Factors That Explain Excretion of Enteric Pathogens by Persons Without Diarrhea

Myron M. Levine[1] and Roy M. Robins-Browne[2]

[1]Center for Vaccine Development, University of Maryland School of Medicine, Baltimore; and [2]Department of Microbiology and Immunology, The University of Melbourne, and Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia

Excretion of enteropathogens by subjects without diarrhea influences our appreciation of the role of these pathogens as etiologic agents. Characteristics of the pathogens and host and environmental factors help explain asymptomatic excretion of diarrheal pathogens by persons without diarrhea. After causing acute diarrhea followed by clinical recovery, some enteropathogens are excreted asymptomatically for many weeks. Thus, in a prevalence survey of persons without diarrhea, some may be excreting pathogens from diarrheal episodes experienced many weeks earlier. Volunteer challenges with *Vibrio cholerae* O1, enterotoxigenic *Escherichia coli* (ETEC), enteropathogenic *E. coli*, *Campylobacter jejuni*, and *Giardia lamblia* document heterogeneity among enteropathogen strains, with some inexplicably not eliciting diarrhea. The immune host may not manifest diarrhea following ingestion of a pathogen but may nevertheless asymptomatically excrete. Some human genotypes render them less susceptible to symptomatic or severe diarrheal infection with certain pathogens such as *Vibrio cholerae* O1 and norovirus. Pathogens in stools of individuals without diarrhea may reflect recent ingestion of inocula too small to cause disease in otherwise susceptible hosts or of animal pathogens (eg, bovine or porcine ETEC) that do not cause human illness.

Clinical studies of 2 different designs, case/control and prospective longitudinal follow-up of a cohort, have historically played important roles in (1) identifying putative new diarrheal pathogens; (2) assessing the degree of pathogenicity of new or established enteropathogens; and (3) estimating the relative burden of different enteric pathogens. In case/control studies, clinical specimens from patients with diarrhea (cases) and properly matched (eg, by age and sex) control subjects without diarrhea are examined to detect the pathogens of interest. Odds ratios (ORs) are calculated to quantify the degree of association of the pathogen of interest with diarrhea. This involves comparing the odds of finding the pathogen in cases with the odds of finding the pathogen in controls; the higher the OR, the stronger the association. As described in the paper by Blackwelder et al in this supplement, the OR is also one key factor in the equation used to calculate the attributable fraction (AF) of a pathogen in a case/control study, thereby elucidating the relative contributions of different enteropathogens to the burden of diarrheal illness. Further statistical methods are employed to adjust for the presence of other enteric pathogens in the cases and controls [1]. When applying these statistical methods, it is evident that the prevalence rate of an enteropathogen in controls influences the estimates. The higher the rate of detection of the enteric pathogen(s) of interest in controls, the weaker the OR association (for pathogenicity) or the smaller the AF for that pathogen as a cause of diarrheal disease at the population level.

Similarly, when cohorts of children or adults are followed prospectively for the occurrence of diarrheal illness, the rate of detection of various pathogens of interest when a subject develops diarrhea is typically compared to serial "routine" specimens from that

subject that were collected systematically when he/she did not have diarrhea [2–4]. A "hybrid" approach is to nest a case/control strategy within the cohort study. Thus, a subject within the cohort who develops diarrhea is matched (usually by age and sex) to another subject within the cohort who at the time is free of diarrhea [5, 6]. In these cohort study strategies, the rate of detection of pathogens in stool specimens from the diarrhea cases is compared, respectively, to the rate of pathogen detection in the routine stool specimens from that person or in specimens from the matched control in the nested case/control approach. In these designs, as well, the rate of isolation of pathogens from the controls (or from the period when the subject is free of diarrheal illness) influences the conclusions that can be drawn about the pathogenicity of specific pathogens or their relative importance compared to other pathogens (as calculated using AF).

