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CHAPTER 2.

METHODOLOGY: BUILDING THE EED LIBRARY AND UNDERTAKING A SYSTEMATIC REVIEW OF EED BIOMARKERS/DIAGNOSTICS

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Denno, DM, VanBuskirk, KM, Nelson, ZC, Musser, CA, Tarr, PI. (2016). Environmental enteric dysfunction: Advancing current knowledge. St. Louis, MO: Washington University Libraries. http://dx.doi.org/10.7936/K7WQ0228

Chapter 2. Methodology: Building the EED Library and Undertaking a Systematic Review of EED Biomarkers/Diagnostics

2.1 EED: A Broad Field, Many Unanswered Questions

First, in collaboration with experts in the field, we developed a set of questions that would be of primary importance to better understand and control EED. The scope was broad and included environmental, nutritional, and other factors that might underlie EED, as well as information related to EED pathogenesis. We framed these questions within six “topic areas” (Table 2). We used these questions to guide our systematic literature search, seeking to identify all references that could contribute to answering them. Prior to searching the literature for relevant EED references, a search of the Cochrane Database of Systematic Reviews found no Cochrane Reviews related to EED.

Table 2. Topic areas and questions.

The left column lists inclusive but circumscribed areas of relevance to EED, and the right column presents questions relevant to each topic area. These formed the basis for the literature search terms (Appendix 1).

Topic area	Questions
I. Epidemiology of EED	What is the burden of disease represented by EED? What is the prevalence of EED (including as measured by tests of gut dysfunction or inflammation)? 1. What proportion of stunted/malnourished children have EED (as measured by gut dysfunction/inflammation or infection with specific microbes or identifiable microbial populations) or have a past history of EED? 2. Other
II. EED, malnutrition as an outcome. Associations, risk factors, protective factors, causes of acquisition of EED, malnutrition	What exposures/variables are associated with EED (including as measured by tests of gut dysfunction or inflammation) or malnutrition/stunting? What are the effect sizes (e.g., relative risk (RR), odd ratio (OR)) of the associations? What are the causal pathways/mechanisms? *Exclude exposures related to food security/caloric density.* Include: 3. Is infection with specific enteric pathogens (e.g., subsets of diarrheagenic *E. coli*, *Cryptosporidium*, *Giardia*) associated with EED or malnutrition/stunting? 4. Are recurrent acute enteric infections/recurrent episodes of diarrhea associated with EED or malnutrition/stunting? 5. Are persistent or chronic enteric infections/persistent diarrheal episodes associated with EED or malnutrition/stunting? 6. Is exposure to/ingestion of fecal microbial populations (e.g., in settings with lack of access to improved sanitation) associated with EED or malnutrition/stunting? 7. Are other diseases/conditions including infections not predominantly enteric in origin/manifestation (e.g., HIV, tuberculosis, malaria) associated with EED or malnutrition/stunting? 8. Are environmental (e.g., water, sanitation, hygiene) factors associated with EED or malnutrition/stunting? 9. Are social (e.g., socioeconomic status (SES), household characteristics) or geographic (e.g., rural/urban) factors associated with EED or malnutrition/stunting? 10. Are genetic factors associated with EED or malnutrition/stunting? 11. Are specific foods or nutrients (e.g., micronutrients (MNs), lack of specific foods or nutrients, or specific feeding practices associated with EED or malnutrition/stunting? 12. Are maternal factors (e.g., anemia in pregnancy, maternal short stature) associated with EED or malnutrition/stunting? 13. Is low birth weight (LBW) or small for gestational age (SGA) associated with EED or malnutrition/stunting?

