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GUT HEALTH DIGEST

UNIVERSITY OF WASHINGTON STRATEGIC ANALYSIS, RESEARCH & TRAINING (START) CENTER

REPORT TO THE BILL & MELINDA GATES FOUNDATION

MARCH 1, 2018

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    - Analysis of possible associations between illness and breastfeeding during the first 2 years of life in children from the MAL-ED birth cohort.

DETAILS OF ARTICLES

1. Promising Biomarkers of Environmental Enteric Dysfunction: A Prospective Cohort study in Pakistani Children.
   Iqbal NT, Sadiq K, Syed S, Akhund T, Umrani F, Ahmed S, *et al.*
   Scientific Reports. 8(1). 2018 February 14.
   PubMed ID: 29445110

ABSTRACT
Environmental Enteric Dysfunction (EED), a syndrome characterized by chronic gut inflammation, contributes towards stunting and poor response to enteric vaccines in children in developing countries. In this study, we evaluated major putative biomarkers of EED using growth faltering as its clinical proxy. Newborns (n = 380) were enrolled and followed till 18 months with monthly anthropometry. Biomarkers associated with gut and systemic inflammation were assessed at 6 and 9 months. Linear mixed effects model was used to determine the associations of these biomarkers with growth faltering between birth and 18 months. Fecal myeloperoxidase (neutrophil activation marker) at 6 months [$\beta = -0.207$, $p = 0.005$], and serum GLP 2 (enterocyte proliferation marker) at 6 and 9 months [6M: $\beta = -0.271$, $p = 0.035$; 9M: $\beta = -0.267$, $p = 0.045$] were associated with decreasing LAZ score. Ferritin at 6 and 9 months was associated with decreasing LAZ score [6M: $\beta = -0.882$, $p < 0.0001$; 9M: $\beta = -0.714$, $p < 0.0001$] and so was CRP [$\beta = -0.451$, $p = 0.039$] and AGP [$\beta = -0.443$, $p = 0.012$] at 9 months. Both gut specific and systemic biomarkers correlated negatively with IGF-1, but only weakly correlated, if at all with each other. We therefore conclude that EED may be contributing directly towards growth faltering, and this pathway is not entirely through the pathway of systemic inflammation.

DOI: 10.1038/s41598-018-21319-8
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2

START COMMENTARY: This longitudinal study in a group of infants with high exposure to enteric pathogens explores possible mechanisms by which EED affects growth. The authors evaluated the associations between EED biomarkers and change in LAZ score for the infants in the study. Delta LAZ was chosen in part because a large proportion of children were stunted at enrollment (~4 days of age). The authors performed a Principal Component Analysis to investigate possible relationships between eight biomarkers representing several physiological processes including gut inflammation, intestinal tissue regeneration, systemic inflammation, and function of the growth hormone axis. At the age of six-months, the variance of these biomarkers loaded onto four principal components (PCs), and at 9 month these biomarkers loaded onto three PCs. At 6 months of age, biomarkers of systemic inflammation and growth hormone axis function (IGF-1) loaded onto PC1, fecal MPO and Reg1B (intestinal inflammation and tissue repair, respectively) loaded onto PC2, neopterin (NEO) and serum Reg1B loaded onto PC3 and PC4 respectively. The PCs were similar at 9 months of age, however IGF-1 loaded onto PC2 along with NEO and serum Reg1B, and MPO and fecal Reg1B loaded onto PC3. Table 5 lists the direction (positive or negative) and magnitude of these biomarker loadings and Figure 4 presents these loadings in a three-dimensional space, although the axes are not entirely consistent with the data from Table 5. Table 4 presents Spearman's correlations between biomarker pairs, showing weak correlations between intestinal inflammation (MPO) or repair (GLP-2) with some of the systemic inflammation biomarkers at 6 months and 9 months. Most intestinal inflammation and tissue repair biomarkers were consistently associated with IGF-1, as were biomarkers of systemic inflammation. Based on the results, the investigators proposed that EED influences infant growth (via IGF-1) through both direct and indirect pathways (through systemic inflammation). The proposed mechanistic framework of their hypothesis is

presented in Figure 3. This study suggests that biomarker measurements can be used to predict stunting in children and used to identify individuals who may benefit from intervention. Strangely, while GLP-2 was considered in the analyses related to infant growth, it was not included in PCA analyses.

