

New Causes and Treatments for Infectious Diarrhea in Children

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Current Gastroenterology Reports 1999, 1:238-244

Current Science Inc. ISSN 1522-8037

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Infectious diarrhea is a universal and important health problem in the pediatric population. An expanding number of potential viral, bacterial, and parasitic pathogens have been associated with diarrheal disease. However, the epidemiologic association of a microorganism with diarrhea is only one step in the process of identifying new pathogens. Once the virulence mechanisms of these organisms are elucidated, a causal relationship can be more readily defined. This article reviews the etiologic agents of diarrhea in the pediatric population and focuses on the newer treatment and prevention modalities, including probiotics and vaccinations, which are used increasingly to combat these diseases.

Introduction

Infectious diarrheal disease is one of the most common ailments in the pediatric population and plays a major role in pediatric mortality from a worldwide perspective. With the advent of rehydration therapy, the mortality rate from acute diarrhea has decreased sharply, although persistent diarrhea (>14 days) remains a significant health burden. In the United States and in developing countries, infectious diarrhea remains a cause of considerable morbidity and health care expenditure. Infectious diarrhea has been estimated to be the major complaint in up to 10% of pediatric outpatient visits, with a cost to society of between \$0.6 and \$1 billion each year in the United States alone [1••].

When an etiologic agent is sought, a microorganism is identified roughly half of the time. The epidemiology of these commonly found organisms has changed little over recent years. With the advent of more sophisticated technology (such as electron microscopy and immunologic antigen testing to identify new organisms), numerous potential pathogens have been implicated as factors in diarrhea. As modes of detection are becoming more advanced, identification of pathobiologic mechanisms is increasingly important because the presence of a microorganism in the stool does not

prove a causal relationship to disease (diarrhea). Furthermore, the self-limiting nature of the majority of diarrheal diseases currently makes this expensive technology impractical for everyday use. Knowledge of the etiologic agents of infectious diarrhea and their pathologic mechanisms is also useful as new therapies in the treatment and prevention of diarrhea are developed. This review, though not intended to be all-inclusive, aims to address the causes of acute infectious diarrhea with emphasis on some of the more newly implicated pathogens and the therapeutic modalities involved in the treatment and prevention of these diseases.

Viral Gastroenteritis

Viruses are the major cause of acute diarrheal illness throughout the world. They remain the presumptive diagnosis of most episodes of short-lived gastroenteritis that are not assigned a specific etiology, either because laboratory evaluation is not performed or pathogens are not identified in the routine microbiology laboratory. With the identification of a viral agent in stools during a gastroenteritis outbreak in Norwalk, Ohio in 1969 (referred to as Norwalk virus) [2] and later identification of rotavirus particles by electron microscopy in biopsy specimens of children suffering from acute gastroenteritis in 1973 by Bishop [3], the search for viral pathogens was initiated. The known pathogens implicated in acute viral gastroenteritis and some of their distinguishing characteristics are listed in Table 1.

Rotavirus is a nonenveloped, double-stranded RNA virus surrounded by a protein capsid shell. It is the most common pathogen identified in most epidemiologic studies of infectious diarrhea worldwide. In the United States, the incidence of rotavirus infection peaks in the winter and early spring. It has a short incubation time and usually causes a nonbloody diarrhea and vomiting that last from 2 to 8 days. Transmission is usually by direct, person-to-person or person-fomite contact. The majority of severe illness occurs in children aged under 2 years, after which natural immunity usually occurs [4]. In addition, breast-fed infants receive some degree of protection from factors in human milk. Diagnosis of rotavirus is usually made by an enzyme-linked immunosorbent assay detecting the capsid protein antigen of the most common disease-caus-

Table 1. The Viral Pathogens

Virus	Family	Description	Seasonality	Incubation period	Duration of symptoms
Rotavirus	Reoviridae	ds-RNA	Winter-spring	2–3 days	2–8 days
Norwalk/SRSV	Caliciviridae	ss-RNA	Winter	1–2 days	1–2 days
Astrovirus	Astroviridae	ss-RNA	No seasonality	1–2 days	1–4 days
Adenovirus 40/41	Adenoviridae	ds-DNA	Summer	8–10 days	Up to 14 days
Torovirus	Coronaviridae	ss-RNA	No seasonality	Unknown	Unknown

ds—double-stranded; SRSV—other small round structured viruses; ss—single-stranded.

ing strains (group A). This test has a sensitivity of approximately 90% [5].