Topic area	Questions
	14. Are microbially contaminated foods/lack of food safety or contaminated bottles, feeding utensils, etc. associated with EED or malnutrition/stunting? 15. Is malnutrition a risk factor for EED? 16. Other
III. EED as an exposure. EED association with, risk factor for, a cause of subsequent other child health problems.	What outcomes are associated with EED (including as measured by gut dysfunction or inflammation or infection with specific microbes or identifiable microbial populations)? 17. Is EED a risk factor for malnutrition/stunting? 18. Is EED a risk factor for MN deficiencies—either multiple deficiencies or isolated deficiencies (including zinc, vitamin A, iron, vitamin D, folate, vitamin B12)? 19. Is EED a risk factor for overnutrition (including overweight and obesity), especially later in childhood/adulthood? 20. Is EED a risk factor for subsequent enteric infections/diarrheal illness, either in general or as caused by specific pathogens? 21. Is EED a risk factor for decreased oral vaccine efficacy or oral drug efficacy? 22. Is EED a risk factor for diminished cognitive function or developmental delay? 23. Other
IV. Assessment, biomarkers, and diagnostics of EED or malnutrition	24. What diagnostic tools or biomarkers are available to assess for EED or malnutrition[1]? What biomarkers are manifest during the EED clinical state that could be utilized to develop a diagnostic test? Subquestions: 24a. How sensitive and specific is the test/biomarker in identifying the child with EED compared to villous blunting with crypt hyperplasia on histologic examination of small bowel biopsy intestinal biopsy? In the absence of comparison to histology, how does the test/marker compare to other diagnostic tests of gut function/dysfunction including permeability, inflammation, or nutrient uptake? 24b. Does the marker or diagnostic allow grading of disease severity or gut dysfunction? 24c. Is the diagnostic or biomarker field-friendly? 24d. What are the costs associated with the diagnostic/marker? 25. Other
V. EED Clinical course, patho-physiology	26. What is the clinical course of EED (e.g., clinical symptoms, signs and laboratory findings)? What are the underlying mechanisms/pathways of these clinical changes? 27. What nutritional changes/abnormalities occur in EED including

Topic area	Questions
	energy metabolism, MN uptake, bioavailability and metabolism? 28. What gut pathophysiology, histology or cellular changes are found in EED? 29. Other
VI. EED, malnutrition interventions—prevention and treatment	What interventions can prevent, treat or mitigate EED (including as measured by tests of gut dysfunction or inflammation) or stunting/malnutrition? What is the effect[2] of interventions in reducing prevalence of EED or malnutrition among children (compared to no intervention or placebo intervention)? What is the effect of interventions on treating or mitigating the impact of EED (*outcomes could include diminished gut inflammation, diminished microbial content in host guts, improved gut function)* or stunting/malnutrition *(outcomes could include improved linear growth or weight gain)* among individual children? For each treatment intervention identified, what is the effect in impacting outcomes compared to no intervention or placebo intervention[3]? Exclude interventions related to increased calorie intake or food security. Interventions include: **Population-based interventions among asymptomatic children in developing-country settings:** 30. Zinc, vitamin A, folic acid, vitamin B12 supplementation or fortification[4] (or supplementation or fortification with other MNs or with multiple MNs) 31. Interventions related to breastfeeding 32. Nutritional interventions such as introduction of or increased consumption of certain foods, including weaning or complementary foods 33. Feeding practices (e.g., responsive feeding practices among caretakers) 34. Improved food safety (e.g., boiling eating utensils, improved food storage and reheating) 35. Improved water, sanitation, hygiene 36. Prebiotics, probiotics 37. Maternal interventions (e.g., prenatal iron/folate supplements in pregnancy and examination of impact on EED or malnutrition in offspring) 38. Health services interventions (e.g., implementation of growth monitoring programs, cash transfers in return for care-seeking) 39. Other **Interventions to prevent EED implemented among children in developing-country settings with specific symptoms (e.g., diarrhea):** 40. Zinc, vitamin A, folic acid, vitamin B12, or other MNs (e.g., for treatment of diarrhea) 41. Prebiotics, probiotics (e.g., for treatment diarrhea)

Topic area	Questions
	42. Management/treatment of other conditions (e.g., antitubercular agents, tuberculosis, antiretrovirals for HIV, antimalarials to treat malaria infections, intermittent preventive treatment of malaria) 43. Other **Among children identified/diagnosed as having EED or malnutrition:** 44. Treatment with specific MNs including: iron, zinc, vitamin A, vitamin D, folic acid, vitamin B12 45. Treatment with multiple MN preparations (e.g., Sprinkles) 46. Treatment with antibiotics 47. Treatment with probiotics 48. Nutritional interventions such as introduction or increased consumption of specific foods; ready-to-use therapeutic or supplementary foods; related nutritional therapeutics 49. Feeding practices (e.g., responsive feeding practices among caretakers) 50. Other

[1] Not including measures of physical growth (e.g., height, weight, mid upper arm circumference), indices calculated from measures of physical growth (e.g., body surface area), or use of growth charts or growth standards.
[2] Including any potential harms identified with the intervention. Furthermore, cost information should be captured where available.
[3] Where possible, also identify the best stage in the EED clinical spectrum in which to intervene and at which stage does therapeutic effect have the greatest impact on outcomes compared to other stages?
[4] Supplementation defined as administration of MNs to a population subgroup based on age or other life cycle factors. E.g., prenatal vitamins, giving 6-36 month-olds vitamin A capsule every six months. Fortification defined as adding MNs to food staples such as the addition of folate to flour.

