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PLOS | NEGLECTED TROPICAL DISEASES

Viewpoints

"Barriers" to Child Development and Human Potential: The Case for Including the "Neglected Enteric Protozoa" (NEP) and Other Enteropathy-Associated Pathogens in the NTDs

Luther A. Bartelt[1]*, Aldo A. M. Lima[2], Margaret Kosek[3], Pablo Peñataro Yori[3], Gwenyth Lee[3], Richard L. Guerrant[1]

1 Center for Global Health and Division of Infectious Disease, University of Virginia, Charlottesville, Virginia, United States of America, **2** Department of Physiology and Pharmacology, Institute of the Brazilian Semi-Arid, School of Medicine, Federal University of Ceara, Fortaleza, Brazil, **3** Johns Hopkins School of Public Health and Hygiene, Baltimore, Maryland, United States of America

The World Health Organization (WHO) has set forth ambitious efforts to control, and where possible, eliminate the neglected tropical diseases (NTDs) that contribute to poverty and "impair the ability of those infected to achieve their full potential, both developmentally and socio-economically" [1,2]. This neglected disease initiative's (NDI) purpose has been to close the existing poverty gap between individuals living in low/middle-income and high-income countries, and thus facilitate the achievement of the 2000 Millennium Developmental Goals [3]. The gap is still large. Yet, some marked achievements of the NDI, including coordinated administration of preventive chemotherapy to nearly 670 million children globally and the imminent elimination of dracunculiasis, give hope that the WHO's NTD paradigm, a "five-pronged" approach of 1) preventive chemotherapy, 2) intensified case-management, 3) vector control, 4) provision of safe water, sanitation, and hygiene, and 5) veterinary public health, are proving beneficial [1].

Malnutrition and unfulfilled human potential are widely prevalent among the 1.4 billion people also afflicted by the principal NTDs. Over the last decade, we have become increasingly aware that alterations in intestinal function not only associate with malnutrition, but are likely one of its driving forces. It was recognized half a century ago that children in developing countries had intestinal mucosa that showed morphological flattening [4] and malabsorption [5] that were reversible upon exposure to a cleaner environment. Similarly, Lindenbaum also showed in the 1960s that Peace Corps volunteers with diarrhea and malnutrition had biochemical markers of malabsorption: 40% had decreased d-xylose levels, and 52% had low Schilling tests. Moreover, 88% of intestinal biopsies from these volunteers showed mild to moderate jejunitis with decreased villus:crypt ratios [6].

Villus blunting along with chronic inflammation associates with impaired intestinal barrier function with resultant increased intestinal permeability [7]. This combination of altered villus architecture and barrier function, so uniquely dependent upon one's environment, has been termed "environmental enteropathy" (EE) [8]. These characteristic EE changes have been epidemiologically linked with growth faltering and are one hypothesis for why intensive nutritional supplementation interventions done under even ideal clinical trial conditions have significant but limited success in improving weight, linear growth, and cognitive function [9–11].

Syndemic with environmental enteropathy are high rates of childhood diarrhea. Although children may display "catch-up" growth following isolated and short-lived (3 days) diarrheal episodes [12], prolonged and persistent diarrheal (>14 days) episodes strongly associate with stunting [13–16]. Testament to the effectiveness of WHO campaigns, the combination of oral rehydration solution (ORS) in the 1980s and its subsequent refinements, and the introduction of rotavirus vaccines in some populations [17], have led to significant reductions in diarrhea-related mortality from 4.5 million/year over a decade ago to 1.5 million/year in 2010 [18,19]. Currently, however, there are still >700,000 deaths per year globally related to diarrheal diseases [20], and conversely, the frequency of diarrheal episodes has not declined but remains unacceptably high [21]. Among the myriad pathogens causing diarrhea in low/middle-income countries, the protozoa *Giardia lamblia* (synonymous with *G. intestinalis*/*G. duodenalis*) and *Cryptosporidium* spp. are among the most commonly isolated [22]. Although present in *PLOS NTDs*' expanded NTD list [23], and added to the WHO's NDI in 2004 [2,24], these organisms are not mentioned in the 2010 WHO NTD report [1]. Despite the call for increased surveillance [2], the true global prevalence of these infections remains poorly defined [25]. In the past few years, we have learned important lessons that make imperative an emphasis on these "neglected enteric protozoa" (NEP) and other enteropathy-associated pathogens within the NDI:

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* E-mail: lab@virginia.edu

1. The enteric protozoa should be classified with the soil-transmitted helminths (STHs) as pathogens associated with stunting.
2. We need to restructure our theoretical framework to broaden our concept of "diarrheal" disease to include "asymptomatic" enteric infections.
3. We must recognize that environmental enteropathy is likely both a common and complex disease, and that infections related to the development or exacerbation of environmental enteropathy deserve prioritization in disease control strategies.
4. Therapeutic strategies against enteropathy-associated pathogens can and should be evaluated for efficacy within the existing NDI programs.

The NEP Belong with the STHs as Stunting Pathogens

The NEP *Cryptosporidium*, *E. histolytica*, and *G. lamblia* (as well as other enteropathogens such as enteroaggregative *E. coli* [EAEC]) are increasingly recognized to be associated with growth shortfalls [26–36]. Mondal et al. recently demonstrated that stunted infants in Bangladesh were at increased risk of severe diarrhea, and in particular, diarrhea-associated *E. histolytica* and *Cryptosporidium* infections [24]. The impact of enteric protozoal infections has a wide geographic distribution with specific associations between *Cryptosporidium* and malnutrition in Jamaica [27], Israel [28], Peru [29], Mexico [30], Uganda [31], Bangladesh [22,32,33], and Brazil [34–36]. Furthermore, both *Cryptosporidium* and variably *Giardia* associate with persistent diarrhea [14,37]. Additionally, we have found that even when controlled for nutritional status, early childhood cryptosporidial infection either with or without diarrhea was associated with reduced fitness at 6–9 years of age and retarded weight gain [37]. Also, similar to the cognitive deficits seen in infestation with STHs [38], *Giardia* has been associated with decreased cognitive performance [39–41]. The overall impact of even "asymptomatic" disease from enteric protozoa, therefore, may be a major contributor to lost human potential. Moreover, the incoming field data from large multicenter trials such as the Global Enterics Multicenter Study (GEMS) [42] are demonstrating a morass of diverse enteric infections. Such infections occur both concomitantly and sequentially with STHs. It is quite plausible that along with their individual impacts, there is a potentially synergistic effect between STHs and NEP whereby sequential hits affect children at their most vulnerable ages and serially interrupt crucial developmental milestones. The growth impairments that directly result from *Cryptosporidium* and other enteropathogens (such as EAEC) are clear not only from these field studies, but also from animal models in which the "vicious cycle" of enteric infection and malnutrition can be causally linked [43–45]. Future investigations assessing the long-term, and possibly permanent, impact of these serial exposures into adulthood could be included in the assessments of STH outcomes through NDI surveillance.

We Need to Restructure Our Conceptualization beyond Diarrheal Disease to Enteropathy

The NEP may be signaling us to expand our understanding of "diarrheal" disease. Beginning with the pioneering work of L. Mata, who showed that repeated episodes of diarrheal disease were temporally linked with associated linear growth shortfalls [46], to the observations associating even "asymptomatic" enteric protozoa infections with developmental impairments, we have been challenged to re-conceptualize our traditional case definition of "diarrheal" disease. The GEMS findings presented at the recent American Society of Tropical Medicine and Hygiene annual meeting revealed that *Cryptosporidium* ranks highly (in some populations, second only to rotavirus) among all viral, bacterial, and parasitic pathogens causing moderate to severe diarrhea in children, and that the parasite also associates with persistent linear growth shortfalls [42]. The GEMS investigators also recently published a systematic review and meta-analysis demonstrating that *Giardia*, present in >90% of children in some populations by 12 months of age [22], was strongly associated with persistent diarrhea, but inversely associated with acute diarrhea in endemic settings [47]. Elsewhere, *Giardia* associates with wasting (WAZ<-2) [48,49] and stunting [50,51], suggesting the parasite (or particular *G. lamblia* strains) may have an underappreciated influence on childhood development. Thus, as we had discovered with the more readily identifiable STHs using stool microscopy, we need to examine not only the overt liquid diarrhea, but also developmental shortfalls that may indicate a more silent and ominous threat to human potential. Indeed, restructured key "case" definitions of disease resulting from enteric infections will be critical to correctly determining the true prevalence and impact of these infections, and thus to prioritizing treatment and prevention strategies in the most appropriate manner.