The Norwalk family of viruses, including the small, round, structured viruses (SRSV), is a heterogeneous group of viruses responsible for epidemic diarrhea in children and adults throughout the winter season. Viruses of this type are classified in the family Caliciviridae and are also RNA viruses surrounded by a protein capsid. Diseases caused by the Norwalk virus and related viruses tend to be less severe than rotaviral illness, lasting only 1 to 2 days. A relative of the Norwalk viruses that was initially classified as an SRSV is astrovirus, an RNA virus now given its own familial classification. Astrovirus infection appears to have no seasonality and may be the second most common cause of viral gastroenteritis. This virus has been studied in various outpatient settings including Thailand [6] and Chile [7] and was prevalent in 8% to 11% of infants and toddlers with diarrhea. In the Chilean study, astrovirus infection was a common copathogen, occurring with another infectious agent, mainly rotavirus and *Giardia*, approximately 50% of the time [7]. The cellular target of human astrovirus infection has not been elucidated, although it has been shown to be present in mature epithelial cells in lambs, where it causes a similar diarrheal illness [8].

Adenoviruses are a large group of viruses that are composed of 47 recognized serotypes. Of these, two enteric serotypes, HadV 40 and 41, are generally accepted as causing diarrheal disease. The incidence of adenovirus infection peaks in the summer months, and the duration of symptoms tends to be somewhat longer than that for other viral gastroenteritides.

Torovirus, a member of the family Coronaviridae, is a recently described pathogen related to the Breda virus that has been shown to invade intestinal epithelial crypt cells in calves [9]. Torovirus has been associated with pediatric diarrhea and found to affect older children, especially the immunocompromised, and as a nosocomial infection. There is a tendency for less vomiting with this infection, but there is an 11% incidence of bloody diarrhea [10]. Other members of the Coronavirus family have been implicated in gastrointestinal disease, although no compelling epidemiologic evidence or pathobiologic basis has been established.

Bacterial Enteritis

Bacterial pathogens continue to play an important role in infectious diarrhea and account for a significant proportion of serious disease. *Campylobacter*, *Salmonella*, and *Shigella* are the most common pathogens implicated in foodborne illnesses reported to the Foodborne Diseases Active Surveillance Network (FoodNet) branch of the US Centers for Disease Control (CDC) [11]. Other pathogens, including *Yersinia*, Enterohemorrhagic *Escherichia coli* O157:H7, and *Vibrio* species are found at a much lower rate. The major bacterial pathogens that cause diarrhea are summarized in Table 2.

E. coli are a diverse group of pathogenic and nonpathogenic commensal organisms. The disease-causing strains are organized into five major groups, largely by pathobiologic features inherent to their virulence. Enterotoxigenic *E. coli* produces infant and traveler's diarrhea. Enterohemorrhagic *E. coli*, serotype O157:H7, is responsible for most instances of hemolytic-uremic syndrome and has been associated with outbreaks of infectious diarrhea in the United States. Enterohemorrhagic *E. coli* can also cause sporadic illness and may be the most common cause of infectious bloody diarrhea in North America [12,13]. Enteroinvasive *E. coli* has been identified as a cause of shigella-like diarrheal illness. Enteropathogenic *E. coli* is a common cause of acute and persistent diarrhea among infants in the developing world. The pathogenic features of this organism include localized enterocyte adherence, microvillus membrane effacement, and initiation of several signal transduction pathways leading to intestinal secretion [14]. Enteroaggregative *E. coli* and diffuse enteroadherent *E. coli* are described on the basis of their adherence patterns to tissue culture cells. Enteroaggregative *E. coli* have been associated with acute and chronic diarrhea in Western Europe [15], while diffuse enteroadherent *E. coli* have been suggested as a cause of diarrhea in Mayan children [16]. In a US multicenter study examining the etiology of outpatient nondysenteric diarrhea, the leading causes of bacterial disease were *E. coli* strains identified by a diffuse Hep-2 cell adherence pattern [1••]. Little is known regarding the pathologic mechanisms in which diffuse and enteroaggregative *E. coli* cause diarrhea. The epidemiologic association of these organisms with diarrhea has led to speculation that they are indeed pathogenic.