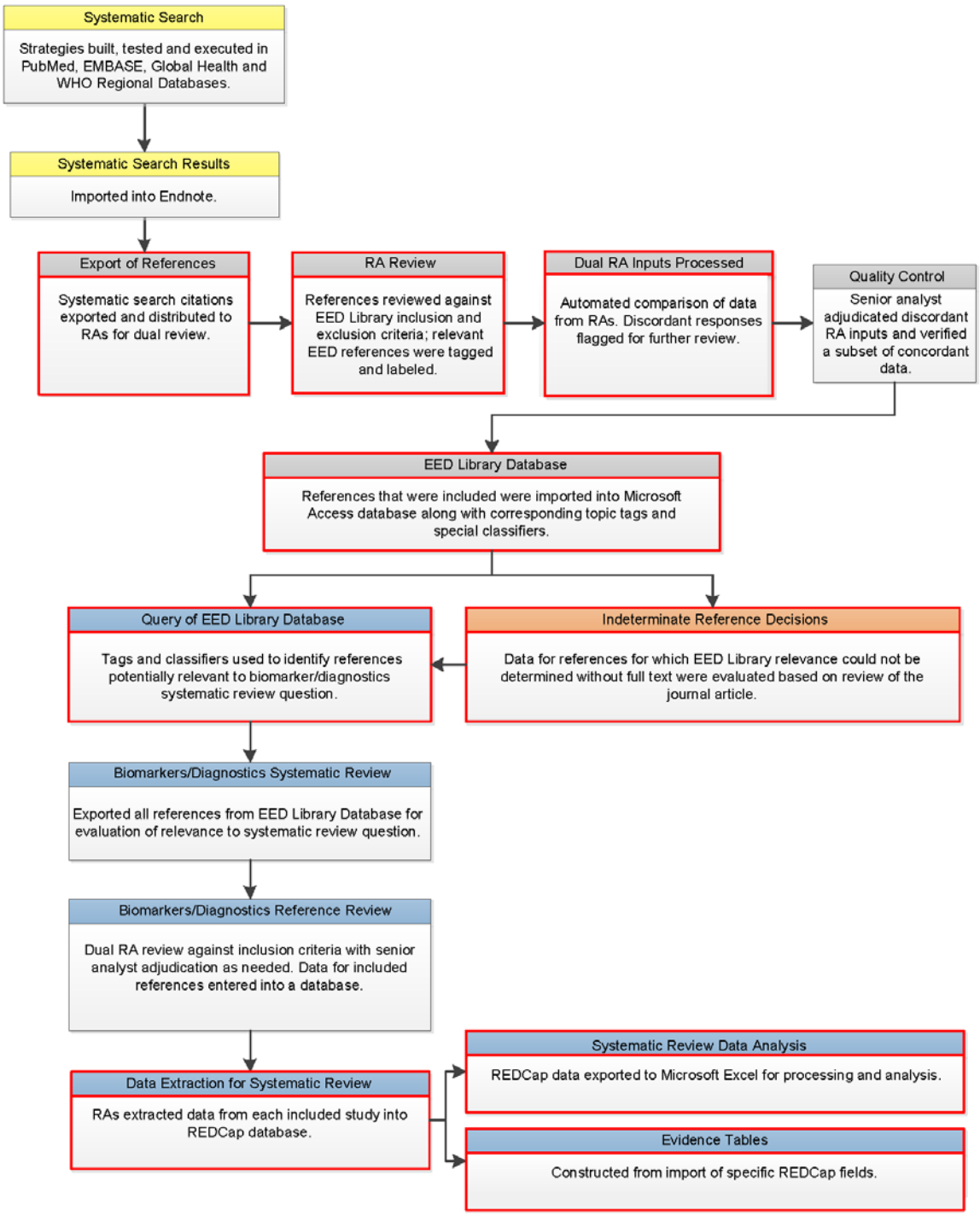
A “wide-net” broadly inclusive systematic search strategy was considered necessary at project inception in order to capture sufficient references of relevance for four reasons: (1) a wide scope and breadth of questions were deemed of interest for potential systematic investigation; (2) we believed that there would likely be modified or derivative questions after the initial review was performed; (3) EED has a broad, indistinct, and historically variable definition; and (4) specific search terms for EED, ED or even enteropathy do not exist in the medical and health databases. Because the effort involved in searching for articles solely related to one EED systematic review question would only be marginally more compared to searching more broadly for articles to address a wide range of EED-related questions, we opted for an infrastructure that could produce a systematic review product efficiently and expediently.

