Globally, 800,000 children under age five die from diarrhea each year, which accounts for 10 percent of all deaths in children under-five.[1] Most of these deaths occur in sub-Saharan Africa and south Asia. Although diarrheal deaths remain unacceptably high, the number of childhood deaths is decreasing by about 4% per year. The number of diarrheal disease cases, however, is decreasing less rapidly.

Over forty enteric (gut) pathogens have been identified to cause moderate-to-severe diarrhea (MSD) in children and adults.[2] The most common pathogens responsible for childhood diarrhea are summarized below (Table 1). These pathogens include bacteria, viruses, protozoa, and helminths. Bacteria are single-celled organisms that are aproximatedly one micron (one millionth of a meter) in size, have a cell wall, and can replicate in the environment. Virus particles are DNA or RNA strands that are generally less than 1 micron in size, have a protein coat and (in some cases) a lipid envelope, and can only replicate inside the cells of other organisms. Protozoa are large, 3-10 micron, single-celled organisms that in the environment are encased in a protective shell, which opens up to infect the intestine. Helminths are large, multicellular worm-like organisms that can live in, and feed on, living hosts; those that live in the human digestive tract are referred to as intestinal parasites. Organisms from these four pathogen classes cause diarrheal disease n children and adults. Diarrheal disease incidence is greatly reduced when piped, treated infrastructure water is provided, as the combination of filtration and chlorination removes the vast majority of bacteria, viruses, protozoa, and parasites.

Bacteria	Viruses	Protozoa	Helminths
Diarrheagenic *Escherichia coli*	Rotavirus	*Cryptosporidium species*	*Strongyloides stercoralis*
Campylobacter jejuni	Norovirus	*Giardia lamblia*	*Angiostrongylus costaricensis*
Shigella species	Adenovirus	*Microsporidia*	*Schistosoma mansoni/japonicum*
Vibrio cholerae O1	Astrovirus	*Entamoeba histolytica*	
Vibrio cholerae O139	Cytomegalovirus	*Isospora belli*	
V. parahameolyticus		*Cyclospora cayetanensis*	
Bacteroides fragilis		*Dientamoeba fragilis*	
C. coli		*Blastocystis hominis*	
C. upsaliensis			
Nontyphoidal *Salmonella*			
Clostridium difficile			
Yersinia eterocolitica			
Y. pseudotuberculosis			

Table 1: Enteric pathogens that cause moderate-to-severe diarrhea in children

Until recently, it was unknown which of these pathogens actually was responsible for the majority of diarrheal disease cases and deaths. The recent GEMS study (summarized in Technical Memorandum 2) found that the five most common pathogens associated with MSD and death – independent of geographic location – are Rotavirus, *Cryptosporidium species*, and *Shigella species,* ST-ETEC, and typical enteropathogenic *E. coli*.[1,3,4]

Although malnutrition is commonly believed to be associated with MSD, there remains considerable debate as to whether malnutrition is caused by multiple MSD episodes or if MSD is simply more common in children who are malnourished.[4–9] Three factors that are thought to potentially play a role in the development of malnutrition are: 1) pathophysiology – the impact the diarrheal disease-causing enteric pathogen has on the intestine itself[1,3,5,10,11]; 2) exposure early in life; and, 3) duration of disease. Children that are exposed earliest to enteric pathogens that cause longer duration disease and intestinal damage are thought to be most likely to develop malnutrition. Subsequently children with malnutrition are more susceptible to infection with diarrheal diseases of longer duration illness and that lead to additional intestinal damage that exacerbates the malnutrition.

In the small intestine (where most nutrient absorption occurs), proteins, fats, and carbohydrates are first broken down into small, absorbable components by digestive enzymes secreted by the pancreas. This digested food is then able to pass into the blood vessels in the intestinal wall through either diffusion or active transport. The inner wall, or mucosa, of the small intestine is lined with epithelial tissue. Structurally, the mucosa is covered in wrinkles or folds called plicae circulares (Figure 2). From the plicae circulares project microscopic finger-like pieces of tissue called villi. The individual epithelial cells also have finger-like projections known as microvilli. The functions of the plicae circulares, the villi, and the microvilli are to increase the amount of surface area available for the absorption of nutrients, and to limit the loss of nutrients to intestinal fauna.

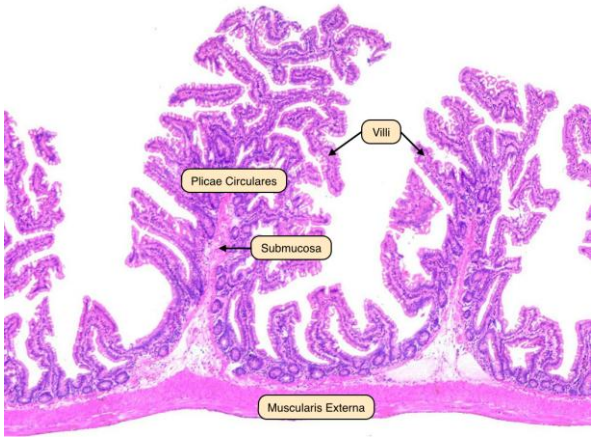

Figure 2: Structure of small intestine mucosa (http://medcell.med.yale.edu)

While all of the enteric pathogens described in Table 1 are likely to cause diarrhea, the impact each has on the intestine – and subsequent nutrient absorption and development or exacerbation of malnutrition – varies greatly.

- *Campylobacter*, *Cryptosporidium*, and *Giardia* all cause inflammation of the intestine. When the intestine becomes inflamed the villi can become stunted and the intestine becomes more porous or "leaky." This increased porosity allows large molecules to pass across the intestinal walls, reducing the absorption of nutrients.[11,12] This inflammation and leakiness is termed 'environmental enteropathy'.
- Enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) cause changes in epithelial cell function, because these organisms adhere to the epithilial cells during an infection.
- Infections caused by pathogens that produce a cytotoxin, such as *Clostridium difficile*, are not likely to be contributors to malnutrition. However, malnourished individuals are regularly seen with these infections.

The potential for development of malnutrition also depends on the duration and severity of disease, which is impacted by whether or not a child is symptomatic (has diarrhea), if there is treatment available, and if the child gains immunity to the disease after exposure. It has been found that children infected with Rotavirus and *Cryptosporidium* can have intestinal inflammation and disruption even when asymptomatic for diarrhea.[13] However, diarrheal caused Rotavirus, except in immune-compromised individuals, is self-limiting (the disease resolves within intervention). Infected individuals also have limited immunity to future infections. Thus, if a child survives Rotavirus infection, they are unlikely to become malnourished. Conversely, *Cryptosporidium* is particularly severe because it persists both symptomatically and asymptomatically and there is no viable treatment option. *Cryptosporidium* infection has been shown to contribute to the development of malnutrition; the data suggest that when an infant (0-11 months of age) has Cryptosporidosis the severity of the disease may be so great that the child's intestinal mucosa never fully recovers.[14] A similar pattern, but less severe, occurs in children up to age three. It has also been suggested, although not yet confirmed by data, that *Shigella* species and ETEC may also play a role in developing malnutrition.[4,15,16] It has been hypothesized that ETEC may contribute to the development of malnutrition because, like *Cryptosporidium*, it is persistent and infected individuals can be asymptomatic. Although data is limited, the reduced uptake of nutrients due to intestinal inflammation and permeability may be associated with the development of malnutrition.

