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## Diagnosis and Treatment of Acute or Persistent Diarrhea



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**Studies of microbial pathogens and the toxins they produce are important for determining the mechanisms by which they cause disease and spread throughout a population. Some bacteria produce secretory enterotoxins (such as cholera toxin or the heat-labile or stable enterotoxins produced by *Escherichia coli*) that invade cells directly. Others invade cells or produce cytotoxins (such as those produced by *Shigella*, enteroinvasive *E coli*, or *Clostridium difficile*) that damage cells or trigger host responses that cause small or large bowel diseases (such as enteroaggregative or enteropathogenic *E coli* or *Salmonella*). Viruses (such as noroviruses and rotaviruses) and protozoa (such as *Cryptosporidium*, *Giardia*, or *Entamoeba histolytica*) disrupt cell functions and cause short- or long-term disease. Much epidemiologic data about these pathogens have been collected from community- and hospital-acquired settings, as well as from patients with traveler's or persistent diarrhea. These studies have led to practical approaches for prevention, diagnosis, and treatment.**

A battle is ongoing between the host microbiome of normal flora and microbial invaders from the outside. When the invaders win, a range of problems can be created for the host—symptomatic infections can alter the intestinal barrier and absorptive functions or lead to rapidly fatal dehydrating diarrhea, toxic megacolon, or shock. Asymptomatic infections can go unrecognized, but they have long-lasting consequences for children's growth and development.<sup>1,2</sup> Therefore, proper diagnosis and treatment are of critical importance, not only for the individual, whose life and cognitive development are at risk, but also for the communities among whom uncontrolled pathogens can spread. Most are acquired through contaminated food or water; however, only small numbers of some pathogens (such as *Shigella*, *Cryptosporidium*, *Giardia*, rotaviruses, or noroviruses) can cause infection. These infections can spread by direct person-to-person

contact, such as in crowded conditions or in institutions such as day care centers. New sensitive and specific diagnostic methods, such as direct polymerase chain reaction (PCR) analysis of fecal specimens, have been used to identify pathogens such as enteroaggregative *Escherichia coli* (EAEC)<sup>3</sup>; this technology is only used in research settings but might someday be used in diagnosis. Currently, careful collection of a patient's history and simple tests, such as analysis of fecal leukocytes or inflammatory markers such as lactoferrin, neopterin, or calprotectin, are used in diagnosis and selection of therapy. This review focuses on pathophysiologies of 3 basic types of bacterial diarrheal diseases: enterotoxigenic upper small bowel infections (such as cholera); invasive or cytotoxin-induced distal, colonic infections (such as *Shigella* dysentery or *Clostridium difficile* colitis); and diarrhea triggered by the host responses to pathogens (such as enteropathogenic *E coli* [EPEC] or EAEC). It also covers viral and parasitic diarrheas and a range of diagnostic methods, epidemiologic settings, and approaches to diagnosis and therapy.

### Pathophysiology of Bacterial Diarrhea

The best diagnostics and therapeutics for diarrheal diseases have been developed based on an understanding of the basic pathophysiology of the pathogens involved (Figure 1 and Table 1). Upper small bowel infections are relatively noninvasive and noninflammatory, causing watery diarrhea. Typically described as secretory, this type of diarrhea results from increased chloride se-

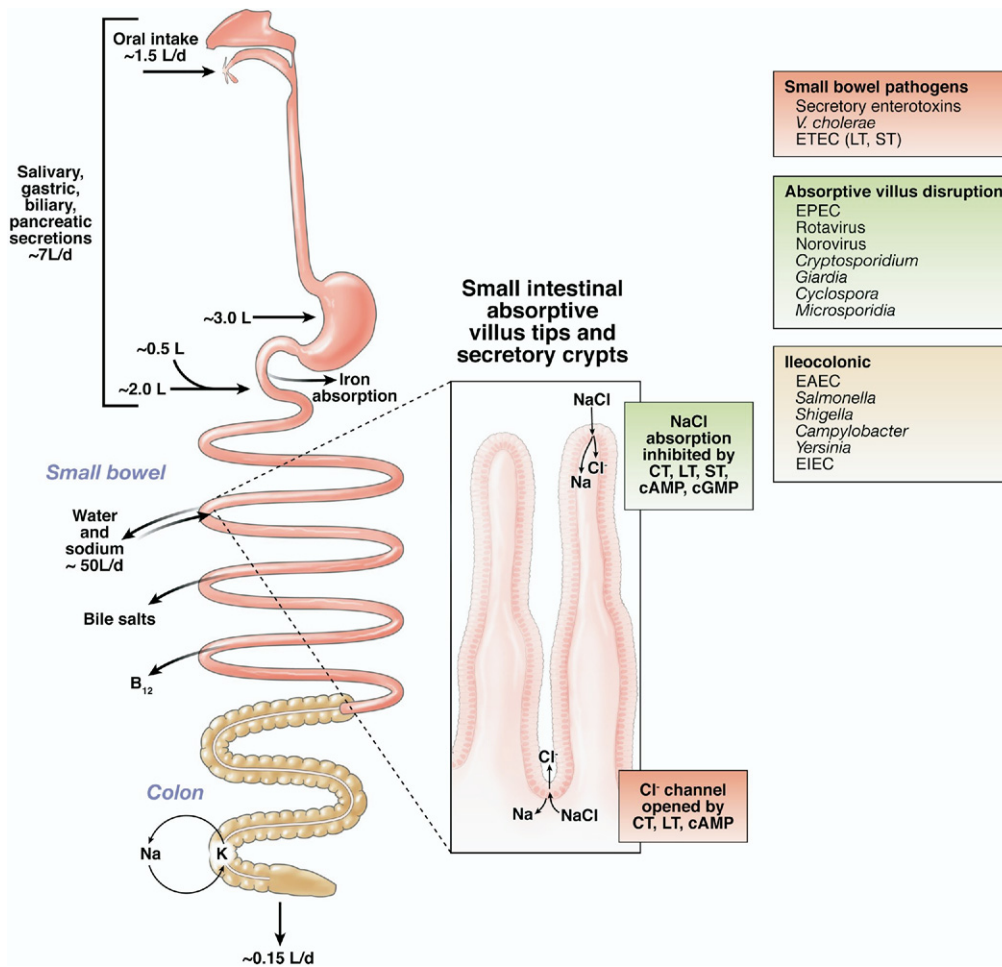
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**Abbreviations used in this paper:** cAMP, cyclic adenosine 3',5' monophosphate; CDI, *Clostridium difficile* infection; EAEC, enteroaggregative *Escherichia coli*; EHEC, enterohemorrhagic *Escherichia coli*; EIEC, enteroinvasive *Escherichia coli*; ELISA, enzyme-linked immunosorbent assay; EPEC, enteropathogenic *Escherichia coli*; ETEC, enterotoxigenic *Escherichia coli*; GI, gastrointestinal; PCR, polymerase chain reaction; PI-IBS, postinfectious irritable bowel syndrome.