Finally, for clinicians who must make judgments about the need for specific therapeutic interventions based on the isolation of a specific diarrheal pathogen from a case of diarrhea, knowledge (from epidemiologic studies) of the relative frequency with which that enteric pathogen is found in healthy subjects without diarrhea provides information that may be helpful in decision making in the clinical situation.

Because the excretion of enteric pathogens in subjects without diarrhea influences our appreciation of the role of those pathogens as causes of diarrhea, it is imperative to consider the reasons why one finds diarrheal pathogens in healthy persons not suffering from diarrhea. Herein we review the characteristics of the pathogens, host factors, and environmental factors that provide explanations for the asymptomatic excretion of known diarrheal pathogens.

CHARACTERISTICS OF THE PATHOGEN

Unusually Long Duration of Excretion After Causing Diarrheal Illness

When subjects recover clinically following diarrheal illness caused by certain pathogens, the pathogens continue to be excreted asymptomatically for an extended period. Thus, when subjects without diarrhea are selected to serve as nondiarrheal controls, some may still be excreting a pathogen consequent to an episode of clinical diarrhea that may have occurred many weeks earlier. Enteric pathogens associated with extended excretion following an episode of acute diarrhea include nontyphoidal *Salmonella* [7, 8], *Campylobacter jejuni* [9–12], norovirus GI and GII [13–16], and, uncommonly, *Shigella* [17].

Heterogeneity of Pathogenicity Among Strains of the Pathogen

Experimental challenge studies in healthy adult volunteers who were fed various strains of known or putative enteric pathogens revealed that some strains caused diarrhea more readily than others at the same challenge inoculum, with some strains failing to cause diarrhea at all. Moreover, among the strains that did elicit diarrhea, the severity and range of symptoms sometimes varied widely. These observations were made with experimental challenge studies involving strains of *Vibrio cholerae* O1, enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *E. coli* (ETEC), *Campylobacter jejuni*, and *Giardia lamblia*. Thus, with many enteropathogens there appears to be heterogeneity among the strains that are circulating in human populations, with some strains being more prone to cause clinical disease than others. When many of these observations were initially made, the virulence attributes and other characteristics that differentiated the "diarrheagenic" strains from the other strains were not readily appreciated; in some instances the explanations are still not available.

In the early years following the identification of ETEC as pathogens, 3 broad categories came to be recognized, with some producing both heat-labile enterotoxin (LT) and heat-stable enterotoxin (ST), while others elaborated only ST or only LT [18]. Early clinical challenge studies showed that LT/ST strains [19, 20] and ST-only strains [21] reliably elicited watery diarrhea in volunteers. In contrast, LT-only strains were inconsistent in inducing diarrheal illness. LT-only strain E2528-C1, which was epidemiologically incriminated as responsible for an outbreak of acute diarrhea on a cruise ship [22], induced diarrheal illness after a relatively short incubation period when fed to volunteers [20]. In contrast, *E. coli* strain H10407P, which was derived from strain H10407 consequent to the loss of a plasmid encoding fimbrial colonization factor antigen I (CFA/I) and ST, did not cause diarrhea in volunteers even though the strain elaborated LT [23, 24], and the parent LT/ST, CFA/I-positive strain induced copious watery diarrhea [23–25]. These clinical trials provided early indications that fimbrial colonization factors play an important role in the pathogenesis of ETEC diarrhea in humans, as they do in ETEC pathogens of piglets and calves.