As intestinal impairment has been shown to limit growth and development, understanding the mechanism by which various pathogens could potentially cause or exacerbate malnutrition is critical to developing a long-term strategy to reduce malnutrition.[15,16] It has recently been suggested that malnourished children have a critical window of the first 1,000 days (from conception to age 2) to prevent malnutrition, and that within this time period children can potentially 'catch up' to a normal well-nourished state. Interventions targeted during the time period from fetal development to the child's second birthday have resulted in improved nutrition for children, reduced diarrheal disease burden, and reduced under-nutrition for mothers[17–19]. Additionally, a recent review suggests that even interventions outside of that window should not be overlooked as there is potentially an additional window of opportunity in adolescence when interventions may be beneficial.[20] Lastly, macronutrient interventions, especially those including Zinc and Vitamin A, may help to reduce the number of episodes or severity of diarrheal disease in malnourished children.[19,21,22]

Key Points and Recommendations

- Diarrheal disease is caused by enteric pathoges (bacteria, viruses, protozoa, and helminthes).
- The most common enteric pathogens that cause moderate-to-severe diarrhea and death in children are Rotavirus, *Cryptosporidium*, and *Shigella species,* ST-ETEC, and typical enteropathogenic *E. coli.*
- There remains considerable debate as to whether malnutrition is caused by multiple moderate-to-severe diarrhea episodes or if moderate-to-severe diarrhea is simply more common in children who are malnourished.
    - Children exposed earliest to enteric pathogens that cause longer duration disease and intestinal damage are thought to be most likely to develop malnutrition.
    - Children who are malnourished are more susceptible to infection, and infection can be of longer duration and more severe than in well-nourished children.
- The physical impact infection with an enteric pathogen has on intestinal function impacts the potential for developing malnutrition.
- *Cryptosporidium* is the only enteric pathogen with evidence showing infection leads to malnutrition (particularly in under-1's and under-3's), although it is postulated this could also be true for ETEC and *Shigella species*.
- The earlier a child is reached with interventions, the greater the chance there is of preventing malnutrition.
- In malnourished children, treatment for moderate-to-severe diarrhea should be aggressive.

References and Further Reading

1. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013;382(9888):209-22. doi:10.1016/S0140-6736(13)60844-2.
2. Farthing M, Salam M a, Lindberg G, et al. Acute diarrhea in adults and children: a global perspective. *J. Clin. Gastroenterol.* 2013;47(1):12-20. doi:10.1097/MCG.0b013e31826df662.
3. Lanata CF, Fischer-Walker CL, Olascoaga AC, Torres CX, Aryee MJ, Black RE. Global causes of diarrheal disease mortality in children <5 years of age: a systematic review. *PLoS One* 2013;8(9):e72788. doi:10.1371/journal.pone.0072788.
4. Ferdous F, Das SK, Ahmed S, et al. Severity of diarrhea and malnutrition among under five-year-old children in rural Bangladesh. *Am. J. Trop. Med. Hyg.* 2013;89(2):223-8. doi:10.4269/ajtmh.12-0743.
5. Briend A, Aziz K, Hasan KZ, Hoque BA. Are Diarrhoea Control Programs Likely to Reduce Childhood Malnutrition? Observations From Rural Bangladesh. *Lancet* 1989;August 5:319-322.
6. States M. MALNUTRITION : the most effective interventions must be prioritised. 2010;(April):2010-2012.
7. Brown KH. Symposium : Nutrition and Infection, Prologue and Progress Since 1968 Diarrhea and Malnutrition 1. 2003:328-332.
8. Macallan D. Malnutrition and Infection. *Medicine (Baltimore).* 2001;29(1):17-19. doi:10.1383/medc.29.1.17.27511.
9. Rice L, Sacco L, Hyder A, Black RE. Malnutrition as an underlying cause of childhood deaths associated with infectious diseases in developing countries. *Bull. World Health Organ.* 2000;78(10):1207-21. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2560622&tool=pmcentrez&rendertype=abstract.
10. Petri WA, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J. Clin. Invest.* 2008;118(4):1277-1290.
11. Kelly P. Diarrhoea in children and Cryptosporidium. *Lancet* 2013;382(9904):1552. doi:10.1016/S0140-6736(13)62321-1.
12. Costa LB, Johnbull EA, Reeves JT, et al. Cryptosporidium -Malnutrition interactions: Mucosal Disruption, Cytokines, and TLR Signaling in a Weaned Murine Model. *J. Parasitol.* 2011;97(6):1113-1120. doi:10.1645/GE-2848.1.CRYPTOSPORIDIUM.
13. Kirkpatrick BD, Noel F, Rouzier PD, et al. Childhood Cryptosporidiosis Is Associated with a Persistent Systemic Inflammatory Response. *Clin. Infect. Dis.* 2006;43:4-8.
14. Molbak K, Anderson M, Aaby P, et al. Cryptosporidium infection in infancy as a cause of malnutrition : a community study from Guinea-Bissau , West Africa. 1996.
15. Van den Broek JM1, Roy SK, Khan WA, Ara G, Chakraborty B, Islam S BB. shigella.pdf. *J. Heal. Popul. Nutr.* 2005;23(3):259-265.
16. Dewan N, Faruque a S, Fuchs GJ. Nutritional status and diarrhoeal pathogen in hospitalized children in Bangladesh. *Acta Paediatr.* 1998;87(6):627-30. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9686653.
17. Bhutta Z a, Das JK, Rizvi A, et al. Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? *Lancet* 2013;382(9890):452-77. doi:10.1016/S0140-6736(13)60996-4.
18. Horton R, Lo S. Maternal and child nutrition: building momentum for impact. *Lancet* 2013;382(9890):372-5. doi:10.1016/S0140-6736(13)60988-5.
19. Bhutta Z a, Ahmed T, Black RE, et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet* 2008;371(9610):417-40. doi:10.1016/S0140-6736(07)61693-6.
20. Prentice AM, Ward KA, Goldberg GR, et al. Perspective Critical windows for nutritional interventions against stunting 1 – 3. *Am. J. Clin. Nutr.* 2013:911-918. doi:10.3945/ajcn.112.052332.1.
21. Robert E Black MD, Prof Cesar G Victora MD, Prof Susan P Walker PhD, Prof Zulfiqar A Bhutta PhD, Prof Parul Christian DrPH, Mercedes de Onis MD, Prof Majid Ezzati PhD, Prof Sally Grantham-McGregor FRCP, Prof Joanne Katz ScD, Prof Reynaldo MRobert E Black the M and CNSG. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet* 2013;382(9890):427-451.
22. Morris SS, Cousens SN, Kirkwood BR, Arthur P, Ross D a. Is prevalence of diarrhea a better predictor of subsequent mortality and weight gain than diarrhea incidence? *Am. J. Epidemiol.* 1996;144(6):582-8. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8797518.

