Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby gran to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

Calbeth Alaribe	Date

Examining the Underlying Role of Environmental Enteric Dysfunction (EED) in Childhood Stunting and Recommending a Diagnostic Method to Improve Detection in Children Living in Developing Countries

By

Calbeth Alaribe
Master of Public Health

Hubert Department of Global Health

_____

Joanne McGriff, MD, MPH
Committee Chair

Examining the Underlying Role of Environmental Enteric Dysfunction (EED) in Childhood Stunting and Recommending a Diagnostic Method to Improve Detection in Children Living in Developing Countries

By

Calbeth Alaribe
BS
University of Nevada, Reno
2015

Thesis Committee Chair: Joanne McGriff, MD, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
In Global Health
2018

Abstract

Examining the Underlying Role of Environmental Enteric Dysfunction (EED) in Childhood Stunting and Recommending a Diagnostic Method to Improve Detection in Children Living in Developing Countries

By Calbeth Alaribe

Background: Childhood stunting is a global health problem affecting children under the age of five years old living in low and middle-income countries (LMIC). The pathophysiology of childhood stunting has been poorly understood for many years and now new studies are suggesting that Environmental Enteric Dysfunction (EED), which impacts the structure and function of the small intestines, may be associated with stunting or failed linear growth. Presently, there is no specific and well-validated diagnostic or detection criteria available to identify and diagnosis EED in children.

Purpose: The purpose of this scoping review is to examine diagnostic testing modalities that are available to detect EED against criteria that includes: study design, social and political context of applying the diagnostic method in the target population, healthcare infrastructure in the community of the target population, cost, ethics, and scalability. The review concludes with a recommendation for the most suitable diagnostic test for children under the age of five years living in low resource settings with inadequate water supply, poor sanitation and hygiene services, high prevalence rates of infectious diseases, and poor healthcare system infrastructure.

Methods: Peer reviewed papers were generated from three databases based on search terms that included "environmental enteric dysfunction," "clinical marker" and "diagnosis". Inclusion criteria included articles that were written in English and discussed a diagnostic or detection test that determined if a child under the age of five years old living in a LMIC, was at risk for developing EED or diagnosing a child with full-blown EED. Exclusion criteria included studies that discussed the association between EED and stunting without incorporating diagnostic methods.

Results: A total of 43 articles were identified for the scoping review after searching the three databases. Thirty-four articles were excluded from the review because they were duplicates; or they were not studies that discussed diagnostic methods for at risk children LMICs; or because they were articles that did not discuss diagnostic methods to detect EED. Nine articles were included in the scoping review. Diagnostic methods that were identified included biomarkers (fecal markers), lactulose mannitol (L:M) ratio, plasma tryptophan, bile acids, and optical biopsies.

Discussion and Recommendation: Based on the analysis of the nine articles against criteria, fecal mRNAs transcript testing could be utilized to determine if a child is at risk for developing EED or for diagnosing a child with full-blown EED. Although further research is needed to determine the validity and reliability of mRNA transcripts, it could serve as a promising non-invasive detection test to diagnose EED in children within low resource settings.

Examining the Underlying Role of Environmental Enteric Dysfunction (EED) in Childhood Stunting and Recommending a Diagnostic Method to Improve Detection in Children Living in Developing Countries

By

Calbeth Alaribe

BS
University of Nevada, Reno
2015

Thesis Committee Chair: Joanne McGriff, MD, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
In Global Health
2018

Table of Contents

Background

Stunting

Childhood stunting, known as growth faltering or linear growth failure, is considered "one of the most significant impediments to human development" [1]. It is a global health problem affecting children under the age of five years old (0-60 months) living in low and middle-income countries (LMICs). Stunting or "being too short for one's age" occurs when a child's height-for-age scores are more than two standard deviations (Z score <-2) below the World Health Organization (WHO) Child Growth Standards median [2].

Although the number of stunted children has declined from 198 million to 158 million from the years 2000-2016, this condition continues to be problematic in developing countries, and the number of stunted children is declining at a very slow rate [3]. In 2016, less than 1 in 4 children globally had stunted growth (22.9%), and out of those stunted children, 1 in 2 lived in South Asia and 1 in 3 lived in Sub-Saharan Africa [3]. Specifically, 35.8% of children in South Asia, 34.4% of children in Eastern and South Africa, and 33.5% of children in West and Central Africa are stunted. These prevalence rates are between 30-39%, making this of high public health significance according to WHO's 1995 Growth Standard's cut-off values for assessing the severity of stunting by prevalence [4]. The percentages in these regions are also substantial: 15.3% of children in the Middle East and North Africa, 9.3% of children in East Asia and the Pacific, and 2.3% of children in North America have stunted growth [3]. Alarmingly, the number of stunted children has increased over time in all regions of Africa from 50.4 million in 2000 to 59 million in 2016 [5]. Western Africa constitutes the highest occurrence of stunting in Africa, where there were 4 million more stunted children in 2016 than in 2000 [5]. Taking gender into consideration globally, males and females are equally likely to be stunted [6]. However, in sub-Saharan Africa, stunting affects males more (42%) than females (36%) [6].

In regards to income level, less than half of children under the age of 5 live in a lower-middle income country, and two-thirds of those children are stunted (Figure 1) [5]. This means that approximately 66% of all stunted children in the world, live in lower-middle income countries (Figure 2) [5].

Figure 1: Distribution of Children Under the Age of 5 in the world, By Country Income Grouping, 2016. Source: Hayashi, C., et al., *Levels and trends in child malnutrition. UNICEF/WHO/World Bank Group joint child malnutrition estimates: key findings of the 2017 edition.* 2017 [5].

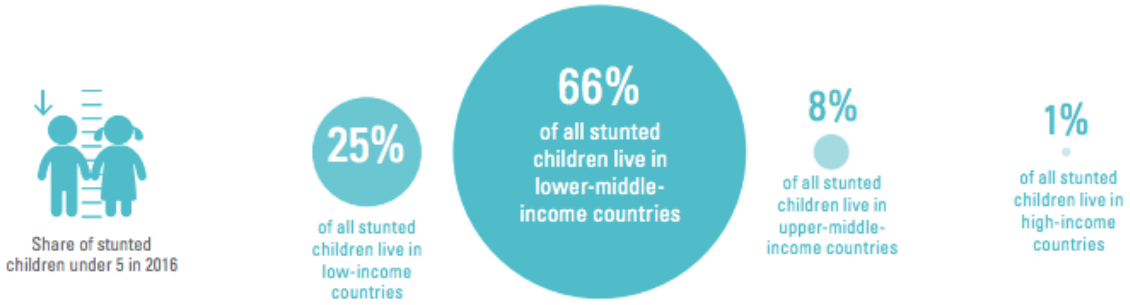

Figure 2: Distribution of Children Under 5 Affected by Stunting Old. Source: Hayashi, C., et al., *Levels and trends in child malnutrition. UNICEF/WHO/World Bank Group joint child malnutrition estimates: key findings of the 2017 edition.* 2017 [5].

Low-income countries have also seen an increase in the number of stunted children. From 2000 to 2016, low-income countries had a 7% increase in the number of stunted children (35.2 million to 37.7 million), while upper-middle-income countries had a 61% decrease (33.4 million to 12.9 million) and high-income countries had nearly a 25% decrease (2.2 million to 1.6 million) [5].

The impact of stunting has short- and long-term negative effects, and can be detrimental to the growth of a child and hinders them from reaching their full potential as they become adults. Adverse outcomes include in underdeveloped brain and irreversible damage, which causes decreased cognitive ability and decreased school performance [7]. A 2008 maternal and child undernutrition study reviewed cohort studies that followed stunted children from late adolescence to adulthood from LMICs such as Brazil, Philippines, South Africa, India, and Guatemala. The study concluded that being stunted at 24 months reduced school attendance by 0.9 years, delayed school enrollment, and increased the risk of failing at least one grade in school by 16% after controlling for confounding variables such as socioeconomic status and sex [8]. In a cross-sectional household study in Brazil, they found that height may be directly related to one's productivity and earnings [9]. In another article by UNICEF Executive Director, Anthony Lake, it is stated that individuals who are stunted have a 22% decrease in earning potential [10].

Child mortality is considered to be one of the best measures of the wellbeing and health of a nation and stunting plays a major factor in childhood mortality in developing countries [11]. Stunting costs developing countries billions of dollars in future economic revenue as a result of decreased productivity and more days away from work due to illness [9]. The World Bank estimates that malnutrition reduces a country's GDP by up to 11% in Africa and Asia each year [12]. Additionally, GDP and childhood mortality are associated. A 2013 systematic review and meta-analysis aimed to quantify the correlation between a country's national income and the mortality of infants and children under five in developing countries and concluded that income is in important factor in a child's survival rate [11]. All in all, undernutrition during childhood increases a child's risk for mortality and can also negatively impact a country's GDP.

Stunting is a subcategory of undernutrition, and undernutrition accounts for nearly half of all deaths in children below the age of five in developing countries [6] due to infections such as pneumonia, diarrhea, malaria, HIV and AIDS, and measles [6]. Because stunted children are nutritionally-deprived their immune function declines [13]. In a systematic review that explored immune function in children with malnutrition, it was discovered that malnutrition is correlated with the decrease in exocrine secretion of protective substances that combat infectious diseases, atrophy of the thymus, and impaired function of the gut-barrier [13]. This decreased immune status puts them at risk for the development, exacerbation, and increased duration of infections. A study among pre-school children in rural Northern Nigeria found that children who were malnourished had longer episodes of a diarrhea [13]. Children who were underweight, had a 33% longer duration in diarrhea episodes and children who were stunted had a 37% longer duration in comparison to children who were not malnourished [14]. Moreover, according to a 2008 multi-country analysis of the effects of diarrhea on childhood stunting, children at 24 months of age have greater odds of being stunted with each diarrheal episode and day of illness [15].

Studies have also shown that malnutrition and infection increases children's mortality risk. In a 1997 cohort study of children in the Philippines from birth to 24 months, data suggested that malnutrition is associated with increased mortality from pneumonia and lower respiratory infections [16]. A longitudinal study was conducted following undernourished children and their risk from dying from pneumonia and diarrhea. Data was gathered from children between the ages of 18 and 24 months old in Bangladesh, Brazil, Guinea-Bissau, Ghana, India, Nepal, Senegal, and the Philippines, and it concluded that stunted and severely undernourished children had a 4.6 greater odds of dying from diarrhea and 3.2 greater odds of

dying from pneumonia than well-nourished children in the selected countries (Figure 3) [17].

	Odds ratio			
	Severe undernutrition	Moderate undernutrition	Mild undernutrition	No undernutrition
Underweight				
Diarrhoea	9.5	3.4	2.1	1.0
Pneumonia	6.4	1.3	1.2	1.0
Stunting				
Diarrhoea	4.6	1.6	1.2	1.0
Pneumonia	3.2	1.3	1.0	1.0
Wasting				
Diarrhoea	6.3	2.9	1.2	1.0
Pneumonia	8.7	4.2	1.6	1.0

* Bangladesh, Ghana, Guinea-Bissau, India, Nepal, Pakistan, the Philippines and Senegal.

Source: Adapted from Black et al., 'Maternal and Child Undernutrition: Global and regional exposures and health consequences', *Lancet*, vol. 371, no. 9608, 19 January 2008, pp. 243–260.

Figure 3: Odds Ratio Among Undernourished Children Under 5 Years Old Dying from Diarrhea or Pneumonia. Source: United Nations Children's Funds (UNICEF) [17].

Further, as stunted children become older, they may have an increased risk of developing nutrition-related chronic diseases such as hypertension. A study with Jamaican children found that stunted children between the ages of 9 to 24 months may have an increased risk of developing elevated systolic blood pressure by the time they are 7-8 years of age [18]. Stunting, which is an indicator of nutritional deprivation, may also be linked to developing diabetes later in life [19].

