CHAPTER 3.

EED LIBRARY AS A BASIS FOR SYSTEMATIC REVIEWS

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Chapter 3. EED Library as a Basis for Systematic Reviews

3.1 Defining Systematic Review Question Priorities

Evidence related to any topic area and addressing questions raised in Table 2 has potential to move the EED field forward. While an argument could be made to pursue any of the topic areas/questions, we had to define a starting question to address and had to develop a prioritization scheme given the importance of many of the topic areas/questions. Descriptive epidemiology (topic area I in Table 2), for example, would certainly be useful to gauge the scope of the problem, but would probably not produce useful recommendations. We considered developing a review that considered EED as a dependent variable (i.e., an outcome) of processes and risk factors (topic area II in Table 2). Such a characterization might be used to develop preventive interventions for EED. We next formulated a model of EED as an event that causes many injuries in the host (topic area III in Table 2), such as stunting and micronutrient deficiencies. A review based on this model could be considered an analysis of its consequences by focusing on host injuries and population impact. Biomarkers of EED as a subject for review (topic area IV in Table 2) could provide a compendium of tools that could be used to detect EED, and possibly to shed light on its origin. Consideration was additionally given to reporting the clinical course and pathophysiology of EED (topic area V in Table 2), to summarize the state of knowledge about cellular and organ processes that underlie its disease course. Finally, we considered reviewing existing treatment or prevention interventions for EED (topic area VI in Table 2).
To provide direction for our initial efforts, we decided that it was important to select areas in which a sufficient body of data is likely to exist. An additional attribute for a useful review is that the resulting analysis can be used for disease control.

With these considerations in mind, we narrowed the set to four lead questions:

1. What is the evidence that EED is caused by (an) identifiable pathogen(s), microbial populations, environmental or other identifiable factors?
2. What is the evidence that EED can be prevented by any interventions?
3. What is the evidence that EED can be noninvasively diagnosed?
4. What is the evidence regarding efficacy/effectiveness of treatment interventions for EED?

Based on deliberations amongst the co-authors, and engagement with the Bill & Melinda Gates Foundation, as well as discussions at the Gut Integrity Workshop held in Seattle, Washington in December 2010, we focused on noninvasive diagnosis of EED as a priority systematic review question.
3.2 Determining Relevance to the Systematic Review

We carefully considered the specifics of the review question and framed the question for consistency with the Population Intervention Comparison Outcome (PICO) framework for systematic review questions [99]:

What biomarkers or diagnostic tests\(^1\) have been used to identify or have been shown to be associated with mucosal dysfunction of the small intestine\(^2\) or host inflammation\(^3\) in children under five years of age from developing-country settings\(^4\)?

For the purpose of this systematic review question, dysfunction was defined as manifestation of increased small intestinal permeability, decreased absorption of nutrients, enteric inflammation, or abnormal enterocyte metabolism or cell function. These conditions could be present in children with environmental enteric dysfunction based on histology or persistent diarrhea or those with malnutrition, or who were clinically asymptomatic. Evaluation of asymptomatic or “normal” subjects without overt clinical evidence of enteric dysfunction or those with acute diarrhea was of interest as long as they were evaluated for tests of mucosal small intestinal dysfunction (e.g., endoscopy, histology, or markers of permeability or absorption from serum, urine, or stool) or they were being tested in the same study as children with persistent diarrhea. Gastrointestinal dysfunction or enteropathy related to celiac disease, cow's milk protein allergy (CMPA), inflammatory bowel disease, or cystic fibrosis, as well as primary

\(^1\) Assessments of host biological materials or imaging assessments (e.g. radiologic) of the host.
\(^2\) Including increased small intestinal permeability, decreased absorption of nutrients, enteric inflammation, or abnormal enterocyte metabolism or cell function among those with enteropathy (e.g. environmental enteric dysfunction (EED) based on histology or persistent diarrhea) or children with malnutrition or clinically asymptomatic children.
\(^3\) Laboratory confirmed generalized or tissue inflammation, but not necessarily specifically measuring gut-specific inflammation, e.g. C-reactive protein (CRP), IL-6.
\(^4\) Defined as low or middle income country as determined by World Bank or among marginalized or indigenous populations in a developed country.
immunodeficiency disorders (e.g., X-linked agammaglobulinemia, common variable immunodeficiency, IgA deficiency, IgG subclass deficiency) were excluded from this systematic review.