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0016-5085/09/\$36.00

doi:10.1053/j.gastro.2009.02.072



**Figure 1.** Normal intestinal physiology and alteration by pathogens and their toxins. The average oral intake for an adult is 1.5 L fluid/day. Combined with salivary, gastric, biliary, and pancreatic secretions 7 L fluid enter the upper small bowel each day, most of which is absorbed by the time it reaches the distal small bowel. However, this small bowel fluid is mixed with a remarkable bidirectional flux of water and electrolytes in the upper small bowel that probably exceeds 50 L isotonic fluid each day, to aid in the absorption of digested dietary intake.<sup>4</sup> This striking bidirectional electrolyte transport is driven by an active, adenosine triphosphate (ATP)-dependent active sodium (Na) absorption pump located on the basolateral membranes of both intestinal crypt and villus tip cells (*middle*). Because the chloride (Cl<sup>-</sup>) channel is located on the luminal surface of the crypt cells, this Na pump mediates secretion of Cl<sup>-</sup> (along with Na and water) from the crypts and neutral NaCl absorption in the differentiated villus tip cells. Thus, a relatively slight shift in this large bidirectional flux can readily overload the colonic absorptive capacity, which rarely exceeds 2–3 L/day. Like cholera toxin (CT), *E coli* heat-labile toxin (LT) opens Cl<sup>-</sup> channels, leading to secretory watery diarrhea or traveler’s diarrhea. The *E coli* heat-stable toxin (ST) activates guanylate cyclase to increase intracellular cyclic guanosine monophosphate (cGMP) levels, inhibiting NaCl absorption and leading to secretory diarrhea. Selective damage of absorptive villus tips, which occurs in viral, protozoal, and other villus-damaging or inflammatory processes, leaves unbalanced secretory crypts that are not offset by healthy villus tip absorption, also leading to watery diarrhea. Small bowel pathogens are presented in the *right panels*: those that produce secretory enterotoxins are in the *red box*, those that selectively disrupt absorptive villus tips are in the *green box*, and ileocolonic pathogens are in the *tan box*.

cretion, decreased sodium absorption, or increased mucosal permeability. Cholera, the prototype of secretory diarrhea, is caused by the enterotoxin of *Vibrio cholerae* (cholera toxin). Cholera toxin binds to the epithelial receptor GM<sub>1</sub> to activate adenyl cyclase, which produces cyclic adenosine 3',5' monophosphate (cAMP). Continuous cAMP production activates chloride channels, resulting in unabated water and electrolyte secretion that leads to voluminous watery diarrhea.<sup>4</sup> Similar to *V cholerae*, enterotoxigenic *E coli* (ETEC; the main cause of traveler’s diarrhea) produce enterotoxins that activate adenylate or guanylate, causing chloride secretion to the intestinal

lumen. In addition, impaired sodium absorption and intestinal permeability have been implicated in this process.<sup>5,6</sup> Other pathogens that cause secretory diarrhea have pathogenic mechanisms that include increased ion secretion, impaired absorption secondary to microvillus blunting, or disrupted intercellular junctions.

Distal small bowel and colonic infections tend to be invasive, causing more inflammatory colitis than upper small bowel infections. Ileocolonic infections are typically caused by invasive or cytotoxic organisms such as *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, invasive *E coli*, EAEC, cytotoxic *C difficile*, or *Bacteroides fragilis*, or the

**Table 1.** Typical enteric pathogens

Location	Pathogens	Pathophysiology
Upper small bowel	<i>Vibrio cholerae</i> Enterotoxigenic <i>E coli</i> (heat-labile toxin, heat-stable toxin, or both) Enteropathogenic <i>E coli</i> (EPEC) Rotaviruses Noroviruses <i>Giardia intestinalis</i> <i>Cryptosporidium parvum/hominis</i> <i>Strongyloides stercoralis</i>	Enterotoxigenic; damaging to absorptive villus tips; usually relatively noninflammatory
Ileocolonic	<i>Shigella</i> <i>Salmonella</i> <i>Campylobacter</i> <i>Yersinia</i> Invasive <i>E coli</i> (EIEC) Enteraggregative <i>E coli</i> (EAEC) Cytotoxigenic <i>C difficile</i> <i>Entamoeba histolytica</i> ? <i>Balantidium coli</i> ? Toxigenic <i>B fragilis</i>	Often invasive or cytotoxicogenic; usually inflammatory
Small and large bowel	Enteraggregative <i>E coli</i>	Triggers host cell inflammatory responses which, in turn, cause secretion

protozoan parasites *Entamoeba histolytica* or *Balantidium coli*. In addition, in immunocompromised hosts, enteric adenoviruses and cytomegaloviruses can cause severe enterocolitis. Cellular invasion by the pathogens (in *Shigella* and others) or the presence of their toxins (in *C difficile* and *B fragilis*) elicits an inflammatory response from the host, causing chemokine secretion and recruitment of immune cells in the intestinal tissue.

Secretory diarrhea is also caused by bacterial pathogens such as EAEC or EPEC, which activate cell signaling pathways that contribute to bowel disease and symptoms. These microbes colonize the gastrointestinal (GI) tract and then trigger inflammatory or “attaching and effacing” responses in host cells. They also produce toxins that can disrupt intestinal absorptive function and cause diarrhea.<sup>7,8</sup>

Viral and protozoan pathogens act through different mechanisms to induce secretory diarrhea. Rotaviruses, noroviruses, and protozoa such as *Cryptosporidium* primarily infect and damage the absorptive villus tips, leaving secretory crypts unbalanced, to cause net secretion and diarrhea. Rotaviruses cause winter- or dry-season diarrhea in young children worldwide, whereas noroviruses are the main causes of winter diarrhea in people of all ages in temperate regions as well as dry-season diarrhea in tropical areas. The protozoa *Giardia intestinalis*, *Cryptosporidium parvum* or *hominis*, and *Strongyloides stercoralis* (the predominant helminth that causes diarrhea in tropical areas) disrupt absorptive villus architecture by direct infection or by triggering host epithelial or inflammatory responses.<sup>9,10</sup>

An overview of the fluid intake and output from the normal GI tract and the mechanisms by which these are

altered by specific pathogens and their toxins are presented in Figure 1.

