods that contain both pro- and prebiotic are known as ‘synbiotics’.

A number of health benefits are claimed in favour of products containing probiotic organisms including antimicrobial activity and gastrointestinal infections, improvement in lactose metabolism, antimutagenic properties, anticarcinogenic properties, reduction in serum cholesterol, anti-diarrhoeal properties, immune system stimulation, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection. Some of the health benefits are well established while other benefits have shown promising results in animal models. However, additional studies are required in humans to substantiate these claims. Health benefits imparted by probiotic bacteria are strain specific, and not species or genus specific. It is important to note that no strain will provide all proposed benefits, not even strains of the same species, and not all strains of the same species will be effective against defined health conditions. The strains *L. rhamnosus* GG, *S. cerevisiae* Boulardii, *L. casei* Shirota, and *B. animalis* Bb 12 have the strongest human health efficacy data with respect to management of lactose malabsorption, rotaviral diarrhoea, antibiotic-associated diarrhoea, and *Clostridium difficile* diarrhoea. There is sufficient evidence to support the view that oral administration of *Lactobacillus* and *Bifidobacterium* is able to restore the normal balance of microbial populations in the intestine.
Results of three interventions to ameliorate EED.

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Background: Effective interventions against EED remain elusive.

Methods: We conducted four double-blinded, randomized, placebo-controlled trials in rural Malawi testing five interventions against EED. All of the trials included urinary lactulose:mannitol (L:M) ratio as a primary endpoint. The interventions include a probiotic (Lactobacillus GG), the non-absorbable antibiotic rifaximin, zinc supplementation or the anti-helminth albendazole, and micronutrient supplementation with or without fish oil. Both the probiotic and rifaximin trials enrolled subjects between 3-5yo, while the zinc/albendazole and micronutrient/fish oil trials included 1-3yos. With the exception of the micronutrient/fish oil trial, which took place over 6 months, each of the remaining trials assessed for change in L:M 3-5 weeks after the intervention was initiated. Both the zinc/albendazole and micronutrient/fish oil trials bridged the annual cycle of hungry and post-harvest seasons that takes place throughout rural Malawi.

Results: Baseline rates of increased L:M ratio exceeded 73% in each included trial, reaching 100% in the micronutrient/fish oil trial. Neither LactobacillusGG for 30 days nor rifaximin for 7 days was found to have an effect on L:M in 3-5 year old children. Zinc for 14 days and albendazole once were each found to attenuate the progression of EED after seven weeks, as the placebo group had a 0.12 increase in L:M ratio while the zinc and albendazole groups increased 0.3 and 0.4, respectively. Micronutrient supplementation, both with and without fish oil, led to modest improvement in L:M at 12 and 24 weeks when compared to placebo. A trial of a package of interventions including zinc, albendazole, and daily multiple micronutrient supplementation recently completed and results are being analysed.

Conclusion: While trials investigating zinc, albendazole, and micronutrient supplementation in younger children showed these interventions to be of modest benefit for EED, further work is needed to identify impactful treatments for the many children for whom prevention is not yet possible.
Environmental Enteric Dysfunction (EED) is an acquired syndrome of impaired gastrointestinal mucosal barrier function that plays a key role in malnutrition and growth failure in early life. It has been conceptualised as a partly adaptive response to excess environmental pathogen exposure in the context of reduced nutrient availability. However, it is clinically similar to other inflammatory enteropathies, which result from both host and environmental triggers, and for which immunomodulation may be advantageous. Our group performed the first controlled trial of a directly immunomodulatory therapy directed against host-sustained mucosal immune activation. We found that mesalazine (5-aminosalicylic acid) was safe: there was no excess of adverse events, evidence of deterioration in intestinal barrier integrity, or impact on nutritional recovery compared to placebo. There were modest reductions in several inflammatory markers with mesalazine. The trial has provided the first evidence of a potential role for pharmacologic immunomodulation in the management of EED.
Benefits of a household WASH package to Community Management of Acute Malnutrition (CMAM) program - The OUADINUT study.

