

Global Impact of Diarrheal Diseases That Are Sampled by Travelers: The Rest of the Hippopotamus

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Travelers who experience diarrhea (i.e., “turista”) are exposed to the same pathogens and illnesses that pose some of the greatest threats to life and development among malnourished children in developing areas around the world, where inadequate water and poor sanitation remain. This article focuses on new findings about the impact, diagnosis, and control of these illnesses and the genetic predispositions of persons who acquire them. Despite the reductions in mortality due to dehydrating diarrhea, the morbidity associated with diarrheal illnesses continues unabated. Furthermore, we increasingly recognize the lasting detrimental effects of enteric infections that occur during early childhood on later physical and cognitive development and, in patients with acquired immunodeficiency syndrome, on the absorption of antiretroviral drugs. Genetic predispositions to inflammation and potential protection associated with such alleles as *ApoE4*, which are not suspected of being involved in diarrhea, remind us of how much we have to learn about the effect and interactions of enteric tropical infectious diseases with regard to our host genome. New diagnostic methods hold promise for improved recognition and, hopefully, control of enteric infections worldwide.

Each year, >50 million people travel from North America, Europe, Japan, and Australia to the less-developed parts of the world where most people live (i.e., Asia, Africa, and Latin America), where, in addition to experiencing the marvelous culture, geography, and climate of these areas, they are exposed to the enteric pathogens that are nearly ubiquitous there. A diarrheal illness that is merely a nuisance for 30%–40% of otherwise healthy, affluent, well-nourished travelers is often devastating for young malnourished children and for patients with AIDS living in many of these areas. Dehydration can be rapidly fatal for frail young children who ingest the common enterotoxigenic *Escherichia coli* that are the predominant cause of traveler’s diarrhea,

and successive or persistent malnourishing infections with *Cryptosporidium* or *Giardia* species or enteroaggregative *E. coli* (EAEC) are increasingly appreciated to have devastating, long-term consequences for the later physical and cognitive development of these children. Furthermore, in patients with AIDS, these infections may result in malabsorption of desperately needed antiretroviral drugs and, possibly, in increased drug resistance. Hence, the common problem of traveler’s diarrhea can be seen as the “tip of an iceberg” (or, in the tropics, the “eyes of the hippopotamus”) of a huge, often ignored or “submerged” problem of endemic diarrheal illnesses. This article will focus on the “rest of the hippopotamus” of endemic diarrhea and enteric infections, which is being increasingly recognized as we begin to appreciate its importance to the lifelong developmental impairment of impoverished children and, potentially, to the development of drug-resistant pathogens, such as HIV and *Mycobacterium tuberculosis*, which increasingly threaten all who share the planet.

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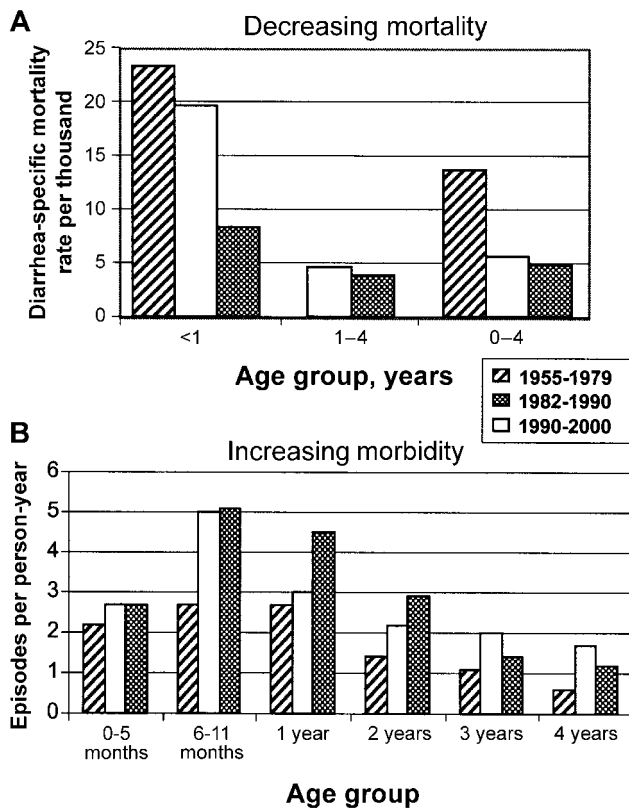


Figure 1. Decreasing mortality and increasing morbidity due to diarrheal diseases, 1955–2000. Data are from [2–4].

