Foods and Food Ingredients for Prevention of Diarrheal Disease in Children in Developing Countries

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ABSTRACT

Increased awareness of the morbidity and mortality attributed to diarrheal disease among children under 5 years of age in developing countries has led to a variety of approaches aimed toward prevention. In this review, foods and food ingredients which appear to be most effective in prevention of diarrheal disease in infants are considered. The effect of each of the following potential food or food ingredient categories on the control or prevention of diarrheal disease is discussed: human milk components, antibodies, probiotics, and fermented foods.

Diarrheal disease is a major cause of morbidity and mortality among children in developing countries who are under 5 years of age. Annually, about 5 to 10 million children die in developing countries as a result of diarrheal disease (45,56,57,114). Peak incidence occurs between the ages of 6 months to 2 years.

Enterotoxigenic Escherichia coli, rotaviruses, and Shigella have been reported to be most commonly involved in outbreaks of diarrheal disease (18,45,68). However, other microorganisms, such as Campylobacter jejuni (45), may be more commonly involved in diarrheal illness in children of developed and developing countries than has been previously reported (4). The incidence of diarrheal disease varies among countries in which studies have occurred as well as across various age, sex, and social sub-groups of the population (17). For example, in a Bangladesh treatment center, the enteropathogens most commonly identified in children with diarrheal disease were rotavirus (45%), followed by E. coli (28%), Vibrio cholerae (8%), and Shigella (5%) (21). However, when a field survey of diarrheal disease was conducted among children in Bangladesh, only 46% of the causative organisms were identified [E. coli (20%), Shigella (15%), and rotavirus (11%)] (18). Other bacterial (Salmonella and Yersinia), viral (Norwalk agent), and parasitic agents (Giardia, Cryptosporidium, and Entamoeba histolytica) have been implicated in diarrheal illness in children (18,60). The significance of these organisms in diarrheal disease appears to be less than that of E. coli, rotavirus, V. cholerae, and Shigella, which are transmitted via a fecal-oral route. Cholera is mainly a waterborne disease.

E. coli is commonly found in early weaning foods, particularly foods contaminated by impure water and unclean utensils, and prepared under insanitary conditions. The problem is further aggravated by rapid multiplication of E. coli in foods which are not consumed soon after preparation. Rotaviruses do not replicate or readily survive in the extraenteral environment. These microorganisms may be transmitted via the person-to-person route and are commonly involved in diarrheal disease because they are highly infectious in small doses (18).

Prevention of diarrhea requires complex and expensive environmental improvements designed to reduce ingestion or contact with fecal microorganisms (94). Clean protected water supplies, effective universal sanitation systems (safe disposal of human excreta), and improved community, personal, and household hygiene are essential in preventing diarrhea (9,18,61,94,104). However, certain types of diarrheal disease in infants, such as that caused by rotaviruses, are difficult to prevent in certain environments including U.S. hospitals and day care centers. Recently, several possible interventions have been examined for potential contribution toward control of diarrheal disease (5,6,24,25,30,32,33). Interventions suggested by these authors included: (a) increased measles immunization (90% coverage of infants 9-11 months old) could theoretically avert 13-26% of diarrheal deaths among children under 5 years of age; (b) supplementary feeding programs to enhance the nutritional status and food intake in preschool children would reduce rates of diarrheal disease; (c) promotion of breast-feeding could reduce diarrhea mortality by 24-27% among infants 0-5 months and by 8-9% among children under 5 years of age; (d) the number of low birth weight infants could be reduced through nutrition programs designed for expectant mothers; (e) administration of drugs and antimicrobials in low-dose nonabsorbable form could reduce diarrheal mortality rates by 0.3-1.2%; (f) rotavirus immunization may reduce diarrhea mortality rates by 6-10% among children under 5 years of age in developing countries; (g) cholera immunization may reduce diarrheal mortality rates by 1-2% among children under 5 years of age in countries like Bangladesh; (h) improved water supplies and excreta disposal facilities to reduce ingestion of en-
Factor | Examples of in vitro activity against:
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Bifidus factor | Favors growth of *B. bifidum*, discourages growth of enteric pathogens
Secretory IgA | Active against mucocoe invading or colonizing bacteria; *E. coli*, *E. coli* enterotoxin, *Vibrio cholerae*, *V. cholerae* toxin, *Salmonella*, *Shigella*, rotavirus
Lactoferrin | Binds iron and inhibits bacterial multiplication, *E. coli*
Lactoperoxidase | *Streptococcus*, *E. coli*, *S. typhimurium*
Lysozyme | Lyses gram-positive and some gram-negative bacteria, *E. coli*, *Salmonella*
Lipid (unsaturated fatty acid) | S. aureus
Lipid (mono-glycerides and unsaturated fatty acids) | Semiliki forest virus, rass river virus, herpes simplex
Non-immunoglobulin | Herpes simplex, vesicular stomatitis virus, rotaviruses
Receptor-like glycoproteins | *E. coli*, *E. coli* enterotoxins, *V. cholerae*, *V. cholerae* toxin
Ribonuclease-like factor | RNA-viruses
Milk cells | By phagocytosis: *E. coli*, By sensitized lymphocytes: *E. coli*