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Clear evidence exists that some Water, Sanitation and Hygiene (WASH) interventions can successfully prevent diarrhoea. For instance, interventions aiming at improving water quality at household level or promoting hand washing with soap to reduce significantly diarrhoea incidence. Estimations showed that WASH interventions have a small but measurable benefit on height, but not on weight or weight/height. Yet, to our knowledge, no impact of WASH interventions has been assessed, neither during nutritional rehabilitation where children are particularly vulnerable to infections, nor after discharge where immune recovery is still incomplete.

In the context of nutritional rehabilitation of SAM (Severe Acute Malnutrition), we hypothesize that improving water quality and hygiene-related care practices at household level would decrease incidence of WASH-related infections, such as diarrhoea, nematode and environmental enteropathy. As such, it would improve weight gain, decrease relapses after successful discharge and, overall, could decrease over time the incidence of acute malnutrition in the community.

In order to test these hypotheses, Action Contre la Faim is currently implementing a cluster randomized controlled trial in Mao and Mondo health districts, Kanem region in Chad, comparing two groups: 1) nutritional rehabilitation; 2) nutritional rehabilitation + “household WASH package”.

The “household WASH package” includes: i) household water treatment and hygiene kit (water container, water disinfection consumables, soap, cup, hygiene promotion leaflet) provided at beginning of SAM treatment; ii) sessions of hygiene promotion provided weekly at health centre level; iii) household visits and hygiene sessions made during the treatment; group discussion on hygiene and care practices made with mother at community level after successful discharge.

2000 children, aged between 6 and 59 months, admitted to 20 OTP centres for SAM (outpatient treatment program for severe acute malnutrition) will be included into the study during 8 months (2 months treatment, and 6 months after successful discharge). Primary evaluation outcomes are length of stay and relapse rate, while secondary outcomes include anthropometric status (weight for height Z-score, height for age Z-score, MUAC), diarrhoea infections, home water quality and hygiene practices. Recruitment of the participants is in progress and the first results of the study are expected in August 2015. The final results and conclusions will be available in March 2016.

This project is conducted within a partnership that includes Action Contre la Faim- France, the Institute of Tropical Medicine in Antwerp, Belgium, and the Sahel Association of applied research for sustainable development (ASRADD) in Chad. Financial support is provided by ACF and the British Department for International Development (DFID).
Malnutrition and childhood infections in Africa: developing strategies against child malnutrition.

The MALINEA project is divided into three main parts:

**Part 1: Evaluation the association between disturbances of the digestive microflora and malnutrition.** This component aims to study differences between malnourished and non-malnourished children in the distal intestinal microbiota and in frequency of pathogens. Analyses will be stratified by country, age and sex of the children, and presence of diarrhoea. Stool collections will be analysed by a metagenomic approach and PCR detection of pathogens.

**Part 2: Improvement of the management of moderate acute malnutrition (MAM) by acting on this microbial component.** This part is a multicenter randomized clinical trial comparing the effectiveness (recovery at 3 months) of 3 re-feeding protocols: 1) CSB ++ standard treatment (Fortified Corn-Soy Blend); 2) CSB ++ associated with antibiotic; 3) CSB ++ associated with prebiotic. Secondary objectives aim to compare the 3 groups on anthropometric measurements, clinical characteristics, adherence to interventions, tolerance to interventions, as well as scales and cognitive measures at 3, 6 and 12 months after study entry. 1,800 MAM children between 6 and 24 months will be included and followed up. The flour used will be produced locally by social enterprises established in the economic fabric of the country.