## Diagnostic Methods

For many years, enteric infections were diagnosed by analysis of bacterial cultures and microscopy to detect ova and parasites. Selective agars allow culture of specific *Salmonella*, *Shigella*, *Vibrio*, *Yersinia*, and *Campylobacter* species. Isolation of cultured organisms is still an invaluable tool for determining sensitivity to antimicrobial agents in clinical settings and for identifying specific strains, virulence factors, or toxins during investigations of outbreaks. In some instances, such as in diagnosis of patients with *E coli*-induced diarrhea, colony-based techniques that use serotyping or HEp-2 adherence assays are used. These traditional methods for differentiating *E coli* strains are less sensitive than PCR analyses of isolates or stool samples; they are also a challenge when the sample produces few colonies, such as a sample from a patient with early-phase gastroenteritis.<sup>11</sup> Bacterial genes can be detected in stool samples with the use of molecular diagnostic techniques, although this method is still limited to research settings. For other pathogens it is more important to identify the toxins than the organisms themselves; this is the case for the enterotoxigenic *Vibrios* and *E coli*, as well as the shiga toxin of enterohemorrhagic *E coli* (EHEC), which is produced by more *E coli* strains than the classic, sorbitol-negative O157:H7. Furthermore, *C difficile* produces toxins A and B. Many *E coli* and *C difficile* isolates do not produce these toxins and are therefore not pathogens. So detection of the toxins is actually more relevant to diagnosis than simply culturing the organism.

Light microscopy to view ova and parasites had been the traditional technique used to diagnose intestinal parasitism. Although microscopy has the advantage of low cost, its sensitivity depends on the burden of infection, the freshness of the specimen, and the experience level of the microscopist. Stains are used to improve detection of specific organisms. Coccidian parasites can be visualized with a variety of stains, including modified Ziehl-Neelsen, Kinyoun acid-fast, Auramine-rhodamine, Gomori's trichrome, or Giemsa. *Cyclospora* and *Isospora* typically autofluoresce; therefore, they can be detected by epifluorescence microscopy. Each organism can be identified through its size and shape: *Cryptosporidium* are round or oval and approximately 4–7  $\mu\text{m}$  in diameter; *Cyclospora* are about twice the size of *Cryptosporidium*; *Isospora* are ellipsoid and approximately 10–20  $\mu\text{m}$  by 20–33  $\mu\text{m}$  in size. Fluorescence-labeled antibodies against specific parasites have improved the sensitivity and specificity of epifluorescence microscopy analyses to 98%–100%.<sup>12</sup> Many laboratories have replaced diagnostic methods, based on microscopy of fecal samples, with more sensitive and specific (and less observer-dependent) enzyme-linked immunosorbent assay (ELISA) methods; ELISA is used to detect protozoa such as *Giardia* and *Cryptosporidium* in fecal samples. PCR analysis can detect most protozoan infections and is more sensitive than antibody detection methods,<sup>13–15</sup> although these assays are still not performed routinely in the clinic.

Enteric viruses are difficult to grow in cell cultures, so when they were first discovered, in the 1970s, definitive diagnoses of infection could only be made based on electron microscopy results. However, the impracticality and inaccessibility of electron microscopes necessitated that rotavirus or norovirus infections be diagnosed on the basis of epidemiologic clues and the clinical presentation of the patients. Currently, sensitive ELISA and latex agglutination analyses can rapidly determine whether a patient is infected with rotavirus.<sup>16,17</sup> However, when specific genotypes must be identified, such as during investigations of norovirus or other outbreaks, additional molecular genetic techniques are required.

Although molecular diagnostics are still used primarily in research laboratories, they are highly sensitive and specific in detecting infections in small samples and can simultaneously identify multiple infections. Multiplex genetic assays are used to detect different toxins, pathogens, and species or genotypes of the same pathogen.<sup>13,18–20</sup> Patients are frequently infected with rotaviruses or noroviruses, especially young children or in winter or dry seasons, respectively. On the basis of data from these assays in diagnostic settings, the most common bacterial pathogen in Baltimore, MD, and New Haven, CT, was found to be EAEC.<sup>3</sup> Metagenomic sequence analysis is being used to determine microbial diversity and to identify new pathogens in human fecal samples, but it is not yet of practical value for clinical

settings.<sup>21</sup> The challenge is to make these highly sensitive and specific molecular assays accessible and affordable for the primary care clinics and community health centers in resource-sufficient and resource-limited areas of the world, respectively.

## Four Epidemiologic Settings

Although many important microbial studies have been performed in resource-sufficient settings, diarrhea and enteric infections, especially among young children, continue to impair growth and development in patients in resource-limited areas. Furthermore, the HIV epidemic has broadened our appreciation of the range of manifestations of enteric infections such as cryptosporidiosis. Patients with symptoms of cryptosporidiosis require prompt rehydration therapy; epidemiologic and clinical analyses should then be performed (Figure 2). In resource-sufficient areas, diarrheal illnesses occur largely in 4 general epidemiologic settings; diagnosis of patients in each of these requires a distinct approach. Diarrheal diseases are most commonly community-acquired (infant, childhood, and adult settings), hospital-acquired, acquired during travel (eg, traveler's diarrhea), or persistent (in normal and compromised hosts). Knowledge of these epidemiologic settings has helped researchers to develop algorithms for the approach, diagnosis, and treatment plans of patients who present with presumed infectious diarrhea (Figure 3). Information about symptoms, the length of time the patients have been sick, the number of individuals affected, the patients' recent histories, and diet guide the practitioner in making a diagnosis. If diarrhea is severe, bloody, or inflammatory or if an outbreak is suspected, fecal specimens should be obtained for further diagnostic and possible therapeutic considerations. Each epidemiologic setting requires a distinct diagnostic and therapeutic approach. It is important to remember that multiple pathogens can be involved; in a study in Tunisia, multiple pathogens were found in 7%–22% of cases,<sup>22</sup> so single therapies might not always be effective. Unfortunately, the cause of infectious diarrhea is not determined in about 80% of cases<sup>23</sup>; through improved diagnostic methods, the yield will increase.