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2. A Longitudinal Study of Household Water, Sanitation, and Hygiene Characteristics and Environmental Enteropathy Markers in Children Less than 24 Months in Iquitos, Peru.
Exum NG, Lee GO, Olórtegui MP, Yori PP, Salas MS, Trigoso DR, *et al.*
American Journal of Tropical Medicine and Hygiene. 2018 February 12. [Epub ahead of print]
PubMed ID: 29436350

ABSTRACT
Poor child gut health, resulting from a lack of access to an improved toilet or clean water, has been proposed as a biological mechanism underlying child stunting and oral vaccine failure. Characteristics related to household sanitation, water use, and hygiene were measured among a birth cohort of 270 children from peri-urban Iquitos Peru. These children had monthly stool samples and urine samples at four time points and serum samples at (2-4) time points analyzed for biomarkers related to intestinal inflammation and permeability. We found that less storage of fecal matter near the household along with a reliable water connection were associated with reduced inflammation, most prominently the fecal biomarker myeloperoxidase (MPO) (no sanitation facility compared with those with an onsite toilet had -0.43 log MPO, 95% confidence interval [CI]: -0.74, -0.13; and households with an intermittent connection versus those with a continuous supply had +0.36 log MPO, 95% CI: 0.08, 0.63). These results provide preliminary evidence for the hypothesis that children less than 24 months of age living in unsanitary conditions will have elevated gut inflammation.

DOI: 10.4269/ajtmh.17-0464
IMPACT FACTOR: 2.549
CITED HALF-LIFE: >10.0

START COMMENTARY: This is one of several studies published this month with an analysis of data from the MAL-ED birth cohort. This multi-level study used data collected through household surveys during the MAL-ED study in Peru along with data from a community survey on water storage practices to evaluate the associations between WASH measurements and biomarkers for EE. Surveys on WASH specific characteristics of the household were administered at baseline and then every six months through the duration of the study. Water storage practices were assessed through a single community survey administered at baseline. Children who lived in households without a toilet facility in their household had significantly lower levels of fecal MPO compared to children with a flush toilet to a septic tank (Table 4). The authors noted that there is no centralized sewage system in the study site, so even households with a pit latrine have no way to safely empty and treat fecal matter. Additionally, frequent flooding in the area often causes septic storage pits to overflow and spill fecal matter into the environment. Therefore, the authors hypothesized that children without access to a toilet facility in their household were farther away from possible fecal contaminants and therefore had less exposure to enteric pathogens. A limitation of the study is that it did not collect data on fecal matter storage and treatment practices to directly test this hypothesis. However, the findings suggest that simply increasing the number of pit latrines or flush toilets in a community will not necessarily reduce EED if the waste remains in close proximity to the household. Additional care needs to be taken to ensure that fecal matter can be safely exposed of and that good hygiene practices are being practiced. This paper did not look at associations between enteropathogen burden and WASH characteristics, however, as this data was collected, additional analyses may be forthcoming.

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3. The mechanistic link between health and gut microbiota diversity.
   Larsen, OFA & Claassen E.
   Scientific Reports. 8(1). 2018 February 01.
   PubMed ID: 29391457

ABSTRACT
Although numerous reports link a decreased diversity of the gut microbiota to a declined health status, to date no mechanistic motivation for this exists. Here, we show by applying first principles basic graph theory on small networks that higher diversity within such a network indeed leads to more efficient systems and redundancy. Our results quantitatively support earlier hypothetical considerations on gut microbiota richness with respect to these parameters. Our simulations show that higher species diversity leads to higher resilience within small microbiological ecosystems, like being present in the gut microbiota. This notion should provide an ingredient when developing new interventional strategies within the domain of microbiota management.