**Part 3: Improvement of local capacity/knowledge on nutrition by promoting the transfer of know-how and partnerships between research institutes and fight against malnutrition programs.** Achievements of the project will target authorities and health structures in each country, as well as the academic world. Data and protocols will be co-published with scientists from the different countries, involving doctoral students. Development of new themes in academic courses (Masters) in African universities will be promoted. Scholarships will allow students and young researchers to be involved in the project, and to be trained in the methods used. Beyond the establishment of scientific protocols, MALINEA project will be a permanent working group to continue to work on this topic "Nutrition and infections" to develop new projects and meet other operational issues. This first project is the basis for a lasting partnership between the International Network of Pasteur Institutes and the two NGO Action Against Hunger International and GRET on the theme “nutrition and infection”.

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Ways to operationalize WASH-Nutrition synergies in implementation.

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Global momentum around emerging evidence of the direct linkages between water, sanitation and hygiene (WASH) and undernutrition as well as high-level advocacy have come to fruition in the Sustainable Development Goals, in which WASH and nutrition targets feature prominently. Development partners, including the World Bank Group, need to seize this opportunity to support country governments in helping to deliver “nutrition-sensitive” WASH services and shaping policies for greater impact on nutrition. Recent attention on the role of WASH in undernutrition has centered on a hypothesis that enteric dysfunction may be a key cause of chronic child undernutrition and the primary pathway linking poor WASH to poor nutrition outcomes (rather than through diarrhea). A burgeoning body of evidence is finding strong linkages between poor sanitation, and open defecation in particular, and stunting. Finally, the strong association between income poverty, child stunting, and lack of access to water supply and sanitation highlights the critical need for interventions that benefit the bottom 40 percent of the population.

Despite this growing momentum, practical guidelines and evidence on how to best work multi-sectorally are sparsely available. A recent review by the World Bank is offering a number of principles that can help shape such nutrition-sensitive WASH services. Firstly, the increased use of geographic and demographic targeting to reach populations where water and sanitation coverage is low and undernutrition is high. Secondly, incorporating state-of-the-art behavior change methods and insights from behavioral economics into World Bank operations could enhance nutritional impacts. Thirdly, WASH interventions can increase nutritional impact by aiming for and monitoring outcomes beyond “access” to services, such as usage, maintenance of infrastructure, and behavioral outcomes, especially related to hygiene practices. Finally, institutional incentives are needed to align development partners and governments agencies in support of multi-sectoral approaches, where results-based incentives can help to align objectives at the programme level. Operationalizing these principles will help governments and development partners to maximize nutritional impacts, however, at the same time knowledge gaps remain. Research and policy knowledge gaps span three broad areas: (i) direct and indirect effects of WASH on child nutrition outcomes; (ii) effectiveness and cost-effectiveness of nutrition-sensitive WASH interventions; and (iii) how to strengthen nutrition impacts in WASH programs. Further experimental and quasi-experimental impact evaluations will be required to answer these questions.
Legumes and growth project.

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Interventions that decrease the burden of childhood malnutrition are urgently needed, as millions of children die annually due to undernutrition and hundreds millions more are stunted. Environmental enteropathy (EE), a pervasive chronic subclinical inflammatory condition among children when complementary foods are introduced, places them at high risk for stunting, malabsorption, and poor oral vaccine efficacy. The project involves two randomized, controlled clinical trials to determine if common beans or cowpeas improve growth, ameliorate EE, and alter the intestinal microbiome during this high-risk period. The first study involves 6-11 month old children who will receive common beans, cowpeas, or standard local complementary foods for 6 months. Anthropometry will be compared among the three groups. EE will be assessed using a urine dual-sugar absorption test and by quantifying human intestinal mRNA for inflammatory messages, and the intestinal microbiota characterized by deep sequencing of fecal DNA to enumerate the host microbial populations and their metabolic capacity. The second randomized, controlled trial will enroll 12-35 month old children and follow them for 12 months; each subject will receive dietary interventions, either legume-based or control. Anthropometric, host inflammatory and gut microbiota analyses will be conducted similar to the first study. Project approach will be discussed.
Speaker Biographies
**Agapova, Sophia**

Sophia Agapova is a medical student at Columbia University College of Physicians and Surgeons. She is currently working with Dr. Mark Manary on a clinical trial studying the impact of legume flour-based interventions on EED and growth in children living in rural Malawi. She worked in 2012 in Dr. Mark Manary’s laboratory at Washington University in St. Louis to develop a method for quantifying human mRNA in stool and used this method to evaluate potential mRNA biomarkers for EED.