DECREASING MORTALITY BUT INCREASING MORBIDITY

Among the great advances in 20th-century medicine was the development of adequate and available oral rehydration therapy, which has saved countless millions of young lives from the effects of dehydrating diarrhea [1]. This achievement is documented in reviews of the impressive World Health Organization Diarrhoeal Diseases Control Programme, which helped disseminate the use of oral rehydration therapy during the last quarter of the 20th century [2].

However, the morbidity rates associated with those illnesses have not declined, but, with the growth of the populations most affected, are actually greater than ever (figure 1) [2]. Furthermore, we are just beginning to recognize the long-term consequences of successive episodes of diarrhea and enteric infection for young children in poor areas.

Long-term effect of diarrhea on malnourished children.

As summarized in table 1, one can calculate that the occurrence of diarrhea and enteric parasitic infections during the first 2 years of life is associated with a growth shortfall of as much as 8.2 cm by age 7 years [5], fitness decrements amounting to 17% of work productivity [6], and a loss of as many as 10 IQ

points and a full year of schooling [7, 9]. Furthermore, directly relevant to the discussion of genetic predispositions below, the specific cognitive defect most affected is semantic, not phonetic, fluency, the same defect that is associated with early Alzheimer disease [10].

Number of diarrhea-associated disability-adjusted life years more than doubled. In the calculation of global disability-adjusted life years, the inclusion of long-term effects of only 1%–2% more than doubles the number of disability-adjusted life years lost to diarrhea worldwide [8]. Thus, interventions such as improved water quality and sanitation or nutritional repair of intestinal injury have far greater “value” than ever calculated.

Whether the clear association of early childhood diarrhea and enteric infections with lasting developmental consequences is actually causal is thus critical to establish. This relationship can be elucidated by either genetic studies that demonstrate a plausible explanation for a causal relationship or by interventions that alter the outcomes in children with successive episodes of diarrhea or enteric infection with malnutrition. Indeed, such associations and interventions are under study and are discussed in the Pathogenesis and Predisposing Factors section.

Malabsorption of critical drugs in immunocompromised patients with diarrhea. In the areas most affected by HIV, enteric infections also predominate. Thus, those individuals who most need their antiretroviral drugs may often present with persistent diarrheal illnesses that are characteristically associated with severely impaired intestinal absorptive function [11]. Thus, it is not surprising that malabsorption of antiretroviral drugs can be severely impaired in patients with diarrhea who live in areas of endemicity [12, 13] and that patients with diarrhea have a higher mortality rate than do patients without diarrhea [14]. Hence, such means as glutamine or alanyl-glutamine or micronutrients that may improve intestinal absorptive function are critical not only for improving responses to antiretroviral or other therapy but, also, for potentially preventing the emergence of drug-resistant virus [13].

Table 1. Evidence for lasting disability effects resulting from early childhood diarrhea.

1. Growth shortfalls (especially HAZ-2; 8.2 cm by age 7 years)
2. Fitness impairment (17% decreased work productivity)
3. Cognitive impairment (~10 IQ points)
4. School performance (~1 year; increased age at starting school and age-for-grade)

NOTE. HAZ-2, height-for-age Z score at age 2 years. Data are from [5–8].