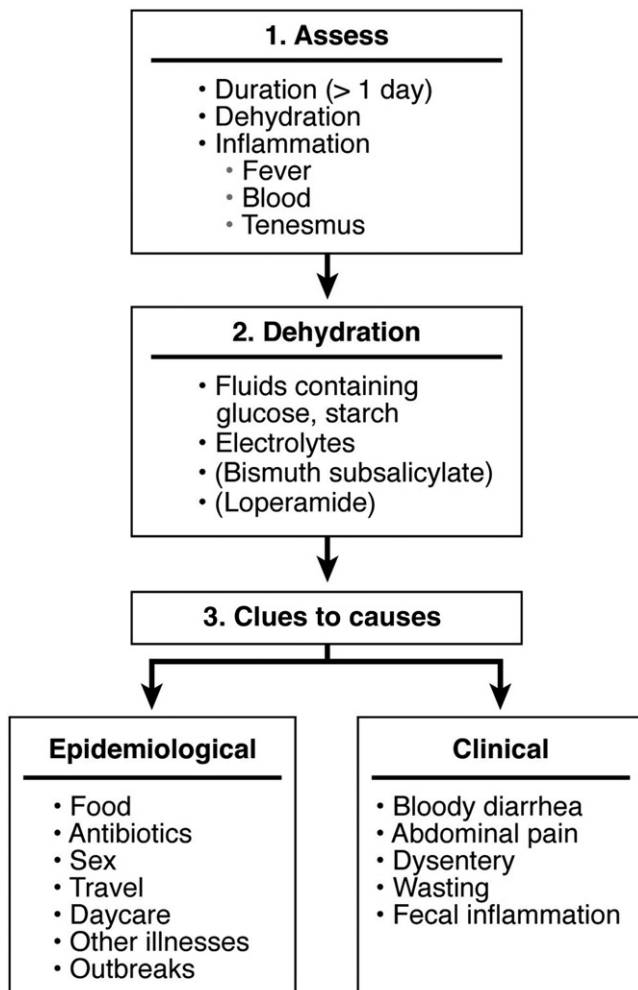
### Community-Acquired Diarrhea

In the United States as well as other developed nations, the leading causes of diarrheal illnesses are rotaviruses and noroviruses; almost every child, worldwide, becomes infected with rotavirus during the first 5 years of life.<sup>24</sup> Norovirus, a member of the human calicivirus family or Norwalk-like virus (NLV) is the leading cause of gastroenteritis in the United States, affecting 23 million people per year.<sup>25</sup> It causes 60% of cases of gastroenteritis, with some reports identifying NLV in 90% of cases of nonbacterial gastroenteritis.<sup>26</sup> Worldwide it is thought to

account for about 50% of gastroenteritis outbreaks, with most patients fully recovering. However, rotavirus is the leading cause of diarrheal-associated death in children younger than 5 years, worldwide. It is estimated that of the 1.6 million worldwide childhood deaths attributed to diarrhea each year,<sup>27,28</sup> rotavirus accounts for 352,000–595,000 cases; evidence also suggests that this is an underestimation.<sup>24,29</sup> Although it is generally thought of as a virus that affects children, rotavirus can affect adults as immunity wanes later in life. Rotaviruses and noroviruses have similar modes of transmission: both are passed person to person, with outbreaks occurring in close settings such as day care centers, long-term care facilities, schools, and within families. Norovirus can also be spread by contaminated food or water. The previous name of norovirus, “winter vomiting disease,” is a graphic reminder of its seasonality and main symptoms, although in adults the principal presentation is diarrheal illness. Unlike rotavirus, which is believed to produce lasting immunity, norovirus is antigenically diverse and produces acquired immunity that is believed to be type specific. Therefore, previous norovirus infection does not

necessarily prevent further illness in a subsequent season, when another variant emerges.<sup>30,31</sup> Other enteric viruses that are less prevalent include sapoviruses, coronaviruses, toroviruses, and enteroviruses.

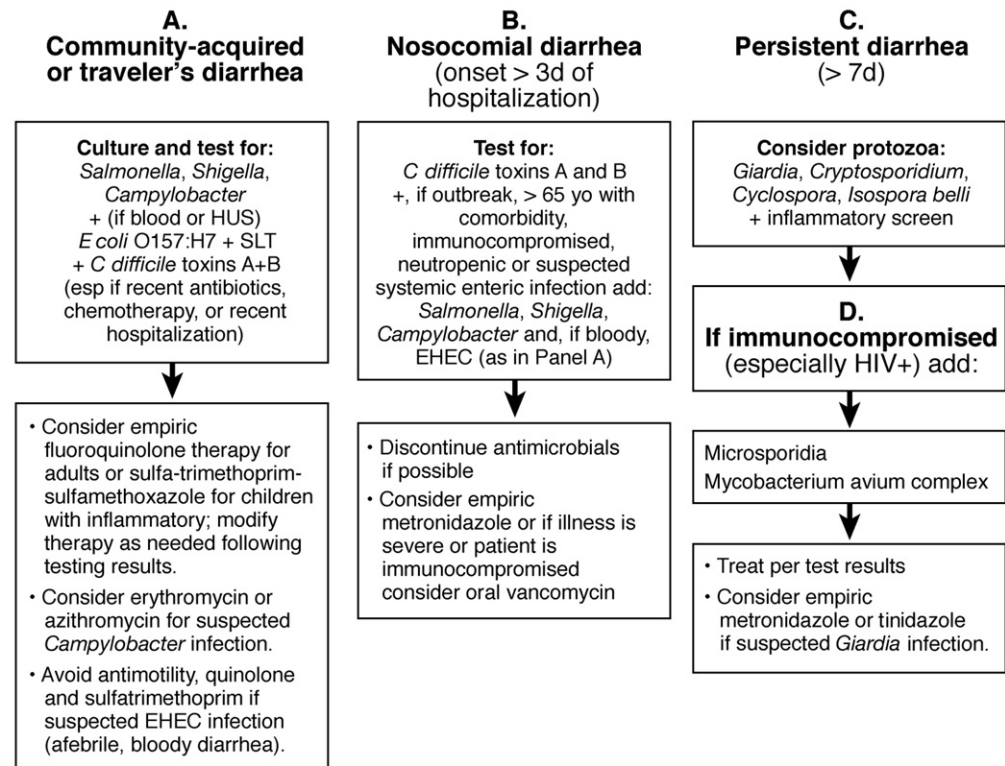
In warmer and wetter months, especially in developing or tropical regions, infections with bacterial and parasitic pathogens become more common. In the United States, the leading causes of bacterial diarrheal illness include *Campylobacter*, nontyphoidal *Salmonella*, *Shigella*, and EHEC. Because of improved diagnostic methods, EAEC (which causes childhood diarrhea in developing countries, traveler’s diarrhea, and diarrheal illnesses in patients with AIDS) is now recognized as a more common, community-acquired enteropathogen; some studies indicate it is the most common bacterial enteropathogen.<sup>3</sup> Although previously thought of as a hospital-acquired illness, *C difficile* infection (CDI) is now recognized as a common cause of community-acquired diarrhea; 22%–44% of cases are thought to occur within the community,<sup>32–34</sup> with many patients lacking the typical risk factors associated with acquisition. Other less common enteropathogens include *Yersinia*, noncholera *Vibrio* spp,