**Altmann, Mathias**

With a background in clinical biology, Mathias Altmann has engaged with humanitarian projects, in Afghanistan and in different African countries. He developed his skills in epidemiology and international public health. He is now an operational research advisor at Action Contre la Faim – France HQ. He is in charge of developing operational research as well as supporting the development of monitoring, evaluation and impact assessment of projects in humanitarian context.

**Ashorn, Per**

Per Ashorn, MD, PhD, works as a Professor of Paediatrics at the University of Tampere School of Medicine, Finland. He is a specialist medical doctor in paediatrics and paediatric infectious diseases and he has worked both as a laboratory scientist, a clinical physician, a global health specialist, an administrator, and a university teacher. He has conducted research in Finland, the US, Malawi, Ghana, and India and been involved in academic teaching and public health in numerous other low- and middle-income countries. Dr. Ashorn has been the principal investigator or active collaborator on approximately 20 clinical trials in Sub-Saharan Africa. His current research is focused around maternal and child health in low-income settings, with a special emphasis on the interaction between infections, inflammation, nutrition and growth failure, both in fetal period and postnatally. Dr. Ashorn has published over 150 scientific articles and supervised approximately 30 graduate or post-graduate students on their thesis work.

**Blaut, Michael**

Professor Blaut studied Biology at the University of Gottingen where he received his PhD. After working as a Postdoc at the University of California in Los Angeles he established his own research group at the University of Gottingen. In 1994 he became Professor at the University of Potsdam and simultaneously Head of the Department Gastrointestinal Microbiology at the German Institute of Human Nutrition (DIfE). His group investigates the role of the gastrointestinal microbiota in nutrition-related diseases. His research aims at understanding the molecular basis of nutrition-dependent diseases, and of developing new strategies for prevention, treatment, and nutritional recommendations. The research is focused on understanding the mechanisms underlying the correlations between disturbances in the gut microbiome and diseases. Professor Blaut has a long standing expertise in
microbial ecology, molecular microbiology and gut microbiota research. He coordinated previous EU-funded projects and has received continuous funding from the DFG. He has been serving as an editor for various scientific journals and as a reviewer for funding organizations in Europe and the US.

**Butler, Ross**

Professor Ross Butler has had over 35 years of experience in gastroenterology and nutrition research. He is currently an Adjunct Professor at UniSA. Past recent appointments have included: Chair for Paediatric Research and Innovation in the Sansom Institute for Health Research at the University of South Australia and a SAHMRI Senior Cancer Research Fellow. He is a recognised expert on nutritional factors and bowel health with diet and large bowel cancer, inflammatory bowel disease, upper gut cancers and irritable bowel syndrome as the focus of his research in the adult arena. He was awarded an ICRETT scholarship for cancer research studies at the International Agency for Research on Cancer (IARC) in Lyon, France in 1991. He has developed a range of breath tests for use as diagnostics in gastroenterology in Australia. In the last 20 years he has concentrated on paediatric gastroenterology and functional nutrition. A significant part of this work concentrated on establishing an array of stable isotope breath tests in Australia beginning with the first $^{13}$C urea breath test kit for detection of *Helicobacter pylori* infection available in this country. This work has now been expanded to include novel new breath tests for a variety of cancers and for helping to define the impact of microbiome activity on disease susceptibility. His interests include the modification and interaction of the microbiome in health and disease, and the role of dietary, probiotic and prebiotic effects in development and in conferring susceptibility to disease. He has established the use of new ways to assess the pathogenesis and eradication of *Helicobacter pylori*. and continues to work in this arena investigating the interaction between *Helicobacter pylori*, the innate immune system and gastric cancer. He has over 150 peer reviewed publications in recognised journals and has attracted significant funding from granting bodies and industry over his career.