PATHOGENESIS AND PREDISPOSING FACTORS

Host Defenses versus Microbial Virulence Traits

As illustrated in table 2, enteric infections are the result of microbial virulence traits overcoming the host defenses. Enteric infections are characteristically acquired by ingestion of an infectious dose of the pathogen, which, if other host defenses (e.g., gastric acid and normal microbial flora) are intact, may range from 1 to 100 organisms (for *Shigella* species or hardy oocysts of *Entamoeba histolytica* or *Cryptosporidium* or *Giardia* species) to 100,000 to 100 million organisms (for *Salmonella* species, enterotoxigenic *E. coli*, or *Vibrio* or *Campylobacter* species). Neutralization of gastric acid can reduce the infectious dose by up to 3 logs. Normal flora is key to preventing not only infection with *Clostridium difficile*, but, also, infection with *Salmonella* species and, likely, with other enteric pathogens. For further discussion of host traits and microbial virulence traits, see reviews that describe each of these host and microbial traits in detail [15].

Genetic Determinants of Host Susceptibility

The availability of molecular genetic tools has provided entirely new opportunities for examining key genetic host (as well as microbial) determinants of susceptibility to infection or disease. Two examples illustrate these important new advances.

EAEC, inflammation, and the -251 IL-8 promotor.

First, in developing tropical areas, EAEC infections are common among both travelers and malnourished children [16, 17]. Indeed, it appears that children with intestinal inflammation and EAEC infection experience growth shortfalls, even in the absence of overt diarrhea [18], and that the unique Fli-C EAEC flagellin may be responsible for inducing the release of IL-8 in the host [19].

In studies of travelers with EAEC infection, Jiang et al. [17] found that only subjects with AT or AA at position -251 in the IL-8 promoter region developed diarrhea, which is significantly different from the 18%–20% of subjects without diarrhea or without EAEC infection who had TT at this allele. Furthermore, subjects with AA at this position also had greater concentrations of IL-8 in feces than did subjects with AT or TT at this position.

ApoE4 (the “Alzheimer gene”) and protection against cognitive impairment associated with heavy diarrhea burdens during early childhood. A second recent set of findings suggesting that a key genetic determinant may influence the outcome of heavy diarrhea burdens during early childhood stems from the findings that diarrhea during early childhood is associated with impaired cognitive and physical development. As noted above, the cognitive impairment involves semantic, not phonetic, fluency, which is the same deficit seen in early Alzheimer dementia [10]. Hence, we examined whether the gene most associated with Alzheimer disease, *ApoE4*, might be as-

Table 2. Host traits vs. microbial virulence traits.

| |
|-------------------------------|
| Host traits |
| Hygiene |
| Gastric acid |
| Humoral and cellular immunity |
| Mucus |
| Normal motility |
| Normal flora |
| Microbial traits |
| Adhesins |
| Enterotoxins |
| Cytotoxins |
| Invasiveness |
| Inflammation inducers |

sociated with cognitive outcomes in children experiencing heavy diarrhea burdens. To our surprise, children with a heavy diarrhea burden and the apolipoprotein E 4 allele actually performed better on cognitive tests, suggesting that this allele may actually protect children who experience heavy diarrhea burdens during early childhood against cognitive impairment [20]. There were no differences in children with light diarrhea burdens, nor is cognitive function correlated with apolipoprotein E alleles in healthy children. Whether this protective effect relates to neuroprotection conferred by improved delivery of cholesterol to developing neurons or to reported effects on the arginine-selective cationic amino acid transporter-1 [21] is under further study and may offer an intervention to prevent these long-term effects on the cognitive development for children with heavy burdens of endemic diarrhea. In addition, in our animal model studies, we discovered that apolipoprotein E knockout mice have impaired adaptations after refeeding when challenged by postnatal malnutrition, compared with wild-type controls [22], suggesting that the apolipoprotein E-cholesterol complex might play an important role in intestinal maturation and recovery after injury to the intestinal mucosa.