The Global Enteric Multicenter Study (GEMS) was a study funded by the Bill and Melinda Gates Foundation to estimate the population-based burden, microbiologic etiology, and adverse clinical consequences (including growth faltering, and death) of moderate-to-severe diarrhea (MSD) among children in developing countries. The study was concurrently conducted in seven countries with known high diarrheal disease burdens, including four countries in Africa (Mali, The Gambia, Kenya, and Mozambique) and three countries in Asia (India, Bangladesh, and Pakistan).

The methodology used in GEMS was a 3-year, prospective, age-stratified, matched case-control study of MSD in children aged 0–59 months residing in censused populations at the seven study sites. GEMS targeted three age strata: infants (0–11 months), toddlers (12–23 months), and children (24–59 months). From 2007-2011, at each site, GEMS staff recruited children with MSD seeking care at health centers ('cases') along with one to three randomly selected age-, sex-, and residence-matched community control children without diarrhea ('controls'). At enrollment, caretakers of cases and controls underwent standardized interviews to solicit demographic, epidemiological, and clinical information. For both cases and controls, height/length was measured, a height-for-age score (HAZ) was calculated, and a stool sample was collected. Following enrollment, clinical records were documented and one follow-up visit about 60 days after enrollment was conducted to assess the case and control vital status, capture interim medical events, and repeat anthropometric measurements. Fecal samples were analyzed for almost 40 bacterial, viral, and protozoal pathogens. Results were analyzed across all children enrolled in the study, and also stratified by age, pathogen, and study site.

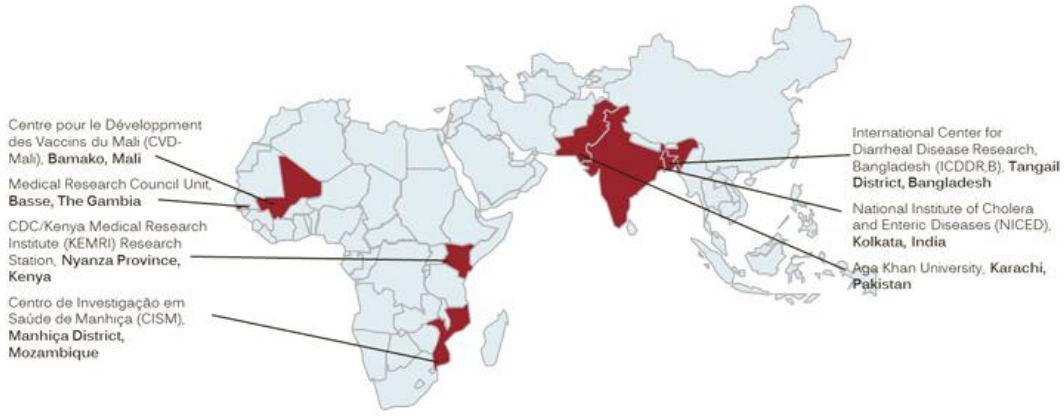

Figure 1: GEMS Study Sites

In all health centers, 12.2% of the visits for infants, 14.7% of the visits for toddlers, and 6.3% of the visits for children were for diarrhea. Overall, 9,439 children with MSD and 13,129 control children without diarrhea were enrolled in GEMS. The overall annual incidence of MSD per 100 child-years was calculated to be 30.8 for infants, 23.1 for toddlers, and 7.7 for children. Demographic and health indicators in cases and controls varied, as controls in several sites and age groups belonged to a higher wealth quintile (India and Pakistan), had greater access to improved water (Mali, Kenya, and The Gambia), and had more educated mothers (India) than did patients with MSD. Overall, one or more pathogens were identified in the stools of 7,851 cases (83%) and in stools of 9,395 controls (72%); two or more pathogens were identified in stools of 4,200 cases (45%) and stools of 4,075 controls (31%).

To represent what percentage of MSD could be attributable to a specific pathogen, GEMS researchers calculated a value termed an adjusted population attributable fraction (AF), which takes into account the fact multiple pathogens were seen in the stool samples of a large minority of cases and controls. The median proportion of episodes attributable to a pathogen was 44% for infants, 47% for toddlers, and 40% for children. Four pathogens were significantly associated with MSD at all seven study sites in one or more age strata: Rotavirus, *Cryptosporidium, Shigella*, and heat stable Enterotoxigenic *E. coli* (ST-ETEC) (Table 1). Rotavirus had the highest AF of any pathogen at every site during infancy. Although its AF generally diminished with age, Rotavirus had the largest AF of any pathogen in toddlers at four sites, and at the Mali and India sites even in the eldest age stratum. *Cryptosporidium* had the second highest AF during infancy at five sites, persisting in importance, albeit at a lower level, during the second year of life at five sites; in the eldest stratum, *Cryptosporidium* was significantly associated with diarrhea only in Kenya. By contrast, the adjusted AF of *Shigella* increased from infants to toddlers at every site, rising to the rank of first or second in AF at four sites in toddlers and five sites in the eldest stratum. ST-ETEC was a significant pathogen at every site in at least one age stratum and in all age strata at four sites.

Table 1: Adjusted attributable fraction (AF) of top four pathogens associated with MSD

	The Gambia	Mali	Mozambique	Kenya	India	Bangladesh	Pakistan
0-11 months (n)	400	727	374	673	672	550	633
Rotavirus	23.5	21.7	27.8	19.7	27.0	16.3	22.6
Cryptosporidium	11.7	14.0	14.7	9.0	11.8	5.3	5.6
Shigella	4.0	---	---	4.5	3.0	13.2	7.6
ST-ETEC	4.9	3.6	---	7.0	2.0	1.4	7.0
12-23 months (n)	455	682	195	410	588	476	399
Rotavirus	17.0	11.8	---	13.3	25.4	18.3	9.8
Cryptosporidium	7.7	4.7	---	8.9	8.4	---	8.2
Shigella	12.8	2.4	6.6	4.6	7.2	52.2	12.5
ST-ETEC	8.0	2.3	9.0	6.9	5.8	---	5.7
24-59 months (n)	174	624	112	393	308	368	226
Rotavirus	12.1	3.0	---	3.5	14.5	---	---
Cryptosporidium	---	---	---	2.5	---	---	---
Shigella	12.6	2.0	14.9	9.6	12.1	67.6	10.0
ST-ETEC	9.2	---	---	4.9	6.1	---	5.8

In addition to the four main pathogens identified as significantly associated with MSD, other pathogens were identified in specific study regions. In Africa, Adenovirus*, Entamoeba histolytica*, *Salmonella* (non-typhoidal), *V. cholerae 01,* and Norovirus were identified. In Asia, Aeromonas, *Entamoeba histolytica*, *Salmonella* (non-typhoidal), *V. cholerae 01*, *Campylobacter jejuni*, Enteroaggregative *E. coli* (EAEC), and Norovirus were identified.