*Compiled from references: 7,11,12,14,21,28,36,43,46,50,51,64,68,72,74,78,85-87,106,116,117,122.*

HUMAN MILK COMPONENTS

Human milk contains numerous non-immunological components which provide protection against diarrheal disease. In this section, many of these components are identified and mechanisms proposed for their protective ability are discussed. Identification of analogous substances which possess similar protective mechanisms could lead to their addition to weaning foods to complement the protection already provided through breast milk.

Many reports have suggested that enteric infections of breast-fed infants have lower rates of morbidity and mortality than do those of other infants (22,29,33,34,66). Recently, Feachem and Koblinsky (33) reviewed studies from 14 countries on breast-feeding as a specific intervention in diarrheal disease. They reported that just the promotion of breast-feeding of infants in the first 6 months of life may reduce diarrheal morbidity by 8-20% and mortality by 24-27%. Breast-feeding protects infants against many infectious agents which invade or colonize the small and large intestine (8,23,39,54,62,64,67,73). Several components of human milk, which offer protection against enterotoxigenic *E. coli*, *V. cholerae*, *Salmonella*, *Shigella*, and rotavirus, as well as many other infectious agents, have been isolated and identified in colostrum and human milk (Table 1). The effectiveness of these human milk components has been demonstrated primarily in in vitro studies involving the diarrheal-causing organisms. The effectiveness of many of these agents under in vivo conditions remains to be determined. Individually, some of these agents may be found to exert a protective effect after ingestion by the infant (116).

The "bifidus factor", which is found in human milk but not cow’s milk or infant formula, favors growth of *Bifidobacterium bifidum*. The bifidus factor in human milk has been identified as a nitrogen containing polysaccharide (72). The bifidobacteria utilize bifidus factors which have not been absorbed in the upper parts of the intestine and produce large quantities of acetic and lactic acids (13,14). Production of these acids appears to exert a protective effect by reducing the pH of the intestinal lumen and thereby inhibiting growth of enterotoxigenic pathogens and intestinal protozoa.

Human milk also contains large amounts of lactoferrin. This human milk component inhibits growth of enterotoxigenic *E. coli* by limiting the availability of iron (116). Additionally, Holmgren et al. (50,51) reported the presence of receptor-like compounds in human milk, which offer a protective effect against enteric infections by *E. coli* and *V. cholerae*. The receptor-like compounds are glycoproteins which exert their protective effect by competition with bacteria for binding sites on intestinal epithelial cells (3,51). Moreover, the receptor-like compounds are stable to boiling but can be destroyed by peridate treatment. Further research is needed in vivo to assess the protective effect of these receptor-like glycoproteins found in human milk to determine whether intestinal absorption or degradation may reduce or increase their protective effect. If found effective, addition of chemical analogues of the bifidus factor and/or receptor-like glycoproteins to foods for children may contribute to protecting against enterotoxigenic *E. coli*, *V. cholerae*, and other enteric pathogens. Both the bifidus factor and receptor-like glycoproteins are stable to boiling for 15 min.