Environmental Enteropathy Is Likely both Common and Complex

The mechanisms accounting for growth impairments following infection are complex, and they are likely intertwined with environmental enteropathy. There is a pathological basis for the NEP to potentially initiate and/or propagate environmental enteropathy. In murine models, *Cryptosporidium* causes weight loss, villus blunting, crypt hyperplasia, and increased IFN-gamma and TNF-alpha reminiscent of EE changes [44,52]. Chronic *G. lamblia* infection in humans has also demonstrated abnormalities in epithelial tight-junction proteins (claudin-1) and altered mucosal morphometry [53]. A better understanding of the pathogenesis of the NEP and EE and their developmental sequlae may help to identify common pathways by which certain enteric pathogens, together with malnutrition, promote lost human potential. Such discoveries may open avenues for new therapeutics that restore gut function. Despite the aforementioned foundational observations linking malnutrition, enteric infections, and alterations in intestinal architecture, inflammation, and function, it has taken many years to begin to dissect the etiologies and mechanisms driving EE. One significant hurdle to overcome is the challenge inherent in studying a disease process that otherwise requires endoscopy and tissue biopsy for diagnosis. The pursuit for reliable non-invasive biomarkers of EE that are readily available and both sensitive and specific is crucial for determining the true prevalence of this condition, and for increasing our understanding of its underlying pathophysiology and disease modulators (i.e., differential effects from various pathogens and co-pathogen infections, nutritional status and micronutrient intake, host genetics/epigenetics, and microbiota). Studies are currently investigating several leading candidate biomarkers found to be elevated in children in low-income countries, including: markers of intestinal inflammation such as fecal myeloperoxidase (MPO), lactoferrin, and neopterin; serum alpha-1 anti-trypsin (A1AT), a marker of hyperpermeability and protein wasting; and serum endotoxin core antibody (EndoCAb), a marker of bacterial

translocation and systemic immune activation. Clinical Investigations in this field have identified that elevated fecal lactoferrin and EndoCAb are present in malnourished children and in certain enteric infections, and that malnourished children have increased lactulose:mannitol ratios (L:M) [22,54]. A combination of these candidate biomarkers or novel approaches such as metabonomics are needed to begin to truly appreciate the global prevalence, spectrum, and impact of EE and the differential influence NEP and other pathogens have on its severity.

Strategies toward Reducing the Burden of the NEP Can Be Incorporated into Existing NDI Programs

Even when not associated with diarrhea, the capacity of the NEP and other enteropathy-associated pathogens to limit human potential in populations living in poverty, and the increasing recognition of the systemic effects of EE, suggest that novel strategies are needed to fully address the burden of enteric pathogens. Though provision of safe water and sanitation should be universal, not all water purification techniques are effective against the chlorine-resistant and environmentally hardy protozoa such as *Cryptosporidium* spp. Therapeutic strategies are also needed. As was recently demonstrated, mass azithromycin distribution for trachoma was associated with reduced all-cause mortality and infectious childhood mortality [55], a benefit that could be partially attributed to decreased intestinal pathogen burden [56]. Albendazole given in a 5-day regimen has similar efficacy to metronidazole for *Giardia* [57], which could be incorporated into mass de-worming campaigns with extended therapy for *Giardia*-endemic regions. The addition of nitazoxanide as an anti-protozoal agent, and more specifically for *Cryptosporidium* [58], may synergize with azithromycin and albendazole in mass preventive chemotherapy campaigns. Novel intestinal repair therapies that could be added to the presently recommended zinc supplementation need to be identified and incorporated as measures to combat the "vicious malnutrition-enteric disease cycle" (Figure 1 [15]) [59,60].