**Crane, Rosie**

Rosie is a paediatrician and a Wellcome Trust Research Training Fellow at KEMRI Wellcome Trust Research Programme in Kilifi, Kenya, where she is supervised by Prof. Jay Berkley. Rosie is conducting a birth cohort in rural Kilifi that aims to improve understanding of the aetiology of Environmental Enteric Dysfunction. Rosie is also involved in a larger project that aims to characterise the spatial, social and environmental determinants of malnutrition amongst under-fives in Kenya.

**Freemark, Michael**

Michael Freemark is the Atkins Professor of Pediatrics and Chief of the Division of Pediatric Endocrinology and Diabetes at Duke University Medical Center.
Jones, Kelsey

Kelsey Jones is a paediatrician pursuing subspeciality training in gastroenterology, hepatology and nutrition. His doctoral work took place between Imperial College, London and the KEMRI-Wellcome Trust Research Programme in Kenya, and was supervised by John Warner and Jay Berkley. He conducted the first controlled trial of a directly immunomodulatory agent in severely malnourished children, and developed and trialled a new therapeutic feed for malnutrition that had a balanced polyunsaturated fatty acid profile. His ongoing research interests are in understanding the complex impacts of poverty on gut health and mucosal immune homeostasis.

Loy, Alexander

Alexander Loy is Associate Professor at the Department of Microbiology and Ecosystem Science, University of Vienna, Austria. He received his PhD in Microbiology at the Technical University in Munich, Germany where he developed phylogenetic microarrays for cultivation-independent analyses of microorganisms and studied the ecology of sulfate-reducing microorganisms. In 2003, he received a Marie Curie postdoctoral fellowship to join the newly founded Department of Microbial Ecology at the University of Vienna in Austria. He established his own research group in 2006 based on third-party grants and was Assistant Professor from 2009 to 2013. He obtained his Habilitation (venia docendi) and the Young Scientist Award of the City of Vienna in 2012. His research focuses on evolution and ecology of sulfur microorganisms, on the function of the intestinal microbiota in animals and humans, and on the development of molecular and isotope-labeling methods for studying uncultivated microorganisms in their natural environment.

Katsis, Alexis

Dr. Alexis Katsis is an immunologist with almost ten years of experience in global health working with the federal government and non-governmental organizations. Alexis obtained her Ph.D. in Microbiology and Immunology as well as a MS in Public Health Microbiology from the George Washington University.

Alexis joined the Foundation in November, 2014 as a Program Officer in the Enteric and Diarrheal Diseases (EDD) team where she supports the effort to develop a strategy for Cryptosporidium and manages a portfolio of grants related to EEO. Alexis comes to the Foundation from the Food and Drug Administration (FDA) where she assisted in developing tools and processes to better communicate the FDA’s benefit-risk assessments to outside stakeholders.

Prior to the FDA, Alexis spent 5 years at the National Institutes of Health (NIH) performing her graduate and post-graduate research in the innate immune response to filarial parasites. She spent two years at the Centers for Disease Control and Prevention (CDC) as an Emerging Infectious Disease fellow, working to understand the effect of mass drug administration on transmission of the causative agents of lymphatic filariasis.
Kelly, Paul

Paul Kelly qualified from Oxford and The London in 1986 and trained in internal medicine and gastroenterology. Since working as a medical officer in southern Zambia he has divided his time between research posts in London and Lusaka, in both of which he carries out clinical medicine. His research interests are enteropathies of the tropics in relation to infectious disease and malnutrition, protozoal and helminth infections, hepatosplenic schistosomiasis, and gastrointestinal cancers.

Kosek, Margaret

I am a physician with a primary interest in enteric diseases and child health. I have spent 15 years doing community based studies in the Peruvian Amazon and my research focus has shifted from identifying which specific enteropathogens and other illnesses impact child growth to the study of enteropathy in order to understand the pathways by which these effects are mediated.