NEW DIAGNOSTIC APPROACHES

Inflammatory versus Noninflammatory Diarrhea

The distinction between inflammatory and noninflammatory diarrhea has long been useful in the diagnosis of diarrhea and in the creation of treatment algorithms for managing diarrhea [23–26]. The highly inflammatory diarrheas (or overt dysenteries) are those caused by cultivable and potentially treatable pathogens, such as *Shigella* species, *Campylobacter jejuni*, and, sometimes, *Salmonella* species. Fecal microscopy can be very helpful if it is done promptly by an experienced microscopist [27]. Alternatively, testing for the presence of fecal lactoferrin offers a simple, quick means to detect this inflammatory marker, which can be quantified and which appears in high

levels in diarrhea due to *Shigella* or *Salmonella* species, *C. jejuni*, or *C. difficile*, all of which are diagnosable and potentially treatable infections.

Furthermore, for children in developing areas, as well as for patients with HIV, infections with *Cryptosporidium parvum* or *Giardia* species result in mild intestinal inflammation that leads to detectable levels of fecal lactoferrin (a sensitive marker for the presence of fecal leukocytes) [11, 28–30]. In contrast, fecal lactoferrin was not observed in healthy US adults experimentally infected with *C. parvum* [31]. In addition, as noted above, evidence of intestinal inflammation (such as the presence of lactoferrin in feces) is associated with the pathogenesis of malnutrition due to EAEC infection in children, as well as with diarrhea in travelers [17, 18].

New Diagnostic Methods for Potentially Treatable Causes of Persistent Diarrhea in Travelers, Malnourished Children, and HIV-Infected Patients: *Giardia* and *Cryptosporidium* Species and EAEC Infections

Since *Cryptosporidium* species was first described as a human pathogen in 1976, it has become recognized as one of the more important pathogens that causes persistent diarrhea in children and immunocompromised patients. Several studies indicate an overall prevalence of *Cryptosporidium* species of 13% in developing countries, and the pathogen is an etiology for up to 25% of cases of persistent diarrhea in immunocompromised patients [32]. *Giardia* species, like *Cryptosporidium* species, have a global distribution and cause ~280 million cases of diarrhea annually [33]. In Asia, Africa, and Latin America, ~200 million people have symptomatic giardiasis, with ~500,000 new cases reported each year [34].

Infection with *Cryptosporidium* or *Giardia* species should be suspected in any patients, particularly travelers, who present with persistent watery diarrhea, abdominal cramps, and nausea; cryptosporidiosis is the more likely disease in immunocompromised patients and in individuals with recreational water exposure. In immunocompromised patients, infection with *Cryptosporidium* species may cause severe and prolonged diarrhea, and, if the biliary tree is involved, there may be elevations in levels of alkaline phosphatase and transaminases, as well as irregular ductal strictures [35].

Microscopy. In cryptosporidiosis, shedding of oocysts can be intermittent, and up to 3 stool specimens may be needed for diagnosis [31, 36]. By use of a modified acid-fast stain (Kinyoun), oocysts appear as red spheres 4–6 μm in diameter; no other organisms should be easily confused with *Cryptosporidium* species on the basis of size and appearance. Unfortunately, acid-fast staining is relatively insensitive, requiring 10,000 oocysts/g of watery stool and 500,000 oocysts/g of formed stool to make the diagnosis. Microscopy remains the best available test for acid-fast *Cyclospora cayentanensis* infections.

Traditionally, giardiasis has been diagnosed by the direct detection of cysts or trophozoites in a stool sample by means of an ova and parasite examination of fecal or small bowel specimens (including small bowel specimens obtained using the “string” test) [37, 38]. The cysts are ovoid or ellipsoid and measure 11–15 μm in diameter. Trophozoites are approximately the same size, with 2 anteriorly placed nuclei and 8 flagella best visualized by staining with trichrome or with the iron hematoxylin method [39, 40].

