

Recent Advances and Evidence Gaps in Persistent Diarrhea

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Despite advances in the understanding of diarrheal disorders and management strategies, globally nearly 1.8 million children die annually as a consequence of diarrhea (1). Of these, a significant proportion dies following prolonged episodes of diarrhea. Persistent diarrhea (PD) is defined as diarrheal episodes that start acutely but last for 14 days or more, usually associated with growth faltering (1,2). Unlike acute diarrhea, in which dehydration is the chief contributor to mortality, PD has multiple and diverse adverse effects on childhood outcomes, including micronutrient deficiencies, stunting (3), and cognitive impairment (4). It is an important contributor to morbidities and mortalities from other diseases. It has been estimated that PD is associated with 3 million disability adjusted life-years lost annually (2).

CURRENT CONTROVERSIES AND ISSUES

Epidemiology and Burden Estimates

Despite the individual and population-based consequences of PD, this disorder poses definitional, epidemiological, diagnostic, and risk analysis challenges. First, it is not clear how best to define PD. Although a working definition of nonresolved diarrhea for at least 2 weeks is a reasonable starting point, it must be recognized that most diarrheal episodes form a continuum and even diarrheal episodes lasting 7 to 14 days can be associated with a nutritional penalty (5). There has been a significant reduction in the number of publications related to PD in the global literature on diarrheal diseases (Fig. 1) in the

past decade. There are little data to indicate that PD has abated, and it is likely that this diminished publication output also reflects reduced research interest in the subject. It is notable that given the continuum between acute and persistent diarrhea it may not be feasible to focus on this delayed and somewhat arbitrary point in illness with the sole goal of preventing PD. It is advisable to attempt interventions once diarrhea exceeds 5 to 7 days, and this category of “prolonged diarrhea” may help us understand the pathogenesis of PD and the impact of interventions. Although an extensive modeling of a diagnostic-driven intervention recently has been published (6), the value of such an effort awaits improvements in technology, and obligates coordination with intervention strategies.

Issues in Pathogenesis

One cannot easily determine the frequency with which PD follows a primary enteric illness, and it is for this reason difficult to design hypothetical interventions at the initiation of this process. Also, although PD is certainly a risk for stunting and other consequences, even when controlling for a variety of potentially confounding factors, it is not known what fraction of these target consequences are caused by PD versus other processes (which may themselves be marked by PD, such as human immunodeficiency virus [HIV] infection). A recent evaluation of the global burden of undernutrition and risk factors (7) revealed that the odds of stunting at 24 months increased by 5% with each diarrheal episode (odds ratio [OR] 1.05; 95% confidence interval [CI] 1.03–1.07). In a subset analysis, after controlling for height-for-age z score at 6 months, the odds of stunting at 24 months increased by 4% with each diarrheal episode between 6 and 24 months (OR 1.04; 95% CI 1.0–1.08; $P = 0.03$). However, it is not

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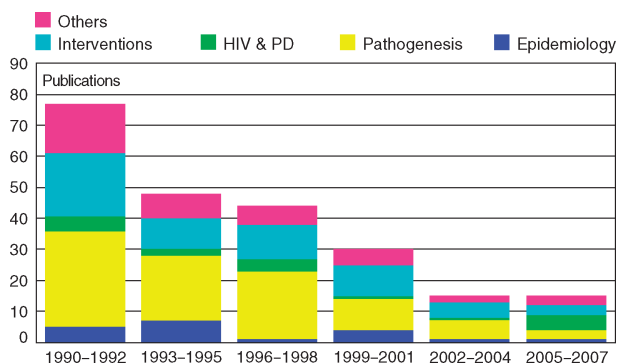


FIG. 1. Trends in indexed publications (number of articles) on persistent diarrhea in children, 1990–2007.

possible to determine the population-attributable fraction of stunting and undernutrition that may be related to PD. Although there seems to be a strong association with several persistently detected pathogens (ie, enteroaggregative *Escherichia coli*, *Cryptosporidium parvum*, and *Giardia lamblia*) and PD in some countries (8,9), sensitive and polymicrobial assessments of the precipitants of PD have been sparse. Therefore, assuming that multiple different agents can produce PD, we do not yet know the relative risk of PD for various infectious etiologies. In addition, the clinical diagnosis of PD can reflect consecutive acute infective episodes, as in-patient studies have recognized, and unless microbial investigations are carried out such intercurrent infections will not be recognized. It is also important to rule out other causes of chronic diarrhea that may appear as persistent diarrhea; for example, coeliac disease (which is now well recognized in developing as well as developed countries), food-related enteropathies, and the rarer cases of intractable diarrhea (congenital enteropathies).