Many causes of childhood stunting include poor nutrition during the first 1,000 days of a child's life [3], non-exclusive breastfeeding, complementary feeding limited in micronutrient quality and quantity, poor hygiene and inadequate sanitation that lead to contracting infectious diseases, and subclinical diseases Figure 4 [1].

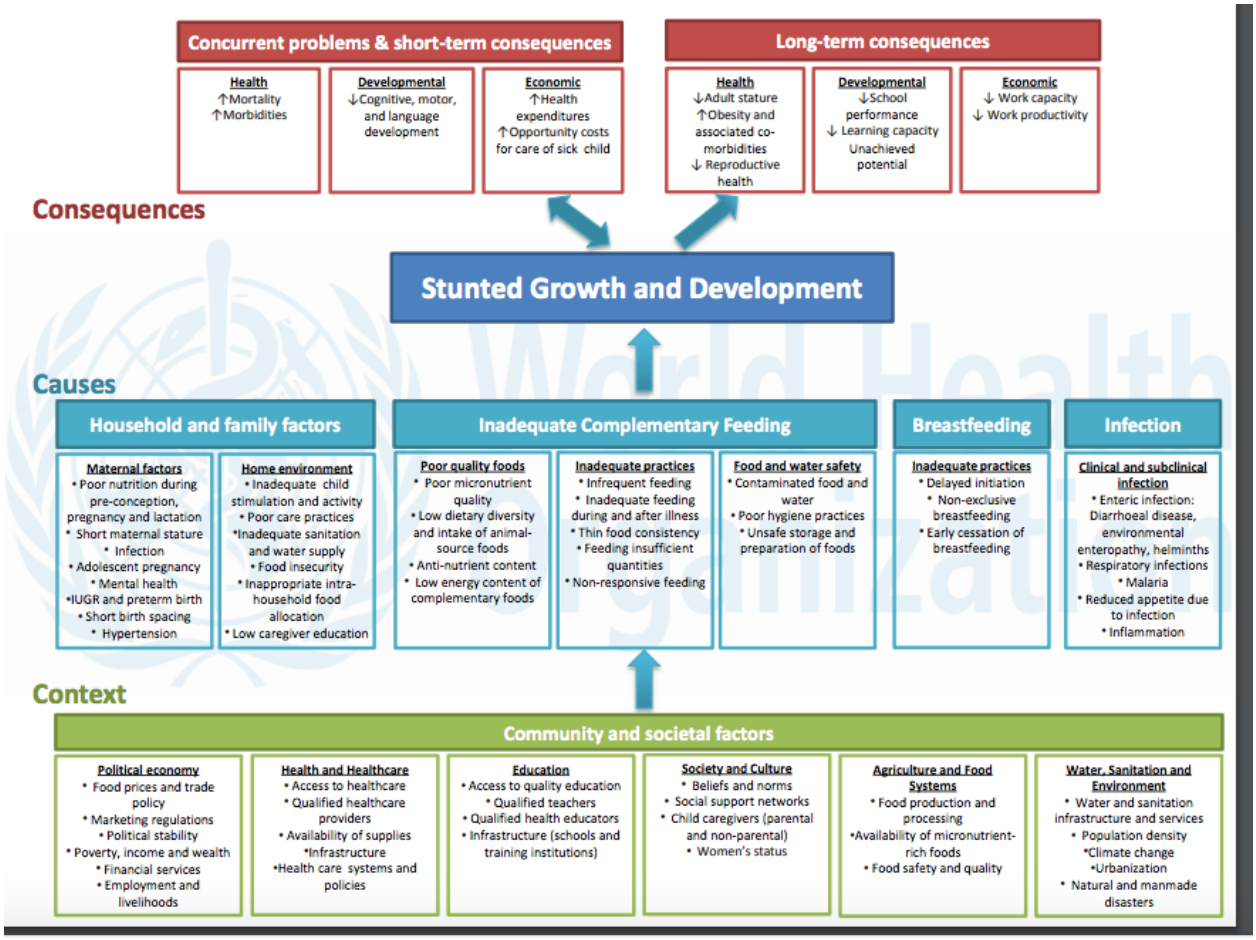

Figure 4: WHO Conceptual Framework on Stunting: Context, Causes and Consequences.

Source: *Contextualising complementary feeding in a broader framework for stunting prevention.* Maternal & child nutrition, 2013 [7].

In 2012, the World Health Assembly Resolution developed a comprehensive plan known as the WHO Global Nutrition Target: Stunting Policy Brief, that included expanding micronutrient supplementation, infant and young child feeding (IYCF), and hygiene and sanitation initiatives to combat these causes of stunting [1]. Breastfeeding is an important source of nutrients and since 2001 the WHO has recommended exclusive breastfeeding for the first 6 months of life and after, complementary foods and breastfeeding till the age of 2 years [20]. Globally, less than 40% of infants are exclusively breastfed and 32% of infants less than 6 months old in sub-Saharan Africa are exclusively breastfed [6]. Similarly, 60% of children between the ages of 6-8 months around the world receive nutritious semi-solid or soft foods [6]. The IYCF initiative's goals were to promote exclusive breastfeeding for the first six months of life and up to the age of 2 and beyond; with the addition of encouraging semi-solid and soft age-appropriate foods at the beginning of 6 months of age [6]. Significant components of the IYCF strategy were communicating with communities for behavioral and social change in regards to breastfeeding, development of curriculum for IYCF, support groups for mothers to discuss the importance of breastfeeding, and the establishment of community-based IYCF counseling services [6]. The initiative also included programs to increase the delivery of micronutrient supplements such as vitamin A and iron supplementation, and the fortification of complementary foods using multiple micronutrient powders (MNPs) [6].

The Stunting Policy Brief also addressed how infectious diseases due to poor water quality and hygienic conditions increases a child's risk for stunting [21]. The framework for action to improve water, sanitation, and hygiene practices (WASH) to reduce the prevalence of stunting included improving household practices of hand hygiene with soap, and increasing access to safe water and affordability to soap [22]. It was estimated that hygiene interventions

with 99% coverage would decrease diarrheal episodes in children by 30% and as a result, lead to a 2.5% reduction in stunting [23]. Examples of WASH initiatives that were implemented were expansion of water and sanitation services in Brazil and in Bolivia [22]. By developing micronutrient supplementation, breastfeeding and complementary feeding promotion, and WASH initiatives, the proposed brief sought to reduce the percentage of stunted children under the age of 5 years old to 40% [1] or about 70 million children by 2025 [6].

Although there have been successful aspects of the proposal in reducing the number of children that are stunted globally such as the micronutrient interventions, the effectiveness of the approach has not clearly met expected outcomes. For instance, micronutrient interventions efforts in addressing child growth is still vague [24]. A 2008 meta-analysis was conducted on the effects of single and multiple micronutrient intervention trials on improving height, length, and weight for children under the age of five-years old and concluded that iron only, zinc only, and vitamin A only micronutrient interventions had no significant effect on childhood stunting [24]. 17 studies were included in the meta-analysis, studying vitamin A only supplementation in 69,320 children between the ages of 5 and 48 months from Asia (11), Africa (4), and Latin America and the Caribbean (2) [24]. Though vitamin A-only supplementation intervention trials showed positive effect sizes for height in comparison to no vitamin A supplementation, the overall weighted mean of the effect size was 0.08 (95% CI: -0.18, 0.34) and considered small and not statistically significant [24]. A total of 27 studies from Asia (16), Africa (3), Latin America and the Caribbean (3), North America (2), and Europe (3), examined iron-only supplementation intervention trials in children between the ages of 1 to 48 months and showed that the weighted mean effect size for height was 0.01 (95% CI: -0.08, 0.10) [24].This illustrates that there was no significant difference in height between children that received iron only

supplementation and children that received a placebo [24]. In regards to zinc-only supplementation intervention trials, 43 studies with children between the mean ages of 0 and 48 months from Asia (15), Latin America and the Caribbean (13), North America and Europe (8), Africa (7) were analyzed [24]. The effect sizes for height in those studies ranged from -0.80 to 1.12 and although 30 of those studies had positive effect sizes, only 11 of them were statistically significant [24]. The overall weighted mean effect size for zinc only supplementation was 0.07 (95% CI: -0.03, 0.17), which indicates that the effect of the supplementation was small and not statistically significant [24].

In the same meta-analysis, there were also studies that evaluated the combination of using two types of micronutrient supplementations in the improvement of a child's height. The weighted effect sizes for height using a combination of iron and zinc was 0.004 (95% CI: -0.21, 0.21), a combination of vitamin A and zinc was 0.10 (95% CI: -0.14, 0.61), and a combination of iron and folic acid was 0.16 (95% CI: -0.05, 0.38) [24]. This would indicate that the height of children using a combination of two types of micronutrient supplements is not significantly different from children not using two forms of micronutrient supplements [24]. Lastly, the meta-analysis analyzed 20 studies that aimed at understanding the effects of the use of multiple micronutrients (3 or more micronutrients) at improving child growth, with 80% of the studies including vitamin A, iron and zinc supplementation [24]. The majority of the studies were conducted in developing countries: 5 in Latin America and the Caribbean, 7 in Africa, and 8 in Asia [24]. The overall mean effect size generated from analysis for height was 0.09 (95% CI: 0.008, 0.17) [24]. When only considering studies that contained at least vitamin A, iron, and zinc the mean effect size was 0.11 (95% CI: 0.02, 0.18) [24]. This information justifies the fact that

the use of supplementation that contains multiple micronutrients has a small impact on improving a child's height.

Another intervention that has been used to prevent childhood stunting is breastfeeding promotion. Although breastfeeding promotion has been effective in improving child mortality rates, it has been very limited in improving childhood stunting [23]. A 2015 systematic review and meta-analysis investigated the impact of breastfeeding promotion interventions on infant nutritional status and based on 35 studies, discovered that these interventions did not have an impactful effect on infant weight or length [25]. The majority of the breastfeeding promotion studies (20) were carried out in a community/outreach settings and took place in majority middle-income countries [25]. Results showed that the pooled mean length/height z-score difference for children notably in the first six months of life, was nonsignificant at 0.03 (95% CI: -0.02, 0.08) [25].

From an environmental perspective, although increased access to water, sanitization, and hygiene (WASH) services, which is part of the Sustainable Development Goals (SDGs), may have potential to reduce stunting, this particular intervention requires additional efforts to achieve universal access [26]. Currently, high coverage of WASH initiatives are inadequate and it will take time to reduce poor water and sanitation globally [27]. It is estimated that 1.8 billion people are using fecal-contaminated water as their primary source of water and due to a high number of people needing WASH services, implementation of hygiene initiatives remain a challenge [16].

In 2018, a cluster-randomized control trial aimed to determine whether WASH and nutrition interventions decreased diarrhea incidence and growth faltering in children in rural Kenya. Results concluded that household upgrades from unimproved latrines to improved

latrines, water treatment, nutrition interventions, and handwashing stations led to small growth benefits in children [28]. The study randomized 1,226 villages and developed 702 clusters that were randomly allocated into 7 groups: a control group, an active group (household visits to measure children's mid-upper-arm circumferences), a passive control group (collecting data without household visits), a group receiving water, sanitation, and handwashing interventions, a group receiving combined water, sanitation, and handwashing interventions, a nutrition-only intervention group, and a combined nutrition, water, sanitation, and handwashing intervention group [28]. Children in the villages were between the ages of 16 and 31 months with a median age of 25 months and were followed for two years to determine if there was increased linear growth as a result of the interventions [28]. At the end of the study, data showed that by the age of 2 years there was no significant improvement in growth in children in the water only, handwashing only, sanitation only or combined water, handwashing, and sanitation group in comparison to the control and control-passive group. Additionally, although there were signs of more improvement in growth in children in the combined water, sanitation, handwashing, and nutrition group versus the control group than in the nutrition only group versus the control group, the differences in growth were of small clinical significance [28].