Studies that used tests or markers specifically related to small intestinal mucosal function (except for the aforementioned excluded specific enteropathies) among children under five years of age from a developing country setting were included. These tests include biopsy, tests of nutrient absorption (e.g., iron absorption), tests of gut permeability and/or absorption (e.g., D-xylose, lactulose:mannitol ratio [L:M]), and stool markers (e.g., fecal fat, reducing substances). Articles describing tests or markers of systemic inflammation that can be affected by mucosal intestinal function (e.g., IL-6, C-reactive protein (CRP), blood counts) were also included as long as they were conducted: a) among children with EED or enteric dysfunction consistent with EED (e.g., those with persistent diarrhea and without an excluded enteropathy), b) among acute diarrhea or asymptomatic patients in a study that compared results to subjects with a small intestinal mucosal disorder of interest, or c) in association with a test of mucosal small intestinal function. Articles that were limited to tests of micronutrient status, celiac or CMPA disease-specific tests, or tests for specific pathogens were excluded from the systematic review.

We decided to restrict this analysis to articles published between 2000 and 2010 in the interest of producing an expedited analysis of a well-defined literature set. We retain the ability to apply this methodology to the literature identified for prior intervals. We also performed an assessment of 10 references chosen at random that were published between 1990 and 1999 to determine the scope of additional information that an analysis of the literature prior to our restricted time block might provide (Appendix 3). Of the 10 articles, only one had a sample size of 100 or more subjects under five years of age. Overall, these articles do not lend substantial or novel data to content already derived from the 2000-2010 analysis.
We acknowledge that delving back to prior decades could provide additional informative data. This is especially true because much study regarding EED occurred in the 1970s and 1980s and waned in the subsequent decades, and because technology is not evolving rapidly in this field. However, secular trends in socioeconomic, environmental, nutritional, and disease conditions as well as improvements in laboratory, epidemiologic, and biostatistical methods complicate comparison of data across studies from different time periods. Also, earlier studies focused on adults.

The team included analysts knowledgeable in German, French, Spanish, Italian, and Portuguese; thus, we were able to thoroughly dissect articles in these languages. References in other languages were excluded as we were not able to translate other languages in detail sufficient for the purposes of thorough extraction and analysis.

A summary of inclusion/exclusion criteria and of the instructions given to analysts is provided in Table 6.
Table 6. Guidelines for systematic review inclusion/exclusion determination and data extraction.

Biomarkers and Diagnostics Systematic Review Question:

What biomarkers or diagnostic tests\(^1\) have been used to identify or have been shown to be associated with mucosal dysfunction\(^2\) of the small intestine or host inflammation\(^3\) in children under five years of age from developing-country settings\(^4\)?

1. Assessments of host biological materials or imaging (e.g., radiologic) assessments of the host.
2. Dysfunction can be related to increased small intestinal permeability, decreased absorption of nutrients, enteric inflammation, or abnormal enterocyte metabolism or cell function among those with enteropathy (e.g., EED based on histology, persistent diarrhea) or children with malnutrition or clinically asymptomatic children.
3. Laboratory-confirmed generalized or tissue inflammation, but not necessarily specifically measuring gut-specific inflammation (e.g. CRP, IL-6).
4. Developing-country setting is defined as a low- or middle-income country (as classified by World Bank) or among indigenous populations in a developed country.