We have here enumerated several reasons why increased awareness of NEP and other pathogens associated with changes resembling EE is necessary to achieve the NDI's goals of eliminating diseases of poverty. Environmental enteropathy, so intimately dependent upon individuals' surroundings, is perhaps our closest physiopathological correlate to poverty. Through its multi-pronged approach, including intensified case recognition and management, the NDI has the existing infrastructure to take over where decades of acute life-saving oral rehydrating therapy has left off, and begin to reverse the trends of increasing diarrhea-associated morbidities [21]. Alongside efforts to combat STHs, we need aggres-

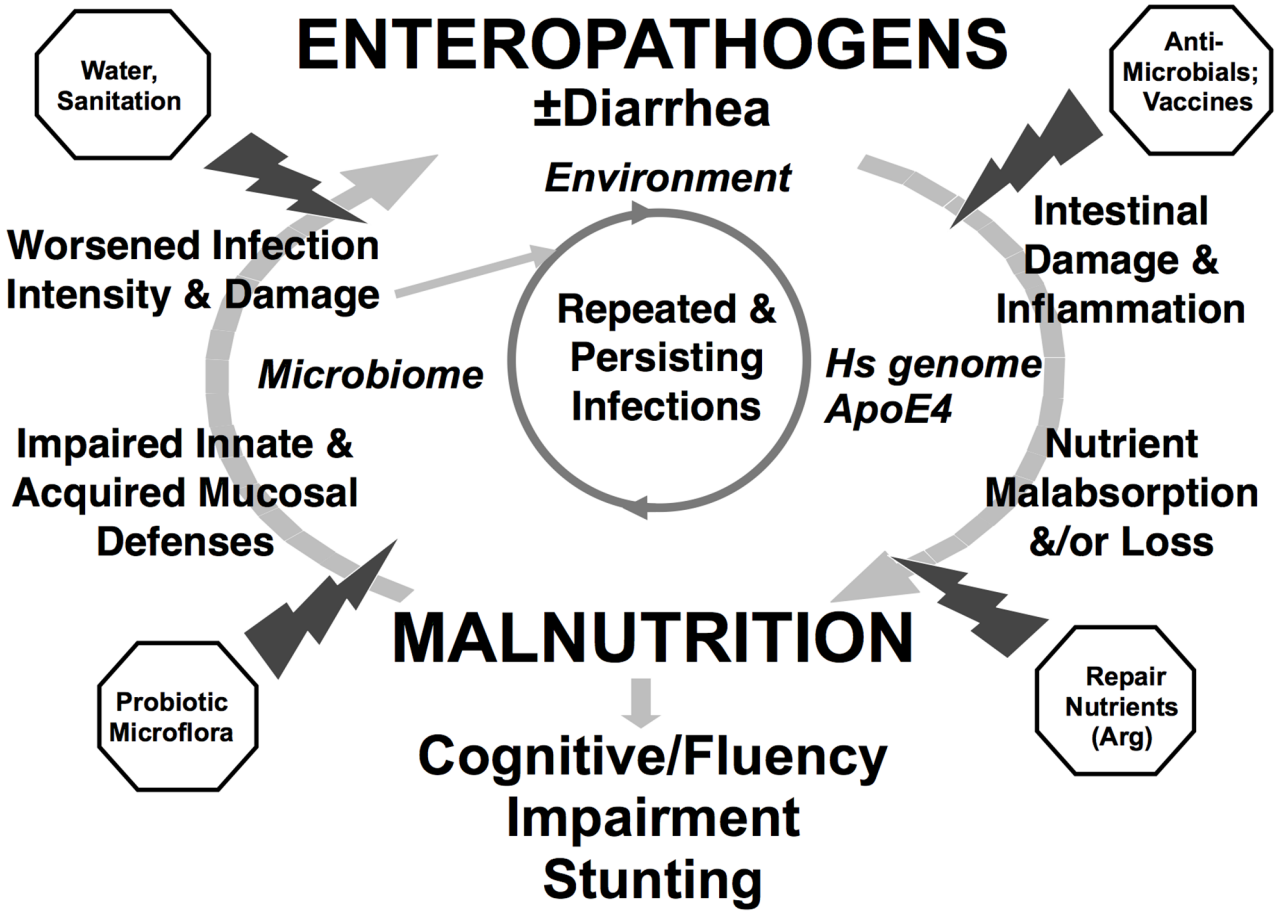

Figure 1. The "vicious cycle" of enteropathogens, malnutrition, and impaired childhood development, and multifaceted opportunities for intervention. Figure adapted from Nutr Rev. 2008 September; 66(9): 487–505 [15].
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sive measures to address "stunting" and "wasting" enterics such as *Cryptosporidium*, *E. histolytica*, *G. lamblia*, and other pathogens (i.e., EAEC) as they are identified. Such measures will prove critical for the more than one-third of the world's children among the "bottom billion" to achieve their full human potential.

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