DOI: 10.1038/s41598-018-20141-6
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2

START COMMENTARY: This paper provides evidence from graph theory estimations that support observations from the field that individuals which more diverse microbiota tend to be healthier than those with less diverse microbiota. The investigators used network theory to evaluate the relationships between microbial guilds: small groups of microbial species that interact with each other via a shared metabolic process(es) which culminates in the production of guild-specific end product(s). For example, one species may produce an intermediate metabolite that is taken up by another species, and converted into another metabolite, etc. The weighted average density is a measure of the energy expended by the gut microbiota to produce the end products. Figure 3 shows that as the number of nodes (microbial guilds) increases, the weighted average density decreases. The investigators suggested that this demonstrates that a more diverse microbial community expends less energy to produce the same end product as a community with fewer guilds. This relationship with functional efficiency and nodes is presented graphically in Figure 4. Figure 4 depicts the authors' hypothesis that there is an area of redundancy that is reached around 6 nodes, in which the presence of additional nodes does not increase the functional efficiency of the microbial community. Simulations such as these will be an important tool to help understand the complex relationships among gut microbiota and how gut microbial diversity and composition is related to health.

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4. Intestinal microbiota development and gestational age in preterm neonates.
   Korpela K, Blakstad EW, Moltu SJ, Strømmen K, Nakstad B, Rønnestad AE, *et al.*
   Scientific Reports. 8(1). 2018 February 06.
   PubMed ID: 29410448

ABSTRACT
The intestinal microbiota is an important contributor to the health of preterm infants, and may be destabilized by a number of environmental factors and treatment modalities. How to promote the development of a healthy microbiota in preterm infants is largely unknown. We collected fecal samples from 45 breastfed preterm very low birth weight (birth weight < 1500 g) infants from birth until 60 days postnatal age to characterize the intestinal microbiota development during the first weeks of life in preterm infants. Fecal microbiota composition was determined by 16S rRNA amplicon sequencing. The main driver of microbiota development was gestational age; antibiotic use had strong but temporary effects and birth mode had little influence. Microbiota development proceeded in four phases indicated by the dominance of *Staphylococcus*, *Enterococcus*, *Enterobacter*, and finally *Bifidobacterium*. The *Enterococcus* phase was only observed among the extremely premature infants and appeared to delay the microbiota succession. The results indicate that hospitalized preterm infants receiving breast milk may develop a normal microbiota resembling that of term infants.

DOI: 10.1038/s41598-018-20827-x
IMPACT FACTOR: 4.259
CITED HALF-LIFE: 2

START COMMENTARY: This study analyzed 262 fecal samples from 45 preterm infants participating in a randomized control trial in Norway. There were 21 infants characterized as extremely premature, and 24 who were moderately/very premature. Figure 1 shows the bacterial composition and dominant organisms found in the stool of the infants over time. In addition to the changing micro-organism composition, investigators found that the microbial diversity and total DNA also increased, as shown in Figure 2. The authors suggested that preterm infants can develop healthy microbiota, as defined by abundance of *Bifidobacterium* species, independent of birth method, if they are breastfed during the first months of life. The authors also observed that relative abundance of *Bifidobacterium* was negatively associated with sepsis. As seen in Figure 5, 10 infants who had sepsis during the study had significantly lower abundance of fecal *Bifidobacterium* compared to infants with no sepsis. A major limitation of the study is a lack of non-exclusively breastfed preterm infants to serve as a control group, and the high reliance on *Bifidobacterium* abundance as a proxy for healthy microbiome formation. Because all enrolled infants were included in a randomized controlled trial and received high-quality hospital-based care for the duration of the study, these findings may not be generalizable to low and middle-income countries where long-term hospitalization of preterm infants may be less available. Additional research is needed to determine if exclusive breastfeeding of premature and very low birthweight infants can improve the gut microbiota composition and decrease morbidity and mortality in less idealized healthcare contexts.