Several of the human milk components (secretory immunoglobulin A (sIgA), lipid (monoglycerides and unsaturated fatty acids), non-immunoglobulin macromolecules, and ribonuclease-like factor have also been reported to have antiviral properties. Secretory IgA is dis-
discussed in more detail in the next section. Several studies have indicated that lipids found in human milk reduce the infectivity of the lipid-coated flaviviruses and alphaviruses both in vivo (35) and in vitro (31,117). Several other viruses (oncoviruses, murine leukemia virus, and mouse mammary tumor virus) have also been shown to be inactivated by human milk lipids (35,99). The human lipids act by disrupting the virus envelope and are effective primarily against enveloped viruses. Cow milk and synthetic milk formula do not possess lipid-mediated activity toward viruses. Otnaess and Orstavik (86,87) indicated that human milk colostrum also contains rotavirus-inhibiting activity which is of non-immunoglobulin origin. The factors in human milk responsible for the rotavirus-inhibiting activities appear to be associated with proteins.

Ribonuclease-like factor from human milk has also been reported to be effective against RNA-viruses (74,78). Addition of ribonuclease-like factor to commercial milks or infant formula might be considered as a means of protecting individuals against infections by RNA-viruses.

The cells found in colostrum and milk appear to have three possible antiviral functions: (a) by secretion of interferon; (b) by direct phagocytosis, and (c) by the production of specific immunoglobulin A (IgA) (116). However, little evidence exists to suggest any in vivo effect of interferon and phagocytosis on enteric viral infections.

Trypsin enhances human rotavirus replication in vitro and it is possible that intestinal trypsin augments virus production in human infants (75,79). Yolken (119) found that alpha-1-anti-trypsin, soy protease inhibitor, egg white protease inhibitor, bestatin, pepstatin, and bis-(5 amidino-2-benzimidazolyl) methane (BABIM) had in vitro efficacy against rotavirus. Efficacy was demonstrated in the presence and absence of proteases. BABIM administered orally or parenterally inhibited murine rotavirus in mice while the other inhibitors had a lower level of efficacy. Protease inhibitors derived from plant sources may be effective in preventing diarrheal illness caused by rotavirus (Yolken, personal communication). The activity of these inhibitors may be affected by the extent of processing (e.g., heating) and the source of inhibitor. Trypsin inhibitors found in human colostrum and milk and other sources may overcome the effect of trypsin and work to prevent human neonatal rotavirus infection either alone or in combination with rotavirus specific slgA. If levels of slgA and trypsin inhibitor in combination or separately are high enough, the infection rate is substantially reduced (75).

ANTIBODIES (PASSIVE IMMUNITY)

Human milk contains slgA which is present in highest concentration (50 mg/ml) in the first few days postpartum (in the colostrum) and then progressively decreases to a level of 1 mg/ml (47). Secretory IgA is resistant to the acid conditions and proteolytic activity of the gastrointestinal tract. Significant amounts of slgA have been found intact in infant feces (71,72,116). Several reports have appeared concerning the activity of slgA against enterotoxigenic E. coli, E. coli enterotoxin, V. cholerae, V. cholerae toxin, Salmonella, Shigella, rotavirus, as well as other viruses and pathogenic bacteria (28, 44, 46, 47, 68, 106, 117). Secretory IgA appears to prevent infection by binding with pathogenic microorganisms to inhibit their attachment to intestinal epithelial cell membranes.