Krebs, Nancy

Nancy F. Krebs, MD, MS, is a Professor of Pediatrics and Vice Chair for Academic Affairs in the Department of Pediatrics at the University of Colorado School of Medicine, and is the Head of the Section of Nutrition. Dr. Krebs is board certified in Pediatrics, Clinical Nutrition, and Pediatric Gastroenterology. She has extensive research experience with the application of stable isotope methodology to determine bioavailability, homeostasis and dietary requirements of zinc and iron in breastfed infants, young children and adults. She and her colleagues have conducted many investigations in international low resource settings, including evaluating the effects of micronutrient biofortification or supplements on zinc and iron status and on the intestinal microbiome. A study of zinc absorption and homeostasis in young children with environmental enteropathy is currently underway in Bangladesh. Other active research is an RCT to test the effects on linear growth of a maternal nutritional intervention initiated pre-conception vs prenatally in 4 low resource settings. Dr. Krebs has approximately 230 research and scholarly publications.

Maleta, Ken

Dr. Maleta is currently professor of public health at the School of Public health and Family Medicine, College of Medicine, Malawi. Previously, Dr. Maleta has been Dean of the College of Medicine University of Malawi. Dr Maleta’s research interests are in the field of maternal and child health focusing on prevention and management of undernutrition, childhood growth and development, and the interactions between nutrition and infection.

Manary, Mark

Dr. Mark Manary currently serves as the Helene B. Roberson Professor of Pediatrics at
Washington University School of Medicine in St. Louis, Missouri. Dr. Manary has committed his life to solving the problem of child malnutrition. He devotes severe months a year supervising therapeutic feeding trials and intervention trials for environmental enteropathy.

**Mbuya, Mduduzi**

Mduduzi Mbuya is an Associate Director with the Zvitambo Institute for Maternal and Child Health Research in Zimbabwe and a co-investigator in the Sanitation/Hygiene Infant Nutrition Efficacy (SHINE) trial. His research focuses on understanding the drivers and deterrents of success in public health interventions in three domains: design, delivery/implementation and uptake/compliance. In SHINE he has led the design of interventions to avoid feco-oral transmission (with the express objective of preventing EED) and optimize nutrient intake, and is, with other investigators, developing process evaluation methods. He also holds the appointments of Courtesy Assistant Professor in the Division of Nutritional Sciences at Cornell University and Associate Faculty at Johns Hopkins Bloomberg School of Public Health.

**Michaelsen, Kim F.**

Professor in paediatric nutrition at Department of Nutrition, Exercise and Sports, University of Copenhagen. Advisor to the National Board of Health on infant nutrition. Has been temporary advisor and performed consultancies for WHO on infant and young child feeding, feeding of moderately undernourished children and long term effects of complementary feeding. Research projects focus on the effect of early nutrition on growth, development and later health in industrialised and low-income countries. Topics include breastfeeding, complementary feeding, growth and body composition, early determinants of obesity, prevention and treatment of undernutrition. Studies are performed in Denmark, Ethiopia, Uganda, Cambodia and Burkina Faso.

**Morrison, Douglas**

Douglas Morrison is a Senior Lecturer in Stable Isotope Biochemistry at the University of Glasgow. His research focuses on understanding the mechanisms which link diet, environment, the gut microbiome and human health. With a background in Biological Chemistry, he uses molecular probes to understand the role of the gut microbiome in human health. Coupled with expertise in stable isotopes and focusing on physiologically relevant human studies, recent work has led to new insights into the role of products of microbiome metabolism in human health.

**Murch, Simon**

Simon Murch is a Consultant Paediatric Gastroenterologist at University Hospital Coventry & Warwickshire and is Emeritus Professor of Paediatrics at Warwick University. His clinical interests include non-IgE-mediated food allergies, coeliac disease and IBD. His research background is in mucosal immunology, and his early studies of the role of macrophage cytokines in intestinal and lung inflammation contributed to the introduction of anti-TNF therapy in
Crohn’s disease. He has subsequently worked in the areas of intestinal immune responses in food allergies and related disorders. His other main areas of research have been into mechanisms of intestinal protein leakage and of the mucosal pathology of environmental enteric dysfunction contributing to childhood malnutrition.