Excludable conditions (non-EED enteropathies)

Celiac disease, IBD, CMPA, cystic fibrosis (CF) (diagnosed by abnormal sweat test), as well as primary immunodeficiency disorders (e.g., X-Linked agammaglobulinemia, common variable immunodeficiency, IgA deficiency, IgG subclass deficiency) were not conditions of interest for this review unless the following circumstances existed:

1. The study had controls or other subjects of interest who underwent diagnostic tests that are of interest to us (see "Category I Tests," below).
2. The condition (i.e., celiac disease, CMPA, IBD) did not meet our systematic review criteria for defining or diagnosing that condition. In other words, these disorders may have been incorrectly diagnosed and could actually have been an enteric dysfunction of interest.

Asymptomatic children and children with acute diarrhea:

Evaluation of asymptomatic or ‘normal’ subjects without overt clinical evidence of enteropathy or those with acute diarrhea was pertinent to our review as long as the included tests of mucosal small intestinal dysfunction (e.g., endoscopy, histology, or serum, urine, or stool markers of permeability or absorption). We were not interested in asymptomatic children or those with acute diarrhea if tested for only systemic markers, unless they were tested in the same study as children with EED or persistent diarrhea (PD). We were interested in the comparison of systemic tests in patients who are asymptomatic and/or have acute diarrhea vs. PD. For example, if a systemic marker was measured in subjects who were asymptomatic or had acute diarrhea, we did not include these data. However, if these tests were also performed in a PD group, then we included the data from all of these subjects— acute diarrhea, PD, and asymptomatic subjects— taking care to separate findings by these categories.

We did not include references about children who presented with abdominal pain, vomiting, anemia, rectal bleeding, gastroesophageal reflux, etc., unless they reported to have also had EED, tropical enteropathy (TE), environmental enteropathy (EE), PD, malabsorption, or other symptoms suggesting small intestinal mucosal dysfunction.
Tests to Include:

Tests specific to intestinal dysfunction: We included biomarkers and diagnostic tests specifically related to small intestinal mucosal function if other inclusion criteria were met (i.e., age under 5, developing-country setting, etc.). We included these types of tests:

- Endoscopy
- Intestinal biopsy or lavage
- Lactose/sucrose load test

Tests of nutrient absorption (not static blood levels; see Excludable Diagnostic Tests, below), such as the following:

- B12 absorption
- Iron absorption
- Calcium absorption
- $^{13}$C sucrose or hydrogen breath test (HBT)

Urine markers of gut permeability or absorption:

- D-xylose
- Creatinine, fraction excretion
- Lactulose, fraction excretion
- Sucrose, fraction excretion
- Sucralose, fraction excretion
- Mannitol, fraction excretion
- L:M (lactulose:mannitol) ratio
- Sucrose:lactulose ratio
- Sucralose:lactulose ratio
- Urea:creatinine ratio
- Lactose:creatinine ratio

Any stool markers (except those testing for specific micro-organisms; see Excludable Diagnostic Tests, below), such as the following:

- Alpha-1-antitrypsin
- Calprotectin
- Fecal fat
- Lactoferrin
- Neopterin
- Myeloperoxidase
- pH
- Reducing substances
- Leukocytes (i.e., white blood cells (WBCs) by microscopy
- Occult blood testing (including guiac)
- Red blood cells (RBCs) by microscopy

Systemic, Non-specific Tests: Many biomarkers and diagnostic tests, including the below list of systemic markers of inflammation, can be impacted by mucosal intestinal function, but they can also be impacted by other non-gastrointestinal disorders.
For these tests, we only included if one or more of the following conditions were met:

1. They were conducted among patients with a mucosal small intestinal disorder of interest (e.g., EED, PD, or among asymptomatic or acute diarrhea subjects in a study that also examined subjects with mucosal small intestinal disorder of interest).