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5. Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling.
   Fuks G, Elgart M, Amir A, Zeisel A, Turnbaugh PJ, Soen Y, & Shental N.
   MIcrobiome. 6(1). 2018 January 26.
   PubMed ID: 29373999

ABSTRACT:
BACKGROUND: Most of our knowledge about the remarkable microbial diversity on Earth comes from sequencing the 16S rRNA gene. The use of next-generation sequencing methods has increased sample number and sequencing depth, but the read length of the most widely used sequencing platforms today is quite short, requiring the researcher to choose a subset of the gene to sequence (typically 16-33% of the total length). Thus, many bacteria may share the same amplified region, and the resolution of profiling is inherently limited. Platforms that offer ultra-long read lengths, whole genome shotgun sequencing approaches, and computational frameworks formerly suggested by us and by others all allow different ways to circumvent this problem yet suffer various shortcomings. There is a need for a simple and low-cost 16S rRNA gene-based profiling approach that harnesses the short read length to provide a much larger coverage of the gene to allow for high resolution, even in harsh conditions of low bacterial biomass and fragmented DNA.
RESULTS: This manuscript suggests Short MUltiple Regions Framework (SMURF), a method to combine sequencing results from different PCR-amplified regions to provide one coherent profiling. The de facto amplicon length is the total length of all amplified regions, thus providing much higher resolution compared to current techniques. Computationally, the method solves a convex optimization problem that allows extremely fast reconstruction and requires only moderate memory. We demonstrate the increase in resolution by in silico simulations and by profiling two mock mixtures and real-world biological samples. Reanalyzing a mock mixture from the Human Microbiome Project achieved about twofold improvement in resolution when combing two independent regions. Using a custom set of six primer pairs spanning about 1200 bp (80%) of the 16S rRNA gene, we were able to achieve ~ 100-fold improvement in resolution compared to a single region, over a mock mixture of common human gut bacterial isolates. Finally, the profiling of a Drosophila melanogaster microbiome using the set of six primer pairs provided a ~ 100-fold increase in resolution and thus enabling efficient downstream analysis.
CONCLUSIONS: SMURF enables the identification of near full-length 16S rRNA gene sequences in microbial communities, having resolution superior compared to current techniques. It may be applied to standard sample preparation protocols with very little modifications. SMURF also paves the way to high-resolution profiling of low-biomass and fragmented DNA, e.g., in the case of formalin-fixed and paraffin-embedded samples, fossil-derived DNA, or DNA exposed to other degrading conditions. The approach is not restricted to combining amplicons of the 16S rRNA gene and may be applied to any set of amplicons, e.g., in multilocus sequence typing (MLST).

DOI: 10.1186/s40168-017-0396-x
IMPACT FACTOR: 8.496
CITED HALF-LIFE: 2.4

START COMMENTARY: This paper proposes the use of a new novel technique, SMURF, to allow high-resolution profiling of the gut microbiota. Figure 1 presents a schematic of the SMURF process, which independently amplifies several sequencing regions along the 16s rRNA gene and then computationally combines them to estimate the microbial diversity. The authors suggest six primary advantages to this technique including (i) using standard sample preparation and easily available primers; (ii) allowing the

use of sub-optimal primers to improve the universality of the primers; (iii) ability to sequence highly fragmented DNA; (iv) averaging bias to particular bacteria by reconstruction small segments based on several amplified regions; and (v) decreasing the computational time required with long-region methods. The technique was first tested with computer simulations and then in mock mixture-sample preparations of ten and then 21 bacterial strains. A limitation of the study is the lack of validation of the technique in human samples.

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6. Enteroaggregative Escherichia coli Subclinical Infection and Coinfections and Impaired Child Growth in the MAL-ED Cohort Study.
   Lima AAM, Soares AM, Filho JQS, Havt A, Lima IFN, Lima NL, *et al.*
   Journal of Pediatric Gastroenterology and Nutrition. 66(2). 2018 February.
   PubMed ID.