Most information related to slgA protection against enterotoxigenic E. coli, V. cholerae, rotaviruses, and other infectious pathogens is based on in vitro or animal model studies (10,15). Fubara and Freter (43) obtained purified slgA from the intestinal lumen of orally vaccinated germ-free mice. The slgA was induced in the germ-free mice by feeding with heat-killed Vibrio. The purified antibody preparations were reported to protect mice against enteric cholera infections by inhibiting binding of Vibrio to the mucosal surface of the intestine. Calves and piglets can be protected against neonatal enteric infections by immunization of dams (53,80,96). Protection appears to be transferred via antibody in maternal colostrum and milk in both species. For example, Rutter et al. (80) vaccinated sows with purified K88 antigen from E. coli and subsequently challenged piglets at birth with K88-positive strains of E. coli. They found that mortality was decreased from 69% in litters from unvaccinated sows to 13% in litters born to vaccinated sows. Further, they reported that vaccinated sows possessed high titers of colostral antibody capable of inhibiting adherence of K88 protein strains to intestinal brush border. The antibody level in sow’s milk rapidly diminished to non-detectable levels by 7 d postpartum.

Considerable interest also exists in protecting infants against infectious pathogens involved in diarrhea by vaccination of mothers with appropriate colonization antigen factors (68). Although much active research is underway in this area, development of vaccines is outside the scope of this report.

Edelman (27) suggested that possible supplementation of infant formula or milk with antibodies raised against selected enteropathogens may provide a way to prevent infectious diarrhea in infants. Two studies have involved production of antibodies in bovine milk against enterotoxigenic E. coli (49,70). Hilpert (49) reported on the production of a protein concentrate containing active non-denatured immunoglobulins (Igs). In this study, cows were hyperimmunized by administration of a successive series of antigens via the parenteral and local routes from about the eighth week to the second week before calving. The vaccine contained antigens from 15 different strains of E. coli which had been implicated in outbreaks of neonatal gastroenteritis. Colostrum, transition milk, and end of lactation milk was collected from hyperimmunized cows and used to prepare protein concentrates containing active Igs (Fig. 1). When packaged under sterile conditions, the protein concentrate can be stored for about 45
Colostrum, transition milk, and end-of-lactation milk

- Skimming
- Coagulation
- Separation of Casein
- Filtration
- Ultrafiltration
- Prefiltration
- Sterile Filtration
- Evaporation
- Freezing and Freeze-Drying
- Packaging

Figure 1. Process for production of protein concentrates containing active immunoglobulins.

d at −30°C without loss of antibody activity. In vitro and in vivo (mice 2 weeks to 1 year old) experiments with protein concentrate containing IgGs indicated that bovine IgGs has the same antibody specificity as that of human IgGs. In clinical tests with infants, the bovine IgGs in the protein concentrate withstood proteolytic degradation in the intestinal tract since intact active bovine IgGs was detected in the feces of infants. Hilpert (49) demonstrated that protein concentrate containing active bovine IgGs against infectious *E. coli* may be incorporated into infant milk formula to protect infants. This approach may also be used to produce protein concentrates containing active factors toward other infectious microorganisms involved in infant diarrhea. However, further development and subsequent evaluation in field trials before application to infants in developing countries of this method are needed.

Recently, Linggood et al. (70) reported on a process for hyperimmunizing cows for production of milk containing antibodies against human enterotoxigenic *E. coli*. Adhesion factors (pili) produced by *E. coli* were continuously administered to pregnant cows for a period of six weeks before expected calving. The process used for preparation of antibody-rich concentrate from bovine colostrum and milk is shown in Fig. 2. The antibody-rich concentrate was effective in preventing diarrhea due to enterotoxigenic *E. coli*. Antibody-rich concentrate was also prepared from bovine sera. Infections caused by *E. coli* could be cleared in 4-5 d in both mice and piglets when the concentrate was administered after infection. Bovine antibodies protected against enterotoxigenic *E. coli* infections by preventing colonization in the intestine. The antibody-rich concentrates recovered in functional form from bovine milk may be added to infant milk formula or fruit beverage mixes in an amount sufficient to provide passive protection against enterotoxigenic *E. coli*. Incorporation of antibody-rich concentrate into filter-sterilized soft drink mixes appears to be a good approach to provide passive protection against infectious microorganisms in infants since cooking or excess heating could be avoided. However, additional information on the stability of antibodies in the soft drink beverage mixes during storage is needed.