Although our understanding of the pathogenesis of intractable diarrhea and intestinal injury has increased (1,10), there are still major gaps in our knowledge of the molecular mechanisms underlying prolonged intestinal injury in PD of infectious etiology (include HIV populations). Small intestinal mucosa injury that becomes prolonged has been named as a central mechanism in the pathophysiology of PD (11), but we must discriminate between persisting infective colonization with enteropathy and a postinfective enteropathy that fails to heal or heals slowly. In the former cases, it is becoming apparent that bacterial effector molecules (eg, in *Salmonella*, *Yersinia*, *Shigella*, and enteropathogenic infections) that are translocated into the host cell and downregulate the host immune system may play a role in prolonged colonization (12). In such circumstances, the interrelation between the normal flora and the host may be altered, leading to prolonged inflammation possibly via altered Toll-like receptor signaling (13). The stem cell compartment is

integral to the response to damaged epithelium, and factors influencing its response to wounding are becoming known, including activated macrophages, Toll-like receptor signaling, and the microbiota (14). Their potential role, if any, in the pathogenesis of PD remains uncertain.

Most work has focused on the categorization of injury (ie, identifying changes in the absorptive, secretory, and reabsorptive capacities for minerals, carbohydrates, protein, and fats) (1). Additional studies have focused on morphological analyses of small intestinal biopsies showing villous blunting and increased cellular infiltrates.

Intestinal regeneration is the process by which intestinal injury is mitigated and an understanding of the process may enable us to improve bowel function in PD through appropriate interventions. The bowel has considerable regenerative and renewal capacities, which would enable recovery from the PD state, but we do not know the kinetics of tissue recovery in PD (15). The regenerative process involves epithelial cell migration and proliferation, changes in cellular function, adaptation of subepithelial tissues. This requires interaction of multiple cell types. This regulation in PD is not well understood. It is believed that a variety of host effectors—such as epidermal growth factor, interferon- γ , and interleukin-8—play important roles in many aspects of regeneration, including mesenchymal–epithelial interactions (16,17).

There is considerable interest in the potential role of mannose-binding lectin (MBL) in the pathogenesis of enteric inflammation. MBL is a collagenous lectin found in serum that primarily binds to multiple mannose and *N*-acetyl glucosamine residues (18). Upon binding, MBL mediates elimination of a wide variety of bacteria, yeasts, viruses, and protozoa through activation of the complement system or direct interaction with phagocyte receptors (19). MBL deficiency has been proposed to be one of the most common forms of immune deficiency (20) and also has been implicated in the pathogenesis of PD in HIV-infected individuals (21,22). Research in this area mainly concentrated on the role of MBL in the defense against *Cryptosporidium parvum* infection and has shown that individuals homozygous for MBL structural gene mutations were at increased risk of cryptosporidiosis (OR 8.2; 95% CI 1.5–42; $P = 0.02$) (21). In a subsequent case-control study in Haitian children with cryptosporidiosis, serum MBL levels were markedly lower in children with cryptosporidiosis ($P = 0.002$) than in controls (22). However, the perceived role of MBL deficiency in the pathogenesis of other enteric pathogens has been inconsistent. Children with MBL deficiency do not have increased predisposition to *E coli* O157:H7 infections. In addition, the precise mechanisms by which MBL deficiency may predispose to cryptosporidiosis are not fully understood (23). The potential roles of common and subtle genetic mutations that may predispose an individual to PD merit further studies in representative settings.

Recent Developments in Diarrhea Prevention and Their Implications for PD

There has been considerable evidence as to the efficacy of zinc for preventing and treating diarrhea (24), and a recommendation has been made to promulgate zinc for its impact on diarrheal disease and growth (25). There are also attempts planned to use zinc to prevent or treat diarrhea, and although there is evidence of significant impact on recovery rates and diarrhea duration in acute episodes, it is not yet known how this will affect PD incidence or prevalence. However, the best modality with which to deliver zinc to populations has not yet been determined.