Immunoassays. There are several immunologic techniques, including immunofluorescence assays and ELISAs, that are commercially available for the improved detection of *Giardia* and *Cryptosporidium* species. These tests are both highly specific and sensitive, compared with the ova and parasite examination. The direct fluorescent antibody (DFA) assay uses fluorescein-labeled monoclonal antibodies directed against cell wall antigens of *Giardia* cysts and *Cryptosporidium* oocysts and allows visualization of the intact parasites. EIAs detect soluble stool antibodies for the qualitative detection of *Giardia* species- and *Cryptosporidium* species-specific antigens in stool specimens. Compared with the conventional modified Kinyoun acid-fast staining method, DFA assays increase the detection rate of *Cryptosporidium* species by 69.9% and that of *Giardia* species by 49.4% [41].

A comparison of the Merifluor DFA assay with the ProSpecT microplate EZ EIA for the detection of *Giardia* species revealed that they had comparable degrees of sensitivity (100% and 97%, respectively) and specificity (>98% for both) [42]. When commercially available DFA assays from different manufacturers were compared, their results were found to be in agreement for all samples tested for *Cryptosporidium* and *Giardia* species [43]. In a similar comparison of several EIA products, the sensitivity for *Giardia* species ranged from 94% to 99%, and that for *Cryptosporidium* species ranged from 98% to 99%, with similar specificities (100%) [43]. Recently, however, pseudo-outbreaks of infection with *Cryptosporidium* species have been associated with false-positive EIA results [44–46].

A nonenzymatic, solid-phase, qualitative immunochromatographic assay (ImmunoCard STAT! *Cryptosporidium*/*Giardia* Rapid Assay; Meridian Bioscience) reportedly detects and distinguishes between *Giardia* species and *C. parvum* in aqueous extracts of human fecal specimens [47]. This test can be completed in 12 min on formalin-fixed or unfixed samples. Compared with reference methods (i.e., modified acid-fast staining and DFA assay), the test yielded sensitivity, specificity, and positive and negative predictive values of 93.5%, 100%, 100%, and 95.5%, respectively, for *Giardia* species and 98.8%, 100%, 100%, and 99.7%, respectively, for *Cryptosporidium* species. False-negative results were seen for *Giardia* species with low parasite numbers (10–100 cysts) or for specimens that contained trophozoites only [47]. In a comparison of 3 commercial

Table 3. Measuring and stemming the staggering cost of inadequate water and sanitation.

| |
|---|
| Diarrhea → stunting at 2–7 years of age (especially HAZ-2) |
| Diarrhea/stunting (HAZ-2) → cognitive impairment at 6–12 years of age |
| Poor sanitation, water source/storage → increase of diarrhea/stunting |
| Strategies to reduce the devastating costs of early childhood diarrhea and its potential long-term impact are imperatives that we can no longer afford to ignore (water, sanitation, hygiene, and breast-feeding) |

NOTE. HAZ-2, height-for-age Z score at age 2 years. Data are from [5, 7, 8, 68–72].

assays—the Merifluor DFA assay, the ImmunoCard STAT! assay, and the ProSpecT microplate EZ EIA (Remel)—the Merifluor DFA assay was found to be the most sensitive for the detection of *Cryptosporidium* and *Giardia* species. With use of the Merifluor DFA assay as the standard, the EIA and the ImmunoCard STAT! assay had a sensitivity of 90.6% and 81.3%, respectively, for *Giardia* species, a lower sensitivity of 70.3% and 67.6%, respectively, for *Cryptosporidium* species, and a specificity of 99.5% for both species. As in the previous study, the 2 assays failed to detect low numbers of parasites and were most reliable when there were >175 organisms/10 μ L, an effect that was most apparent with *Cryptosporidium* infections [48]. However, problems with false-positive results of rapid EIAs have led to wasteful pseudo-outbreaks of cryptosporidiosis in Wisconsin and recall of the 3 lots of ImmunoCard STAT! tests in Colorado [44, 45, 49].