ABSTRACT
OBJECTIVE: We evaluated the impact of subclinical enteroaggregative Escherichia coli (EAEC) infection alone and in combination with other pathogens in the first 6 months of life on child growth.
METHODS: Non-diarrheal samples from 1684 children across 8 Multisite Birth Cohort Study, Malnutrition and Enteric Diseases (MAL-ED) sites in Asia, Africa, and Latin America were tested monthly; more than 90% of children were followed-up twice weekly for the first 6 months of life.
RESULTS: Children with subclinical EAEC infection did not show altered growth between enrollment and 6 months. Conversely, EAEC coinfection with any other pathogen was negatively associated with delta weight-for-length ($P < 0.05$) and weight-for-age ($P > 0.05$) z scores between 0 and 6 months. The presence of 2 or more pathogens without EAEC was not significantly associated with delta weight-for-length and weight-for-age. The most frequent EAEC coinfections included Campylobacter spp, heat-labile toxin-producing enterotoxigenic E coli, Cryptosporidium spp, and atypical enteropathogenic E coli. Myeloperoxidase levels were increased with EAEC coinfection ($P < 0.05$). EAEC pathogen codetection was associated with lower neopterin levels compared to those of no-pathogen control children ($P < 0.05$). Mothers of children with EAEC coinfections had lower levels of education, poorer hygiene and sanitation, lower socioeconomic status, and lower breast-feeding rates compared to mothers of children in whom no pathogen was detected ($P < 0.05$).
CONCLUSIONS: These data emphasize the public health importance of subclinical EAEC infection in early infancy in association with other pathogens and the need for improved maternal and child care, hygiene, sanitation, and socioeconomic factors.

DOI: 10.1097/MPG.0000000000001717
IMPACT FACTOR: 2.799
CITED HALF-LIFE: 7.3

START COMMENTARY: This is one of several articles published this month from the MAL-ED birth cohort. This study analyzed non-diarrheal stool samples collected from 1684 infants at household visits during the first 6 months of life. *E. coli* was detected in samples using PCR. Gut function was evaluated using the lactulose: mannitol test when infants were 3 and 6 months of age. In addition, fecal alpha-1-antitrypsin (A1AT), MPO, and NEO were measured monthly. Infants were divided into 7 groups based on the enteric pathogens detected in their stool. Compared to the group with no pathogens, MPO levels were significantly elevated in infants in whom EAEC was co-detected with at least 2 other pathogens. Conversely, median NEO was significantly lower in such infants compared to the no-pathogen group. Median NEO levels in the EAEC plus 3 other pathogens group was significantly lower than all other groups with the exception of those infants with 3 or more pathogens detected without EAEC. The results of this study suggest that coinfection with enteric pathogens may result in growth impairment in young infants. As all stool samples in this study were from seemingly asymptomatic infants, the results highlight the need for interventions and treatments that target all children in communities at high risk for enteric infections, and not just those who present with diarrhea.

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7. High SMAD7 and p-SMAD2,3 expression is associated with Environmental Enteropathy in children.
   Syed S, Dinallo V, Iqbal NT, Di Iorio L, Di Fusco D, Guleria S, *et al.*
   PLoS Neglected Tropical Diseases. 12(2). 2018 February 07.
   PubMed ID: 29415065