The extent to which prevention of infectious diarrhea by vaccination will reduce PD and malnutrition is also unknown. Rotavirus is the most common cause of severe dehydrating diarrhea in young children, globally accounting for an estimated 610,000 deaths, 2.4 million hospital admissions, and 24 million outpatient visits annually (26). Of an estimated 10.6 million deaths in children ages younger than 5 years during the period 2000–2003, diarrheal diseases account for 1.76 million deaths (17%) (http://www.who.int/whr/2005/annex/annexes_3-4_en.pdf). The anticipated use of vaccines against rotavirus and other enteric diseases in immunization programs provides an opportunity to determine the role that infectious diarrhea plays in the development of PD and malnutrition. It is probable, but not yet demonstrated, that the prevention of these primary infections will diminish the frequency of PD. Also, there are economic considerations for the use of enteric vaccines, which have not yet been resolved in resource-poor countries where the impact of PD is the greatest. At this time, considerable emphasis is being placed on scaling up rotavirus vaccine strategies through novel fiscal support mechanisms, including advanced marketing commitments and financing initiatives.

A major additional question is the concern that rotavirus and other vaccines may have suboptimal impact in regions such as south Asia, where mucosal vaccines may be potentially less effective. Given the need to assess the impact of the vaccine on diarrhea and nutrition outcomes, it also is important to have ongoing and systematic surveillance of diarrheal disease burden after the introduction of these vaccines, including assessment of the rates of PD. This surveillance, through existing disease enumeration systems, could be critical to decision making for the introduction of new diarrheal vaccines and other preventive strategies. Such cost-benefit analyses should not be limited to acute diarrhea, but also include estimates of PD.

Additional strategies for preventing, treating, and enhancing recovery from diarrhea and PD include the use of probiotics and functional foods. These modalities have been used to prevent diarrhea and PD in developing countries (27,28). However, there have been relatively

few studies determining the mechanisms underlying their effects and their potential role in population-based interventions. Embellishments of these interventions may include genetically engineered molecules that could improve host intestinal function. Recently, the use of butyrate has been recommended as a treatment for shigellosis via the upregulation of cathelicidin, an antimicrobial peptide of the innate immune system. The pathway for this action is unknown, but isoleucine and vitamin D also appear to induce these antimicrobial peptides, raising the possibility of treating infection by stimulating host defences (29).

RESEARCH AGENDA IN PERSISTENT DIARRHEA

There are many research gaps in this field, and the section below considers some salient areas in which additional data from designed studies could provide timely and useful information.

Research Priorities in Epidemiology

Despite the advances listed above, our understanding of the epidemiology and pathogenesis of PD has major lacunae. These gaps include:

- Clear delineation of the link between prolonged and persistent diarrhea and a syndrome-based definition of categories of diarrhea with prognostic specificity for progression to PD
- Delineation of the subset of children with PD that progresses to consequential and adverse outcomes, including long-term deficits in growth and neurodevelopment
- A robust evaluation of factors (secular, nutritional, or interventional) associated with reduction in PD in communities where this disorder has receded

Research Priorities in Pathogenesis of PD

Above, we highlighted our perception of the research gaps that need to be filled to understand the mechanisms associated with delayed intestinal mucosal regeneration and development of PD. We propose that major areas should be emphasized in further study, as follows:

- Assessment of mucosal immunopathology in PD and its relationship with malnutrition in HIV-infected and noninfected children
- Interaction between intestinal microbiota, immune responses, gene expression, and intestinal absorption in children with malnutrition and PD
- Improved understanding of the regulation of intestinal regeneration so as to develop therapeutic

approaches to promote intestinal healing in PD both related and not related to HIV

- Identification of microbial precipitants and perpetrators of PD and the molecular mechanisms underlying these trends

Research Priorities in PD Management in Health Systems

In consideration of current recommendations for the recognition and treatment of PD, we identify several areas that merit additional operational research:

- Should all prolonged diarrhea episodes be treated aggressively as potential PD? If not, how should we triage those that warrant treatment?
- What enteral nutrition regimens or parenteral nutrition approaches, if any, are optimal for the nutritional sustenance of children with PD?
- Given the remarkable success of interventions such as green banana and rice lentil diets in PD (1,30), the potential role of luminal nutrients in the management and prevention of PD needs further elucidation.