After estimating the annual incidence rates of MSD at all sites combined, Rotavirus dominated during the first 2 years of life, with an incidence of MSD during infancy (7.0 episodes per 100 child-years) that was more than double that of any other pathogen. Overall, there were two pathogens per age stratum whose incidence markedly exceeded the others: Rotavirus and *Cryptosporidium* in infants; Rotavirus and *Shigella* in toddlers; and *Shigella* and Rotavirus in children. Regardless of the age stratum, the estimated incidence of MSD was highest in India, next highest in Kenya and Mali, and lowest in The Gambia, Pakistan, Bangladesh, and Mozambique.

During household visit follow-up within 90 days of enrollment, 190 (2.0%) deaths were detected in the 9,439 cases and 37 (0.3%) deaths were detected in the 13,129 control children. Thus, the odds of dying within 90 days after enrollment were 8.5-fold higher in cases than in controls, with most of these deaths (167 [87·9%]) occurring during the first 2 years of life. Additional, 61% of these deaths occurred more than one week after children were diagnosed with MSD, when they may no longer be receiving care, and 56% of deaths among cases occurred at home, suggesting that focusing only on deaths occurring in health centers may underestimate the total mortality burden of MSD. The pathogens associated with increased risk of case death were ST-ETEC and typical enteropathogenic *E. coli* in infants aged 0–11 months, and Cryptosporidium in toddlers aged 12–23 months.

The mean HAZ at enrollment in cases and controls was considerably below the World Health Organization reference for infants and, with one exception, deviated further from the reference at older ages. The mean enrollment HAZ was significantly lower in cases as compared to controls in only two of the 21 age- and site-comparisons. However, HAZ scores of cases decreased between enrollment and follow-up, and with only one exception in the 21 comparisons this decline was significantly greater in cases than controls. Thus, while in general cases and controls had similarly low HAZ scores at enrollment, increased growth faltering occurred in cases after an MSD episode.

Based on these results, it was recommended that interventions targeting five specific pathogens (Rotavirus, *Shigella*, ST ETEC, *Cryptosporidium*, typical enteropathogenic *E. coli*) could substantially reduce the morbidity and mortality burden of MSD. In particular, it was recommended to place the Rotavirus vaccine on the regular vaccine schedule. Additionally, two of these five pathogens – *Cryptosporidium* and ETEC – are of particular concern for potentially contributing to malnutrition due to pathophysicological changes in the intestine and the long duration of illness. The GEMS data also strongly shows that growth faltering after MSD is significant, and can lead to death. Thus, nutritional rehabilitation was recommended to be part of case management for MSD.

Key Points and Recommendations

- Four pathogens were significantly associated with MSD at all seven study sites in one or more age strata: Rotavirus, *Cryptosporidium, Shigella*, and ST-ETEC.
    - Rotavirus had the highest attributable fraction of any pathogen at every site during infancy.
    - Overall, there were two pathogens per age stratum whose incidence markedly exceeded the others: Rotavirus and *Cryptosporidium* in infants; Rotavirus and *Shigella* in toddlers; and, *Shigella* and Rotavirus in children.
- Growth faltering after MSD is significant, and can lead to death.
- The odds of a child dying after MSD are 8.5 times higher than in a child not with MSD.
- The pathogens associated with increased risk of case death were ST-ETEC and typical enteropathogenic *E. coli* in infants, and Cryptosporidium in toddlers.
- Interventions targeting five specific pathogens (Rotavirus, *Shigella*, ST ETEC, *Cryptosporidium*, typical enteropathogenic *E. coli*) could substantially reduce the morbidity and mortality burden of MSD.
- Nutritional rehabilitation is recommended to be part of case management for MSD.

References and Further Reading

Kotloff et al (2013). *Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case control study.* The Lancet; 382:209-222.

GEMS Fact Sheets, http://medschool.umaryland.edu/GEMS/ Accessed March 2014.

A recent review and model found that scaling-up ten nutrition-specific interventions to 90% coverage would cost 9.6 billion USD worldwide per year, and reduce mortality in children younger than 5 years by 15%, stunting by 20.3% and severe wasting by 61.4%[1]. The ten nutrition-specific interventions considered in this analysis were: periconceptional folic acid supplementation or fortification, maternal balanced energy protein supplementation, maternal calcium supplementation, multiple micronutrient supplementation in pregnancy, promotion of breastfeeding, appropriate complementary feeding, vitamin A and preventive zinc supplementation in children 6-59 months of age, management of severe acute malnutrition (SAM), and management of moderate acute malnutrition (MAM).

While these numbers highlight the impact nutrition-specific interventions can have, they also highlight that not all acute malnutration and less than half of chronic malnutrition can be alleviated with only nutrition interventions. Nutrition-sensitive programs – such as programs in agriculture and food security, social safety nets, early child development, maternal mental health, women's empowerment, child protection, schooling, water, sanitation, and hygiene, and health and family planning services – also play a role in the reduction of malnutrution. Specifically, "Nutrition-sensitive programmes can help scale up nutrition-specific interventions and create a stimulating environment in which young children can grow and develop to their full potential."[2]

The logical framework for water, sanitation, and hygiene (WASH) interventions in malnutrition is that WASH interventions reduce diarrheal disease incidence, and this can then reduce malnutrition. There is strong evidence that all four of the main WASH interventions – water treatment, water supply, sanitation, and hygiene – do reduce diarrhea by approximately one-third to one-half, although there is active debate on the relative magnitude of each of the specific interventions.[3] However, the link between diarrheal disease incidence reduction and malnutrition reduction is more tenuous, as malnutrition is caused by multiple nutrition-specific and nutrition-sensitive factors. Additionally, as covered in other Technical Memorandum's in this series, the link between diarrhea and malnutrition is actually related to development of gut damage and environmental enteropathy. Thus, the link between WASH interventions and malnutrition is complex, and not simple to evaluate.

When evaluating the impact of programs, experimental studies such as randomized controlled trials are considered to provide the strongest data as the presence of a control group allows for causation to be attributed. Observational studies, including econometric analysis, provide less strong data, as the correlations that are seen could be due to non-controlled confounding factors (such as socio-economic status or maternal education). Other evidence, such as biological mechanism, also provides information on the relationship between WASH interventions and malnutrition.

As can be seen in Table 1 below, there has been substantial work of various qualities that investigates the relationship between WASH interventions and nutritional outcomes. In subsequent paragraphs, the details of the studies leading to this summary table are described.