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**Figure 2.** Diagnosis, treatment, and causes of diarrheal diseases. For patients with diarrheal illnesses, it is important to first adequately assess the patient and determine the level of dehydration (panel 1). Important factors include duration of illness; alertness, skin turgor, mucous membrane dryness, sunken eyes or fontanelles, and postural hypotension all indicate moderate or severe hypovolemia and dehydration; signs of inflammation include fever, bloody stool, and tenesmus. In cases of dehydration (panel 2), patients can be rehydrated by oral administration of fluids that contain glucose, sugar or starch, and electrolytes; and patients can be given bismuth subsalicylate or loperamide if their diarrhea is not bloody (panel 2). If patients are unconscious or vomiting, fluids should be replaced intravenously. Pediatric patients can be given an oral rehydration solution made of sodium chloride, sodium bicarbonate, potassium chloride, and glucose or glucose polymer (such as sucrose) in water. Or, table salt and sugar can be added to orange juice, and bananas can be added to provide potassium. There are a number of epidemiologic and clinical clues that can be used to identify the infectious agent (panel 3). Epidemiologic factors that should be considered include the food that patients have eaten (raw, undercooked meats, raw seafood), antibiotics taken (especially for *C difficile* infection), sexual practices (men who have sex with men might have proctitis or colitis with sexually transmitted pathogens), and recent travel (indicate bacterial pathogens such as enterotoxigenic *E coli*, *Salmonella*, *Shigella*, and *Campylobacter*; persistent diarrhea can be caused by protozoans such as *Giardia* or *Cryptosporidium*). Outbreaks of viral-related (norovirus or rotavirus) disease or those caused by organisms with relatively low-infectious inocula that are spread by fecal oral contact, such as *Shigella*, *Giardia*, and *Cryptosporidium*, are most commonly observed in day care settings. Other epidemiologic clues include immunoglobulin A deficiency, which predisposes to giardiasis, and immunosuppression (particularly in patients with AIDS), which can lead to protozoal infections, *Mycobacterium avium* complex, and intestinal cytomegalovirus infections. Clinical features can also be used to identify the infectious agent. Bloody diarrhea is associated with Shiga toxin-producing *E coli*, *Shigella* and *E histolytica* infections; abdominal pain with *Yersinia* and some *C difficile* infections; dysentery with *Shigella* or *Campylobacter* infection; wasting with *Giardia* or *Cryptosporidium* infection; and fecal inflammation with *Shigella*, *Campylobacter*, *Salmonella*, and some *E coli* and *C difficile* infections.

**1. Obtain fecal specimen for one or more panels, if severe, bloody, inflammatory or outbreak suspected:**



**Figure 3.** An algorithm for severe, bloody, inflammatory, or outbreak-related infectious diarrhea. In addition to the management of fluid and electrolyte imbalances, nonsecretory or outbreak-related diarrhea may need further workup for definitive causative diagnoses and pathogen-directed therapy. Depending on the epidemiologic setting involved and likely causes, specific diagnostic tests are suggested as discussed in the text. Specific bacteria such as *Campylobacter*, *Shigella*, or *C difficile* and protozoa such as *Cryptosporidium*, *Giardia*, or *Cyclospora* may require specific antibacterial or antiparasitic agents, respectively. Empiric antimicrobial therapy, while awaiting laboratory test results, is usually appropriate for these pathogens. However, antibacterial treatment may worsen diarrhea secondary to EHEC (eg, *E coli* O157:H7), which usually presents with non-inflammatory, bloody diarrhea.

**2. Consider antimicrobial therapy for specific pathogens.**

**3. Report diarrheal diseases promptly to public health department authorities.**

- In suspected outbreaks, save culture plates, isolates and freeze fecal and suspected specimens at -70°C.
- Diarrheal diseases designated as notifiable at the national level in the U.S. include cholera, cryptosporidiosis, cyclosporiasis, giardiasis, salmonellosis, shigellosis, and infection with STEC.

Additional reporting requirements are available from the Council of State and Territorial Epidemiologists (<http://www.cste.org>) and the CDC (<http://www.cdc.gov/epo/dphsi/phs/infdis.htm>).

and enterotoxigenic *B fragilis*. Cases can be sporadic and isolated or part of a large-scale outbreak, such as the recent outbreaks of salmonellosis. Symptoms of diarrheal illness in the United States range from mild, noninflammatory diarrhea to severe diarrhea that leads to shock, colectomy, and death; symptoms are often the worst in immunocompromised, very young, and elderly patients.