As shown in Table 1, similar experiences were observed when several different strains of *V. cholerae* O1 El Tor [26], EPEC [27], *C. jejuni* [28], and *G. lamblia* [29, 30] were fed to volunteers, even though all the strains were all isolated from patients with diarrheal illness. Thus, *V. cholerae* O1 El Tor strains N16961 and E7946, EPEC strains E2348/69 and E851/71, *C. jejuni* strain 81–176, and *G. lamblia* strain Gsm caused higher attack rates and more severe diarrhea, whereas *V. cholerae* O1 El Tor strain N16117, EPEC strain E74/68, *C. jejuni* strain A3249, and *G. lamblia* strain Isr either did not cause diarrhea or elicited lower attack rates or markedly milder clinical illness. Thus, in case/control studies of diarrhea in developing countries, it is possible that a proportion of controls with asymptomatic infection are carrying nonpathogenic or less pathogenic strains such as *V. cholerae* O1 N16117, EPEC

Table 1. Experimental Challenge Studies in Volunteers Documenting Variability in the Pathogenicity of Circulating Strains of Bacterial and Protozoal Pathogens Isolated From Patients With Diarrhea

Enteric Pathogen	Challenge Strain	Dose (CFU for Bacteria; No. of Trophozoites for Protozoa)	Diarrhea Attack Rate (%)	Positive Stool Culture or Pathogen Detection (%)	Ref.
Vibrio cholerae O1 El Tor	Inaba N16961	10^5	3/5 (60)	4/5 (80)	[26, 85]
	Inaba N16961	10^6	9/10 (90)	10/10 (100)	[26, 85]
	Ogawa E7946	10^6	6/6 (100)	6/6 (100)	[86]
	Ogawa N15870	10^5	3/5 (60)	4/5 (80)	[26, 85]
	Ogawa N15870	10^6	8/8 (100)	8/8 (100)	[26, 85]
	Ogawa N16117	10^5	0/4 (0)	2/4 (50)	[26, 85]
	Ogawa N16117	10^6	0/5 (0)	1/5 (20)	[26, 85]
EPEC	E851/71 (O142:H6)	10^{10}	5/5 (100)	5/5 (100)	[27]
	E2348/69 (O127:H6)	10^{10}	3/5 (60)	5/5 (100)	[27]
	E2348/69 (O127:H6)	10^{10}	11/11 (100)	11/11 (100)	[87]
	E74/68 (O128:H2)	10^{10}	0/5 (0)	5/5 (100)	[27]
ETEC	B2C (O6:H16)	10^8	2/5 (40)[a]	5/5 (100)	[19]
	B2C (O6:H16)	10^{10}	3/5 (60)[b]	5/5 (100)	[19]
	B7A (O148:H28)	10^8	1/5 (20)[a]	4/5 (80)	[19]
	B7A (O148:H28)	10^{10}	4/5 (80)[b]	5/5 (100)	[19]
	B7A (O148:H28)	10^6	3/6 (50)	6/6 (100)	[20]
	B7A (O148:H28)	10^8	7/11 (64)	11/11 (100)	[20]
	263 (pig strain)	10^8	0/5 (0)	5/5 (100)	[19]
	263 (pig strain)	10^{10}	0/5 (0)	3/4 (75)	[19]
Campylobacter jejuni	81–176	10^6	3/7 (43)	7/7 (100)	[28]
	81–176	10^8	6/10 (60)	10/10 (100)	[28]
	A3249	10^6	2/19 (11)	15/19 (79)	[28]
	A3249	10^8	0/5 (0)	5/5 (100)	[28]
Giardia lamblia	GS/M (genotype B)	5×10^4	4/10 (40)	10/10 (100)	[30]
	Isr (genotype A)	5×10^4	0/5 (0)	0/5 (0)	[30]

In all bacterial challenge studies, the inocula were administered to fasting subjects with 2.0 g of NaHCO$_3$ (to neutralize gastric acid) except for reference [19], in which the inocula were administered in 45 mL of milk. *Giardia* trophozoites were administered directly into the proximal small by means of an intestinal tube (130-cm distance from the subject's mouth).

Abbreviations: CFU, colony-forming units; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*.

[a] Described as mild diarrhea.

[b] Described as severe diarrhea.