Many of the interventions used to tackle childhood stunting have not been as effective to reduce the rates. While the World Health Assembly has proposed a plan to reduce global stunting by 40% by 2025, requiring a 3.9% yearly reduction rate [1], it has been projected that at current progress, the target goal would miss its mark and by 2025, there would only be a 26% reduction rate [13]. With this in mind, there would need to be more focus on determining other underlying factors that could potentially contribute to childhood stunting such as, subclinical manifestations. One of those manifestations is known as Environmental Enteric Dysfunction (EED).

Stunting & EED

The pathophysiology of childhood stunting has been poorly understood for many years and now new studies are suggesting that EED, also known as environmental enteropathy, which impacts the structure and function of the small intestines needed for nutrient absorption, may be correlated with childhood linear growth [29]. Currently, EED is not a priority health issue because it does not cause evident symptoms and it can be found in healthy individuals [30]. Although it is unclear whether stunting causes EED or vice versa [31], the connection between EED and stunting is undervalued and should be at the forefront of discussion.

EED

Environmental Enteric Dysfunction (EED), is defined as an asymptomatic and reversible [29] subclinical disorder that impacts the structure and function of the distal small intestine structure and generally affects children that live in low-resource settings who are exposed to pathogens in contaminated water and food [32]. As children are exposed to environmental factors such as poor water and sanitation, it creates an inflammatory response in the small intestine that causes lymphocytes to infiltrate the intestinal epithelium and the lamina propria connective tissue that supports the intestinal epithelium (Figure 5) [32]. Increased intestinal permeability as a result of the inflammatory response causes microbes to enter the bloodstream, causing chronic immune activation (Figure 6) [33]. The inflammatory response that occurs is T-cell mediated and similar to a response that would be seen in someone who has celiac disease [30]. Additionally, the small intestine's villi atrophy, and crypts, which are grooves in between the villi that line the small intestine [34], elongate (hyperplasia) [35]. The small intestine therefore loses its ability to act as a barrier to preventing pathogens and toxins from entering the enteric system and the intestines are unable to absorb nutrients because of intestinal

inflammation [32]. As a result of these circumstances, the body is unable to utilize energy reserves due to lack of nutrients [32]. The limited energy available is used during the inflammatory response to combat microbes and toxins that enter the body [32]. Because there is no available energy for the growth and development of a child, EED is considered a major factor in chronic malnutrition, particularly stunting in children in developing countries [32].

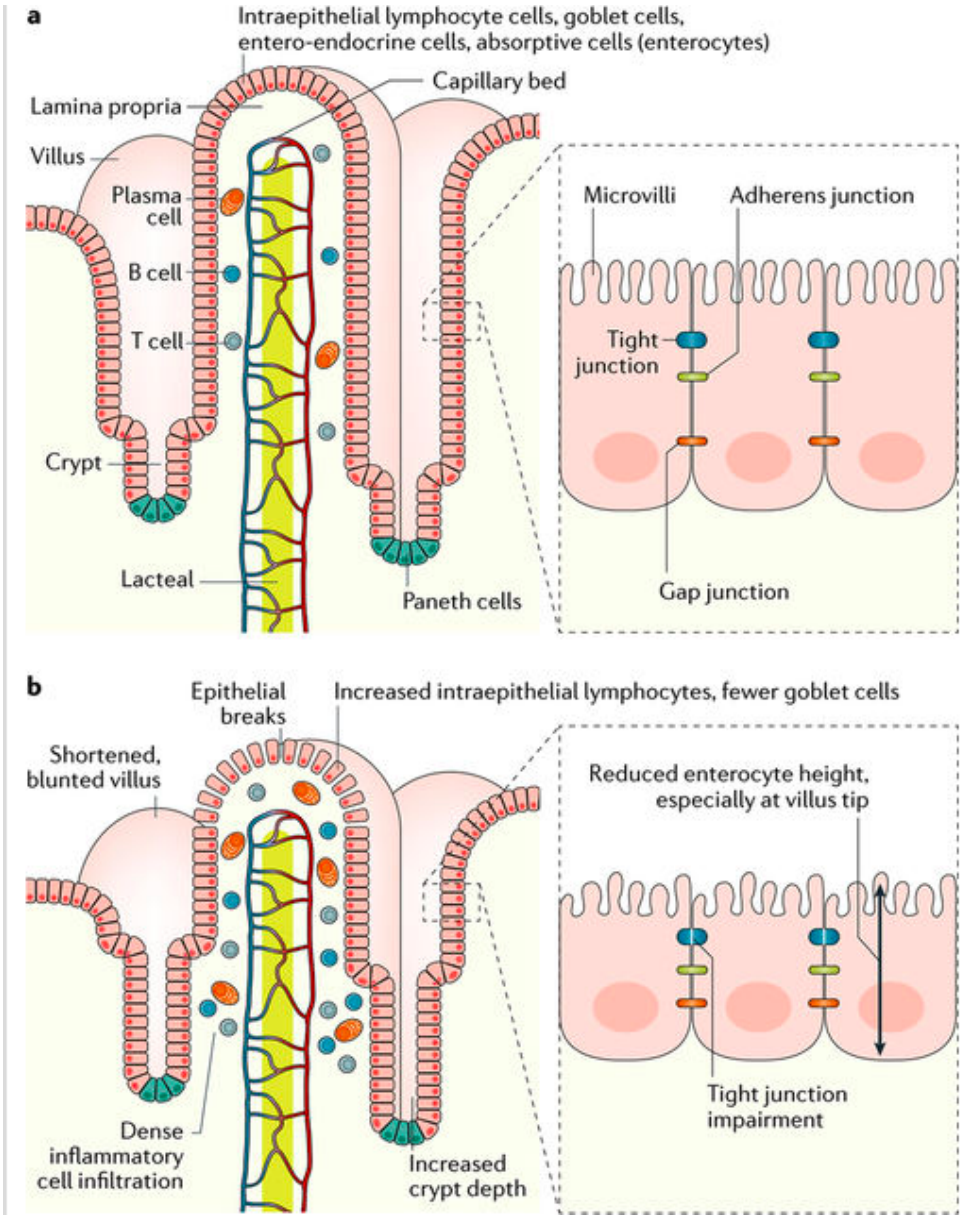

Figure 5: Epithelium of a Healthy Small Intestine (a) and the Epithelium of an Individual with EED. Source: Thompson, A.J., et al., *The potential role of optical biopsy in the study and*

diagnosis of environmental enteric dysfunction. Nature Reviews Gastroenterology & Hepatology, 2017. **14**(12): p. 727-738 [32].

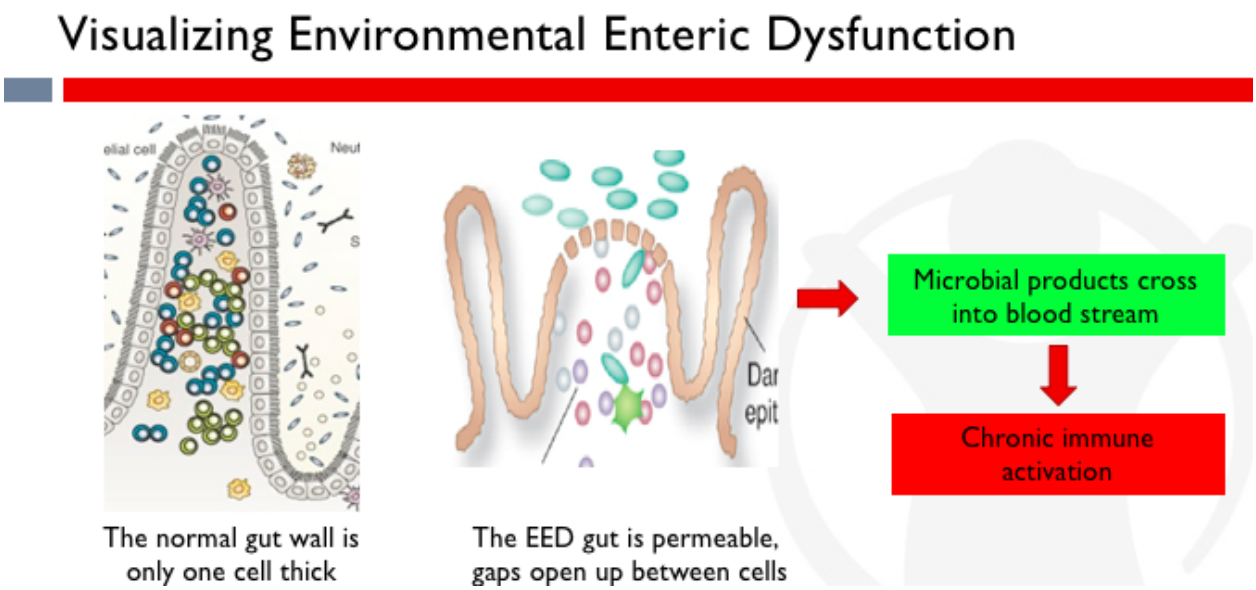

Figure 6: Visualizing Environmental Enteric Dysfunction. Source: Mbuya, Mduduzi, *Environmental Enteric Dysfunction and Child Nutrition: Exploring the Potential Effects of WASH. Presentation. SHINE Study. Istanbul,* 2015 [36].

History of EED

EED was first recognized in adults and is considered a condition of the tropics [31]. It was known as "tropical enteropathy" in the 1960s and was renamed "environmental enteropathy" after it was discovered in the late 2000s that environmental factors played a more significant role in someone developing EED than climate or latitude [31]. It was eventually changed to "environmental enteric dysfunction" [31]. In the 1960s a microscopic observation identified small intestinal villi atrophy leading to a decrease in surface area for absorption of nutrients in adults in low- and middle-income countries [37]. In a 1969 study on the morphology of the jejunum of the small intestine in Ugandan adults, researchers brought attention to what appeared to be convoluted ridges and patterns of the small intestine that was affecting the nutrient

absorption of a small group of Ugandan adults [38]. Similar appearances were also identified in adult populations in South India, Thailand, and Singapore [38].

In 1971, an observational study of 41 US Peace Corps volunteers demonstrated that structural damage of the intestines due to EED could be reversed [39]. For 18 to 24 months, Peace Corps volunteers who were previously living in India or Pakistan were studied after their return to the United States. Study participants included 30 men and 11 women between the ages of 23-33 years old who had previous episodes of diarrhea and weight loss while in India or Pakistan [39]. Further, five of the participants had abnormal jejunal biopsies of rigid and convoluted villi of the small intestine while in Pakistan [39]. Results concluded that out of 31 participants who had previous diarrheal episodes while in the Peace Corps, all them had no subsequent episodes after their return to the U.S. [39]. Additionally, out of the 34 participants who lost weight, 33 of them had regained their weight back approximately one month after their return [39] and participants who had abnormal intestinal biopsies, had biopsies within normal limits in the U.S. [39]. The study indicated that a recovery of the function of the small intestine within 1-2 years of return to the U.S. was possible [39]. This finding implied that EED could result from environmental factors [39].

In the early 1990s, studies showed that EED could occur in infants and children [31]. A longitudinal study was conducted between January 1997 and August 1998 on 73 rural Gambian infants between 8 and 64 weeks of age, and concluded that at 8 weeks post birth, infants were close to normal values in regards to weight but over time the children grew in weight and height at a very slow pace [40]. Their mean weight at 64 weeks was 8.14 ± 0.94 kg and their height was 73.0 ± 2.0 cm, which indicates that although there was growth, it was at a poor rate [40]. Also, there was increased intestinal permeability by one year old ($r=0.44$, $p<0.001$) measured by

increase lactose and decreased mannitol excretion [40]. The study further demonstrated that increased intestinal permeability contributed to 22% of growth failure in the children who began to show intestinal enteropathy after 3 months old , the period when the children began to be introduced to weening of foods [40].