In resource-limited and war-torn countries with extreme poverty, poor sanitation, and crowded living conditions, *Vibrio cholerae* remains an important cause of community-acquired diarrheal illness, death, and large-scale outbreaks. Recent outbreaks (in 2008 and 2009) affected locales such as Iraq, Guinea Bassau, and most recently Zimbabwe; >45 outbreaks (mostly in Africa) have been documented by the World Health Organization since 2000.<sup>35</sup> Poor sanitation and crowded condi-

tions also perpetuate the spread of the more common bacterial causes of infectious diarrhea, such as diarrheagenic *E coli* (ETEC, EAEC, EPEC, and enteroinvasive *E coli* [EIEC]), *Campylobacter*, *Shigella*, *Salmonella*, and *B fragilis*. Although they rarely cause death in the developed world, these infections are still a main cause of mortality in resource-limited regions.<sup>36</sup> Mortality has decreased dramatically since the 1950s, but the illness and morbidity rates from diarrheal illnesses remain unchanged globally<sup>36</sup>; these have profound effects on the development of children and economies of nations. Studies have shown a correlation between the number of diarrheal illness episodes in children younger than 2 years and lower intelligence and physical fitness scores 4–8 years later, an increased age of starting school, reduced growth, and an increase in disability-adjusted life years.<sup>1,37</sup>

Crowded living conditions and poor sanitation also spur the spread of parasitic infections among communities within resource-limited countries. *Giardia* and *Cryptosporidium* are the most common enteric protozoan infections worldwide.<sup>38</sup> It has been reported that 40–50 million cases of amebic colitis and liver abscess are caused by *Entamoeba histolytica* annually, leading to as many as 100,000 deaths.<sup>39</sup> Other less-common parasitic infections include *Blastocystis*, microsporidia (*Enterocytozoon* spp, *Encephalitozoon* spp), *Isospora*, *Cyclospora*, *Schistosoma*, and *Strongyloides*. Symptoms range from self-limited diarrheal illness (mild cases of cryptosporidiosis) to invasive intestinal amebiasis with liver abscesses, septicemia, and death. The seemingly mild symptoms, which also occur with persistent bacterial enteropathogens, become more problematic as poor sanitation perpetuates repetitive infections, leading to persistent diarrhea. In addition, persistent diarrhea and coinfection with helminths reduce growth in children.<sup>37,40</sup>

*Cryptosporidium* and *Giardia* are the most common parasitic causes of diarrheal illness in the United States and other developed nations, where they were associated with 50.8% and 40.6% of waterborne outbreaks, respectively.<sup>39</sup> *Strongyloides* is endemic in some regions of the United States but is rarely implicated in disease, except in immunocompromised patients. *Cyclospora* has been associated with numerous outbreaks in the United States,<sup>41–44</sup> most notably with Guatemalan raspberries.<sup>45,46</sup> *Isospora* and *Microsporidia* are generally only detected in immunocompromised hosts or returning travelers. *Cryptosporidium*, which is found in most public water sources before purification, usually manifests as a self-limited illness. It is often associated with outbreaks caused by contaminated water sources (drinking sources, water parks, and swimming pools), foods, and with person-to-person spread. In immunocompromised patients, children, or the elderly, it can cause a persistent dehydrating diarrhea that causes voluminous, inflammatory, or non-inflammatory watery diarrhea; weight loss; and malnutrition. *Cryptosporidium* is found in stool samples of 16%–28% of patients with AIDS and diarrhea; although the rate of *Cryptosporidium* infection during outbreaks is not higher in patients with HIV, compared with the rest of the population, the duration and severity of illness is directly proportional to the CD4<sup>+</sup> T-cell count.<sup>47</sup> However, *Cryptosporidium* infection can also be asymptomatic, even in patients with low CD4<sup>+</sup> T-cell counts.<sup>48</sup> *Cryptosporidium* and *Giardia* are also common causes of sporadic diarrheal illness, especially in outdoor water recreationalists (anglers, river rafters, and swimmers); outbreaks have been correlated with wet weather events and months with high amounts of precipitation.<sup>49</sup> *Entamoeba* has been detected in higher numbers in men who have sex with men.<sup>50</sup>

### Hospital-Acquired Diarrhea

Diarrheal illnesses that occur after 3 days of hospitalization are often considered to be nosocomial or hospital acquired. This 3-day rule is controversial however, because some preexisting infectious causative agents only become apparent after a patient's arrival at the hospital. Distinguishing between a community-acquired infection and a hospital-acquired infection can be difficult, especially in the case of *C difficile*, the pathogen most frequently implicated in hospital-acquired diarrheal illness in developed nations. No clear guidelines are available for differentiating between community-acquired and hospital-acquired CDIs. One reason for this is that an estimated 4%–20% of nursing home residents<sup>51</sup> and 3% of the healthy population<sup>52</sup> are asymptomatic carriers of *C difficile*; many other infectious agents often considered to be nosocomially acquired might also be spread by asymptomatic carriers.<sup>52</sup> Nosocomially acquired infections could become even more important and be of greater cost to the US health care system as Medicare, Medicaid, and other insurance companies place restrictions on payment for some hospital-acquired infections; this is likely to reduce detection and reporting of these outbreaks.

Community-acquired and hospital-acquired *C difficile* have become more common and serious, with a 2-fold increase in CDI-related hospitalizations and a 4-fold increase in mortality over a 5-year period, with most of the increase occurring within the older population.<sup>53,54</sup> The reasons for the increased incidence and mortality might include the emergence of an epidemic BI/NAP1 strain; this strain is resistant to fluoroquinolones, produces large amounts of toxins A and B as well as a binary toxin, and might hypersporulate.<sup>55</sup> These infections are also observed in otherwise healthy, young, or pregnant individuals, without the classic risk factors.

Although diarrhea that occurs in hospitalized patients can be caused by medications or hyperosmolar feedings, it is not safe to assume that all hospital-acquired infectious diarrheal diseases are secondary to *C difficile*. Other infectious causes include viruses, enteropathogenic bacteria, bacteria not typically associated with diarrheal illness (*Klebsiella oxytoca* and *Staphylococcus aureus*<sup>56</sup>), and parasites such as *Cryptosporidium*. In addition to community outbreaks, hospital-wide diarrheal outbreaks can occur; a study in England found that 15% of intestinal disease outbreaks occur in the hospital setting.<sup>57</sup> With the global spread of HIV, increased use of chemotherapies and other immunosuppressants, and the aging population, it is important to consider these factors in making a differential diagnosis.

Rotavirus is the most common cause of infectious diarrheal illness in the developed and developing world; infected children often require hospitalization for adequate rehydration and treatment. Symptomatic patients place others at risk of nosocomial viral infection, so isolation and contact precautions are believed to be the



most effective method to reduce spread. However, sub-clinical asymptomatic carriers, which can make up 11% of the cases during an outbreak,<sup>58</sup> have also been implicated as one reason for nosocomial spread. Hospitals in both the developed<sup>59–61</sup> and developing world<sup>60–62</sup> have reported outbreaks of rotavirus infection; many of these patients are asymptomatic while they are in the hospital and only develop symptoms after discharge. Although in resource-sufficient nations nosocomial infections are generally considered to be a nuisance and a cause of a prolonged hospital stay, in resource-limited nations they are a significant cause of mortality. For adults, noroviruses are most often implicated in community and hospital outbreaks of infectious diarrheal illness. Additional outbreaks occur in health care-associated facilities such as long-term nursing facilities, psychiatric hospitals, and day care centers.