Table 2. The Major Bacterial Pathogens

Organism	Major pathologic mechanisms	Duration	Blood in stool	Systemic complications
Salmonella	Invasion Intra-epithelial proliferation	Acute carrier state	Sometimes	Bacteremia
Shigella	Intracellular invasion Shiga toxin elaboration	Acute	Common	Seizures HUS
Campylobacter	Adherence Toxin production Invasion	Acute	Common	Bacteremia Guillain-Barré syndrome
Yersinia	Invasion of epithelium Accumulation in lymph nodes	Acute Persistent	Common	Arthritis Pseudoappendicitis, mesenteric adenitis
Aeromonas	Adherence Cytotoxin elaboration	Acute Persistent	Uncommon	No
EHEC	Adherence Elaboration of shiga-like toxins	Acute	Common	HUS Hemorrhagic colitis
EPEC	Adherence Microvilli effacement	Acute Persistent	Uncommon	No
ETEC	Heat labile, heat stable toxin production	Acute	No	No
EIEC	Invasion Toxin elaboration	Acute	Sometimes	No
EaggEC	Adherence in "stacked brick" configuration Pathogenicity poorly understood	Acute Persistent	Uncommon	Possible growth attenuation
DAEC	Diffuse adherence Pathogenicity poorly understood	Acute	No	No
Vibrio species	Toxin elaboration Adherence, mucinase production	Acute	No	Non-O1 vibrios: sepsis
<i>Clostridium difficile</i>	Toxin A, toxin B production	Acute	Sometimes	Pseudomembranous colitis, Recurrent infection

DAEC—diffuse-adhering *E. coli*; EaggEC—enteroaggregative *E. coli*; EIEC—enteroinvasive *E. coli*; EHEC—enterohemorrhagic *E. coli*; EPEC—enteropathogenic *E. coli*; ETEC—enterotoxigenic *E. coli*; HUS—hemolytic uremic syndrome.

Aeromonas species are gram-negative, oxidase-positive rods that have been associated with diarrheal disease. They have been found with a frequency of 2% or higher in children with diarrhea but not in asymptomatic controls [17,18]. Infection is more common in warmer months. The diarrhea is usually watery and self-limited, although bloody stools and persistent diarrhea may occur [17]. At least one human isolate of *Aeromonas hydrophila* has demonstrated a high degree of cytotoxicity [19•]. *Pleisomonas shigelloides*, a related organism once classified as an *Aeromonas* species, can also cause human diarrheal illness.

Bacteroides fragilis is an anaerobe that is found in normal intestinal flora. In 1984, an enterotoxic strain in animals that caused a diarrheal illness was described. Since that time, enterotoxigenic strains have been isolated from patients with diarrhea and from asymptomatic control subjects. Several descriptions have been given of an association between *B. fragilis* and diarrhea, the most recent showing a significant increase in stools from patients compared to control subjects aged over 1 year [20]. The significance was lost when children aged under 1 year were included. This circumstance suggests either

asymptomatic carriage in the very young, as seen with *C. difficile* infections, or the presence of strain-specific virulence factors. Although this is an evolving story, there are not yet enough data to conclude that *B. fragilis* is a human enteropathogen.