ABSTRACT
Enteropathies such as Crohn's disease are associated with enteric inflammation characterized by impaired TGF-β signaling, decreased expression of phosphorylated (p)-SMAD2,3 and increased expression of SMAD7 (an inhibitor of SMAD3 phosphorylation). Environmental enteropathy (EE) is an acquired inflammatory disease of the small intestine (SI), which is associated with linear growth disruption, cognitive deficits, and reduced oral vaccine responsiveness in children <5 y in resource-poor countries. We aimed to characterize EE inflammatory pathways by determining SMAD7 and p-SMAD2,3 levels (using Western blotting) in EE duodenal biopsies (N = 19 children, 7 from Pakistan, 12 from Zambia) and comparing these with healthy controls (Ctl) and celiac disease (CD) patients from Italy. Densitometric analysis of immunoblots showed that EE SI biopsies expressed higher levels of both SMAD7 (mean±SD in arbitrary units [a.u.], Ctl = 0.47±0.20 a.u., EE = 1.13±0.25 a.u., p-value = 0.03) and p-SMAD2,3 (mean±SD, Ctl = 0.38±0.14 a.u., EE = 0.60±0.10 a.u., p-value = 0.03). immunohistochemistry showed that, in EE, SMAD7 is expressed in both the epithelium and in mononuclear cells of the lamina propria (LP). In contrast, p-SMAD3 in EE is expressed much more prominently in epithelial cells than in the LP. The high SMAD7 immunoreactivity and lack of p-SMAD2,3 expression in the LP suggests defective TGF-β signaling in the LP in EE similar to a previously reported SMAD7-mediated inflammatory pathway in refractory CD and Crohn's disease. However, Western blot densitometry showed elevated p-SMAD2,3 levels in EE, possibly suggesting a different inflammatory pathway than Crohn's disease but more likely reflecting cumulative protein expression from across all compartments of the mucosa as opposed to the LP alone. Further studies are needed to substantiate these preliminary results and to illustrate the relationship between SMAD proteins, TGF-β signaling, and inflammatory cytokine production, all of which may be potential therapeutic targets.

DOI: 10.1371/journal.pntd.0006224
IMPACT FACTOR: 3.834
CITED HALF-LIFE: 4.253

START COMMENTARY: This study investigated possible biochemical pathways that lead to inflammation in 19 children with environmental enteropathy (EE), 7 of whom were subjects in a Pakistani prospective birth cohort, and 12 children from the malnutrition ward of the University Teaching Hospital in Lusaka, Zambia. The results are novel in part because investigators used small-gut specific inflammation data from the analysis of duodenal tissue, rather than gut biomarkers assessed in stool samples. As shown in Figure 2, there was no significant difference in mean TGF-β levels between EE patients and controls. The authors hypothesized that these results suggest the inflammation caused by EE is not a result of a lack of TGF-β synthesis, which controls inflammation. However, the study is limited by its small sample size, and the great variability of TGF- β expression in the EE subjects. Additional insights could be made in the future, should the authors compare these data to the infants' enteropathogen data and non-invasive stool and serum EE biomarker data (e.g. GLP-2, MPO, etc.).

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8. *Bifidobacterium longum* BB536 alleviated upper respiratory illnesses and modulated gut microbiota profiles in Malaysian pre-school children.
Lau AS, Yanagisawa N, Hor YY, Lew LC, Ong JS, Chuah LO, *et al.*
Beneficial Microbes. 9(1). 2018 January 29.
PubMed ID: 29065707

ABSTRACT:
This 10-months randomised, double-blind, parallel and placebo-controlled study evaluated the effects of *Bifidobacterium longum* BB536 on diarrhoea and/or upper respiratory illnesses in 520 healthy Malaysian pre-school children aged 2-6 years old. The subjects randomly received a one-gram sachet containing either BB536 (5×109 cfu) or placebo daily. Data analysis was performed on 219 subjects who fully complied over 10-months (placebo n=110, BB536 n=109). While BB536 did not exert significant effects against diarrhoea in children, Poisson regression with generalised estimating equations model indicated significant intergroup difference in the mean number of times of respiratory illnesses over 10 months. The duration of sore throat was reduced by 46% (P=0.018), with marginal reduction for duration of fever (reduced by 27%, P=0.084), runny nose (reduced by 15%, P=0.087) and cough (reduced by 16%, P=0.087) as compared to the placebo. Principal coordinate analysis at genus level of the gut microbiota revealed significant differences between 0 and 10 months in the BB536 group (P<0.01) but not in placebo group (P>0.05). The abundance of the genus *Faecalibacterium* which is associated with anti-inflammatory and immuno-modulatory properties was significantly higher in the BB536 group (P<0.05) compared to the placebo group. Altogether, our present study illustrated the potential protective effects of BB536 against upper respiratory illnesses in pre-school Malaysian children, with gut microbiota modulating properties.