Diagnosing EED

Presently, there is no specific and well-validated diagnostic or detection criteria available to identify and diagnosis EED in children [19]. The most common histopathological analyses used to test for EED include biomarkers of intestinal permeability and barrier function, intestinal and systemic inflammation, and bacterial translocation. Although histopathological analysis of the small intestines are considered the gold standard for diagnosing existing EED [32], there is currently no biological indicator or marker that can identify early bowel dysfunction that may contribute to growth faltering [41].

Despite that fact there is no well-defined diagnostic test, the most frequently used test for EED is the dual sugar absorption test [31]. The lactulose mannitol (L:M) ratio test is the most common compared to other sugar absorption tests such as xylose and rhamnose [31]. The L:M ratio test is used to measure gut absorption and barrier function. Lactulose and mannitol are administered orally and urine samples are subsequently collected for up to six hours [42]. Lactulose, however, is a large disaccharide molecule and is not normally absorbed by the healthy small intestine [42]. On the other hand, mannitol is a smaller monosaccharide that easily passes the healthy intestinal barrier and is found in the urine [42]. Thus, an increased proportion of lactulose compared to that of mannitol in the urine, which is determined by either liquid chromatography or mass spectrometry, is indicative of reduced absorption or impaired intestinal barrier function [42]. Poor linear growth is usually evident between 1-2 years of life and using an

L:M ratio test for diagnostic purposes may be complicated especially for children because the test requires fasting before the procedure and the collection of urine over five hours [30].

There needs to be further research to accurately develop a diagnostic criterion. With an available case definition and effective diagnostic method, interventions for EED could be developed and potentially have an impact on global prevalence rates of childhood stunting.

Research Question/Purpose of the Review

The purpose of this scoping review is to examine available diagnostic testing modalities used to detect EED. The review will analyze the study design of the testing modalities, participant selection, cultural and social context of where the test was applied (i.e. religion, culture, socioeconomic status, political climate of the country, and healthcare infrastructure of the community where the diagnostic/detection test was utilized). Additionally, ethics, the scalability of the intervention, and cost of using the /diagnostic test will be considered. By determining the available diagnostic tests and analyzing them against criteria, the review will recommend the most suitable test for children under the age of five years living in low resource settings with inadequate water supply, poor sanitation and hygiene services, high prevalence rates of infectious diseases, and poor healthcare system infrastructure.

Overall, the scoping review will aim to answer the following questions:

1. What diagnostic or detection methods are available to diagnose EED in children under the age of five?
2. How does each diagnostic/detection test take into consideration:
    a. Study design
    b. Social and political context of applying the test in the community of target population
    c. Healthcare infrastructure of the specific community
    d. Cost-effectiveness and feasibility
    e. Ethics relevant to the target population
    f. Evaluability assessment & scalability

Methods

Inclusion Criteria

The primary inclusion criteria for selecting the peer-reviewed literature included in this scoping review are as follows:

1. Articles must include a discussion on a diagnostic or detection test that is utilized to determine if a child is at risk for developing EED or for diagnosing a child with full-blown EED. Tests can include biomarkers, clinical markers, and biological markers.
2. Diagnostic tests must be used in children under the age of five years old living in upper-middle, lower middle, and low middle-income countries (Appendix 1).
3. Articles must be in English.

Search Terms

Article searches for this scoping review were conducted on the following search engines: PubMed, Embase, and Web of Science. A combination of terms such as “environmental enteric,” “environmental enteric dysfunction,” “environmental enteropathy,” “biological marker,” “clinical marker,” “biomarker,” “diagnosis,” “diagnostic,” “testing,” “test,” “causality,” and “etiology” were used to conduct a search of literature in these databases. The order of the terms that were used to search for literature in each database included:

PubMed Search:

(environmental enteric OR "environmental enteric dysfunction" OR "environmental enteropathy") AND ("Biomarkers"[Mesh] OR biomarker* OR clinical marker* OR biological marker*) AND (diagnosis OR diagnostic OR testing OR test OR causality OR etiology)

Embase and Web of Science:

(environmental enteric OR "environmental enteric dysfunction" OR "environmental enteropathy") AND ('biological marker'/exp OR biomarker* OR clinical marker* OR biological marker*) AND (diagnosis OR diagnostic OR testing OR test OR causality OR etiology)

After generating the articles from the databases based on the search terms, the title of the articles were reviewed to determine if they fit the inclusion criteria. If the articles included any of the search terms above, they were included in the scoping review. In the next phase, the abstracts of the chosen articles were reviewed by this author. Articles that met the inclusion criteria were selected for full-text reading. Lastly, any article that met the inclusion criteria after full text reading, was included in the scoping review. A flow chart demonstrating the inclusion and exclusion pathway is illustrated in Figure 7. A literature summary table for the selected articles that includes title, study design, target population, diagnostic test used in the article, and study results are shown in Appendix 2.

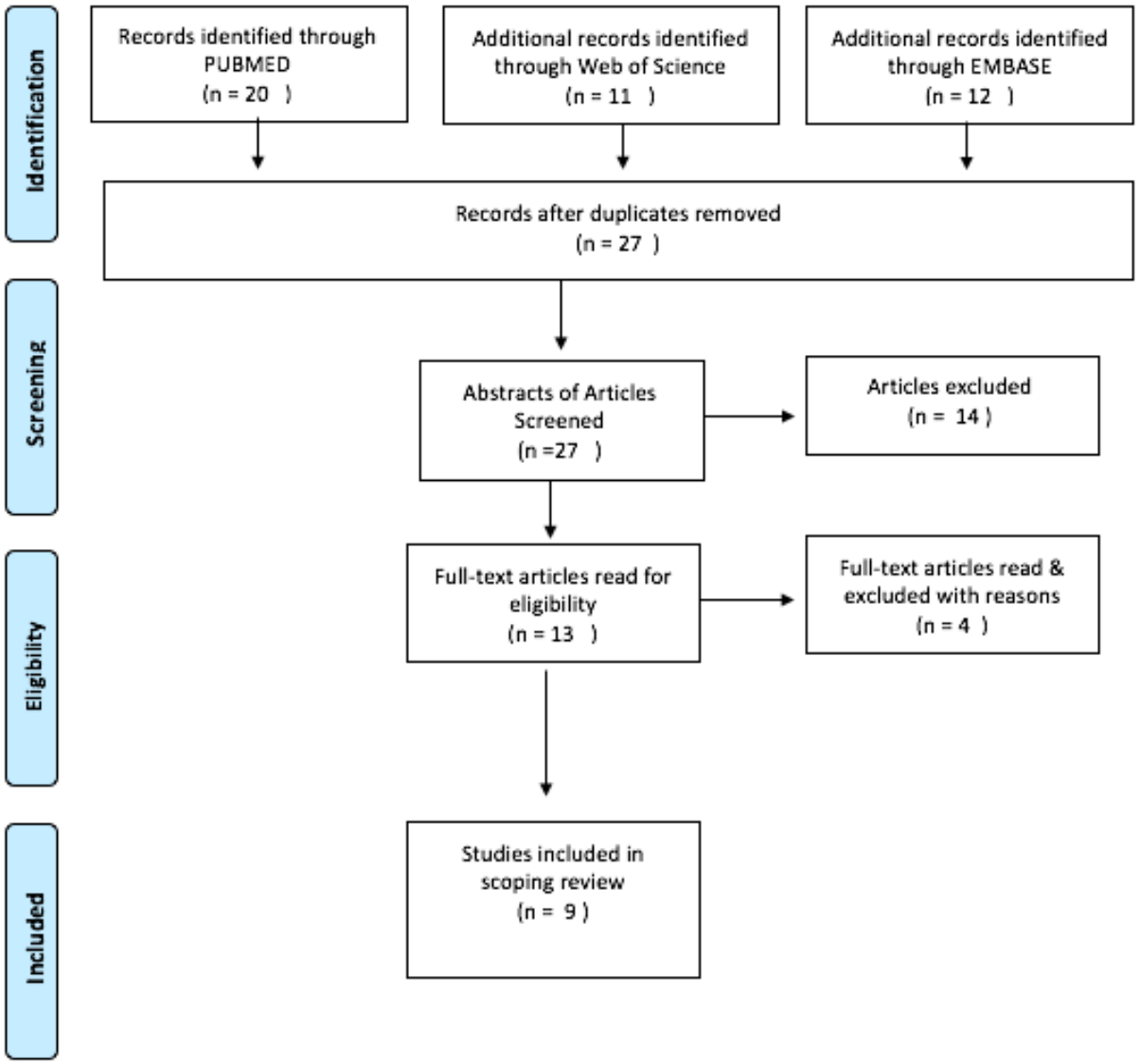

Figure 7: Inclusion & Exclusion Criteria Pathway for Databases

Results

A total of 43 articles were identified for the scoping review after searching the three databases. Sixteen articles were excluded from the review because they were duplicates. Twenty-seven abstracts were then screened and fourteen studies were excluded from the review because they were not studies that discussed diagnostic methods for children at risk or who have EED. In addition, the excluded studies were not conducted in upper-middle, lower middle, or low middle income countries. Then, thirteen articles were read for full-text. As a result, four articles were excluded because they discussed the association between EED and stunting and did not include diagnostic methods to detect EED. Further, other articles discussed treatment methods for EED instead of detection methods. After the elimination of duplicates, screening abstracts, and assessing full-text articles, nine articles were included in the review. Diagnostic methods consisted of biomarkers, including fecal markers, plasma tryptophan, bile acids, L:M ratio, and optical biopsies. Studies were conducted in Bangladesh, Gambia, Brazil, South Africa, Malawi, Nepal, Peru, Tanzania, and Malawi. Four out of 10 of the studies were also conducted in rural Malawi. The age ranges of children that participated in studies were between the ages of less than 17 days old to 5 years old.

Studies Included in the Review

A critical summary of nine studies were selected for the scoping review. Major elements that were discussed in these studies included study design, sample size, age of children in the target population, country the study was conducted, the diagnostic method/technique that was utilized, and conclusion of study findings. Other elements such as costs for using the diagnostic test, strengths and limitations from conducting the study; as well as suitability of utilizing this

specific diagnostic method in children under 5 years old or in developing countries, were considered. Below are the descriptions of the studies.

Biomarkers & Fecal Markers

Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh aimed to use a panel of biomarkers such as myeloperoxidase (MPO) and neopterin (NEO) (markers of intestinal inflammation) and alpha-1 antitrypsin (AAT), which measures a protein enteropathy loss and intestinal permeability, to measure EED in 539 18-month old children [43]. Additionally, the Lactulose Mannitol (L:M) ratio test was used to compare the biomarkers efficiency in diagnosing EED, since L:M ratio is mostly used to detect EED. The study was conducted after the completion of an existing randomized control trial for complementary food supplements. All the children lived in households that had an improved source of drinking water, 82.5% had access to improved sanitation facilities, and 78.4% had mothers that received some form of schooling.

Children were randomized based on the geographical region that they resided in and were put into five groups: (1) child feeding counseling for mothers only, or (2) child feeding counseling plus 1of 4 formulations of complementary food supplements (resulting in four groups). Immediately following the child's 18-month birthday, children were given an L:M ratio urine test. Blood and stool samples were also collected to test for biomarkers. The L:M ratio urine test and blood samples were performed in field clinics, while fecal samples were collected at the children's homes. To analyze the urine test for lactulose and mannitol, high pressure ion chromatography was performed at a laboratory called icddr,b in Dhaka, Bangladesh. Study results showed that blood serum samples and fecal samples showed poor agreement with the L:M ratio test. This poor agreement could result from the complexities of intestinal and systemic

inflammation etiologies in poor-resource settings. Overall, a subset of biomarkers that closely approximated the L:M ratio test was not identified in this study. These results suggested a need for better diagnostic methods of EED and validated biomarkers. Strengths of the study included a large sample size that was already randomized due to a pre-existing research study. Limitations in this study were that the L:M ratio test was used to measure two aspects of intestinal pathology in relation to EED, increased permeability and decreased absorptive ability. However, the biomarkers were selected to assess EED in a broader manner. Also, there are concerns that mannitol can be naturally present in urine. Lastly, the high-pressure ion chromatography may lack sensitivity in indicating low concentration levels of lactulose.