Table 1: WASH and Nutrition Evidence Summary

WASH Intervention	Nutritional Outcome	Quality of Evidence	Strength of Evidence
Open defecation	Stunting	Econometric analysis	Strong
Water and hygiene	Stunting	Experimental studies	Suggestive
WASH	Stunting/Wasting	Observational studies	Suggestive
Undernutrition through diarrhea and environmental enteropathy	Stunting/Wasting	Observational studies and biological mechanisms	Strong on stunting Suggesting on wasting
Water treatment and enhanced nutritional recovery	Improved nutritional status	Experimental studies	Suggestive

Open defecation and stunting

Open defecation is the practice of defecating outside or in public. Approximately 1 billion people (14% of the world's population) are estimated to openly defecate, with open defecation rates of 38% in South Asia and 25% in sub-saharan Africa.[4] In Lao PDR and Vietnam, regression analysis was conducted on nationally-representative survey data to assess the impact of improved latrines on nutritional outcomes.[5,6] In both countries, unimproved sanitation and open defectation were correlated with increased stunting up to age 5 (Figure 1). Children were 1.1-3.7 cm shorter in areas with open defecation and unimproved sanitation as compared with children who lived in communities with improved sanitation. Additionally, in both countries, a child remained at risk if community members used unimproved sanitation or open defecation, even if their family used improved sanitation.

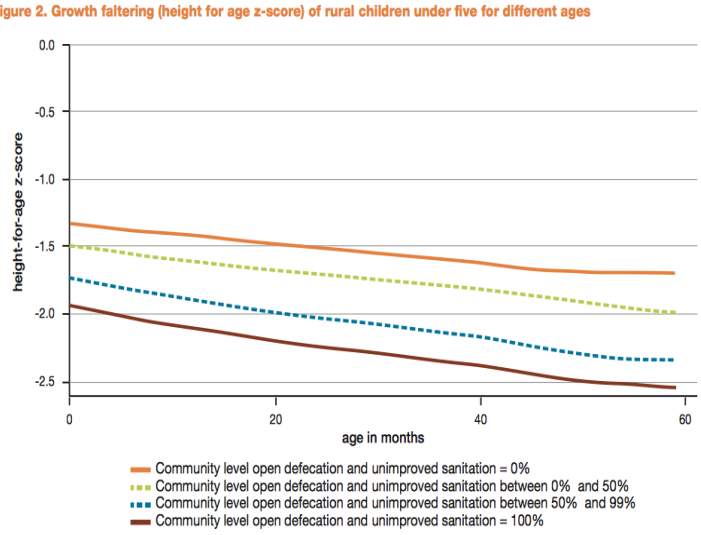

Figure 1: Relationship between HAZ and open defecation/unimproved sanitation[5]

Water and hygiene and stunting

To assess the impact of WASH interventions on nutritional outcomes, researchers conducted a Cochrane Collaboration systematic review. A total of 10 English language databases and three Chinese language databases were systematically searched, and all randomized, quasi-randomised and non-randomised controlled trials, controlled cohort or cross-sectional studies, and historically controlled studies comparing WASH interventions among children aged under 18 years were included in meta-analysis. After reviewing 20,548 studies, 14 studies from 10 low- and middle-income countries were included in the analysis. Weight-for-age, weight-for-height, and height-for-age z-scores were available from five cluster-randomised controlled trials with a duration between 9 and 12 months. Meta-analysis identified no evidence of an effect of WASH interventions on weight-for-age z-score, no evidence of an effect of WASH interventions on weight-for-height z-score, and a borderline statistically significant effect of WASH interventions on height-for-age z-score. The available evidence from meta-analysis of data from cluster-randomised controlled trials with an intervention period of 9-12 months is suggestive of a small benefit of WASH interventions (specifically water and hygiene interventions such as solar disinfection of water, provision of soap, and improvement of water quality) on length growth in children under five years of age. Of note is that the duration of the intervention studies was relatively short, none of the included studies was of high methodological quality, and few studies provided information on intervention adherence, attrition and costs. Additional results from several ongoing studies were noted as necessary to to provide robust evidence to inform these findings.

WASH and stunting

The Sanitation Hygiene Education and Water Supply, Bangladesh (SHEWA-B) program is a large, multi-donor project targeting 20 million people with hygiene programming, including awareness raising, social mobilization, arsenic mitigation, infrastructure building, and school programming. As part of a project evaluation, the following question was asked: "Would children living in relatively clean households have less environmental enteropathy that children in dirtier households?" To answer this question, 10 households from 50 intervention clusters and 50 control clusters were surveyed every month for two years.[8] Households were evaluated and categorized as 'clean' or 'dirty'. Households classified as dirty practiced open defication or had a toilet that did not separate feces from the environment, median *E.coli* levels exceeding 10 CFU/100 mL in household stored water, and lack of a handwashing station or the station did not have water and/or soap. Households classified as clean contained a toilet that separated feces from the environment, had median *E.coli* levels below 10 CFU/100 mL in household stored water, and had handwashing stations with water and soap. Preliminary results show children from cleaner households are 0.9 standard deviations taller than children from dirty households. In total, 5.4% of study households were classified as dirty and 6.4% were classified as clean. Children in households classified as clean had 41% less stunting and 91% less wasting than children in households classified as dirty.

A study in Ethiopia compared the impact of WASH interventions (including protected water supply provision at the ocmmunity level and sanitation education (personal and environmental hygiene practices including soap use, hand-washing practices, sanitary facility construction, cleanliness of house and construction of separate housing for animals, keeping water clean) with health programs, nutritional interventions, and the combination of all three with a control

group.[9] The WASH intervention group was the only group to show a significant increase in mean height-for-age z-score, with a 12.1% decrease in the prevalence of stunting, compared with the baseline group. This group also showed significant improvements in mothers' knowledge of causes of diarrhoea and hygiene practices. The other intervention groups saw non-significant impacts for childhood stunting but improvements in knowledge relating to specific intervention education messages given. The lack of impact in the integrated group was attributed to the group having received a large number of health and nutrition messages, which could have resulted in information overload and compromised the effectiveness of the services delivered.

Undernutrition through diarrhea and environmental enteropathy

The logical framework that postulates WASH interventions reduce diarrhea and thus reduce malnutrition is likely mediated by environmental enteropathy, a physicological condition where the intestine is damaged and unable to absorb nutrients as efficiently. Two recent studies have provided evidence of this causal chain. In Guinea-Bissau, it was found that cryptosporidium infection in children under-2 led to an average 392-gram weight loss in boys and 294-gram weight loss in girls.[10] Over time, there was no recovery (or catch-up of this weight), and the authors concluded that "cryptosporidosis in infancy has a permanent effect on growth". This permanent effect is likely due to the permanent damage on the intestine from prolonged infection with cryptosporidium. Additionally, in the SHEWA-B evaluation mentioned above, markers for environmental enteropathy – incuding lactulose:mannitol (L:M) ratios in urine and immunoglobulin G endotoxin core antibody (IgG EndoCAb) titers – were collected.[11] Both these markers were lower in children from clean households as compared to children from dirty households. After adjusting for age and sex, a 1-unit increase in the ln L:M was associated with a 0.33 SDs decrease in HAZ. These results are consistent with the hypothesis that environmental contamination causes growth faltering mediated through environmental enteropathy.