2.2 Constructing a Systematic Search Strategy: Optimizing Sensitivity

We devised a systematic and comprehensive search, extraction, and analysis strategy. Our overall procedure is depicted in Figure 4. We now describe the process components.

The first step was the construction of a comprehensive, systematic search strategy. We developed individual search strategies for each database with the assistance of a research librarian at the World Health Organization in Geneva. We devised a two-step search strategy that was extensive due to the broadly defined nature of EED and the lack of robust indexing of search terms across databases (as described above) (Appendix 1). In the first step, we used broad terms to capture all references related to EED, including similar or identical disorders (‘tropical enteropathy’, ‘environmental enteropathy’, ‘tropical sprue’, ‘tropical malabsorption syndrome’, and ‘malabsorption’, ’enteropathy’, or ‘intestinal dysfunction’ in the tropics). At first pass, we included any age group and any setting (e.g., returned travelers), because these publications could conceivably contain data that provide some understanding of aspects of EED. We were also interested in other enteropathies among children under five years of age in developing countries, such as celiac disease or Crohn’s disease, because these disorders might have been misdiagnosed EED, or because tests employed could be relevant to EED in children at risk in resource-poor areas of the world.

Figure 4. EED systematic review processes flow chart.



As the second step, we identified references about malnutrition or nutritional status (as measured by anthropometrics) among children under five years of age in developing countries. The goal was to identify scenarios where EED is an intermediary: nutritional status as an outcome of enteric dysfunction, biochemical or radiologic biomarkers/diagnostics of nutritional status, or interventions to prevent or treat malnutrition. For nutritional outcomes, we limited our search to effects on anthropometric indices (e.g., height-for-age, weight-for-age, weight-for-height, mid-upper arm circumference, and growth velocity). We excluded articles strictly about prevalence or incidence of malnutrition (i.e., containing no information about risk factors associated with nutritional status), articles about non-EED outcomes of malnutrition/nutritional status, and articles examining the utility of anthropometric measures.

Next, we constructed search strategies to query the most relevant medical and health databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Embase (http://www.embase.com/), Global Health (database published by Centre for Agriculture and Biosciences International (CABI)), and the WHO regional databases. We included Global Health and the WHO databases because of their higher proportions of articles from resource-poor regions, which are often not published in journals that are indexed in PubMed and Embase. Additionally, the WHO regional databases contain much “gray” literature from government and nongovernmental organizations that could have been of relevance.

To ensure that our search adequately identified relevant references, we developed a test set of references obtained by identifying 20 key EED references in collaboration with two external advisors with content expertise (Appendix 2).

We sought to retain as many relevant references in our search results as possible, while minimizing extraneous and irrelevant information. To maintain specificity, we filtered the references for data from developing countries, tropical settings, or indigenous populations.

However, universal use of this filter resulted in test EED references being missed. We were, however, able to use the filter on the malnutrition search strategy without losing relevant articles. We were able to apply another filter to restrict malnutrition articles to those related to children. We did not restrict language or year of publication. After these modifications to our search strategy, 18 of the 20 test references were captured. To detect the remaining two articles, we would have needed to use a strategy that yielded a ten-fold increase in the number of articles returned in the search. On further scrutiny, this problem was caused by a lack of sensitive index terms for one article [87] and lack of child terms for the other [57] (Appendix 2). We accepted this compromise, recognizing that the "snowball" technique (described below) would increase our search sensitivity.

With our approach, we were able to interrogate the literature on a topic that is both poorly defined and poorly indexed. Our comprehensive, rigorously evaluated, and reproducible methodology can be utilized as a systematic search model approach for other topics that are similarly broad and/or diffusely defined or cataloged, for which standard systematic search techniques would be insufficient and imprecise.

2.3 Reference Volume Mitigation

The systematic search, completed June 2010, identified a total of 85,334 references (after identifying and removing references that were duplicated within the four databases that were searched), dating back to 1910 (discussed further in Results section). This overwhelming volume of potentially relevant literature was unexpected, and we briefly considered using only references from recent review articles on the topic. This was not possible, however, because no previous systematic reviews had been published with which to identify biomarkers that could be used to prevent and treat, or to guide rehabilitation from, intestinal dysfunction in children in resource-limited countries. Some reviews [8, 45, 88-93] either focused on adults or on narrow

components of the problems, and not diagnostic strategies. Hence, this “look back” literature review strategy would not have yielded the information we were seeking.