2. The tests were reported in relation to a test of mucosal small intestinal function (see list above).

Examples of systemic, non-specific tests are the following:

- Hemoglobin (HGB), hematocrit (HCT) (blood cell counts)
- Total serum proteins and other serum proteins such as albumin, pre-albumin
- Serum lipids and lipoproteins
- Liver function tests (e.g., alanine transaminase)
- Urine sodium (Na)
- Urine pH
- Systemic inflammatory markers such as:
  - C-reactive protein (CRP)
  - Erythrocyte sedimentation rate (ESR)
  - Tumor necrosis factor (TNF)
  - Interleukin-6 (IL-6)
  - Interferon-gamma (IFN-gamma)
  - Alpha-1-acid glycoprotein (AGP)
  - Serum immunoglobulins
  - Immune cell subsets
  - Ferritin

Algorithm for our inclusion/exclusion decisions on tests/markers:

1. Was the test performed on children under five years in a developing-country setting? If no, exclude. If yes, continue.

2. Is the test on the list of excludable tests? If yes, exclude. If no, continue.

3. Is the test potentially related to small intestinal mucosal function? If no, exclude. If yes, continue.

4. Is the test specific for small intestinal mucosal function? If yes, include and extract data. If no, continue.

5. Is the test a more general test that could be related to dysfunction of other organ systems? If no, exclude. If yes, continue.

6. Was the test assessed among children with mucosal small intestinal dysfunction or among children who have been assessed for mucosal small intestinal dysfunction? If no, exclude. If yes, include and extract data.
3.3 Acquisition of References and Copyright Fair Use Compliance

References potentially relevant to the systematic review were determined by querying the EED Library Access database. The query identified references tagged as explicitly EED-related and relevant to or possibly relevant to topic area IV (i.e., diagnostic tests and biomarkers).

Starting in reverse chronological order, full texts of references that were identified as potentially relevant to the systematic review were obtained as Portable Document Format files (PDFs) and deposited into a central repository on Google Drive.

We maintained compliance with Fair Use obligations of U.S. Copyright Law, watermarking all PDFs and making the Google Drive repository available only to team members. Furthermore, analysts who performed data extraction indicated their compliance with fair use when logging into the data entry system, via a checkbox that stated “I agree to use this article according to US copyright law.”

3.4 Documenting Relevance to the Systematic Review

Two principal investigators (DMD, PIT) and/or lead analysts (ZCN, KMV) reviewed discordant decisions made by research analysts (RAs) to determine relevance of references to the systematic review according to written guidelines (Table 6). In addition, a subset of concordant decisions (with an emphasis on excluded references) was reviewed for quality control.
After the systematic search of the EED Library, we employed the "snowball technique" to identify further articles relevant to the systematic review. The snowball technique involves review of bibliographies of references determined as relevant to the systematic review, and cited articles were cross-checked against the EED Library. If not already included in the Library, the article was evaluated for inclusion in the Library and the systematic review.

3.5 Data Extraction for the Systematic Review

For data extraction, presentation, and analysis, we utilized the REDCap (Research Electronic Data Capture) system (http://project-redcap.org/). REDCap is a secure, web-based application for construction and management of online surveys and databases from multiple users [100]. A sample REDCap template for data extracted from systematic review references can be found in Appendix 4.

After inclusion/exclusion decisions for the review were finalized, six analysts extracted data from studies into REDCap. The analysts were provided written guidelines on the type of data to be extracted (Table 6). Conference call training sessions were employed to reinforce guidelines and to address questions. Analysts were instructed to extract data on relevant facets including: study objectives, outcome of relevance to review question, setting, study design, subject description, case definition for subjects of interest, age groups and age range, study population, sample size for review question, biomarkers or diagnostic tests, test conditions and specifications, and results, as well as provide their impression of the evidence quality and a study synopsis. Extracted data were reviewed for accuracy and completeness by lead analysts, who made edits as needed and provided feedback to the RAs to increase efficiency and accuracy.
We exported specific fields of data from REDCap, facilitating analyses and data presentation in evidence table format. From these characterizations, we portrayed the spectrum of responses in quantitative and free-text formats as needed.

It is important to note that the EED Library, with references from PubMed, EMBASE, Global Health, and WHO Regional databases that were published between 1980 and 2010, remains available for research relevant to enteric dysfunction in children in resource-poor environments.