A methodologic framework for modeling and assessing biomarkers of environmental enteropathy as predictors of growth in infants: an example from a Peruvian birth cohort, is a cohort study that followed 303 Peruvian infants from birth to 36 months from January 2010-November 2014 [44]. The study assessed the association of biomarkers such as AAT, MPO, and NEO and nutritional status. With the analyses of biomarkers and nutritional status, researchers hoped to assess possible biomarkers of EED. Study participants were part of the Interactions of Malnutrition and Enteric Infections: Consequences for Child Health and Development (MAL-ED) project that recruited and monitored birth cohorts from eight different sites in low and middle-income countries. The Peruvian children were from a peri-urban community that was 15km from the city of Iquitos. In order to analyze biomarkers, stool samples were collected from the infants from birth to 12 months on a monthly basis, and then on a quarterly basis from 12 to 36 months.

Biomarkers were measured using an enzyme-linked immunosorbent assay (ELISA) test in MAL-ED site laboratories in Peru. Study results concluded that the AAT and MPO biomarkers

showed small but highly statistically significant differences in the biomarkers abilities to predict future growth of participants in the study. This study concluded that these biomarkers may potentially be used to diagnose EED because they provide further evidence to the EED hypothesis that increased permeability and inflammation in the intestines can affect nutritional status.

Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy was a cohort study that enrolled 70 Malawian children between the ages of 2-5 years old [45]. 36 of the children had Environmental Enteropathy (EE) , which was indicated from high L:M test levels, while 34 children did not have EE which was indicated by normal L:M test levels. Their stool samples were analyzed to test mRNA biomarkers that could potentially be used to identify EED. The study stated that although mRNA in low copy numbers in human feces is very difficult detect, researchers believe that mRNA may be useful in detecting EED. Study participants were part of an already existing study of intestinal microbiota. Children who participated in the study originated from families who were famers and lived in mud and thatch homes in rural areas in Southern Malawi. Stool samples were collected and sent back to the U.S. for analysis. Study results concluded that out of the 70 stool samples, >20 copies of glyceraldehyde-3-phosphate dehydrogenase per 200 mg of stool was detected and the biomarker REG4, best differentiated children who had EED and children who didn't. These results indicate that mRNA that is present in stool in low copy numbers can be reproduced and analyzed to detect biomarkers, especially REG4, which could potentially be used to detect EED.

Limitations to the study include: lack of a generalizable population, potential misclassification of study participants, and the difficulty of analyzing stool. The study participants came from one population in rural Africa. There was no biopsy done to confirm

cases of EED, so it cannot be confirmed whether children identified as having EED based on an abnormal L:M ratio test truly have EED; and whether children who were classified as not having EED based on a normal L:M ratio test truly had no EED. The researchers also stated that one important limitation was using stool samples. Stool has a mixture of other biological substances which can interfere with isolating biomarkers and Polymerase Chain Reaction (PCR) amplification. This could ultimately hinder the isolation of RNA from other substances and prevent the analysis of mRNA biomarkers.

The study, *Fecal markers of environmental enteropathy and subsequent growth in Bangladeshi children,* was a cohort study that followed 265 children from Dhaka, Bangladesh 17 days after birth till they were 2 years old [46]. Children came from one setting in the Bauniabadh area of Dhaka and from areas of poor sanitation and low socioeconomic status. The purpose of the study was to analyze potential EED biomarkers using the L:M test. Fecal markers that were assessed included NEO and MPO, which measures intestinal inflammation; as well as AAT, which measures intestinal permeability, were analyzed to determine their association with short-term linear growth. NEO is a molecule that is created and released by macrophages and dendric cells when T lymphocytes are activated and MPO is a lysosomal protein that is released into the small intestine when neutrophils and other phagocytes are activated. On the other hand, AAT is large protein molecule that only passes the intestinal lumen when there is an increase in permeability. In the study, 1,125 stool samples were used to test the three fecal markers and concluded that children with high levels of MPO were associated with a decrease in Length-for-age z-score (LAZ) when they were 12, 15, 18, or 21 months at an average of 0.100 in the subsequent 3-month level than children who had low levels of MPO. This suggests that MPO could potentially be a marker for EED. Additionally, AAT and NEO fecal markers were not

associated with linear growth in this study. Limitations to the study include a 15% loss to follow up of children before 24 months old, and a lower number of stools being tested quarterly during the second year of the study.

Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs was a cohort study that followed 798 Malawian children between the ages of 12-61 months to determine if an L:M ratio test can predict future linear growth, if fecal mRNA can predict L:M ratio test results; which demographic, dietary, and household sanitation practices are associated with L:M ratio [47]. By collecting and analyzing data related to these outcomes, researchers aimed to determine if fecal mRNA transcripts were related with EED and whether these transcripts and clinical characteristics could be used to predict EED and the severity of EED. The children that participated in this study had already been enrolled in one of three clinical studies. One of the studies monitored the growth of twins from four villages in a monthly basis, two of the other studies were randomized, double-blind, clinical trials that were placebo-controlled and the goal of the study was to ameliorate EED. Children that were enrolled in the study were at risk for EED and came from families who were farmers, lived in mud huts without electricity, and drank water from boreholes or wells. The cost of conducting the L:M ratio urine test was approximately $90 and the cost of conducting the host transcript test with four transcripts was $35. Urine samples and stool samples were collected and sent to be analyzed in at Mayo Clinic in Rochester, Minnesota.

The study concluded that increased intestinal permeability measured via the L:M ratio test is a predictor of linear growth and that small host fecal mRNAs can predict severe EED with 80-85% sensitivity by using random forest modeling. The study takes into consideration that using L:M ratio tests on children has a high risk of causing osmotic diarrhea which could put infants at

risk for fluid loss. The L:M ratio test adds osmotic load to the lumen of the intestines and creates excessive paracellular leakage. This causes fluid shifts between the gut lumen and intestinal tissue, which ultimately change the composition of the intestinal microbial community. These reasons reveal that performing an L:M ratio test on consecutive days can yield varying results. The study concludes that using fecal mRNA transcripts instead of the L:M ratio test would be a better solution to measure EED because using host fecal transcripts does not interfere with intestinal microbiology, but further research is needed to determine if these transcripts can detect EED in younger populations and other countries. Also, fecal host proteins such as alpha-1-antitrypsin, calprotectin, myeloperoxidase, neopterin, and lithostathine could be a noninvasive method of detecting EED. However, finding these proteins in feces is based on whether there is significant quantities of protein being released into the extracellular space. It is important to note that the study focused on small bowel dysfunction and not general growth limiting factors that occur in unsanitary environments that are also described as EED. As a result, there could potentially be a spectrum range of L:M ratio measurements and thus, the biomarkers in the study may not be appropriate biomarkers for general EED.

Lactulose Mannitol (L:M) Ratio

Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review, was a study that aimed to address what biomarkers or diagnostic tests are used to identify or are associate with small intestine mucosal dysfunction or host inflammation in children under the age of five years in developing countries [48]. The systematic review specifically analyzed the L:M ratio test and identified 25 studies from countries such as Gambia, Brazil, South Africa, Malawi, Nepal, Bangladesh, and Peru. The systematic reviewed showed that although the L:M ratio test may determine the absorptive and

permeability functionality of the small intestine and the association between childhood growth and intestinal dysfunction, it cannot be a stand-alone test in diagnosing intestinal dysfunction due to various concerns and discrepancies.

For example, to refine the L:M ratio test, there needs to be considerations such as proper dosage of the L:M ratio sugar load based on body size. The study mentions that lactulose and mannitol can cause diarrhea and vomiting, and a standard dosage is needed to prevent these adverse effects. Secondly, there are practical difficulties and inconveniences in collecting urine samples for a minimum five hours, especially from children. Further, reference norms are also not established for L:M ratio tests and this becomes problematic when using this test to diagnose intestinal dysfunction. Most studies that were reviewed, reported elevated L:M ratio in their populations based on UK childhood values. However, the accuracy of the assigned normal and abnormal values could be challenged because the intestinal response to environmental factors could be adaptive rather than a pathological process. Additionally, the study mentions that although values of L:M ratio change with age, none of the studies stratified for age or made adjustments. Also, comparison of results was difficult because none of the studies had standard reporting of central tendencies for L:M ratio values.

Plasma Tryptophan

Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy was a longitudinal cohort study that enrolled 494 infants in Tanzania and Peru that were less than 17 days old, who were >1,500 grams at birth [49]. These infants were followed until they were 36 months. These infants were also assessed to evaluate indoleamine 2,3 dioxygenase 1 (IDO1) activity to determine if it was associated with statural

growth deficit and environmental enteropathy, specifically failed mucosal immune response to oral vaccines. The study measured the tryptophan and kynurenine concentration plasma levels and the kynurenine-tryptophan (KTR) ratio at 3, 7, 15, and 24 months of age; as well as performed anthropometric measurements from 0-36 months of age. As tryptophan is converted to kynurenine by IDO1, a low tryptophan, high kynurenine, and an elevated KTR ratio would indicate an increased activity level of IDO1. Citrulline, a biomarker that measures small intestine functional mass was also measured. Study results show that plasma concentrations of tryptophan and kynurenine are consistent with future statural growth deficits in children living under poverty in Tanzania and Peru. For example, the effect size for tryptophan concentration levels and its association was large in that in Peru, a child who had an increased tryptophan concentration by 1 standard deviation, would have a 1 cm increase in linear growth. This study finding could suggest that tryptophan can be a potential biomarker for environmental enteropathy. Also, plasma concentration levels of tryptophan and KTR is strong and could be a more specific prognostic biomarker for enteropathy since IDO1, which is signified by a decreased plasma tryptophan levels and elevated tryptophan metabolites, facilitates systemic immune activation. It is important to note that tryptophan concentrations are also influenced by one's dietary intake and intake. Since maize is a staple food in Tanzania, low tryptophan consumption was documented in the study population. Citrulline levels on the other hand, had no association with linear growth after 3 months of age. Additionally, Citrulline was inversely associated with systemic inflammation.

Bile Acids

Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism was a cross-sectional study that included 313 children between the ages of 12-59 months in six

villages: Masika, Mitondo, Mbiza, Chamba, Mayaka, and Makhwira, in rural Malawi [50]. In these regions of Malawi, 71% of children that participated in the study came from households that had access to a clean water source and 79% of children used pit latrines. Bile acids are important detergents that assist in the intestinal absorption of the fat-soluble vitamins and dietary fats. Also, they are involved in the signaling pathways for nutrient metabolism. It is hypothesized that EED could affect the absorption of bile acid and this study aimed to address the hypothesis that children with EED have serum bile acid metabolism abnormalities. In the study, anthropometric measurements were performed by trained field workers and blood was drawn by nurses and doctors. The L:M ratio test was used to measure the intestinal integrity of the children and children with an L:M ratio ≥0.15 were considered to have EED.