E74/68, *C. jejuni* A3249, and *G. lamblia* strain Isr rather than fully virulent strains. Until the specific virulence characteristics are identified that can differentiate highly pathogenic strains from strains that lack or have minimal pathogenicity, one cannot develop diagnostic tests to detect reliably the "true" pathogens which are expected to be found more often in cases of diarrhea, whereas the nonpathogenic varieties may be overrepresented among isolates from controls.

For Some Pathogens, Clinical Illness May Require Interaction With a Second Pathogen, Whereas a Single Infection is Usually Asymptomatic

In the veterinary field, there are examples where, through a synergistic interaction, clinically overt or more severe diarrheal illness ensues when 2 specific enteric pathogens (such as ETEC and rotavirus) are present [31, 32]. In contrast, when the pathogens are present as single infections, diarrhea is milder or may not occur. Heretofore, examples of similar interactions of enteric pathogens in immunocompetent humans have not been convincingly described, but the possibility remains that they exist. Analyses of data from the Global Enteric Multicenter Study (GEMS) will offer the possibility of exploring that hypothesis.

HOST FACTORS

Host Susceptibility Factors

Host risk factors can play a critical role in the propensity to develop diarrheal illness or more severe illness following ingestion of a known enteropathogen. Many bacterial, viral, and

protozoal enteropathogens utilize molecules exposed on the surface of human intestinal cells as specific receptors to which they attach and initiate pathogenesis. The intestinal cell receptors include sugar moieties as well as proteins. Thus, susceptibility to infection and disease may be affected by the presence or absence of these receptors or the expression of variant receptors. Two striking examples of susceptibility based on genetic factors that involve blood group antigen expression are seen with cholera and norovirus infections. Human blood group antigens are expressed not only on erythrocytes but also on intestinal and other mucosal surfaces by genetically endowed persons ("secretors").

Cholera
Persons of blood group O and individuals with hypochlorhydria are much more prone to develop cholera gravis following the ingestion of a food or water vehicle containing *V. cholerae* O1 or *V. cholerae* O139. Blood group O has been recognized as a risk factor for cholera gravis both in epidemiologic field studies [33–36] and in volunteer challenge studies [37, 38]. In volunteer challenge studies a total purge of >5.0 liters of diarrheal stool is used as the definition of severe cholera and indicates a degree of purging that if not promptly and properly treated with aggressive rehydration would lead to cholera gravis, manifested by severe dehydration and hypovolemic shock. Another host risk factor for development of severe cholera is hypochlorhydria, with evidence deriving from clinical observations [39], epidemiologic studies, and volunteer challenge studies [40, 41].

Norovirus Gastroenteritis
Susceptibility to the Norwalk agent, the prototype GI-1 norovirus, is related to ABO blood group antigens. Volunteer studies showed that some individuals were highly resistant to Norwalk virus, whereas persons of blood group O exhibit increased risk of developing clinical illness upon exposure [42]. Norwalk virus binds to subjects whose intestinal secretions contain blood group O antigen H type 1 [43, 44], while norovirus GII-3 and GII-4 bind to cells of individuals who secrete blood group antigen A. Human hosts with null mutations of the gene encoding FUT2, the fucosyltransferase that determines secretor status, cannot synthesize ABH blood antigens in secretions. Such nonsecretors are in general not susceptible to norovirus disease [45], although recent epidemiologic studies suggest that some norovirus GII viruses can infect and cause disease even in nonsecretors [46, 47].

Other Nonspecific Host Factors That Affect Resistance to Diarrheal Pathogens
Various nonspecific but highly functional barriers protect the human intestine by impeding an enteric pathogen's ability to complete its pathogenesis that would otherwise result in clinical diarrheal illness [48]. One consequence of these barriers remaining intact is that the pathogen may end up colonizing the human intestine for a variable (short or long) period of time without causing overt diarrhea; this may explain some randomly selected matched control subjects in case/control studies who harbor pathogens in the absence of diarrhea. Barriers that a diarrheal pathogen must overcome include the intestinal microbiota (normal flora), the mucus layer, the epithelial cell layer, and various innate immune responses. These will be briefly mentioned in the ensuing paragraphs and recent reviews will be cited, should readers wish to delve deeper into these topics.