3.6 EED Library: Search Results Overview

The systematic search of PubMed, Embase, WHO Regional, and Global Health databases yielded 85,334 references of potential relevance to the EED Library. 17,431 references that were published before 1980 have not been assessed for inclusion in the Library. 67,903 references published between 1980 and 2010 are depicted in Figure 6. A small portion of this set was not reviewed because full text was necessary for determination, but was not available (i.e., we were unable to retrieve 89 articles published between 2000 and 2010).

66,541 references were dual-reviewed against EED Library inclusion criteria with 9,669 admitted to the project Library. Fifteen percent of those included were reviews, commentaries, abstract proceedings, books, or editorials, and the remainder were references with primary data. To conserve project resources, approximately 1,350 articles from the original systematic search that were published before 2000 were not reviewed for library inclusion.
3.7 Quality Control

Accuracy and completeness in coding inclusion/exclusion and labels, tags, and topic areas by analysts were closely monitored. Means for the percent of inaccurate exclusion and inclusion and for kappa statistics were weighted based on the number of reference spreadsheets reviewed by each analyst. The overall inaccurate exclusion and inclusion rates were 2.2% and 2.7%, respectively. The kappa average for the group of analysts was 0.76, which is considered to be in the "substantial concordance" range [98]. In addition, 1,200 references that were concordantly excluded by two analysts were reviewed by a lead analyst; the exclusion error rate for these references was 0.5% (Table 7).
Table 7. Accuracy rates for inclusion and exclusion.

Concordance/discordance between analysts and study investigators on an evaluation set of 12,000 references. Analysts who completed only a limited number of references are not included.

<table>
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<tr>
<th>Analyst</th>
<th>Inaccurate Exclude (%)</th>
<th>Inaccurate Include (%)</th>
<th>Kappa (mean)</th>
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Group metrics:

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<th>Weighted average inaccurate include</th>
<th>Weighted average kappa</th>
<th>Concordant exclusion error rate</th>
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<td>0.76</td>
<td>0.5 %</td>
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3.8 EED Library Status

Twenty percent of all of the references derived from our initial systematic search of the PubMed, Embase, and Global Health databases were published between 2006 and 2010. The discordance between the abundance of references that we found in our search and the paucity of references found in the ISI query suggest that relevant literature is indexed with search terms...
that are neither sensitive nor specific. The inclusive approach using terms that broadened the scope of papers identified was therefore warranted, even though such broadening obligated the inclusion of over 85,000 references.

Furthermore, careful documentation of our search terms allows reproducibility despite the complex nature of our strategy. The search strategy can be replicated and resultant references run through our project procedures to update the Library at any time. In addition, the search strategy designed for this project can be modified if related searches are needed.

The EED Library, as derived from PubMed, EMBASE, Global Health and WHO Regional databases and published between 1980 and 2010, was designed to be a resource for scientists, public health and clinical practitioners working on a variety of EED investigations. In fact, we have interrogated our EED Library for several groups of researchers in the field:

1. Dr. David Rudnick at Washington University in St. Louis requested assistance in his work on liver function and growth in resource-limited settings, and we queried the database as regards the role of aflatoxin and growth as reflected in the literature.
2. We provided a list of references from the last decade that reported use of biopsies among children in resource-limited settings to Dr. James Lavery’s team in Toronto to assist in their examination of ethical considerations of invasive and noninvasive assessments of what they termed “tropical enteropathy/enteric enteropathy.”
3. We provided data from our database to Dr. Gerald Keusch’s team (which includes co-authors Drs. Denno and Tarr) who were building a working definition of EED.
4. The master evidence table was made available to all of the participants of the Bill and Melinda Gates Foundation Grand Challenges Gut Function Biomarker Shaping Meeting in London in June 2012.
5. We performed a pilot project for the Bill and Melinda Gates Foundation to determine the number of studies in the EED database that involved interventions. We further determined how many of these were clinical trials vs. treatment studies, categorized the interventions, and tallied the number of studies per category.

The EED Library can be searched using the codes, labels and tags that our Research Analyst team assigned to EED Library records. Continued assembly of literature post-2010 would add value if the database is to be further utilized to address other queries.