Although not a common phenomenon, nosocomial parasitic infections and in-hospital outbreaks have been reported. Cryptosporidial infection remains the most common parasitic cause of nosocomial diarrhea; coinfection with *C. difficile* has been reported.<sup>63</sup> Cryptosporidiosis is most often spread from patient to health care worker to patient,<sup>64,65</sup> although one hospital outbreak was linked to ice contaminated by an infected symptomatic patient.<sup>66</sup> In the community setting, most outbreaks occur through contaminated public water sources. Like outpatient outbreaks, in-hospital outbreaks most often affect immunocompromised, elderly, and pediatric patients. Although most nosocomial parasitic infections have been associated with *Cryptosporidium*, reports from developing countries have implicated *Giardia*, *Blastocystis*, and *Entamoeba histolytica*.<sup>67,68</sup>

Although *C. difficile* remains the most common bacterial pathogen, causing hospital-acquired diarrhea in resource-sufficient nations, typical bacterial enteropathogens are more often implicated in developing nations. Although not a common phenomenon in developed nations, *Salmonella* has been implicated as a prominent cause of nosocomial diarrhea in more resource-limited settings.<sup>57</sup> The most severe cases reported often occur in immunocompromised hosts, who are at greater risk of developing septicemia. Cases have been linked to contaminated food,<sup>69</sup> infected food handlers,<sup>70</sup> person-to-person spread,<sup>71</sup> enteral nutrition or baby formula,<sup>72,73</sup> and chemotherapy-induced reactivation.<sup>74</sup> Alarm has been raised by a number of hospital outbreaks of *Salmonella* strains that have developed resistance by acquiring an extended-spectrum  $\beta$ -lactamase.<sup>75,76</sup> Other implicated bacterial pathogens that have been reported to cause nosocomial infections or outbreaks include *Shigella*, *E coli* O157, EPEC, *K oxytoca*, and *S aureus*.

### **Traveler's Diarrhea**

It is estimated that approximately 170 million travelers visit developing nations each year, with approx-

imately 50 million people traveling from developed nations to developing regions. In 2007, the US Department of Commerce estimated that  $\geq 30$  million US citizens traveled to developing regions, the majority to Mexico. In a cohort of US travelers visiting developing areas, 64% reported to have acquired a travel-related illness; almost half of these cases reported diarrhea.<sup>77</sup> This finding is in agreement with other studies reporting that 20%–50% of travelers from developed nations to developing nations experience traveler's diarrhea, depending on the region visited.

Traveler's diarrhea is defined as having  $\geq 3$  unformed bowel movements (taking the shape of a container in which it is collected) that occur within a 24-hour period, often accompanied by other symptoms, including cramps, nausea, fever, blood in stools, and vomiting.<sup>78</sup> It is most often acquired in the first 2–3 weeks of travel through ingestion of contaminated foods and less often drinks; some studies show that the inoculum size increases the risk of infection.<sup>79</sup> Most affected travelers have self-limited illnesses, but illnesses incapacitate others for  $\sim 24$ –48 hours, affecting travel plans. Although  $< 1\%$  of affected travelers require hospitalization,<sup>80</sup> some studies have shown that 4%–11% of affected travelers develop postinfectious irritable bowel syndrome (PI-IBS).<sup>81,82</sup>

Traveler's diarrhea is associated with travel from low-risk to high-risk regions; risk areas are stratified as low risk (4% risk of developing diarrhea), intermediate risk (15% risk of developing diarrhea), and high risk (40% risk of developing diarrhea).<sup>83</sup> According to the US Centers for Disease Control and Prevention, high-risk regions include Africa (excluding South Africa), South and Central America (excluding Chile and Argentina), as well as most of the Middle East, Southern and Southeast Asia, and Oceania. Intermediate-risk regions include the Caribbean nations, South Africa, Argentina, Chile, Eastern Europe, Russia, China, and Portugal.<sup>84</sup> Additional risk factors include young age, length of stay, adventure travel, immunosuppression, genetic susceptibility,<sup>7</sup> low gastric acidity,<sup>85,86</sup> and, surprisingly, staying in a 5-star hotel.<sup>78</sup> An early study performed by Kozicki et al,<sup>87</sup> in which travelers were counseled to "boil it, cook it, peel it, or forget it," showed that despite these warnings, high-risk eating habits were common. A recent meta-analysis concluded that the incidence of diarrhea was similar in travelers who followed the old adage and those that engaged in riskier eating habits.<sup>88</sup> The reasons for this are probably due to poor restaurant hygiene; one study showed that even though restaurants in Guadalajara, Mexico, cooked and served cooked foods hot, high levels of coliform bacteria could still be cultured from them.<sup>89</sup> Cooking foods does not always kill pathogens, leading to recommendations that food should be steaming hot before it can be considered safe for consumption.<sup>90</sup> These studies highlight the difficulties in preventing traveler's

diarrhea; despite much research into risks and causes, little change as occurred in the frequency of travelers' diarrhea illnesses during the past 50 years.

Several studies have shown that antibiotics can reduce the rates of diarrhea in travelers to resource-limited countries; however, preventive antibiotic therapy is not recommended because of side effects and the availability of rapidly effective, single-dose antimicrobial therapy, if needed. Probiotics have shown benefit in some studies.<sup>91</sup> Prophylactic use of the nonabsorbable chemoprophylactic agent bismuth subsalicylate reduces the rates of diarrhea by 40%–65%.<sup>92–94</sup> In a study of subjects infected with ETEC and then given placebo or prophylactic bismuth, ETEC was recovered less frequently from the subjects given prophylactic bismuth.<sup>95</sup> Rifaximin, a poorly absorbed antimicrobial, can also reduce the risk of traveler's diarrhea by 70%,<sup>96</sup> although the development of resistance causes some concern.