We next considered two options relevant to processing the ca. 85,000 titles. The first option was to scrutinize this very long list with only one or two questions in mind, and vote on each as presumptive “include” or “exclude” related only to the limited inquiry, and ignore all irrelevant topic areas and corresponding questions (“limited-use scrutiny”). The second option was to build an EED Library with references with notation of its relevance to any of the potential topic area(s) and questions (“future use scrutiny”). The third option was to scrutinize each reference, and then note the relevance only at the topic level, and not identify the specific questions that each reference might address. Table 3 summarizes the advantages and disadvantages of each approach.

After careful consideration, we decided to use a “modified future use” approach, i.e., examine each reference for its relevance to each of the six target areas, but not drill down to the question level. However, we left questions available to the analysts as guides to the potential utility of each document. We recognized that this approach entailed more analyst labor, i.e., approximately 200 additional person hours (assuming two readings of each listing) compared to the first strategy in Table 3 (these estimates are limited to the review of the reference lists). However, the hours of effort per question potentially answered would be considerably fewer, when considering that the comprehensive scoring would encompass topic areas that include a total of 49 potentially useful questions.

Table 3. Summary of analysis options.

Advantages and disadvantages of comprehensive versus targeted analysis of literature produced by search terms are portrayed.

Strategy	Advantages	Disadvantages
1. Targeted ("single use") scrutiny of 85,000 references (focus on only 1 or 2 possible uses of/questions for database)	Reduced time per reference, based on an estimate of 0.75 hour for 1 analyst to scrutinize 200 references, and all references are independently reviewed by two analysts, for a total of 637 person hours	Cannot be used for more than a very limited number of questions (estimated 2 at most)
2. Broad ("future use") scrutiny (focus on all possible topic areas, and denote particular questions)	Diminishes need to repeat scrutiny of references, and generates candidate documents for all potential topic areas and questions	Extended time per reference compared to strategy 1 (based on an estimate of 1.75 hours for 1 analyst to scrutinize 200 references, and all references are reviewed by two analysts), for a total of 1488 hours
3. Broad ("modified future use") scrutiny (focus on all possible topic areas, but do not denote particular questions)	Diminishes need to repeat scrutiny of references, and generates candidate documents for all potential topic areas, but not questions	Extended time per reference compared to strategy 1 (based on an estimate of 1.25 hours for 1 analyst to scrutinize 200 references, and all references are reviewed by two analysts), for a total of 1,063 person hours

2.4 Building the EED Library

The initial list of approximately 85,000 references from the processes described above contained the following information from the databases searched: title, authors, journal, and to varying extents, abstracts. We exported the references from PubMed, EMBASE and Global Health into EndNote software (ca. 81,000); this was not possible with the references from the WHO Regional libraries (ca. 4,000) because of the format in which these references were exported from the search engine.

References from all of these sources were reviewed by research analyst (RA) pairs comprised of individuals trained in epidemiology or nutritional sciences, and who received extensive training in our protocol for inclusion/exclusion of studies.

Per standard systematic review methodology, each reference (title, keywords, and abstract when available) was reviewed by two analysts to determine inclusion status for the EED Library. Dual review reduces bias and inaccuracies in the systematic review process. The principal investigators (DMD and PIT) initially piloted the Library inclusion process, until 100% concordance was achieved upon independent review of 1,300 consecutive references. A series of training sessions to convey the goals of the project, along with a protocol with which to determine inclusion of a reference in the EED Library, were provided to the team of RAs. Verbal and written instructions were conveyed to the analysts (Table 4), and a schematic regarding articles to include, and under what category, was also provided (Figure 5).

Table 4. Summary of EED Library inclusion/exclusion instructions to research analysts.

Each analyst was instructed in the scope of interest of the EED Library, and how to code, tag and label data related to each reference included in the EED Library.

A reference was included if it pertained to any of the following conditions:

- Environmental Enteric Dysfunction
- Tropical Enteropathy
- Tropical Sprue
- Environmental Enteropathy
- Tropical Malabsorption Syndrome
- Malabsorption/Enteropathy/Enteric Dysfunction in resource-limited settings

Any age group and any setting (e.g., travelers) were eligible for this filter step.

References about other enteropathies were included *only if* among children under five in developing countries (e.g., celiac disease, inflammatory bowel disease (IBD)).