PCR. A variety of PCRs have been described for *Cryptosporidium* and *Giardia* species. The sensitivity of detection by PCR is greater than that by microscopy, making it of great use for detection of low numbers of parasites in stool samples [50].

In our laboratory, PCR for the detection of *Cryptosporidium* species has a sensitivity of 93% and a specificity of 95%, compared with 67% and 99%, respectively, for the DFA assay and 68% and 58%, respectively, for EIA [51]. In addition to identifying protozoa, the use of real-time PCR–restriction fragment–length polymorphism (RFLP) analysis can detect as few as 5 *Cryptosporidium* oocysts and can differentiate between 5 genotypes and, more recently, subtypes [52]. PCR–RFLP analysis is more sensitive, as it may detect 50–500 oocysts/mL of liquid stool or <1 pg of DNA and <10 oocysts from environmental samples [53–55]. *Giardia* PCR–RFLP analysis is a highly sensitive technique that can detect 1 cyst [56] and can also be used to detect and distinguish *Giardia* assemblages A (with 2 groups) and B [57, 58]. Recently, a multiplex quantitative PCR has been developed using scorpion probes that are capable of genotyping as few as 1.25 trophozoites in stool by use of an oligonucleotide capture technique [59].

In addition to enterotoxigenic *E. coli*, which produce either the heat-labile, cholera-like toxin and/or the heat-stable toxin and which cause most cases of acute traveler’s diarrhea and childhood diarrhea in the tropics, EAEC have emerged as sig-

nificant causes of persistent diarrhea and malnutrition in children, HIV-infected patients [18, 60], and, possibly, travelers [17]. However, detection of EAEC has required a specific test for one of the characteristic virulence traits of this group of organisms. Because an entire cassette of potential virulence traits is regulated by the transcriptional activator AggR, some have proposed that genetic probes for this trait may be the single best test for EAEC at the present time, and such genetic probes have been incorporated into a multiplex PCR test [61].

In addition to bacterial and parasitic causes of both travelers’ and endemic diarrhea, viral agents cause huge burdens of illness, especially among children throughout the world [62, 63]. Rotaviruses constitute major causes of dehydrating diarrhea in young children worldwide, and noroviruses (the cause of winter vomiting disease) not only infect children, families, and travelers throughout developed and developing areas, but also infect up to 70% of children living in northeastern Brazilian “favelas” each year, often causing repeated symptomatic infections, as well as infections with new strains [64, 65].

With the introduction of easier, more-sensitive methods that reduce labor, time, and reagent costs, the possibility of combining assays for the detection of different targets into one assay has become a possibility. A multiplex real-time PCR and an oligonucleotide microarray may be new methods for the detection of *E. histolytica*, *Giardia lamblia*, and *C. parvum*, with excellent, perhaps unprecedented, sensitivity and specificity in either fecal or water samples [66, 67]. Work on these and potential new methods to detect fecal contamination in water may help to identify and ameliorate inadequate sanitation and contaminated water that perpetuates the devastating illness burdens associated with enteric infections around the world [68].

In conclusion, common diarrheal illnesses in travelers remind us of the devastating consequences of poor water quality and sanitation that plague the growing population of impoverished people around the world. As shown in table 3, diarrhea that occurs during early childhood predisposes to stunted physical and impaired cognitive development, which are, perhaps, most readily assessed by the height-for-age Z scores around the second year of life [5–8, 69]. Furthermore, poor sanitation and inadequate water supplies predispose children to increased diarrhea and stunting [70, 71]. Hence, strategies to reduce the devastating costs of diarrhea during early childhood and its potential long-term effects (with improved water, sanitation, hygiene, and breast-feeding) are imperatives that we can no longer afford to ignore [72]. With renewed awareness of the long-term consequences of diarrhea during childhood and improved methods for detection and control of water sanitation, we should expect, at last, that the sanitary revolution can now be driven throughout Asia, Africa, and Latin America as it once was driven by cholera in western Europe and North America in the 19th and 20th centuries [1, 68].

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