Contaminated foods can pose an even greater risk than contaminated water. Studies in Mexico have shown that high levels of coliform bacteria, most often ETEC or EAEC, are present in typical restaurant fare,<sup>89,97</sup> with additional studies implicating street vendors and supermarkets.<sup>98</sup> Interestingly, foods prepared in homes had the highest levels of bacterial contamination; visiting foreign students eating >80% of their meals at their host families' homes had the highest rates of traveler's diarrhea.<sup>98</sup> Bacterial enteropathogens account for 80% of the cases of traveler's diarrhea. Diarrheagenic *E coli*, ETEC, EAEC, and EIEC are implicated in approximately 50% of the cases, followed by *Campylobacter*, *Salmonella*, and *Shigella*; some regions have seasonal variations.<sup>99</sup> Less-common enteropathogens include *Aeromonas*, *Plesiomonas*, and *Vibrio* spp.

Although implicated as the causative agent in only  $\leq 12\%$  of cases of acute traveler's diarrhea, parasites are the most common organism detected in travelers with persistent diarrhea.<sup>100</sup> *Cryptosporidium* and *Giardia* are most often implicated.<sup>101,102</sup> Travel to Asia seems to particularly predispose travelers to *E histolytica* as well as other parasites,<sup>102,103</sup> whereas it is rarely found in short-term travelers to Mexico.<sup>104,105</sup> Other parasites implicated as causative agents of traveler's diarrhea include *Cyclospora*, *Isospora*, and microsporidia, whereas the effects of infection with *Blastocystis*,<sup>106</sup> *Dientamoeba fragilis*, *Balantidium*, and *Endolimax* are not clear.

Viruses also cause travel-related diarrheal illness. Groups traveling in close proximity are at particular risk (eg, on cruise ships). Recent studies have shown that infection with norovirus was second only to that of ETEC in causing diarrhea in students traveling to Mexico and travelers to Guatemala.<sup>107,108</sup> Rotavirus, adenoviruses, and astroviruses have also been implicated.<sup>102,109</sup> Although dengue fever does not normally cause diarrhea, 2 cases with "febrile diarrhea" have been reported.<sup>110</sup> It is also important to remember that malaria can cause di-

arrhea and fever, so this disease must always be considered, especially in febrile travelers during or after visiting endemic regions.

### **Persistent Diarrhea**

Persistent diarrhea is generally defined as the passage of loose stools for >2 weeks, with progression to chronic diarrhea at the 4-week mark. The causes of persistent or chronic diarrhea include: persistent infection; repeated infection, which occurs primarily in resource limited regions with poor hygienic conditions; and PI-IBS after an infection has cleared. Again, the responsible organisms depend on endemicity or recent travel. Persistent diarrhea can lead to long-term morbidity, probably because of malabsorption of key nutrients caused by the blunting of villi, disruption of the epithelium, and submucosal inflammation.

EPEC and EAEC are the most commonly implicated bacterial pathogens in persistent infections in developing countries, especially among children. The exact mechanism by which EAEC leads to persistent diarrhea is not known, whereas EPEC's pathogenesis lies in its ability to disrupt the brush border through the adherence and effacement process, leading to loss of absorptive areas. CDIs are increasingly more difficult to treat as they become more prevalent in the community. This organism is now recognized as a cause of persistent diarrhea in developed countries. Other bacterial pathogens that cause persistent diarrhea include *Campylobacter*, *Salmonella*, and, on rare occasions, *Tropheryma whippelii*. In immunocompromised patients, atypical mycobacteria infections should also be suspected. Although they are prominent causes of acute diarrhea, ETEC, EHEC, and *Shigella* usually do not cause persistent diarrhea.

Intestinal parasites are another main cause of persistent diarrhea in developing regions, and in many areas they are the most common cause of persistent infectious diarrhea. *Giardia* and *Cryptosporidium* are most often implicated, along with *Entamoeba*, *Isospora*, and microsporidia. Studies of expatriates in Nepal have shown seasonal variation in infection; *Cyclospora* is most often implicated between the months of May and October.<sup>111</sup> Overall, intestinal parasites are the most commonly implicated pathogens, causing persistent diarrhea in patients with HIV.<sup>112</sup> *Cryptosporidium*, *Entamoeba*, and *Isospora* have been most-frequently identified, followed by *Giardia* and *Strongyloides*.<sup>113,114</sup> Microsporidia are another important cause of persistent diarrhea.<sup>115</sup> In the United States, persistent diarrhea most often affects the immunocompromised and the elderly; therefore, physicians should test these patients for *Cryptosporidium* and *Giardia*.

Viruses, which are generally thought of as causes of acute dehydrating diarrheal illness, have been found in a minority of immunocompetent patients with persistent diarrhea. In a recent study of children with persistent diarrhea in the US, however, norovirus, rotavirus, and

sapovirus were the only pathogens isolated, rather than bacterial or parasitic pathogens.<sup>23</sup> In immunocompromised individuals, cytomegalovirus and other enteric viruses are important causes of persistent diarrhea.

Acute infectious gastroenteritis can also predispose individuals to PI-IBS. A recent meta-analysis concluded that the odds of developing PI-IBS increased 6-fold after an episode of acute GI infection.<sup>116</sup> Other studies concluded that the risk was even higher. However, additional analyses indicated that individuals who developed PI-IBS had a greater number of psychological disorders and stressful events before their episode of acute gastroenteritis, so it is possible that there is still a prominent psychologic component to PI-IBS.<sup>117-119</sup> In animal models of IBS, rodents are exposed to enteropathogens, including *Campylobacter*, *Trichinella*, or nematodes. Although the exact mechanism of PI-IBS is unknown, patients and animal models have alterations in serotonin transporter system function and increased amounts of inflammatory cells, mast cells, inflammatory markers, intestinal permeability, and colonic transit time. Most studies have implicated *Salmonella*, *Campylobacter*, and *Shigella* in PI-IBS, although studies have reported the development of PI-IBS in patients with traveler's diarrhea caused by ETEC and EAEC.<sup>82</sup>

In conclusion, patients with the common problem of infectious diarrhea require prompt rehydration; then clinical and epidemiologic assessments should be done. Gaining a better understanding of the pathophysiology of infectious diarrhea and the factors that promote the dissemination of infectious agents that cause it will lead to practical approaches for preventing and responding to outbreaks.

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Received November 3, 2008. Accepted February 25, 2009.

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**Conflicts of interest**

The authors disclose the following: Dr Guerrant has licensed fecal lactoferrin to Techlab Inc, Blacksburg VA.