Petri, William

William A. Petri, Jr., M.D., Ph.D. is Chief of the Division of Infectious Diseases & International Health at the University of Virginia where he practices internal medicine and the subspecialty of infectious diseases.

Petri studies enteric infections and their consequences on the health of children. He leads the PROVIDE study of the Bill & Melinda Gates Foundation that is exploring in Bangladesh and India new solutions for the problem of oral poliovirus and rotavirus vaccine failures in the developing world. With his collaborators, he has discovered that zinc deficiency heightens susceptibility to rotavirus diarrhea, and demonstrated that oral polio vaccine underperformance is associated with malnutrition, diarrhea and shortened duration of exclusive breastfeeding.

Petri also studies amebiasis, which is one of the top 10 causes of diarrhea in children in the developing world. He has molecularly defined its ability to kill cells, developed the first FDA cleared test for its diagnosis, and was the first person to discover that children were immune to reinfection. He has discovered that the obesity hormone leptin plays a critical role in defense of the gut from ameba, with mutations in the receptor for leptin a major determinant of susceptibility to infection.

Petri in 2014 received from Governor Terry McAuliffe the Commonwealth of Virginia Outstanding Faculty Award. He has been recognized at UVa with awards for All-University Teaching, Excellence in Faculty Research, Distinguished Mentor (for both the Departments of Biology and Medicine), and Inventor of the Year. International recognition includes election as President of the American Society of Tropical Medicine and Hygiene, Editor of *Infection and Immunity*, receipt of the Squibb Award of the Infectious Diseases Society of America and Burroughs Wellcome New Investigator and Scholar Awards in Molecular Parasitology as well as Lucille P. Markey Scholar in Biomedical Research. He has continuously served on advisory committees for the NIH since 1993, and is recognized as a “Best Doctor in America”.

Priebe, Marion

Dr. M.G. Priebe is a nutritional scientist and her main focus of research is how digestion and fermentation of carbohydrates can affect health. To investigate the rate and extent of digestion of carbohydrates she applies stable isotope techniques. She was involved in the development of a dual label test to diagnose lactose malabsorption. In human studies she investigates how starchy products with slowly or rapidly digestible starch influence the postprandial metabolic response.

Shah, Nagendra Prasad

Nagendra P. Shah is currently a Professor of Food Science and Technology at the University of
Hong Kong. He worked at Victoria University, Melbourne, Australia for 22 years at various capacities including Associate Lecturer, Lecturer, Senior Lecturer, Associate Professor and Full Professor.

Prof. Shah has a long and intensive research history in probiotics, prebiotics and functional foods that has led to a distinguished international reputation in this area. He has published 239 research papers, 27 book chapters, and 205 conference abstracts. Additionally, he has edited 2 books on Dairy Products and Quality Control, and Probiotic and Prebiotic. He has also edited 2 special issues of the International Dairy Journal. His work has been highly cited in peer-reviewed journals.

Prof. Shah’s work has been internationally recognized and has received several prestigious international awards and accolades for his contributions to research including the 1999 American Dairy Science Association Foundation Scholar Award, the 2003 Marschall Rhodia International Dairy Science Award, the 2008 Dairy Industry Association of Australia Loftus Hill award, the 2009 California Dairy Research Foundation William Haines International Dairy Science Award, the 2011 Australian Institute of Food Science and Technology (AIFST) Keith Farrer Award of merit and the 2013 American Dairy Science Association Distinguished Service Award. He is Fellow of the Australian Institute of Food Science and Technology and American Dairy Science Association. He has served as the editor of the Journal of Food Science and Technology and ASEAN Food Journal and has been serving as editor of International Journal of Food and Nutrition and Advances in Chemical Science and associate editor of Journal of Food Science. Additionally, he is on the editorial board of several journals including International Dairy Journal and Journal of Dairy Science.