A rotavirus specific slgA as well as non-immunoglobulin factors inhibitory to rotavirus have been identified in human milk. Both immunoglobulin and non-immunoglobulin factors function to inhibit viral replication (121). Rotavirus specific slgA and non-immunoglobulin factors are able to neutralize human rotavirus infection in tissue culture cells (86). Moreover, animal studies have indicated that the ingestion of colostrum slgA antibody was effective in protecting neonates against rotavirus infection (65,83,105,118).

Contradictory results have been obtained concerning the role of breast-feeding in preventing rotavirus diarrhea. Weinberg et al. (115) reported no substantial reduction in the incidence of diarrhea caused by rotavirus in breast-fed neonates (less than 1 year of age) and that only the frequency of vomiting decreased. However, these researchers did not report anti-rotavirus slgA levels in the milk provided breast-fed infants. Conversely, in other studies when anti-rotavirus slgA was present at sufficient levels, breast-fed infants were less likely to become infected with rotavirus (16,40,63,75,108). Human colostrum contains the highest levels of anti-rotavirus slgA which decreases to lower but detectable levels in most milk specimens collected for up to 24 months after parturition (21,122). Researchers have suggested the possi-
Figure 2. Process for preparation of antibody rich concentrate from immune bovine milk.

ability of developing an inactivated rotavirus vaccine for expectant mothers to increase colostral and especially milk antibody levels (58,59).

Use of colostrum and milk from cows immunized against rotavirus may be effective for prevention of rotavirus-induced diarrhea. In a limited study conducted in Japan, Holstein cows in their eighth month of pregnancy were infected with human rotavirus to produce a protease-resistant IgA-rich cow colostrum containing neutralizing antibody to human rotavirus which was referred to as “Rota colostrum” (26). In a specific epidemic area of Japan, oral administration of Rota colostrum helped to prevent diarrhea in 5 of 6 infants. According to Ebina et al. (26), Rota colostrum with a higher neutralizing antibody titer might be needed to completely prevent diarrhea. Infecting cows to produce neutralizing antibody against human rotavirus strains has potential as a means of preventing rotavirus-induced diarrhea in infants.

If bovine antibodies are found to prevent or vary the clinical course of naturally acquired human rotavirus infection, attempts should be made toward production of milk formula appropriate for human infants that contain satisfactory levels of anti-rotavirus antibody (121). Recently, Yolken et al. (121) investigated the levels of rotavirus antibody IgG1 in raw cow milk, pasteurized cow milk, and milk-based formula preparations that are commonly fed to infants. The rotavirus antibody was naturally present in pooled raw milk samples from 200 herds. No attempt was made to increase titers by immunizing the cows. A beneficial property of the IgG1, the principal subclass in bovine milk, is its resistance to proteolysis by intestinal enzymes (76,93). Both raw and pasteurized milk were capable of decreasing or preventing rotavirus infection and diarrhea in a mouse model of rotavirus infection; commercial formula preparations did not prevent infection. Loss of rotavirus antibody activity was attributed to high-temperature processing used to prepare milk-based formula preparations. Although pasteurized milk contained a lower level of antibody activity, the level was still sufficient to neutralize the in vitro replication of simian, bovine, and human rotaviruses and provide protection in the in vivo model of murine rotavirus infection. These results suggest that additional studies be conducted to determine whether alterations in processing methods might allow further retention of antibody activity while still providing protection against pathogenic organisms.