On occasion, intestinal spirochetes have been implicated in colonic disease, resulting in abdominal symptoms and diarrhea. Spirochetes have been found in stools of healthy subjects and incidentally on colonic examination for other diseases. Human intestinal spirochetosis is usually caused by *Brachyspira aalborgi*, though little is known about this family of organisms. Recently, a case has been made for pathogenicity based on a series of three cases including the electron microscopic description of intestinal microvillus blunting and epithelial invasion of the spirochetes [21].

Finally, *Clostridium difficile* has been clearly identified as playing a causal role in diarrhea. *C. difficile* is a gram-positive, spore-forming anaerobe that has been long known to be involved in antibiotic associated colitis. Infection with the spore, acquired through an oral-fecal route, may result in a range of clinical outcomes including

asymptomatic carriage and a fulminant pseudomembranous colitis that can require surgical intervention. Normal colonic flora can suppress colonization with this microorganism, which is almost always associated with previous antibiotic therapy. *C. difficile* exerts its effect mainly through toxin production. Toxin A is thought to be the predominant mediator of human disease by binding to an enterocyte surface receptor and activating an intracellular G-protein-dependent signal transduction pathway. Toxin B is tested for the most frequently in routine microbiology laboratory procedures. Recently, it has been suggested that, in children, testing for toxin B alone will miss some toxigenic *C. difficile*. In one study, 18.5% of patients with *C. difficile* were positive for toxin A alone, and 48% were positive for toxin B alone [22•]. The frequency of misdiagnosis, therefore, decreases considerably when testing is performed for both toxins. Interestingly, 10% of term infants and 55% of neonates in intensive care units are *C. difficile* positive by cytotoxic assay and lack symptomatology [23]. It is possible that the infant intestine developmentally lacks the appropriate receptor or signaling pathway to mediate the toxic effect. The incidence of asymptomatic carriage in children aged over 2 years drops to approximately 3%, which is the colonization rate in adults. Symptoms, when they are present, usually include watery, nonbloody diarrhea accompanied by crampy abdominal pain and anorexia. In more severe cases this can be accompanied by systemic symptoms such as fever, leukocytosis, hypoalbuminemia, dehydration, and electrolyte imbalance. Occasionally, colitis develops and is characterized by bloody stools and the development of pseudomembranes. A fulminant colitis and toxic megacolon may then develop, ultimately requiring surgical intervention [24•]. Resolution of symptoms usually occurs within 3 days of initiating therapy.

Protozoa

Intestinal parasitism continues to play a role in infectious diarrhea in the United States, with protozoans causing the vast majority of infections and giardiasis constituting the majority of parasitic diarrhea. In an effort to document patterns of parasitism in the United States, results of 216,275 stool specimens collected by state laboratories were reviewed by the CDC. *Giardia* was present in 7.2% of stool specimens submitted to state diagnostic laboratories in 1987. This represents an almost twofold increase in the identification of *Giardia lamblia* over what was seen in 1979, with 40 states reporting increases [25]. More recently, in a limited multicenter epidemiologic study, *Giardia* was found in 22 of 147 pediatric patients (15%) with nondysenteric diarrhea, a finding that reflects a higher incidence in children [1••]. CDC's FoodNet identified an incidence rate of *Cryptosporidium* and *Cyclospora* infections at 2.8 and 0.3, respectively, per 100,000 population in

1997 [11]. Data on the long-term trends of these infections are pending and important because they may reflect isolated outbreaks of these parasites during this time period. Other known pathogens, including *Entamoeba histolytica*, *Isospora bella*, and *Balantidium coli*, occur much less frequently.