Water treatment and enhanced nutritional recovery

Ready to use therapeutic foods (RUTF's) are commonly provided to treat moderate acute malnutrition and severe acute malnutrition. In 2004 in Ethiopia, the organization CARE identified that children recovered more quickly from severe acute malnutrition when they had water treated with the flocculant/disinfectant PuR Purifier of Water®. In 2010-2011 in Pakistan, a study documented a 20-day reduction in recovery time (from 4.15 months in the control group to 3.5 months in the intervention group) in children with moderate acute malnutrition who received PuR Purifier of Water® in addition to Plumpy-Nut® in a UNICEF program.[12] In 2013 in DRC, Action Against Hunger documented that children with severe acute malnutrition provided with RUTF and water treated with PuR Purifier of Water® recovered faster than control children receiving only RUTF (26.4 days compared to 30.4 days) and gained weight more quickly (7.3 g/kg/day compared to 6.6 g/kg/day).[13] The summation of these results indicates that clean water provision may enhance nutritional recovery in therapeutic feeding programs.

Implementation and Further Research

Implementation of integrated WASH and nutrition programs can be challenging, both in ensuring that recipients have the resources to change their WASH and nutrition behaviors and that implementers can complete integrated program. Researchers conducted sixteen semi-structured interviews with both WASH and nutrition experts to help determine successes, failures, and integration issues between the fields.[14] Key barriers the respondents noted were insufficient and siloed funding, staff capacity and interest, knowledge of the two sectors, coordination, and limited evidence on the impact of integrated programs. To achieve more effective integration, respondents recommended more holistic strategies that consider both sectors, improved coordination, donor support and funding, a stronger evidence base for integration, and leadership at all levels.

To address the need for a stronger evidence-base, there are a number of ongoing studies investigating the impact of WASH and Nutrition interventions. One of these studies is the WASH Benefits study funded by the Bill and Melinda Gates Foundation.[15] The WASH Benefits study includes two cluster-randomized trials in rural households with pregnant women in Kenya and Bangladesh. Geographically matched clusters (groups of household compounds in Bangladesh and villages in Kenya) will be randomized to control or one of six intervention arms, including: water quality; sanitation; handwashing; nutrition; combined water, sanitation, and handwashing; and combined water, sanitation, handwashing, and nutrition. Outcome measurements include child length-for-age z-scores, caregiver reported diarrhea, stunting, blood markers for environmental enteropathy, and child development after two years of intervention. This study, and other similar studies, may show which combinations of interventions are most effective, and thus help guide future intervention strategies.

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Key Points and Recommendations

- Nutrition-sensitive interventions, such as WASH interventions, are necessary to fully reduce the worldwide burden of malnutrition.
- There is sufficient evidence (from a variety of studies) of a link between WASH programming and nutritional outcomes – particularly for stunting – to promote WASH as nutrition-sensitive interventions.
- The link between WASH and nutritional outcomes is mediated via environmental enteropathy.
- There are barriers to concurrently implementing WASH and nutrition interventions.
- Large trials are currently being conducted to determine the impact of combined WASH and nutrition interventions.

References and Further Reading

1. Bhutta ZA, Das JK, Rizvi A, et al. *Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost?* Lancet 2013. 382(9890):452-77.
2. Ruel MT, Alderman H. *Nutrition-sensitive interventions and programmes: how can they help to accelerate progress in improving maternal and child nutrition?* Lancet 2013. 382(9891):536-51.
3. Waddington H, Fewtrell L, Snilstveit B, White, H. *Water, Sanitation and Hygiene Interventions to Combat Childhood Diarrhea in Developing Countries* 2009: 3ie Review, London, UK.
4. UNICEF/WHO. Progress on Drinking Water and Sanitation: 2012 Update, United Nations Children's Fund; World Health Organization, New York, NY, USA; Geneva, Switzerland. 2014.
5. WSP. *Investing in the Next Generation: Children grow taller, and smarter, in rural, mountainous villages of Vietnam where community members use improved sanitation.* 2014. Available at: https://www.wsp.org/sites/wsp.org/files/publications/WSP-Vietnam-Stunting-Research-Brief.pdf. Water and Sanitation Program of the World Bank.
6. WSP. *Investing in the Next Generation: Children grow taller, and smarter, in rural villages of Lao PDR where all community members use improved sanitation.* 2014. Available at: https://www.wsp.org/sites/wsp.org/files/publications/WSP-LaoPDR-Stunting-Research-Brief.pdf. Water and Sanitation Program of the World Bank.
7. Dangour A, Watson L, Cumming O, et al. *Interventions to Improve Water Quality and Supply, Sanitation and Hygiene Practices, and Their Effects on the Nutritional Status of Children*; 2013. Cochrane Collaboration Review. Available at: http://onlinelibrary.wiley.com/store/10.1002/14651858.CD009382.pub2/ asset/ CD009382.pdf?v=1&t=i32go1xk&s=1f54d25d312e26640d4f9244bba6dfa140c7e03e.
8. Luby S. *Reducing environmental enteropathy and child growth faltering: An intervention trial update.* Presentation to Family Health International, Washington DC, June 2014. Available at: http://blogs.washplus.org/washnutrition/wp-content/uploads/2014/06/luby-environmental_enteropathy2014.pdf
9. Fenn B, Bulti AT, Nduna T, Duffield A, Watson F. *An evaluation of an operations research project to reduce childhood stunting in a food-insecure area in Ethiopia.* Public Health Nutr. 2012. 15(9):1746-54.
10. Molbak K, Andersen M, Aaby P, et al. *Cryptosporidium infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, West Africa.* American Journal of Clinical Nutrition. 1997. 65:149-152.
11. Lin A, Arnold BF, Afreen S, et al. *Household environmental conditions are associated with enteropathy and impaired growth in rural Bangladesh.* Am. J. Trop. Med. Hyg. 2013. 89(1):130-7.
12. Agboatwalla M, Ahmed R, Reza A. *CMAM in response to 2010 floods in Pakistan: experiences from a national NGO.* Nutrition Exchange of the Emergency Nutrition Network, 2013. (3):15.
13. Pietzsch S, Gressard F, Radin N, Villeminot N. *The Effects of adding PUR® water purifier to the treatment of Severe Acute Malnutrition.* 2014. 37th WEDC International Conference, Hanoi, Vietnam.
14. Teague J, Johnston EA, Graham JP. *Water, sanitation, hygiene, and nutrition: successes, challenges, and implications for integration*. Int. J. Public Health 2014. 59(6):913-29.
15. Arnold BF, Null C, Luby SP, et al. *Cluster-randomised controlled trials of individual and combined water, sanitation, hygiene and nutritional interventions in rural Bangladesh and Kenya: the WASH Benefits study design and rationale*. BMJ Open 2013. 3(8):e003476.