Research Priorities for Micronutrient Interventions for PD

To extend the benefits of zinc and micronutrients for the prevention and treatment of acute diarrhea to PD, several questions should be addressed in well-designed cohort studies:

- What is optimal micronutrient intake (zinc alone or in combination with others) in PD (31,32), and can a clinical algorithm such as is portrayed in Fig. 2 be used worldwide in most circumstances?
- What accounts for variable response to micronutrients, between and within populations?
- What is the optimum mode (ie, supplementation vs fortification) and timing of administration of micronutrients?

CONSENSUS APPROACH TO THE PREVENTION AND TREATMENT OF PD

There is general consensus on the approaches to the prevention and management of PD in developing countries. Early and unhygienic introduction of milk other than breast milk and recurrent acute diarrheal episodes that are poorly managed are important predisposing factors to the development of PD, and it is important that these are prevented. These risk factors

are generally prevalent in poor communities where poverty alleviation and social sector support mechanisms to ensure optimal complementary feeding are fundamentally important. Thus, promotion of exclusive breastfeeding for at least 6 months, avoidance of formula feeding, and timely and adequate weaning with hygienic nutritious foods will help to prevent episodes of post-infectious PD. These must be coupled with environmental control measures and provision of safe water. In the absence of the latter, interventions to promote hand washing strategies and point-of-use water purification are needed (25).

There is in general no reason to provide specialized formula or low lactose diets because, despite alterations in digestive and absorptive mechanisms, analysis of metabolic balance studies in children with PD indicates that satisfactory carbohydrate, protein, and fat absorption can take place on a variety of diets. A variety of diets have been tested and used for the treatment of PD, including specialized formulas and traditional diets (30,33); the key challenge is to scale up these interventions for use in health systems. A process of dietary therapy using inexpensive, home-available, and culturally acceptable ingredients that can be used to manage children with PD in ambulatory settings is appropriate for most developing countries, but may need to be augmented with referral and hospital-based management in complicated cases with parenteral feeding and special formulas as required (1,33).

CONCLUSIONS

PD continues to pose enormous challenges globally. In view of the overall reduction in childhood mortality from acute diarrhea, PD now represents a larger portion of the burden of childhood enteric diseases, and may be associated with a significant proportion of the 1.8 million child deaths due to diarrhea annually. It is critical that we learn more about this disorder, most particularly how best to define, diagnose, triage, and implement interventions. Interventions to be evaluated include evidence-justified strategies such as treating or preventing precipitating and concurrent infections, identifying optimal diets to prevent or treat PD, dietary therapy, micronutrient supplements, and zinc therapy. Research into epidemiology, host response, and etiology should be performed in parallel. The assessment and ultimate implementation of these strategies will include infrastructure development and maintenance. The robust research agenda summarized above is important for improving our understanding of the pathogenesis of PD and future strategies for control. Addressing these issues will require a collaboration of health care professionals, scientists, public health professionals, economists, and policymakers.