Dientamoeba fragilis, a parasite found only in its trophozoite form, colonizes the large intestine. It has a quoted prevalence of 2% to 4% in the general adult population and can be found more frequently in closed populations with poor living conditions. In a retrospective analysis in children, *D. fragilis* was found in 0.3% of all stool specimens over a 5-year period [26•]. In 10 of 11 cases, this parasite was the sole pathogen identified and usually presented as a persistent diarrhea with anorexia, abdominal pain, and vomiting. One case presented as an eosinophilic colitis that responded to therapy with iodoquinol. *D. fragilis* is generally not thought to invade intestinal epithelium, and its pathogenicity has been largely suggested by epidemiology alone.

Many other parasites have been isolated in human stools and implicated in human disease. A summary of intestinal parasites, including pathogenicity and treatment, is presented in Table 3.

Therapy

Fluid and nutritional support remains the mainstay of therapy for both viral and bacterial gastroenteritis. This is the case because no effective antiviral agents are available, and antibiotics, in most cases, will either not alter the course of diarrhea or will shorten the duration of the illness by only a small amount. Oral or intravenous fluid therapy is essential in the care of children with a potentially dehydrating illness. Oral rehydration fluids designed to replace the acute electrolyte and fluid losses in diarrhea have gained increasing acceptance. Attempts to modify the basic formula of electrolytes and a low-dextrose composition with supplements such as glycine [27] and alanine [28] to increase sodium absorption through the amino-acid- Na^+ cotransporter have not resulted in improved clinical efficacy. After acute rehydration therapy, rapid reintroduction of the standard diet improves nutrition, promotes mucosal healing, and shortens the duration of illness.

In recent years, many new modalities have been developed to decrease the morbidity of the infectious diarrheas. In 1985, Gorbach [29] identified a lactobacillus organism as a result of screening bacteria present in fermented milk products thought to be beneficial to human health. This lactobacillus species was acid- and bile-resistant, adhered to human intestinal epithelial cells, and had growth characteristics necessary for commercial development. This strain, identified as *Lactobacillus GG*, is one of several probiotics, or nonpathogenic organisms, used to benefit human

Table 3. The Parasites

Parasite	Pathogen	Drug of choice	Pediatric dosage
Giardia	Yes	Metronidazole	15 mg/kg/dose divided 3 times daily for 5–7 days.
		Furazolidone	6 mg/kg/dose divided 4 times daily for 7–10 days.
Cryptosporidium	Self-limited in immunocompetent patients	Uncertain	
Cyclospora	Yes	TMP-SMX	TMP 5 mg/kg dose divided 2 times daily for 7 days.
<i>Isospora bella</i>	Yes	TMP-SMX	TMP 5 mg/kg dose divided 2 times daily for 7 days.
<i>Balantidium coli</i>	Yes	Tetracycline	40 mg/kg dose divided 4 times daily for 10 days.
		Iodoquinol	40 mg/kg dose divided 3 times daily for 20 days.
		Metronidazole	35–50 mg/kg dose divided 3 times daily for 5 days.
<i>Dientamoeba fragilis</i>	Yes	Iodoquinol	40 mg/kg dose divided 3 times daily for 20 days.
<i>Blastocystis hominis</i>	Maybe*	Metronidazole	15 mg/kg dose divided 3 times daily for 5–7 days.
<i>Entamoeba histolytica</i>	Yes	Metronidazole	35–50 mg/kg dose divided 3 times daily for 10 days.
<i>Entamoeba hartmanni</i>	No		
<i>Entamoeba polecki</i>	No		
<i>Entamoeba coli</i>	No		
<i>Endolimax nana</i>	No		
<i>Iodamoeba butshlii</i>	No		

*Questionable pathogen. Recommend treatment for symptoms if this is the sole organism identified.
TMP-SMX—trimethoprim-sulfame thoxazole.