Aspergillus species are fungi found on grains that have been contaminated during production, harvest, storage, and/or food processing.[1] The fungi grow when temperatures are between 24 and 35°C, and the moisture content of the grain is greater than 7% (or 10% if stored in a ventilated space). *Aspergillus species* are ubiquitous and can contaminate dietary staples such as rice, corn, cassava, nuts, peanuts, chilies, and spices. The *Aspergillus flavus* and *Aspergillus parasiticus* species also produce mycotoxins – toxic chemical products – named Aflatoxins. Originally identified in the 1960's, there are now 14 types of Aflatoxin known to exist in nature. It is estimated 4.5 billion people may be chronically exposed to uncontrolled levels of Aflatoxin; mostly through foodborne exposure which is high in Sub-Saharan Africa and Asia due to consumption of contaminated maize, nuts, rice, sorghum, and cassava[2] (Figure 1).

Aflatoxin is a well-studied toxin in agriculture. Animals with prolonged exposure to food contaminated with *Aspergillus species* are known to have liver damage (including cancer), decreased milk and egg production, embryo toxicity, and recurrent infection as a result of immune system suppression (caused by reducing both cell mediated (non-antibody response) and phagocytic (cell engulfing) immunity).[3] Naturally occuring mixtures of Aflatoxins (and the indiviudal Aflatoxins of B1, M1, and G1) are considered by the International Agency for Research on Cancer (IARC) to have sufficient evidence for carcinogenicity in experimental animals. Aflatoxins are considered as having sufficient evidence in humans to be considered a carcinogen, particularly in the liver.[4,5]

In addition to cancer, there are other health effects from *Aspergillus species* and Aflatoxin in humans.[1] First, inhalation of *Aspergillus species* can cause Aspergilliosis disease (lung infections and allergic reactions) in people with weakened immune systems or lung disease. Second, acute or chronic Aflatoxicosis can be caused by consuming food crops contaminated with Aflatoxin. Acute Aflatoxicosis is caused by exposure to a large Aflatoxin dose and can cause death via cirrhosis of the liver. Chronic Aflatoxicosis is caused by low dose exposure in staple food crops, and can lead to negative effects on immunological function and nutritional uptake. Both chronic and acute Aflatoxin doses have a cumulative effect on the cancer risk.

Figure 1: Maize contaminated with *Aspergillus*

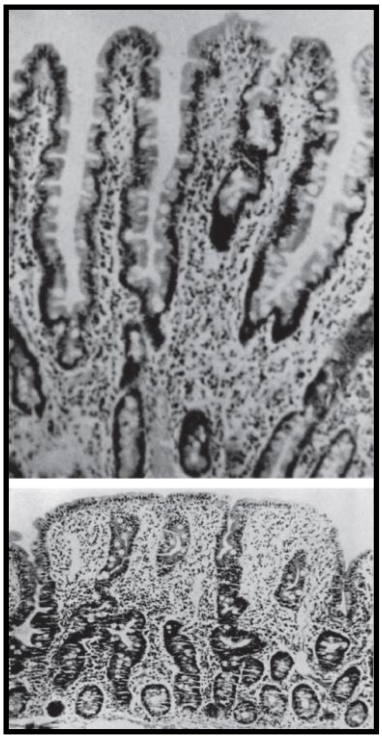

Figure 2: Healthy intestinal villa and EE-affected intesinal villa

Chronic Aflatoxin exposure can impact the human intestine and be a contributing factor to environmental enteropathy (EE) (Figure 2). [6,7] Aflatoxin disrups protein synthesis by binding to RNA, DNA, and the protein itself, and inhibits the immune system as the Immunoglobulin A (IgA) protein is supressed. IgA is the predominant antibody for fighting early gastrointestional infections, as IgA binds to bacterial and viral surface antigens in the mucosal barrier in the intestine. Thus, IgA suppression from Aflatoxin exposure can lead to prolonged infections from enteric pathogens, and subsequent development of EE.

The impact of Aflatoxin exposure is measured in the same terms as malnutrition (stunting and wasting using height-for-age (HAZ) and weight-for-age (WAZ) scores). Aflatoxin exposure is quantified by measuring Aflatonix-albumin adducts in blood; studies have shown significant relationships between Aflatoxin-albumin adducts blood levels and low birth weight, HAZ scores, and WAZ scores for children exposed to Alflatoxin in utero, in breastmilk, and in weaning foods.[8-13] Exposure to Aflatoxin in utero is correlated with low birth weight and reduced height and weight at 1 year.[8-9] Exposure increases greatly when children are weaned off breastmilk and transitioned to solid food; studies in Benin and Togo found that children who were weaned had twice the levels of Aflatoxin-albumin adducts in their blood than those that continued breastfeeding and that there was a signficant correlation between increased Aflatoxin-albumin adducts in blood and decreased HAZ and WAZ scores.[10-12] These findings suggest that Aflatoxin exposure occurs in utero, in breastmilk, and in weaning foods, and thus interventions to reduce Aflatoxin exposure need to start even before conception, and continue through breastfeeding and weaning onto solid foods.[13]

Although the knowledge base on the relationship between Aflatoxin exposure and malnutrition is still developing, Aflatoxin exposure should be considered one factor in the development of malnutrition based of the impacts that exposure to Aflatoxin (in utero, in breastfeeding, and in weaning foods) has on immune response and gut function. Thus, interventions to reduce the amount of Aflatoxin in staple food sources should be undertaken in areas where *Aspergillus* contamination and malnutrition are common, including: 1) pre-harvest interventions such as timing of planting and harvesting, crop planted, genotype of seed, irrigation, and insecticide use; 2) post-harvest (drying and sorting) interventions such as hand sorting, drying on mats, sun drying, storing bags on elevated off ground, use of insecticides, and rodent control; and, 3) post-harvest (cooking) interventions including hand sorting, winnowing, washing, crushing and dehulling, nixtamalization, acidification, chemoprotectant, and enterosorption.[14]

Key Points and Recommendations

- *Aspergillus species* in common staple crops can lead to the presence of the toxin Aflatoxin.
- Aflatoxin exposure starts early (potentially even in utero) and continues through exposure to contaminated breastmilk, and increases after weaning onto contaminated solid food.
- Aflatoxin impacts IgA immune response, the primary response of the gut. This negatively impacts the body's ability to fight gastrointestional infections.
- Aflatoxin causes environmental enteropathy, a physiological change in the lining of the intestine which reduces the ability of the intestine to absorb nutrients.
- Decreased immune response and environmental enteropathy can both exacerbate and cause malnutrition.
- The impacts of Aflatoxin exposure are, like malnutrition, reported in terms of wasting and stunting.
- There is sufficient evidence to support considering Aflatoxin exposure as a contributing factor to malnutrition.
- In areas with malnutrition and Aflatoxin exposure, pre-harvest and post-harvest interventions should be completed to reduce the potential for exposure to Aflatoxin.