Intestinal Microbiota
The intestinal microbiota refers to the complex ecosystem of resident microorganisms (overwhelmingly either strict or facultative anaerobic bacteria) found in the mucus layer along the mucosal surface; enormous numbers (approximately 10^{12-14}) of bacteria are found in the colon and terminal ileum [49]. In addition to performing symbiotic physiological functions for the host (eg, assisting in digestion, producing vitamin K and biotin, and promoting maturation of the mucosal immune system) [49–54], the microbiota constitute a formidable barrier that confronts pathogens [49–51, 54]. Besides competition for attachment sites on the epithelial surface and for nutrients, the end products of sugars metabolized by resident flora include short-chain fatty acids (eg, lactic, butyric, propionic) and other substances that are highly inhibitory for many bacterial enteropathogens such as *V. cholerae* O1 [55], *Salmonella*, and *Shigella* [56, 57].

Mucus Layer
The human intestine is covered by mucus, a product of goblet cells [58]. The mucus covering of the colon, composed of the mucin Muc2, is double layered, with the outer mucus layer being loosely adherent and replete with microbiota. In contrast, the inner mucus layer is highly adherent to the epithelium and is free of microorganisms [58, 59]. A healthy intact outer mucus layer constitutes a potent protective barrier that impedes enteropathogens. Beneath the mucus layer resides another defense barrier, the epithelial glycocalyx, consisting of diverse glycoproteins and glycolipids on the apical surface of enterocytes and colonocytes [60]. Both the mucus layer and the glycocalyx of the human intestine are continually replenished. The small intestine has only a single mucus layer. The mucus layer diminishes pathogen contact with the epithelium and carries bacteria distally [58].

Epithelial Cell Layer
The epithelial layer provides a 1-cell-thick physical barrier connected by tight junctions that separates pathogens in the

intestinal lumen from the lamina propria. In addition to the physical barrier, epithelial cells produce various antimicrobial peptides (defensins, cathelicidins, lysozyme, etc) [48]. Paneth cells, specialized secretory cells located in the crypts of the small intestine, are the primary source of the antimicrobial peptides [61, 62].

Various Innate Immune Responses

Epithelial cells and dendritic cells of the intestinal mucosa are replete with pathogen recognition receptors (PRRs) that detect the presence of pathogens and initiate a cascade of nonspecific innate immune responses that inhibit the pathogen. The PRRs include Toll-like receptors, nucleotide oligomerization domain–like receptors, retinoic-acid-inducible gene–like receptors, and the C-type lectin receptors [62].

Immune Status of the Host That Prevents Clinical Illness but Does Not Prevent Intestinal Colonization

Immune defenses such as intestinal secretory immunoglobulin A (sIgA) antibodies, breast milk sIgA antibodies or other nonspecific properties present in breast milk, or maternally derived serum immunoglobulin G (IgG) antibodies can prevent adherence of enteropathogens to enterocytes or mucosal invasion without killing the pathogen [63, 64]. Therefore, clinical illness is precluded, while still allowing asymptomatic intestinal carriage of the pathogen. The pathogens isolated from such asymptomatic individuals are nevertheless true pathogens. If these individuals are randomly selected healthy controls, they will be scored as control subjects carrying the pathogen(s) of interest. Below, several examples are given to illustrate these points.