Study results concluded that the three most significant bile acids that were discovered in the blood serum were glycochenodexocycholic acid (GCDCA), glycocholic acid (GCA), and glycodeoxycholic acid (GDCA). Taurocholic acid (TCA),taurochenodeoxycholic acid (TCDCA), and deoxycholic acid (DCA), were other serum bile acids that were discovered. Glycoursodeoxycholic acid (GUDCA), Taurodeoxycholic acid (TDCA), and tauromurocholic acid (TMCA) were also found in small amounts in blood serum. Adjusting for age, serum bile acid concentration levels were compared between children with EED and children without EED. TDCA, TMCA, and GUDCA bile salts were found to be significantly lower in serum in 244 children with EED than in 69 children without EED. Total bile acid serum was 12% lower in children with EED in comparison to children without EED. These research findings indicated that EED is associated with altered bile metabolism in children in rural Malawi. Although the article mentions that the strength of this study is that the children who participated in the study come from a setting typical for EED, it also states that the study cannot be generalizable to other

pediatric populations because of the cultural, environmental, and dietary variations that differ in settings outside of Malawi. Also, the study cannot be generalizable because there is a shortage of studies that discuss serum bile salts in young children. The study concludes that there needs to be further research about whether bile acid malabsorption is confirmed in EED cases by measuring with fecal bile acid concentrations.

Optical Biopsy

Position paper: The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction is a position paper that discusses how biomarkers as a tool for diagnosing EED such as the L:M test, can be challenging because it is too invasive to be used in large-scale research studies, particularly in children [51]. The research article states that optical biopsy techniques , which can be minimally invasive or noninvasive, are alternative methods for diagnosing EED in children. Optical biopsy techniques included high-definition (HD) and narrow band imaging (NBI) endoscopy, capsule endoscopy, fluorescence confocal endomicroscopy, optical coherence tomography (OCT), external fluorescence spectroscopy, and external photo-acoustic probe. Each test was evaluated based on factors (Appendix 3) such as technology readiness level, which measures the maturity of the technology, cost, invasiveness, and suitability for developing countries. Further, deployment timescale, which indicates how long it takes to bring the technology into action, was evaluated. Study results conclude that endoscopies show intestinal villi, which indicates that it can identify EED features. An endoscopy utilizes fiber-optic or distal light emitting diodes (LEDs) to illustrate tissue and capture live video of internal tissue. Endoscopy has also been used in celiac disease which has more structural changes to the intestines in comparison to EED. The intestinal villi can be enhanced and shown vis HD endoscopy and NBI endoscopy. Although endoscopies have a

higher technological readiness level in comparison to the other optical biopsy techniques that are presented in the study, they have a low -medium rate for suitability for developing countries, the deployment timescale is short-term, and endoscopic examinations have been proven to have high risk in pediatric populations because children have to be sedated or under anesthesia. Additionally, endoscopic equipment are more expensive to purchase in comparison to the other optical methods.

Capsule endoscopies are less invasive than other endoscopies such as HB and NDI because a capsule with a video chip (camera) is ingested, passed through the body via peristalsis, and it take photographs of tissue. As the capsule is passed through the gastrointestinal tract via peristalsis, it prevents intestinal epithelium damage. However, they are expensive, have a short-term deployment scale, and are invasive.

Fluorescence confocal endomicroscopy provides high resolution images in vivo and is the most commonly used optical biopsy technique. A dye is injected intravenously and a fluorescent stain appears on tissue to show tissue microstructure. Endomicroscopy can be used to changes in intestinal function and permeability. Limitations to utilizing endomicroscopy includes the fact that it is not a commercially available instrument and requires the use of an endoscopy. Additionally, it is limited to use in research studies in small children or adults.

OCT is an endoscopic method that utilizes high speed and high resolution to visualize cross-sectional images of tissue. In diagnosing celiac disease, this technique reported a 100% sensitivity and 82% specificity. OCT has a medium-term deployment timescale and is expensive. Also, it is only limited to use in children 2 years old and above. External fluorescence spectroscopy is when tissue is illuminated with light of a known wavelength and the returning signal is recorded. Fluorescence spectroscopy is a noninvasive technology method that could be

useful in determining morphological intestinal changes in EED like cellular infiltration or breaks in the epithelial layer of the intestines. Although it appears to be a promising method for EED because it is noninvasive, this method requires validation from another test such as an L:M ratio test. Once there is validation, the test will be able to be applied to large settings.

Lastly, photoacoustic imaging is a technique which can provide information about the structure and function of tissues via acoustic waves. A material that absorbs light, emits acoustic waves which sends laser pulses to tissues, and then generates heat to the tissues, and as a result, there is ultrasonic emission. The ultrasounds are generated and detected by transducers and then analyzed to produce tissue images. It has the lowest technological readiness level in comparison to the other optical biopsy methods and has a long-term deployment timescale.

Discussion

Childhood stunting or linear growth failure is a grave public health issue that continues to have high priority because it affects many children under the age of 5 years old globally. Stunting is often a predictor of a child's well-being [52] and future social inequalities, particularly in developing countries. Linear growth failure is associated with pathological disorders such as diabetes, hypertension, and obesity [53], which have increased morbidity and mortality; decreased cognitive function; and a loss of potential earnings as an adult [52]. Research studies have shown that EED, an asymptomatic condition that causes intestinal inflammation and permeability, has a strong association with childhood stunting because it impairs intestinal integrity and function [54]. Currently, there is no defined diagnostic testing criteria used to detect EED and this scoping review aimed to address this issue. The goal of the review was to identify which diagnostic test would better detect EED cases in children under the age of five years living in poor-resource settings within low and middle-income countries with inadequate water supply, poor sanitation and hygiene services, high prevalence rates of infectious diseases, and poor healthcare system infrastructure.

Study Design

Nine studies were included in the review and were analyzed based on criteria that included study design, social and political context of utilizing the diagnostic method in the target population, available healthcare infrastructure to implement the testing modality, cost, ethical issues raised while applying the diagnostic test in the specified target population, and as well as scalability. Based on study design, most studies (5) were cohort studies that followed children prospectively to determine if use of the specified diagnostic or detection tests would result in identification of EED. One study, *Biomarkers of Environmental Enteric Dysfunction Among*

Children in Rural Bangladesh, was conducted after the completion of an already existing randomized control trial study; *Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction,* was a systematic review; *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism*, was a cross-sectional study; and *The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction*, was a position paper. Taking into account limitations of certain study designs, while the cross-sectional study titled *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism,* showed promising results about EED being associated with bile acid metabolism in Malawian children, a limitation of this study design is that temporality is poor. Because exposure and outcome are assessed at one point in time simultaneously, this does not provide strong enough evidence of the direction of the relationship between altered bile metabolism and EED. Without longitudinal information, a true cause and effect relationship is difficult to establish (orthodontics).

Social & Political Context

The social and political setting was also considered when analyzing the research studies. The scoping review looked at whether the studies were conducted in communities where EED would exist such as high poverty areas, areas with no or limited access to WASH and regions with increased prevalence of infectious diseases. The study, *Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh,* had child participants from families where there was access to improved drinking water and sanitation facilities. Furthermore, 78.4% of participants' mothers had some schooling [43]. Based on this information, this study would not fit the social and political criteria where EED would potentially occur. In the *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism* cross-sectional study, the

majority of the children who were enrolled in the study, came from households with access to clean water and pit latrines, which are considered to be an inexpensive and improved form of sanitation [55]. Based on the socioeconomic status (SES) that the scoping review was looking for, this study did not meet that part of the criteria.

Healthcare Infrastructure

The presence of an existing healthcare infrastructure in the community was a criterion used to evaluate how the specified diagnostic test was implemented. As an indicator of quality healthcare, healthcare infrastructure includes adequate numbers of healthcare workers and personnel, training capacity, facilities to deliver care, reliable supplies and medical equipment, and laboratories [56]. The *Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh* study had high pressure ion chromatography equipment and a laboratory in Dhaka, Bangladesh to analyze blood and stool samples. Also, the *Fecal markers of environmental enteropathy and subsequent growth in Bangladeshi children* study conducted laboratory procedures and analyzes at the same laboratory used in the *Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh* study. *A methodologic framework for modeling and assessing biomarkers of environmental enteropathy as predictors of growth in infants: an example from a Peruvian birth cohort* study measured and analyzed biomarkers using an ELISA in site laboratories in Peru.

Two studies, *Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy* and *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs*, transported lab samples to be analyzed in the U.S. The transportation of lab samples to the U.S. would indicate that there is no existing healthcare infrastructure in the area that these studies were being

conducted. If the studies were to be scaled-up in their specific country, there may not be adequate medical equipment and infrastructure necessary to analyze data in those areas. The *Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy* study did not mention if there was any laboratories in the area that the study was being conducted. Although the *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism* study also did not state whether health infrastructure existed in the community, it was the only study out of all the studies reviewed that indicated that there were nurses and doctors available to draw blood samples for testing. None of the other studies indicated whether or not there was adequate healthcare professionals available to collect lab samples for analysis.

The position paper titled, *The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction*, discussed several optical biopsy technological equipment and their use in developing countries. While they developed a scale that measured how suitable the technology could be within a developing country context, the paper never discussed specific countries where it could be applied. Equally, they never discussed whether established healthcare infrastructure would be important in utilizing these biopsy technologies in developing countries.

Cost

The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction, goes on to discuss the cost of each optical biopsy technique ranging from low cost to expensive. In the article, the authors do not explicitly state the exact cost (or U.S. dollars) of purchasing the technology. Continuing on the subject of cost, the only other article that mentions these factors is the study *Environmental Enteric Dysfunction Is Associated With Poor Linear*

Growth and Can Be Identified by Host Fecal mRNAs. The study states that it costs approximately $90 to conduct an L:M ratio urine test and $35 to conduct the host transcript test with four transcripts [47]. This study did not mention whether the cost to conduct the L:M ratio and transcript were per person.

Ethics

Ethical issues in conducting research in the target population of each of the studies was also analyzed. Crucial ethical issues to consider when conducting research are informed consent, beneficence (or do no harm), respect for confidentiality and privacy, and respect for anonymity (nursing). All but one study stated that informed consent was received before conducting the study. The study, *Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy,* mentions that the research protocol was approved by an Institutional Board and an ethics committee [49], but does not mention whether informed consent was received from the parents of the study participants.

Considering beneficence, some of the studies touched on how the diagnostic tool used in conducting the study could potentially cause harm to the study participants. Some studies such as *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs* and the *Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review* state that the L:M ratio test could potentially cause fluid loss and diarrhea that may alter the microbial community in the intestines of children. The *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs* study further states that fecal mRNA transcripts instead

of the L:M ratio test would be a better solution to measure EED because using host fecal transcripts does not interfere with the intestinal microbiology community [47].

Scalability

Scalability was also analyzed among the nine studies. There were two studies: *Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental* enteropathy and *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism* that discussed whether the diagnostic tests used in the studies could be generalizable to other populations. According to the authors, both of these studies were not generalizable to other contexts in different communities outside of the area that the study was conducted and in other developing countries. It is also a caveat to mention that the study, *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs*, focused on small bowel dysfunction and not general EED. Due to the nature of EED, there could be a spectrum of outcomes for the L:M test. Because the study focused on small bowel dysfunction, it is possible that the biomarkers in this study may not be appropriate biomarkers to detect other degrees of EED. With this in mind, it could implied that this study may not be generalizable to a general EED population in other contexts. None of the other articles, discussed how the diagnostic test would be scaled.

Future Direction

Although invasiveness of each diagnostic test was not a criterion in this scoping review, based on analysis of research studies, in the future it should be investigated further. Invasive diagnostics are tests that require the use of an instrument to enter the body [57]. The most common diagnostic tests include blood tests and biopsies [57]. Urine and fecal tests would be considered non-invasive tests. Two studies: *Biomarkers of Environmental Enteric Dysfunction*

Among Children in Rural Bangladesh and *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism* used a combination of invasive (blood samples) and non-invasive (stool samples) procedures and two studies: *Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy* and *The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction*, used invasive procedures such as blood samples and biopsies. Five of the other studies used a single or a combination of non-invasive procedures such as urine tests and fecal samples.