health by improving microbial balance [29]. Following this discovery, multiple candidate microorganisms were developed to aid in human disease. Of these organisms, *Lactobacillus GG* remains the most popular strain to be tested in controlled trials. The reader is referred to review articles by Vanderhoof *et al.* [30••] and Golden [31] for a more comprehensive discussion of probiotics in pediatric disease. *Lactobacillus* has been used to study the prevention of diarrheal illness in undernourished infants in Peru. [32]. *Lactobacillus GG* used daily for a period of 6 to 7 days over a 15-month period has been shown to reduce the occurrence of acute diarrhea in undernourished, non-breast-fed infants aged 18 to 29 months but did not significantly affect younger infants or infants receiving breast milk. In addition, *Lactobacillus GG* has been shown to decrease the incidence of traveler's diarrhea when given as a prophylactic measure [33]. *Lactobacillus* was an adjunctive therapy in the treatment of rotavirus diarrhea in a study of 71 Finnish children [34]. Results were modest, including decreased duration of diarrhea by approximately 1 day. *Lactobacillus* has been successful in the treatment of recurrent *C. difficile* diarrhea after

standard therapy in four of five adults [35] and four children [36]. In the latter study, retreatment was necessary in two children after stools became toxin positive. Long-term follow-up demonstrated no relapse after 8 to 18 months. As our experience grows, probiotics are likely to find a place in the treatment of specific problems related to diarrheal illness.

Another new modality used in infectious diarrhea is enteral immunoglobulin. This strategy, while very expensive, has been successful in treatment of children with prolonged rotaviral illness [37]. In general, this strategy is likely to be reserved for immunodeficient children with protracted diarrhea and has limited value in the treatment of classical rotavirus infection.

As pathogenicity becomes better-elucidated and antigenic proteins or portions of proteins are identified, immunization becomes a feasible means of preventing diarrheal disease. A new addition to the armamentarium against rotaviral induced disease is the rotavirus vaccine. The antigenic proteins located on the viral capsid are classified as rotaviruses. Group A rotavirus is generally thought to cause the majority of human disease. Within this group are four serotypes based on the inner capsid

protein, VP7. These four serotypes are the basis of the rhesus-reassortment virus tetravalent oral vaccine recently approved for use in children aged 2, 4, and 6 months. Multiple trials in developed countries have assessed the efficacy and safety of this vaccine [38, 39, 40, 41]. These studies have demonstrated an approximately 50% efficacy against all episodes of rotavirus diarrhea, a decrease in over 80% of serious rotaviral disease, and complete protection against dehydration. The protection rates observed after three doses of vaccine are comparable to observed rates of protection after natural rotavirus infection in children aged under 2 years [42]. Because the vaccine causes a low-grade fever, and increased reactivity occurs when immunizations are begun past the age of 6 months, immunization is currently not recommended beyond this age. A recent cost-effectiveness analysis has determined that a national immunization program would be beneficial from both a health care and a socioeconomic perspective [43]. In addition to the rotavirus vaccine, immunization strategies are being developed or have been developed for numerous enteric pathogens.

The ways in which vaccines are developed is also evolving. The hypothesis that an immune response can be invoked by antigens found in foodstuffs, along with the technology of plant recombinant genetics, have led to the concept of transgenic foods as vehicles for active immunization. Fourteen human volunteers in a preliminary trial were asked to eat a potato product transformed with the B subunit of heat labile toxin of enterotoxigenic *E. coli*. This activity resulted in mucosal and serologic immune responses similar to those induced by the organism itself [44]. In addition, several animal studies have validated the concept of using plant products transformed with the Norwalk virus capsid protein as potent vaccines [45]. Human trials with this immunization strategy are pending. The use of transgenic foodstuffs as immunogens against enteric pathogens is in its infancy but shows great potential. These vaccines could be produced inexpensively and in bulk for use in developing areas and thus have significant impact on pediatric diarrheal disease.

Conclusions

A working knowledge of the enteric pathogens, the biological mechanisms they utilize to infect the intestine, and the way in which the host responds to their presence has proven valuable in our understanding of how infectious diarrhea is perceived. These diseases have significant morbidity and mortality. An understanding at a molecular level has expanded the emphasis of treatment to include prevention as an effective modality. New strategies must be developed with both efficacy and economic feasibility in mind in order to have a global impact.

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