Mucosal Immunity

The phenomenon of mucosal immunity providing clinical protection while still allowing asymptomatic excretion of pathogen is best illustrated with observations made in volunteer studies. North American volunteers who were vaccinated with a high dose (5×10^{10} colony-forming units [CFU]) of ETEC strain E1392-75-2A (O6:H16, LT/ST, CS1, CS3) mounted strong sIgA anti-CS1 and -CS3 antibody responses detected in jejunal fluids [65]. When 12 of these volunteers were challenged 1 month later with 5×10^{8} CFU of wild-type strain E24377A (O139:H28, LT/ST, CS1,CS3), only 3 of 12 subjects developed diarrhea vs 6 of 6 unimmunized control subjects (75% vaccine efficacy; $P = .009$) [65]. An innovative facet of this study was the collection of jejunal fluids from the challenged vaccinees and control volunteers during late incubation and early in clinical illness to determine the presence and load of E7946 ETEC organisms in the proximal small intestine, the critical site of host–pathogen interaction. It is in the proximal small intestine that ETEC attaches to enterocytes by means of

colonization factors and elaborate enterotoxins that culminate in diarrhea; stool culture positivity was also monitored. All 18 challenged subjects had positive stool cultures for the wild-type challenge organism, and all 6 controls had positive jejunal fluid cultures (with a mean of 7×10^{3} CFU/mL). In contrast, only 1 vaccinee had a positive jejunal fluid culture following challenge ($P < .004$) and the colony count was only 10 CFU/mL [65–68]. Thus, in endemic areas where individuals are repetitively exposed to ETEC, individuals who have antiadhesin immunity in the proximal small intestine may be protected from ETEC diarrhea but may excrete the ETEC organisms in their stools.

Further observations supporting this phenomenon were made with infection-derived immunity to wild-type ETEC. Ten of 17 adult community volunteers developed watery diarrhea following ingestion of a dose of either 10^{6} or 10^{8} CFU of ETEC strain B7A with $NaHCO_3$ buffer [20] (Table 1). Eight of the 10 subjects who developed ETEC diarrhea were rechallenged 2 months later with 10^{8} CFU (with buffer), along with 12 naive control subjects. Diarrhea developed in 7 of 12 controls but in only 1 of the 8 rechallenged "veterans" (75% efficacy, $P = .05$). Despite a significantly lower diarrhea attack rate, all 8 rechallenged veterans as well as all 12 controls had positive stool cultures for the ETEC challenge strain. A similar observation was also made during rechallenge studies with *Shigella flexneri* 2a [69]. A level of 70% clinical protection from prior clinical shigellosis was observed upon rechallenge, but all protected individuals shed *Shigella*, as did all naive controls. One must assume that a similar phenomenon of asymptomatic excretion among clinically protected persons living in ETEC and *Shigella*-endemic areas also occurs. If such individuals without diarrhea are randomly selected to serve as controls at a point when they are asymptomatically excreting ETEC, they will appear as culture-positive controls.

Breast Milk

Breastfeeding can protect infants and toddlers from developing more severe forms of diarrhea or even diarrhea at all [70, 71], without preventing intestinal colonization. Protection may be mediated by specific anti-pathogen sIgA antibodies in breast milk [72, 73] or by known nonspecific mechanisms such as lactoferrin [74, 75] and enterotoxin-binding oligosaccharides [76].

Transplacental Transfer of Maternal Antibodies

High titers of IgG maternal antibody against certain enteropathogens transferred transplacentally may prevent young infants from developing more severe forms of clinical illness infection or severe diarrheal disease until the titers wane [77, 78]. Because young infants in developing countries are also breastfed, it is challenging methodologically to isolate the relative contributions to protection that each of these confers.

Environmental Enteropathy

The syndrome of environmental enteropathy characterized by low-grade intestinal inflammation, blunted villi, increased numbers of intraepithelial and lamina propria lymphocytes, and proximal small bowel bacterial overgrowth is evident in a notable proportion of toddlers and preschool-aged children living in underprivileged conditions in developing countries [79–81]. The gut mucosa of these children is believed to have chronic activation of the innate immune system. In such children the ingestion of inocula that might be sufficient to cause diarrheal illness in a child without environmental enteropathy may be diminished by innate defenses such that colonization occurs but clinical disease does not. Environmental enteropathy may also play a role in diminishing the immune response of young children in developing countries to oral vaccines [81].