DOI: 10.3920/BM2017.0063
IMPACT FACTOR: 2.923
CITED HALF-LIFE: 2.7

START COMMENTARY: This is one of several studies that have evaluated the potential impact of probiotic supplementation on gut composition and health outcomes in children. While several previous studies have looked at supplementation in infants, this study focused on young children. Monthly health questionnaires were given to care takers and asked about respiratory and gastrointestinal disease in the previous month. In addition, stool samples were collected from children at baseline and the end of the study period (month 10). Only children who had all health questionnaires completed were included in the analysis. Even fewer children, 116, provided fecal samples at both time points and were included in the comparison of gut microbial composition. The low level of compliance (20% of children had missing outcome data) is a major limitation of the study and may have reduced its ability to detect additional differences in outcomes between the two arms. As seen in Table 6, there appear to be larger differences in the median bacterial genus proportions in the fecal microbiota between the two arms at month 10 compared to baseline. However, the relatively small sample size made it difficult to determine if such differences were significant. The authors also noted that the period prevalence of diarrhea in the study population overall was low, which may have factored into the non-significant difference in diarrhea period prevalence. Additional studies with larger sample sizes are needed to further demonstrate the potential benefits of probiotic therapy with this species of *Bifidobacterium longum*.

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9. Environmental enteric dysfunction pathways and child stunting: A systematic review.
Harper KM, Mutasa M, Prendergast AJ, Humphrey J, & Manges AR.
PLoS Neglected Tropical Diseases. 12(1). 2018 January 19.
PubMed ID: 29351288

ABSTRACT
BACKGROUND: Environmental enteric dysfunction (EED) is commonly defined as an acquired subclinical disorder of the small intestine, characterized by villous atrophy and crypt hyperplasia. EED has been proposed to underlie stunted growth among children in developing countries. A collection of biomarkers, organized into distinct domains, has been used to measure different aspects of EED. Here, we examine whether these hypothesized relationships, among EED domains and between each domain and stunting, are supported by data from recent studies.
METHODOLOGY: A systematic literature search was conducted using PubMed, MEDLINE, EMBASE, Web of Science, and CINAHL between January 1, 2010 and April 20, 2017. Information on study objective, design, population, location, biomarkers, and results were recorded, as well as qualitative and quantitative definitions of EED. Biomarkers were organized into five EED domains, and the number of studies that support or do not support relationships among domains and between each domain with stunting were summarized.
RESULTS: There was little evidence to support the pathway from intestinal permeability to microbial translocation and from microbial translocation to stunting, but stronger support existed for the link between intestinal inflammation and systemic inflammation and for intestinal inflammation and stunting. There was conflicting evidence for the pathways from intestinal damage to intestinal permeability and intestinal damage to stunting.
CONCLUSIONS: These results suggest that certain EED biomarkers may require reconsideration, particularly those most difficult to measure, such as microbial translocation and intestinal permeability. We discuss several issues with currently used biomarkers and recommend further analysis of pathogen-induced changes to the intestinal microbiota as a pathway leading to stunting.

DOI: 10.1371/journal.pntd.0006205
IMPACT FACTOR: 3.834
CITED HALF-LIFE: 4.253

START COMMENTARY: This systematic review summarizes evidence related to EED biomarkers and respective domains published over the past 7 years. Table 1 highlights observations that support and do not support an association between the different EED biomarker domains, and Table 2 shows the observations that do and do not provide evidence supporting an association between EED markers (categorized by domain) and stunting. The authors note that a large limitation in the systematic review and EED research is a lack of a consistent definition of EED. Of the studies included in this report, 40% of them used diarrhea as the case definition for EED, but several recent studies have shown there is a large proportion of children with EED who are asymptomatic. There were a few inconsistencies in this review. For example, two articles that report AAT/MPO association were not listed in Table 1 (Becker-Dreps 2017 and Kosek 2013). In the methods section, the authors indicate that biomarker-biomarker associations were highlighted only if they were in different domains, but an association between calprotectin with MPO was highlighted in table 1 (both are markers of intestinal inflammation). The "Kosek score" is listed as a marker of intestinal inflammation, but it is a mixed marker of both intestinal inflammation and permeability (incorporates AAT). Finally, it is not clear why malabsorption and permeability were included in the same domain as they are different processes. This meta-analysis presents the universe of relatively recent evidence surrounding EED, and highlights the need to create a

standardized definition for EED in order to move the field forward. The authors encouraged researchers to develop a standardized set of biomarkers when studying EED in order to better understand the possible relationships between EED domains, infectious disease risk, and health outcomes such as stunting.