Nutrition-related articles were included if:

- Malnutrition or nutritional status (as measured by anthropometrics) among children under five in developing country was an outcome
- The study pertained to biochemical diagnostics or biomarkers or radiographic or other imaging among children under five in a developing country
- The study used interventions to prevent or treat malnutrition, even if the outcome was something other than change in nutritional status or prevalence of malnutrition. Interventions were eligible only if they started among children under five years, even if outcomes were measured at a later age.

A separate category of inclusion captured relevant reviews; this was defined broadly to include review articles, meta-analyses, editorials, commentaries, compendia or conference proceedings, letters, books, or book chapters.

Exclusions:

- prevalence, incidence, etc. of malnutrition if there was no information about factors (other than caloric insufficiency/food insecurity) associated with nutritional status
- outcomes specifically due to malnutrition/nutritional status
- the utilization of anthropometric measures

Further delineation of relevance to our systematic review

Topic areas:

I. Epidemiology of EED
II. EED or malnutrition as an outcome
Any associations, risk factors, protective factors, causes of acquisition of EED, malnutrition (except for food insecurity/inadequate calories associated with malnutrition).
III. EED as an exposure
EED as an association with, risk factor for, or cause of subsequent other child health problems.
IV. Assessment, biomarkers, and diagnostics of EED or malnutrition
V. EED clinical course, pathophysiology
VI. EED or malnutrition interventions

Relevant studies included in the EED Library:

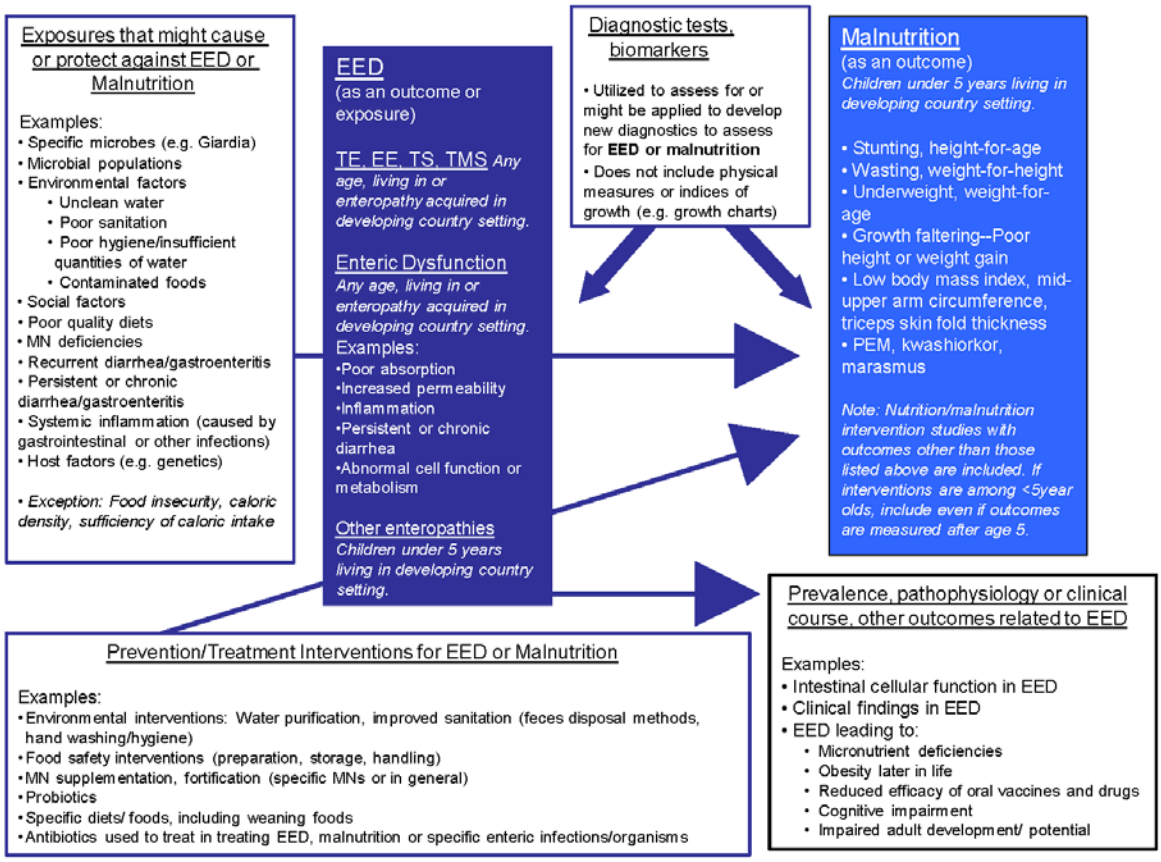
- Exposures, risk factors, protective factors, host factors, prevention or treatment interventions (other than those related to caloric density or food security), and their impact on EED or malnutrition outcomes.
- Diagnostic tests or biomarkers related to, or to assess for, EED or malnutrition.
- Interventions (other than those related to caloric density or food security) to prevent or treat malnutrition in children under five years, even if the outcome was something other than change in prevalence of malnutrition or change in nutritional status of individual children. Outcomes could include, for example, change in case fatality rate. Outcomes measured beyond five years were also included.
- Prevalence, clinical course, and pathophysiology of EED.