References and Further Reading

1. Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D. *Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions.* Am. J. Clin. Nutr. 2004;80(5):1106-22. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15531656.
2. Yard EE, Daniel JH, Lewis LS, et al. *Human aflatoxin exposure in Kenya, 2007: a cross-sectional study*. Food Addit. Contam. Part A. Chem. Anal. Control. Expo. Risk Assess. 2013;30(7):1322-31.
3. Robens JF, Richard JL. *Aflatoxins in human and animal health.* Rev. Environ. Contam. Toxicol. 1992(127):69-94.
4. International Agency for Research on Cancer (IARC). http://www.inchem.org/documents/iarc/vol56/09-afl.html Accessed November 2014.
5. International Agency for Research on Cancer (IARC). http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-23.pdf. 2012
6. Smith LE, Stoltzfus RJ, Prendergast A. *Food Chain Mycotoxin Exposure, Gut Health, and Impaired Growth: A Conceptual Framework.* Adv. Nutr.. 2012 3(4);526-31.
7. Turner PC, Moore SE, Hall AJ, Prentice AM, Wild CP. *Modification of Immune Function through Exposure to Dietary Aflatoxin in Gambian Children*. Environ. Health Perspect. 2002;111(2):217-220.
8. Shuaib FMB, Jolly PE, Ehiri JE, et al. *Association between birth outcomes and aflatoxin B1 biomarker blood levels in pregnant women in Kumasi, Ghana.* Trop. Med. Int. Health 2010;15(2):160-7.
9. Turner PC, Collinson AC, Cheung YB, et al. *Aflatoxin exposure in utero causes growth faltering in Gambian infants.* Int. J. Epidemiol. 2007;36(5):1119-25.
10. Gong YY, Cardwell K, Hounsa A, et al. *Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study.* BMJ 2002;325(7354):20-1.
11. Gong Y, Egal S, Hounsa A, et al. *Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: the critical role of weaning.* Int. J. Epidemiol. 2003;32(4):556-562.
12. Gong Y, Hounsa A, Egal S, et al. *Postweaning Exposure to Aflatoxin Results in Impaired Child Growth: A Longitudinal Study in Benin, West Africa*. Environ. Health Perspect. 2004;112(13):1334-1338.
13. Khlangwiset P, Shephard GS, Wu F. *Aflatoxins and growth impairment: a review.* Crit. Rev. Toxicol. 2011;41(9):740-55.
14. Public Health Strategies for Preventing Aflatoxin Exposure: Final workgroup report from the International Mycotoxin Workshop: Public Health Strategies for Preventing Aflatoxin Exposure. July 2005. Sponsored by CDC and WHO. Available at: http://www.who.int/ipcs/events/2005/workshop_report.pdf.

WASH interventions can contribute to the reduction of malnutrition by breaking part, but not all, of the malnutrition cycle (Figure 1). As can be seen, environmental enteropathy is caused by exposure to Aflatoxin (top chain) and a poor diet leading to immunosuppression combined with exposure to pathogens causing long-term disease (bottom chain). Once a child has enteropathy, a downward spiral occurs, as the clinical manifestations of increased severe infection and low HAZ and WAZ are exacerbated by poor diet, and pathogen and Aflatoxin exposure.

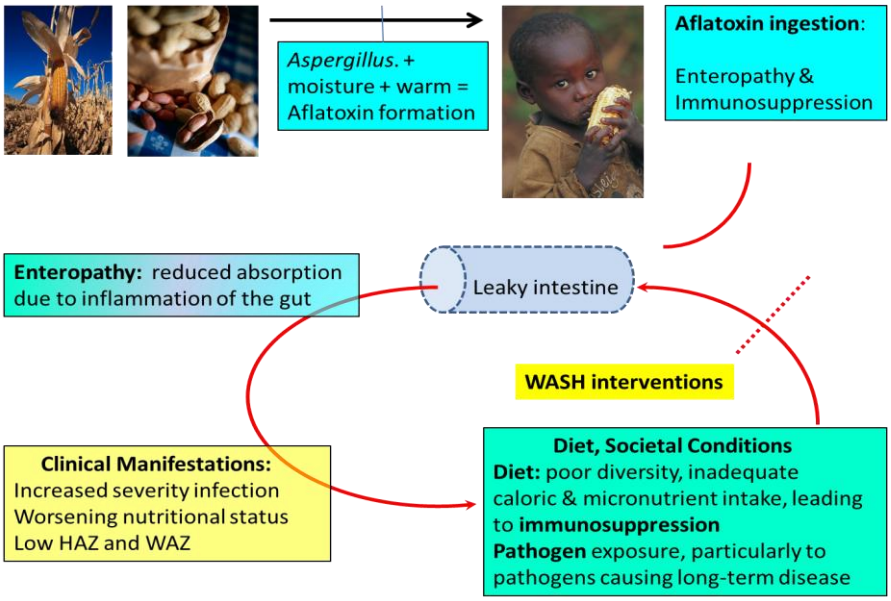

Figure 1: Diagram of factors contributing to enteropathy (credit: Jeff Griffiths, Tufts University)

The link between WASH and nutrition is complex, and we do not yet fully understand this link. However, we do have sufficient evidence to support implementing WASH, particularly in the first 1000 days from conception to age 2, to reduce stunting, and some evidence to support water treatment implementations in acute malnutrition. As can be seen in Figure 1, WASH interventions have the potential to partially break the cycle of malnutrition.

Recommendations that can currently be made to implementers in regards to WASH and nutrition include:

- For chronic malnutrition, interventions should focus on the first 1,000 days, from conception to age 2. Interventions should focus on keeping the gut healthy by preventing environmental enteropathy, and include:
  - Preventing exposure to enteric pathogens causing long-term damage such as *Cryptosporidium*, ETEC, and *Shigella* through improvement in environmental hygiene, including provision of water supply and treatment, reduction of open defecation, and handwashing.
  - Maternal support during the pre-natal and breastfeeding times to reduce in utero and breastfeeding exposure to Aflatoxin.
  - Reducing exposure to Aflatoxin in foods using pre-harvest and/or post-harvest interventions
- For acute malnutrition, sufficient nutrient dense foods should be provided with safe drinking water to assist in absorbing that food. These interventions should be targeted to those with acute malnutrition and to <2's who have recently had moderate-to-severe diarrhea.

Lastly, it is worth nothing that – because of the complex relationships between the factors that cause malnutrition – single intervention assessments are unlikely to show impact. The logical framework behind WASH interventions in malnutrition is more complex than simply WASH intervention reduce diarrhea, which reduces malnutrition. However, some large trials currently being conducted (such as the WASH-Benefits trial in Bangladesh and Kenya) have the potential to provide insight into the most appropriate combination of WASH and nutrition interventions needed to reduce malnutrition. For the latest information on WASH and Nutrition, please visit the WASHPlus WASH and Nutrition blog at http://blogs.washplus.org/washnutrition/home/.