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10. Relationships among Common Illness Symptoms and the Protective Effect of Breastfeeding in Early Childhood in MAL-ED: An Eight-Country Cohort Study.
Richard SA, McCormick BJJ, Seidman JC, Rasmussen Z, Kosek MN, Rogawski ET, *et al.*
The American Journal of Tropical Medicine and Hygiene. 2018 January 29. [Epub ahead of print]
PubMed ID: 29380724

ABSTRACT
Children in low-income countries experience multiple illness symptoms in early childhood. Breastfeeding is protective against diarrhea and respiratory infections, and these illnesses are thought to be risk factors of one another, but these relationships have not been explored simultaneously. In the eight-site MAL-ED study, 1,731 infants were enrolled near birth and followed for 2 years. We collected symptoms and diet information through twice-weekly household visits. Poisson regression was used to determine if recent illness history was associated with incidence of diarrhea or acute lower respiratory infections (ALRI), accounting for exclusive breastfeeding. Recent diarrhea was associated with higher risk of incident diarrhea after the first 6 months of life (relative risk [RR] 1.10, 95% confidence interval [CI] 1.04, 1.16) and with higher risk of incident ALRI in the 3- to 5-month period (RR 1.23, 95% CI 1.03, 1.47). Fever was a consistent risk factor for both diarrhea and ALRI. Exclusive breastfeeding 0-6 months was protective against diarrhea (0-2 months: RR 0.39, 95% CI 0.32, 0.49; 3-5 months: RR 0.83, 95% CI 0.75, 0.93) and ALRI (3-5 months: RR 0.81, 95% CI 0.68, 0.98). Children with recent illness who were exclusively breastfed were half as likely as those not exclusively breastfed to experience diarrhea in the first 3 months of life. Recent illness was associated with greater risk of new illness, causing illnesses to cluster within children, indicating that specific illness-prevention programs may have benefits for preventing other childhood illnesses. The results also underscore the importance of exclusive breastfeeding in the first 6 months of life for disease prevention.

DOI: 10.4269/ajtmh.17-0457
IMPACT FACTOR: 2.549
CITED HALF-LIFE: >10.0

START COMMENTARY: This is one of several MAL-ED birth cohort papers published this month. For this analysis, data on illness and breastfeeding during the first 2 years of life was analyzed. Due to the relatively small number of children who were truly exclusively breastfed in the study (only given breastmilk and nothing else), the authors defined exclusive breastfeeding as any child who was exclusively breastfed for at least 50% of the time during the previous 30 days. This is a limitation of the study that may bias the results and attenuate the estimate of the protective effect of exclusive breastfeeding; children who are only breastfed 50% of the time may not have the full benefits of breastfeeding and therefore have less protective effects and more illness. By including them in the exclusively breastfed category, it may spuriously appear that breastfeeding is less effective than it truly is. While the study looked at associations between diarrhea and breastfeeding, authors did not assess whether breastfeeding protects against infection with specific enteric pathogens. Figure 2 shows the prevalence of diarrhea, ALRI, and fever during the first 24 months of life in children enrolled in the 8 country cohorts. Pakistan, which reported the lowest amount of exclusive breastfeeding (0.4%), had the highest prevalence of all three diseases. However, South Africa and Tanzania, two other sites with low amounts of exclusive breastfeeding (2% and 10%, respectively), have markedly lower disease prevalence, suggesting that the relationship between breastfeeding and disease is complex and needs to be further studied.

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