References we excluded from the EED Library:

- Malnutrition as a risk factor for other morbidities/outcomes (e.g., malnutrition as a risk factor for pneumonia, stunting as a risk factor for obesity or mortality in adulthood, malnutrition as a risk factor for childhood mortality) unless the outcome was EED (e.g., malnutrition as a risk factor for poor intestinal function was included)
- Malnutrition prevalence studies--unless they also examined risk factors for malnutrition or were intervention studies where change in prevalence of malnutrition is an outcome.
- Measures/indices of physical growth such as growth charts or use of new indices calculated based on height, weight, or other physical measurements.

Figure 5. EED Library inclusion schematic.

Analysts used this as a guide in determining which references should be included in the EED Library.



Abbreviations: EE-environmental enteropathy, EED-environmental enteric dysfunction, PEM-protein energy malnutrition, TE-tropical enteropathy, TMS-tropical malabsorption syndrome, TS-tropical sprue

Feedback on nuances of the inclusion protocol was communicated to the analysts on a regular basis for the duration of the project. Table 5 contains samples of such guidance.

Table 5. Additional EED Library inclusion/exclusion guidance and tips.

The EED category was intentionally defined broadly. The term environmental enteric dysfunction (EED) has several potential equivalents in the literature, including tropical enteropathy, environmental enteropathy, tropical sprue, and tropical malabsorption syndrome. We also included references about enteric dysfunction and any other enteropathy impacting children in developing countries. These enteric conditions included kwashiorkor enteropathy, HIV enteropathy, tuberculosis enteropathy, celiac disease, inflammatory bowel disease, and other enteropathies, assuming they occurred in a developing-country setting.

References were included even if our outcomes of interest were not the study's primary focus.

If the title or abstract clearly indicated that the outcome was acute diarrhea, acute gastroenteritis or an acute enteric infection, we excluded it. If the reference noted they specifically examined persistent or chronic diarrhea as an outcome, it was included. Even if the main outcome studied was acute diarrhea, the reference was included if it examined EED or persistent/chronic diarrhea as a "minor" outcome. If an outcome of "diarrhea" was not specified as either acute or persistent/chronic, we assumed the article referred to acute diarrhea and we excluded it. References about acute diarrhea were included when they examined the impact of acute diarrheal illness or acute gastroenteritis on EED or malnutrition.

References about children with IBD or celiac disease originating in a developing country were included (but excluded if the study was conducted in a developed country). Even though the origin of IBD and celiac disease is distinct from EED, we included it from developing-country settings for two reasons:

a. Celiac disease or IBD in developing countries may truly be misdiagnosed EED, a fact we will be better able to judge when reading the study methodology.

b. We are attempting to look at enteric dysfunction in developing settings more broadly and with fresh perspectives, to allow new observations of underlying patterns.

While enteropathy is not always a manifestation of tuberculosis, HIV, or kwashiorkor, if a study in a developing country discussed enteric dysfunction or enteropathy related to these conditions, we included it.

We excluded studies that reported prevalence of infection with a specific pathogen. If a study examined a pathogen's association with EED or other outcomes of interest as previously specified, then we included it.

We focused on small intestine pathology; therefore, we excluded studies looking at gastric/colonic pathology unless they also examined outcomes pertaining to the small intestine.

References about gastrointestinal problems that are not EED-related were excluded; a non-exhaustive list of commonly encountered conditions not included in our review includes:

- Appendicitis
- Blind loop syndrome
- Colonic atresia
- Duodenal atresia
- Dyspepsia

- Hemolytic uremic syndrome
- Henoch-Schonlein purpura
- Hirschsprung's disease
- Intestinal obstruction
- Intussusception
- Irritable bowel syndrome
- Malrotation
- Necrotizing enterocolitis
- Perirectal abscess
- Peritonitis
- Primary bile acid malabsorption
- Pseudomembranous colitis
- Rectal prolapse
- Short bowel syndrome
- Volvulus

We included studies examining potential risk/protective factors for stunting, wasting or other forms of malnutrition, except those related to food security or caloric density. We excluded studies where any type of malnutrition was considered the exposure, unless EED was an outcome.