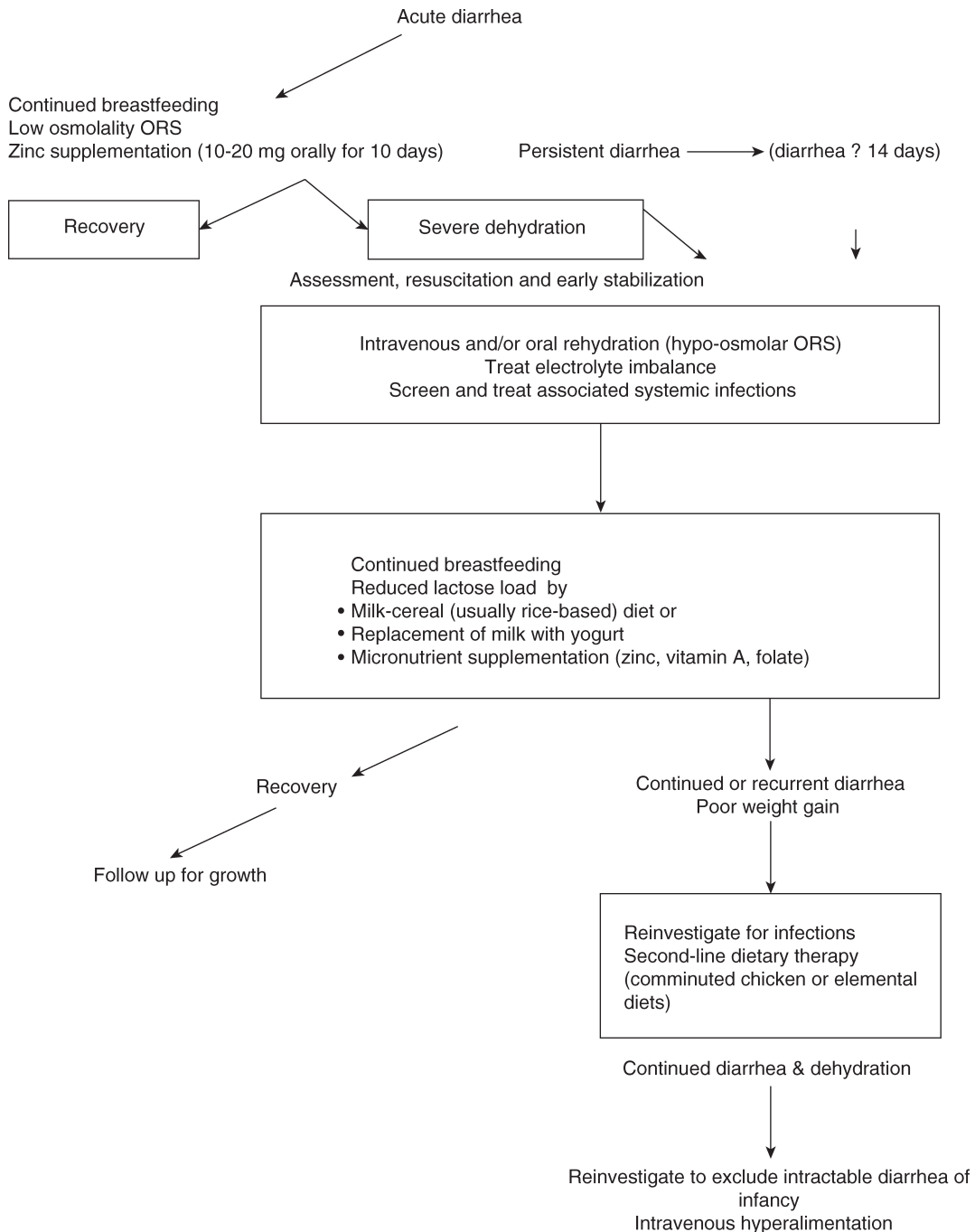


FIG. 2. Suggested algorithm for diagnosis and management of persistent diarrhea.

REFERENCES

1. Bhutta ZA, Ghishan F, Lindley K, et al. Persistent and chronic diarrhea and malabsorption: working group report of the Second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2004;39:S711–6.
2. Guerrant RL, Kosek M, Lima AA, et al. Updating the DALYs for diarrheal disease. *Trends Parasitol* 2002;18:191–3.
3. Lima AA, Moore SR, Barboza MS Jr et al. Persistent diarrhea signals a critical period of increased diarrhea burdens and nutritional shortfalls: a prospective cohort study among children in northeastern Brazil. *J Infect Dis* 2000;181:1643–51.