The Control Subject Is Incubating the Disease

The isolation of an enteropathogen from a control subject without diarrhea may in fact simply reflect identification of a recently exposed susceptible subject who is incubating the infection and will in 1 or more days develop diarrhea.

ENVIRONMENTAL FACTORS

Ingestion of an Inoculum Sufficient to Cause Subclinical Infection but Not Clinical Illness in a Susceptible Host

The presence of the pathogen in the stool of a healthy individual without diarrhea may reflect the recent ingestion of an inoculum too small to cause disease in an otherwise susceptible host; that is, if that individual had ingested a larger inoculum, diarrhea would have occurred. This may be particularly relevant for pathogens such as ETEC and *Salmonella* that are typically transmitted by food vehicles and that exhibit a clear dose-response curve (Table 1).

Ingestion of Host-Restricted Animal Pathogens

Porcine ETEC strain 263 causes severe dehydrating diarrhea in susceptible piglets. Following ingestion of 10^{10} CFU of this strain by adult volunteers, the strain was excreted but no subjects developed diarrhea. This is because the fimbrial colonization factor of this strain is specific for pigs but humans lack the receptors for attachment of the porcine fimbriae. In developing country niches where humans and animals such as pigs and bovines share close quarters, ingestion of animal ETEC incapable of causing human disease may be a common event. If animal ETEC is detected in a control subject without diarrhea by testing colonies for LT and ST and the colonies are not further characterized, they will be scored as ETEC.

OTHER FACTORS

Diagnostic Tests Vary Greatly in Their Sensitivity

Some diagnostic tests for enteropathogens, particularly molecular-based assays, may be so sensitive that they detect the passage through the gut of minute inocula of ingested pathogens that are insufficient to cause diarrhea. The peculiarities of different microbiological assays, including on detection of pathogens in control subjects, are discussed in the article by Robins-Browne and Levine in this supplement.

Disruption of the Intestinal Microbiome

Oral antibiotic use is promiscuous in developing countries and can alter the normal flora to render a human host susceptible to full-blown clinical infection, whereas in the absence of antibiotics, that host's unaltered flora might have interrupted the progression to diarrhea [82, 83]. Similarly, diet can markedly affect the composition of the microbiota [84].

Micronutrient Deficiency

Deficiency of zinc and vitamin A can increase the propensity of a child to develop clinically overt or more severe diarrheal illness following the ingestion of enteropathogens [84]. Conversely, pediatric subjects who do not manifest micronutrient deficiencies may be more likely to respond to the ingestion of enteropathogens by successfully limiting the infection to a subclinical state.

DISCUSSION

With modern, highly sensitive microbiologic methods and tests for pathogens that were unrecognized just a few decades ago, a wide array of enteropathogens can be recovered from cases of diarrhea in the GEMS. Indeed, the vast majority of GEMS patients with diarrhea can be expected to yield 1 or more possible etiologic agents. However, because of the pervasive fecal (human and animal) contamination that constitutes the underprivileged environment in which many young children are living in developing countries, facile transmission of pathogens readily occurs. It is therefore also imperative to assess the prevalence of various enteropathogens among appropriately selected subjects without diarrhea (ie, among matched controls). In a project such as GEMS, one expects to find a proportion of controls asymptomatically excreting known enteric pathogens. In this article we have attempted to review a series of plausible explanations for why healthy subjects without diarrhea may be excreting enteropathogens. To the best of our knowledge, this is the first time that these scenarios have been presented in a comprehensive way and from this perspective. Analyses of the GEMS epidemiologic, clinical, and microbiologic data in conjunction with detailed

characterization of specimens in the GEMS repository will allow us to address many of the hypotheses and commentaries raised in this review.

Notes

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