Articles examining factors associated with anthropometric/growth outcomes were included even if not related to malnutrition. We did not include articles that solely examined the outcomes of overweight and obesity (unless related to EED), but studies of changes in growth status among children under five in developing countries were included. We excluded anthropometric data collected for the purpose of evaluating national statistics (e.g., in relation to WHO child growth standards) and studies of malnutrition or nutritional status prevalence unless the studies also looked at risk or protective factors associated with EED.

Growth outcomes among children with common chronic infectious diseases such as HIV or hepatitis were considered outcomes of interest.

We included any potential risk or protective factors for EED, malnutrition, or other outcomes of interest, even if they are not necessarily directly related to gut dysfunction, e.g., poverty, domestic violence, maternal anemia, small for gestational age (SGA), or low birth weight (LBW). We excluded studies where SGA or LBW was the study outcome, however.

We included genetic risk factors for EED, malnutrition, or another related outcome of interest as long the study was conducted in a developing-country setting.

Many studies contain relevant information about children under five even though they are not restricted to— or even focusing on— that age group. If any children under five were included, we included the reference.

If a relevant study was conducted in a year when the study country was on the developing country list, it was included.

We excluded case reports (or in vitro lab or animal model studies) even if relevant to EED.

The analysts were provided guidelines on inclusion and exclusion criteria. They were further instructed to: include only references from work performed in low- and middle-income countries (per World Bank definitions during the time that the data in the reference were collected) or among marginalized or indigenous populations in developed countries (e.g., Aboriginal Australian children) [94, 95]; to include references related to EED or conditions identical to or very consistent with EED (e.g., environmental enteropathy, tropical enteropathy, persistent diarrhea) among any age group in a setting of interest, and references related to other enteropathies or to nutritional conditions of interest among children under five years of age in a setting of interest.

The refinements in the inclusion/exclusion instructions regarding other enteropathies were implemented because there is accumulating evidence that celiac disease is not confined to individuals of northern European descent residing in industrialized countries, but is instead a worldwide problem, including in regions in which EED is endemic, such as South Asia [96]. Second, there is increasing recognition of inflammatory bowel diseases (i.e., Crohn's disease and ulcerative colitis) in these regions [97], though most cases of intestinal inflammation in these populations are not related to idiopathic inflammatory bowel diseases in children under five years of age. Third, we wished to include references on malnutrition and nutritional status where EED could act as an intermediary while excluding references that examined other aspects of malnutrition. For example, while food insecurity commonly affects populations at risk for EED, studies of nutritional deficits by themselves, including surveys of such deficiencies, were designated to be beyond the scope of our project. In another example, iron deficiency can be caused by a multitude of factors including defective absorptive capacity in the small bowel. To capture only the references relating to intestinal absorptive function, we excluded references if iron deficiency was studied outside the context of intestinal uptake assessment or another process related to EED.

Progressing in reverse chronological order, virtually all references between 1980 and 2010 were evaluated as to whether or not they should be included or excluded from the EED Library, or whether or not additional information from the full text (particularly when the abstract was not initially available from the medical/health databases searched) was needed to make the determination. If included in the Library, references were assigned tags as to whether the reference contained information about: 1) enteropathy or enteric function/dysfunction, 2) nutritional status or malnutrition, and/or 3) enteric microbes. By using the topic areas and tags, we could then formulate queries to apply to our EED Library to identify articles potentially relevant to specific review questions that are contained in Table 2 or identified in the future. Additionally, topic areas (Table 2) covered in each reference were noted, as well as an indication whether the reference was a review or otherwise did not present primary data.

Each reference published between 1980 and 2010 was reviewed for inclusion by two analysts according to the written guidelines and instructions. A principal investigator or lead analyst reviewed all references for which the analysts were discordant on Library inclusion, topic area, or other determinations, and provided final decisions. Furthermore, to verify that systematic errors did not occur in the exclusion of references, a random subset of references excluded by both analysts was scrutinized by a lead analyst. Percent error rate for this subset was calculated.

The kappa statistic was used to evaluate reliability of individual analyst responses against final inclusion/exclusion determinations. Interpretation of kappa was performed using the following guidelines as described by Koepsell and Weiss [98]: agreement of >0.80 was deemed excellent, 0.61-0.80 substantial, 0.41-0.60 moderate, 0.21-0.40 fair, 0.00-0.20 slight, and <0.00 poor.