Smets, Susanna

Susanna Smets is a Sr. Water Supply and Sanitation Specialist with the Global Water Practice of the World Bank. For the past four years, she has been the regional advisor for the World Bank’s Water and Sanitation Program in East Asia and Pacific for rural sanitation, supporting governments in Vietnam, Cambodia, Philippines, Indonesia, and Lao PDR with policy advice and technical assistance on behaviour change, and market development for sanitation. She works closely with the Health and Nutrition Global Practice in the World Bank and with other development partners on operationalizing WASH-nutrition linkages.

Susanna has over 15 years of professional experience in water supply, sanitation and water resources management. Prior to joining the World Bank she worked in the Middle East, Asia and Europe for GiZ, DFID, the private sector and a Dutch water utility. She has a Masters in Water Resources Management from Wageningen University (NL) and a Masters of Business Administration from the Open University (UK).

Stephenson, Kevin

Kevin Stephenson is a fourth-year medical student at Columbia University College of Physicians and Surgeons in New York. He is currently in a year-long research program with Washington
University in St. Louis studying the potential impact of legume flours on EED and growth in Malawi under Dr. Mark Manary. He also studied the effect of Zinc or Albendazole on EED under Dr. Manary in Malawi from 2011-2012.

**Stewart, Christine P.**

Christine Stewart is an Assistant Professor in the Department of Nutrition and Associate Director of the Program in International and Community Nutrition at the University of California, Davis. Her research spans the broad area of maternal and child nutrition with a focus on the immediate and long-term effects of nutrition and health interventions during pregnancy or early childhood. Dr Stewart received her PhD in International Health and Nutrition at the Johns Hopkins Bloomberg School of Public Health and her MPH from the Tulane University School of Public Health and Tropical Medicine. Her research draws from the disciplines of nutrition, epidemiology, environmental health, behavioural sciences, and developmental biology to better understand how to develop and evaluate the efficacy of scalable nutrition interventions targeting vulnerable populations in a variety of settings.

**Vargas Brizuela, Antonio**

Senior Health and Nutrition Adviser / ACF Foundation / Madrid International, non-Governmental Organization.

Medical doctor specialised in Public Health and Tropical Medicine. More than 17 years of professional experience as a general practitioner and health adviser, out of which more than 14 within the context of development programs and projects in Latin America and Africa. Broad practice in formulation, implementation, monitoring and evaluation of projects and actions. Specific focus on strategic planning as well as monitoring and evaluation of HIV/TB national programs, increasingly within a health-as-a-right framework.

Extensive expertise in human resources management and coordination at both field and headquarters level. Proven experience in relationship with different stakeholders, including partners and donors. Researcher coordinator and experienced trainer in THA and HIV related matters. Skilled in planning, design and delivery of HIV-TB capacity building programs to a wide-ranging audience. Result-oriented, adaptable and used to work in a multicultural environment, under challenging conditions and time constraints.

**Zimmermann, Michael**

Michael B. Zimmermann is currently Professor and Head of the Human Nutrition Laboratory in the Department of Health Sciences and Technology, at the Swiss Federal Institute of Technology (ETH) in Zurich, Switzerland. He is jointly a Visiting Professor in Endocrinology, Diabetes and Nutrition at the University of Zurich Hospital, Switzerland. Prof. Zimmermann has published over 180 peer-reviewed papers, many in the area of micronutrient deficiencies, with a focus on
iodine and iron deficiency. His research has won the 2004 Mead Johnson Prize for Nutrition Research from the American Nutrition Society, the 2005 Endocrine Society Award for Excellence in Published Clinical Research, the 2013 International Endocrinology Award of the American Society of Endocrinology and the 2015 Princess Sirindhorn Health Award from the Royal Family of Thailand.