Recommendation

Based on the analysis of the nine articles against the specified criteria, the recommended diagnostic or detection test that could be utilized in a child under 5 years old living in an LMIC and at risk for developing EED would be **fecal mRNA transcripts testing**. Two studies in this review discussed mRNA fecal transcripts and concluded that it has potential to detect EED cases in children. The study, *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs,* infers that mRNA transcripts would be a better solution to measuring EED in children than the L:M ratio test because it does not interfere with the microbiology of the intestines. It also concluded that mRNA transcripts testing can predict severe EED with 80-85% accuracy based on their sample population [47]. The second study, *Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy*, believed that mRNA that is present in stool in low numbers could be used to detect biomarkers, especially REG4, and possibly identify EED cases [45].

Both studies met most of the review criteria with favorable results: study design, political and social context, cost-effectiveness, and ethical considerations. Both studies were cohort studies and had a target population that included Malawian children under the age of 5 years old who came from families who were farmers and lived in mud homes in rural Southern Malawi. The collection of mRNA transcripts is non-invasive because only a stool sample is needed for analysis. In one of the studies, *Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal*, it is mentioned that it cost $35 to conduct an mRNA fecal test with four transcripts in comparison to an L:M ratio urine test which cost $90 [47]. The limitation to both studies were that they were not generalizable to the larger population.

To conclude, further research would need to be conducted, especially in children in other developing countries, to determine the validity and reliability of mRNA transcripts being used to detect EED. A 2010 study by Bennett et al., suggests that although mRNA in stool has been understudied, isolating mRNA is useful in characterizing a wide range of intestinal responses and disease in adults and children [58]. With technology available such as droplet digital PCR (ddPCR), that has been shown to improve detection of fecal host mRNA [59], mRNA transcripts could serve as a promising non-invasive detection test to diagnose EED in young children.

References

1. Organization, W.H., *Global nutrition targets 2025: Stunting policy brief.* 2014.
2. *<WHO _ Stunting in a nutshell.pdf>.*
3. UNICEF, *Undernutrition contributes to nearly half of all deaths in children under 5 and is widespread in Asia and Africa. UNICEF*. 2016.
4. Organization, W.H., *Nutrition Landscape Information System (NLIS) country profile indicators: interpretation guide.* 2010.
5. Hayashi, C., et al., *Levels and trends in child malnutrition. UNICEF/WHO/World Bank Group joint child malnutrition estimates: key findings of the 2017 edition.* 2017.
6. Unicef, *Improving child nutrition. The achievable imperative for global progress. 2013.* Google Scholar, 2016.
7. Stewart, C.P., et al., *Contextualising complementary feeding in a broader framework for stunting prevention.* Maternal & child nutrition, 2013. **9**(S2): p. 27-45.
8. Dewey, K.G. and K. Begum, *Long-term consequences of stunting in early life.* Maternal & child nutrition, 2011. **7**(s3): p. 5-18.
9. Thomas, D. and J. Strauss, *Health and wages: Evidence on men and women in urban Brazil.* Journal of econometrics, 1997. **77**(1): p. 159-185.
10. Devlin, K., *Stunting Limits Learning and Future Earnings of Children.* Washington DC: Population Reference Bureau, 2012.
11. O'Hare, B., et al., *Income and child mortality in developing countries: a systematic review and meta-analysis.* J R Soc Med, 2013. **106**(10): p. 408-14.
12. *The World Bank and Nutrition*. Nutrition: Overview Available from: http://www.worldbank.org/en/topic/nutrition/overview?
13. Rytter, M.J.H., et al., *The immune system in children with malnutrition—a systematic review.* PloS one, 2014. **9**(8): p. e105017.
14. Tomkins, A., *Nutritional status and severity of diarrhoea among pre-school children in rural Nigeria.* The lancet, 1981. **317**(8225): p. 860-862.
15. Checkley, W., et al., *Multi-country analysis of the effects of diarrhoea on childhood stunting.* International journal of epidemiology, 2008. **37**(4): p. 816-830.
16. Rice, A.L., et al., *Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries.* Bulletin of the World Health Organization, 2000. **78**(10): p. 1207-1221.
17. UNICEF, *IMPROVING CHILD NUTRITION: The achievable imperative for global progress*, in *Improving Child Nutrition* 2013.
18. Gaskin, P., et al., *Early linear growth retardation and later blood pressure.* European journal of clinical nutrition, 2000. **54**(7): p. 563.
19. McMillen, I.C. and J.S. Robinson, *Developmental origins of the metabolic syndrome: prediction, plasticity, and programming.* Physiological reviews, 2005. **85**(2): p. 571-633.
20. Organization, W.H. *Breastfeeding*. Nutrition Available from: http://www.who.int/nutrition/topics/exclusive_breastfeeding/en/.
21. Organization, W.H., *Reducing stunting in children: equity considerations for achieving the global nutrition targets 2025.* 2018.
22. *<globaltargets_stunting_policybrief.pdf>.*

23. Bhutta, Z.A., et al., *What works? Interventions for maternal and child undernutrition and survival.* The lancet, 2008. **371**(9610): p. 417-440.
24. Ramakrishnan, U., P. Nguyen, and R. Martorell, *Effects of micronutrients on growth of children under 5 y of age: meta-analyses of single and multiple nutrient interventions–.* The American journal of clinical nutrition, 2008. **89**(1): p. 191-203.
25. Giugliani, E.R., et al., *Effect of breastfeeding promotion interventions on child growth: a systematic review and meta-analysis.* Acta Paediatrica, 2015. **104**(S467): p. 20-29.
26. Cumming, O. and S. Cairncross, *Can water, sanitation and hygiene help eliminate stunting? Current evidence and policy implications.* Maternal & child nutrition, 2016. **12**(S1): p. 91-105.
27. Denno, D.M., P.I. Tarr, and J.P. Nataro, *Environmental Enteric Dysfunction: A Case Definition for Intervention Trials.* Am J Trop Med Hyg, 2017. **97**(6): p. 1643-1646.
28. Null, C., et al., *Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Kenya: a cluster-randomised controlled trial.* The Lancet Global Health, 2018. **6**(3): p. e316-e329.
29. Owino, V., et al., *Environmental Enteric Dysfunction and Growth Failure/Stunting in Global Child Health.* Pediatrics, 2016. **138**(6).
30. Watanabe, K. and W.A. Petri, Jr., *Environmental Enteropathy: Elusive but Significant Subclinical Abnormalities in Developing Countries.* EBioMedicine, 2016. **10**: p. 25-32.
31. Crane, R.J., K.D. Jones, and J.A. Berkley, *Environmental enteric dysfunction: an overview.* Food and nutrition bulletin, 2015. **36**(1_suppl1): p. S76-S87.
32. Ali, A., N.T. Iqbal, and K. Sadiq, *Environmental enteropathy.* Current opinion in gastroenterology, 2016. **32**(1): p. 12-17.
33. Drummond, L.a.S.S. *WASH and Nutrition: Recent Research and the Save the Children Experience*. PowerPoint Presentation]. Available from: Mbuya, Mduduzi, (2015). Environmental Enteric Dysfunction and Child Nutrition: Exploring the Potential Effects of WASH. Presentation. SHINE Study. Istanbul, 2015.
34. Fernández Bañares, F., et al., *Type 1 Marsh celiac disease: diagnosis and response.* OmniaScience Monographs, 2014.
35. Harper, K.M., et al., *Environmental enteric dysfunction pathways and child stunting: A systematic review.* PLoS neglected tropical diseases, 2018. **12**(1): p. e0006205.
36. Mbuya, M. *Environmental Enteric Dysfunction and Child Nutrition: Exploring the Potential Effects of WASH SHINE Study.* . Visualizing Environmental Enteric Dysfunction. 2015 PowerPoint Presentation]. Available from: https://www.savethechildren.org/...nutrition/WASH-NUT-WEBINAR-SLIDES.PPTX.
37. Crane, R.J., K.D. Jones, and J.A. Berkley, *Environmental enteric dysfunction: an overview.* Food Nutr Bull, 2015. **36**(1 Suppl): p. S76-87.
38. Cook, G., S. Kajubi, and F. Lee, *Jejunal morphology of the African in Uganda.* The Journal of pathology, 1969. **98**(3): p. 157-169.
39. Lindenbaum, J., C.D. Gerson, and T.H. Kent, *Recovery of small-intestinal structure and function after residence in the tropics: I. Studies in Peace Corps Volunteers.* Annals of internal medicine, 1971. **74**(2): p. 218-222.

40. Campbell, D., M. Elia, and P. Lunn, *Growth faltering in rural Gambian infants is associated with impaired small intestinal barrier function, leading to endotoxemia and systemic inflammation.* The Journal of nutrition, 2003. **133**(5): p. 1332-1338.
41. Keusch, G.T., et al., *Environmental enteric dysfunction: pathogenesis, diagnosis, and clinical consequences.* Clinical Infectious Diseases, 2014. **59**(suppl_4): p. S207-S212.
42. Thompson, A.J., et al., *The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction.*
43. Campbell, R.K., et al., *Biomarkers of environmental enteric dysfunction among children in rural Bangladesh.* Journal of pediatric gastroenterology and nutrition, 2017. **65**(1): p. 40-46.
44. Colston, J.M., et al., *A methodologic framework for modeling and assessing biomarkers of environmental enteropathy as predictors of growth in infants: An example from a Peruvian birth cohort.* American Journal of Clinical Nutrition, 2017. **106**(1): p. 245-255.
45. Agapova, S., et al., *Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy.* J Pediatr Gastroenterol Nutr, 2013. **56**(1): p. 66-71.
46. Arndt, M.B., et al., *Fecal markers of environmental enteropathy and subsequent growth in Bangladeshi children.* The American journal of tropical medicine and hygiene, 2016. **95**(3): p. 694-701.
47. Ordiz, M.I., et al., *Environmental enteric dysfunction is associated with poor linear growth and can be identified by host fecal MRNAS.* Journal of Pediatric Gastroenterology and Nutrition, 2016. **63**: p. S3-S4.
48. Denno, D.M., et al., *Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review.* Clin Infect Dis, 2014. **59 Suppl 4**: p. S213-9.
49. Kosek, M.N., et al., *Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy.* Am J Trop Med Hyg, 2016. **95**(4): p. 928-937.
50. Semba, R.D., et al., *Environmental Enteric Dysfunction Is Associated With Altered Bile Acid Metabolism.* J Pediatr Gastroenterol Nutr, 2017. **64**(4): p. 536-540.
51. Thompson, A.J., et al., *Position paper: The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction.* Nat Rev Gastroenterol Hepatol, 2017. **14**(12): p. 727-738.
52. de Onis, M. and F. Branca, *Childhood stunting: a global perspective.* Matern Child Nutr, 2016. **12 Suppl 1**: p. 12-26.
53. UNICEF. *Introduction* Stunting Available from: http://unicef.in/Whatwedo/10/Stunting.
54. Semba, R.D., et al., *Metabolic alterations in children with environmental enteric dysfunction.* Scientific reports, 2016. **6**: p. 28009.
55. Graham, J.P. and M.L. Polizzotto, *Pit latrines and their impacts on groundwater quality: a systematic review.* Environmental health perspectives, 2013. **121**(5): p. 521.
56. Foundation, I. *Healthcare Infrastructure* [cited 2018; Available from: http://izumi.org/about/how-we-work/health-care-infrastructure/.