4. Berkman DS, Lescano AG, Gilman RH, et al. Effects of stunting, diarrheal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. *Lancet* 2002;359:564–71.
5. Niehaus MD, Moore SR, Patrick PD, et al. Early childhood diarrhea is associated with diminished cognitive function 4 to 7 years later in children in a northeast Brazilian shantytown. *Am J Trop Med Hyg* 2002;66:590–3.
6. Ricci KA, Girosi F, Tarr PI, et al. Reducing stunting among children: the potential contribution of diagnostics. *Nature* 2006;444:29–38.
7. Black RE, Allen LH, Bhutta ZA, et al. Maternal and Child Undernutrition Study Group. Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet* 2008;371:243–60.
8. Checkley W, Epstein LD, Gilman RH, et al. Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol* 1998;148:497–506.
9. Pereira AL, Ferraz LR, Silva RS, et al. Enteroaggregative *Escherichia coli* virulence markers: positive association with distinct clinical characteristics and segregation into 3 enteropathogenic *E. coli* serogroups. *J Infect Dis* 2007;195:366–74.
10. Nataro JP, Sears CL. Infectious causes of persistent diarrhea. *Pediatr Infect Dis J* 2001;20:195–6.
11. Leberthal E, Ed. Prolonged small intestinal mucosal injury as a primary cause of intractable diarrhea of infancy. *Chronic Diarrhea in Children. Nestlé Nutrition Workshop Series, Vol. 6*. New York: Raven Press; 1984.
12. Islam D, Bandholtz L, Nilsson J, et al. Downregulation of bactericidal peptides in enteric infections: a novel immune escape mechanism with bacterial DNA as a potential regulator. *Nat Med* 2001;7:180–5.
13. Abreu MT, Fukata M, Arditi M. TLR signaling in the gut in health and disease. *J Immunol* 2005;174:4453–60.
14. Pull SL, Doherty JM, Mills JC, et al. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci USA* 2005;102:99–104.
15. Thompson JS, Saxena SK, Sharp JG. Regulation of intestinal regeneration: new insights. *Microsc Res Tech* 2000;51:129–37.
16. Azim T, Ahmad SM, Sefat-E-Khuda, et al. Immune response of children who develop persistent diarrhea following rotavirus infection. *Clin Diagn Lab Immunol* 1999;6:690–695.
17. Steiner TS, Lima AA, Nataro JP, et al. Enteroaggregative *Escherichia coli* produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. *J Infect Dis* 1998;177:88–96.
18. Turner MW. Mannose-binding lectin: the pluripotent molecule of the innate immune system. *Immunol Today* 1996;17:532–40.
19. Neth O, Jack DL, Dodds AW, et al. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immunol* 2000;68:688–93.
20. Steffensen R, Thiel S, Varming K, et al. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding gene by polymerase chain reaction with sequence-specific primers. *J Immunol Meth* 2000;241:33–42.
21. Kelly P, Jack DL, Naeem A, et al. Mannose-binding lectin is a component of innate mucosal defense against *Cryptosporidium parvum* in AIDS. *Gastroenterology* 2000;119:1236–42.
22. Kirkpatrick BD, Huston CD, Wagner D, et al. Serum mannose-binding lectin deficiency is associated with cryptosporidiosis in young Haitian children. *Clin Infect Dis* 2006;43:289–94.
23. Proulx F, Wagner E, Toledano B, et al. Mannan-binding lectin in children with *Escherichia coli* O157:H7 haemorrhagic colitis and haemolytic uraemic syndrome. *Clin Exp Immunol* 2003;133:360–3.
24. Aggarwal R, Sentz J, Miller MA. Role of zinc administration in prevention of childhood diarrhea and respiratory illnesses: a meta-analysis. *Pediatrics* 2007;119:1120–30.
25. Bhutta ZA, Ahmed T, Black RE, et al. What works? Interventions for maternal and child undernutrition and survival. *Lancet* 2008;371:417–40.
26. Glass RI, Parashar UD, Bresee JS, et al. Rotavirus vaccines: current prospects and future challenges. *Lancet* 2006;368:323–32.
27. Basu S, Chatterjee M, Ganguly S, et al. Effect of *Lactobacillus rhamnosus* GG in persistent diarrhea in Indian children: a randomized controlled trial. *J Clin Gastroenterol* 2007;41:756–60.
28. Lei V, Friis H, Michaelsen KF. Spontaneously fermented millet product as a natural probiotic treatment for diarrhea in young children: an intervention study in Northern Ghana. *Int J Food Microbiol* 2006;110:246–53.
29. Zasloff M. Defending the epithelium. *Nat Med* 2006;12:607–8.
30. Rabbani GH, Teka T, Saha SK, et al. Green banana and pectin improve small intestinal permeability and reduce fluid loss in Bangladeshi children with persistent diarrhea. *Dig Dis Sci* 2004;49:475–84.
31. Taneja S, Bhandari N, Strand TA, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. *Am J Clin Nutr* 2007;86:1302–9.
32. Sazawal S, Dhingra U, Dhingra P, et al. Effects of fortified milk on morbidity in young children in north India: community based, randomised, double masked, placebo controlled trial. *BMJ* 2007;334:140.
33. Bhutta ZA, Hendricks KM. Nutritional management of persistent diarrhea in childhood: a perspective from the developing world. *J Pediatr Gastroenterol Nutr* 1996;22:17–37.