57. Center, U.H.M. *Diagnostic Testing* Available from: https://uhmchealth.com/service/diagnostic-testing.
58. Bennett Jr, W.E., et al., *Proinflammatory fecal mRNA and childhood bacterial enteric infections.* Gut Microbes, 2010. **1**(4): p. 209-212.
59. Stauber, J., et al., *Droplet digital PCR quantifies host inflammatory transcripts in feces reliably and reproducibly.* Cellular immunology, 2016. **303**: p. 43-49.
60. Nations, U., *World Economic Situations and Prospects*

Appendices

Economies by per capita GNI in 2012[a]

High-income		Upper middle income		Lower middle income	Low-income
Australia	Lithuania[b]	Albania[b]	Jordan	Armenia	Bangladesh
Austria	Luxembourg	Algeria	Kazakhstan	Bolivia	Benin
Bahrain	Malta	Angola	Lebanon	Cameroon	Burkina Faso
Barbados	Netherlands	Argentina	Libya	Cape Verde	Burundi
Belgium	New Zealand	Azerbaijan	Malaysia	Congo	Central African Republic
Brunei Darussalam	Norway	Belarus	Mauritius	Côte d'Ivoire	Chad
Canada	Oman	Bosnia and Herzegovina	Mexico	Djibouti	Comoros
Chile[b]	Poland	Botswana	Montenegro	Egypt	Democratic Republic of the Congo
Croatia	Portugal	Brazil	Namibia	El Salvador	Eritrea
Cyprus	Qatar	Bulgaria	Panama	Georgia	Ethiopia
Czech Republic	Republic of Korea	China	Peru	Ghana	Gambia, The
Denmark	Russian Federation[b]	Colombia	Romania	Guatemala	Guinea
Equatorial Guinea	Saudi Arabia	Costa Rica	Serbia	Guyana	Guinea-Bissau
Estonia	Singapore	Cuba	South Africa	Honduras	Haiti
Finland	Slovak Republic	Dominican Republic	Thailand	India	Kenya
France	Slovenia	Ecuador	The former Yugoslav Republc of Macedonia	Indonesia	Kyrgyz Republic
Germany	Spain	Gabon	Tunisia	Lesotho	Liberia
Greece	Sweden	Hungary[c]	Turkey	Mauritania[b]	Madagascar
Hong Kong SAR[d]	Switzerland	Iran, Islamic Republic	Turkmenistan	Moldova	Malawi
Iceland	Taiwan Province of China	Iraq[b]	Venezuela, RB	Morocco	Mali
Ireland	Trinidad and Tobago	Jamaica		Nicaragua	Mozambique
Israel	United Arab Emirates			Nigeria	Myanmar
Italy	United Kingdom			Pakistan	Nepal
Japan	United States			Papua New Guinea	Niger
Kuwait	Uruguay[b]			Paraguay	Rwanda
Latvia[b]				Philippines	Sierra Leone
				São Tomé and Principe	Somalia
				Senegal	Tajikistan
				Sri Lanka	Tanzania
				Sudan	Togo
				Syrian Arab Republic	Uganda
				Ukraine	Zimbabwe
				Uzbekistan	
				Vietnam	
				Yemen, Rep.	
				Zambia	

Appendix 1: Economies by Per Capita Gross National Income (GNI) in 2012. Source: *World Economic Situations and Prospects*. United Nations, 2014 [60].

Appendix 2: Description of Articles Used In Scoping Review

Study	Design	Target Population	Sample Size	Diagnostic/Detection Test	Conclusion	Comments
Arndt, M.B., et al., *Fecal markers of environmental enteropathy and subsequent growth in Bangladeshi children*. The American journal of tropical medicine and hygiene, 2016. **95**(3): p. 694-701.	Cohort	Infants in Dhaka, Bangladesh followed from 17 days of life till age 2 years	265	Fecal markers: Neopterin (NEO), Myeloperoxidase (MPO), Alpha-1-antitrypsin (AAT)	MPO could potentially be an EED marker because children with high levels of MPO were associated with a decrease in Length-for-age z-score (LAZ) when they were 12, 15, 18, or 21 months at an average of 0.100 in the subsequent 3-month level, than children who had low levels of MPO	15% loss to follow up of children before 24 months old in the study
Biomarkers of Environmental Enteric Dysfunction Among Children in Rural Bangladesh	Children from a previous randomized food supplementation study	18-month old children from Rural Bangladesh	539	Biomarkers: MPO, NEO, and AAT	EED prevalence (L:M>0.07) was 39.0% Agreement with intestinal biomarkers and L:M test were low There needs to better diagnostic methods of EED and validated biomarkers.	Large sample size that was already randomized due to a pre-existing research study. Limitations were that the L:M test was used to measure two aspects of intestinal pathology in relation to EED and the biomarkers were selected to assess EED in a more broad manner. High pressure ion chromatography may lack sensitivity in indicating low concentration levels of lactulose.

A methodologic framework for modeling and assessing biomarkers of environmental enteropathy as predictors of growth in infants: an example from a Peruvian birth cohort	Cohort	Infants from birth to 36 months from a per-urban community in Peru	303	Biomarkers: MPO, NEO, and AAT	Biomarkers may potentially be used to diagnose EED and provide further evidence to the EED hypothesis that increased permeability and inflammation in the intestines can affect nutritional status	AAT and MPO were associated with small but highly statistically significant differences in the continued statural growth of infants in the cohort
Detection of low-concentration host mRNA transcripts in Malawian children at risk for environmental enteropathy	Cohort	2-5 years old Malawian children 36 of the children had Environmental Enteropathy (EE) indicated high L:M ratio test levels 34 children did not have EE indicated by normal L:M ratio test levels.	70	Host mRNA fecal transcripts	Out of the 70 stool samples, >20 copies of glyceraldehyde-3-phosphate dehydrogenase per 200 mg of stool was detected The biomarker REG4, best differentiated children who had EE and children who didn't	The study was conducted in one population in rural Africa and does not have a generalizable population Stool has a mixture of other biological substances and this can interfere with isolating biomarkers and Polymerase Chain Reaction (PCR) amplification
Environmental Enteric Dysfunction Is Associated With Poor Linear Growth and Can Be Identified by Host Fecal mRNAs	Cohort	Malawian children between the ages of 12-61 months (1-5 years old)	798	Host fecal mRNAs	host fecal mRNAs can predict severe EED with 80-85% sensitivity by using random forest modeling.	Fecal mRNA transcripts instead of the L:M test would be a better solution to measure EED because using host fecal transcripts does not interfere with intestinal microbiology

Use of the lactulose to mannitol ratio to evaluate childhood environmental enteric dysfunction: a systematic review	Systematic Review	25 studies from countries such as Gambia, Brazil, South Africa, Malawi, Nepal, Bangladesh, and Peru	N/A	Lactulose mannitol (L:M) ratio	L:M ratio test may determine the absorptive and permeability functionality of the small intestine and intestinal dysfunction but cannot be a stand-alone test in diagnosing intestinal dysfunction	Proper dosage of the L:M ratio sugar load based on body size need to be considered Lactulose and mannitol can cause diarrhea and vomiting, and a standard dosage is needed to prevent these adverse effects Reference norms are not established for L:M ratio test and this may be an issue when using test to diagnose intestinal dysfunction
Plasma Tryptophan and the Kynurenine-Tryptophan Ratio are Associated with the Acquisition of Statural Growth Deficits and Oral Vaccine Underperformance in Populations with Environmental Enteropathy	Cohort	Infants from Tanzania and Peru less than 17 days old and were followed until they were 36 months old 244 children with EED and 69 children without EED	494	Plasma tryptophan	The effect size for tryptophan concentration levels and its association was large in that in Peru, a child who had an increased tryptophan concentration by 1 standard deviation, would have a 1 cm increase in linear growth This finding could suggest that tryptophan can be a potential biomarker for environmental enteropathy	Tryptophan concentrations are influenced by one's dietary intake and intake Maize is a staple food in Tanzania and low tryptophan consumption was documented in study population
Environmental Enteric Dysfunction Is Associated With Altered Bile	Cross-sectional	Children between the ages of 12-59 months in six	313	Bile Acids: glycochenodexocycholic acid (GCDCA), glycocholic acid	Adjusting for age, TDCA, TMCA, and GUDCA bile salts were found to be significantly	The study cannot be generalizable to other pediatric population because of the cultural,

Acid Metabolism		villages of rural Malawi: Masika, Mitondo, Mbiza, Chamba, Mayaka, and Makhwira		(GCA), glycodeoxycholic acid (GDCA), Taurocholic acid (TCA),taurochenodeoxycholic acid (TCDCA), deoxycholic acid (DCA), Glycoursodeoxycholic acid (GUDCA), Taurodeoxycholic acid (TDCA), and tauromurocholic acid (TMCA)	lower in serum in 244 children with EED than in 69 children without EED Total bile acid serum was 12% lower in children with EED in comparison to children without EED Research findings indicated that EED is associated with altered bile metabolism in children in rural Malawi	environmental, and dietary variations that could be different in settings outside of Malawi The study cannot be generalizable because there is a shortage of studies that discuss serum bile salts in young children There needs to be further research about whether bile acid malabsorption is confirmed in EED cases by measuring with fecal bile acid concentrations.
Position paper: The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction	Position paper	N/A	N/A	Optical biopsy techniques: high-definition (HD) and narrow band imaging (NBI) endoscopy, capsule endoscopy, fluorescence confocal endomicroscopy, optical coherence tomography (OCT), external fluorescence spectroscopy, and external photo-acoustic probe	Endoscopies are able to show intestinal villi, which indicates that it can identify EED features Endoscopies have a higher technological readiness level in comparison to the other optical biopsy techniques that are presented in the study, but are high risk in pediatric populations because children have to be sedated or under anesthesia	Endoscopy has also been used in celiac disease which has more structural changes to the intestines in comparison to EED

					Capsule endoscopies are less invasive than other endoscopies such as HB and NDI but are expensive, have a short-term deployment scale, and are invasive Fluorescence confocal endomicroscopy can be used to view changes in intestinal function and permeability but requires an endoscopy OCT is only limited to use in children 2 years old and above Fluorescence spectroscopy could be useful in determining morphological intestinal changes in EED like cellular infiltration or breaks in the epithelial layer of the intestines, but requires validation from another test such as an L:M ratio test Photoacoustic imaging is a technique which	

					can provide information about the structure and function of tissues via acoustic waves and has the lowest technological readiness level in comparison to the other optical biopsy methods	

Optical biopsy technique	TRL	Cost (approx.)	Invasiveness	Suitability for developing countries	Other applications of technology	Information provided	Deployment timescale
HD and NBI endoscopy	9	$$$	High	Low-medium	High	Villous morphology (plus biopsy collection)	Short-term
Capsule endoscopy	8	$$	Medium	Medium	High	Villous morphology	Short-term
Fluorescence confocal endomicroscopy	8	$$$	High	Low	Medium-high	Villous morphology, gut leakiness, bacterial translocation	Short-term
Optical coherence tomography capsule	7	$$$	Medium	Medium	Medium	Villous morphology	Medium-term
External fluorescence spectroscopy	5	$	Low	High	Low-medium	Gut leakiness, bacterial translocation	Medium-term
External photo-acoustic probe	4	$$	Low	Medium	Low-medium	Gut structure, gut leakiness, bacterial translocation	Long-term

Technological readiness level (TRL) is a scale indicating the maturity of a technology. A score of '1' represents an initial concept, whereas a TRL of '9' indicates a mature, commercialised technology. The remaining factors have been determined in a qualitative manner as described in the Methods section. Deployment timescales are tentatively defined as follows: short term – suitable for deployment immediately or within approximately 1 year; medium term — suitable for deployment in 1–3 years; long term — suitable for deployment in >3 years. HD, high-definition; NBI, narrow band imaging.

Appendix 3: Optical Biopsy Techniques for EED. Source: Thompson, A.J., et al., *Position paper: The potential role of optical biopsy in the study and diagnosis of environmental enteric dysfunction*. Nat Rev Gastroenterol Hepatol, 2017. **14**(12): p. 727-738.