Eggs may be better for production of antibodies against rotavirus (Yolken, personal communication). Yolken et al. (120) tested naturally infected eggs and found 94% contained >100 ng of anti-rotavirus antibodies/ml. They also detected significant levels of anti-rotavirus antibodies in pasteurized egg yolk preparations. The egg anti-rotavirus antibodies prevented in vitro replication of a variety of human rotavirus strains in tissue culture. In a mouse model of rotavirus infection, orally administered egg preparations containing anti-rotavirus antibodies prevented the intestinal replication of rotaviruses and thus prevented development of symptomatic rotavirus diarrheal illness (120).

Pasteurization processes for milk or eggs may decrease the antibody effectiveness. Therefore, processing parameters, alternate processing methods or isolation of the antibodies need to be developed if eggs or milk are to be used as a source of antibodies.

FERMENTED FOODS

Milk products fermented by lactobacilli have been reported to be beneficial to human health because of their high nutritional quality and their ability to inhibit growth of various microorganisms involved in gastrointestinal disorders. These fermented milk products contain not only viable lactobacilli, but also lactic acid, and antibiotic-like substances, which have been proposed to control intestinal flora by inhibiting growth of undesirable organisms, including those involved in infantile diarrhea (52). The lactic acid bacteria most often cited as beneficial dietary adjuncts are Lactobacillus acidophilus, L. casei, and Bifidobacterium bifidum (41). These organisms possess special characteristics that would permit their survival and growth in the intestinal tract. Further, these organisms produce antagonistic actions toward enteric pathogens (41,42). For example, L. acidophilus exerts an antagonistic action on Salmonella typhimurium and enterotoxigenic E. coli when grown in associate cultures (42). The antagonistic action was re-
ported to be due to a combination of antibiotic-like substances, lactic acid, and hydrogen peroxide produced by lactobacilli. Similarly, L. casei has been reported to inhibit E. coli, Vibrio sp., and Salmonella sp.

Twinning-McMath (109) suggested use of L. acidophilus to produce a stable protective intestinal flora which would help eliminate pathogenic microorganisms. Several others have also reported the effectiveness of L. acidophilus against enteric pathogens (98,103). Feeding of L. acidophilus as therapy for restoring a healthful microbial balance in swine has also been reported (84,88,92). In Sweden, a commercial preparation called Majdres (lyophilized L. acidophilus preparation) was used for prophylaxis and therapy of intestinal disturbances in pigs (88). Daily feeding of Majdres to weaning pigs eliminated diarrhea. Tomic-Karovic and Fanjek (107) demonstrated the ability of L. acidophilus milk to inhibit pathogenic E. coli in vitro. When 20 infants suffering from diarrhea caused by E. coli were fed acidophilus milk, the infants made a rapid recovery from diarrhea and E. coli disappeared from their feces within 1 to 5 d of starting therapy. The influence of acidophilus milk on the carrier state of Salmonella in humans and children was investigated by Zychowicz et al. (123) and Alm (1). Feeding acidophilus milk resulted in shortened duration of the Salmonella carrier state. The favorable effect of acidophilus milk on the carrier state of Salmonella was attributed to antibiotic-like substances produced by L. acidophilus (1,123).

Schmidt et al. (100,101) reported that recurrence of diarrhea in infants could be reduced significantly by feeding chemically acidified whole milk formula. The incidence of diarrhea was 10% in children fed acidified whole milk formula compared to 24.7% in children receiving non-acidified whole milk formula. They suggested that acidified milk may be used in prevention of diarrhea in children.

Yogurt has been reported to have an antagonistic effect against growth of several pathogens (E. coli, Vibrio, Salmonella, Shigella, Clostridium, etc.) under in vitro conditions (91). Both the lactic acid and antibiotic factors in yogurt are involved in the inhibitory effects on the various enteropathogens. These factors produce favorable conditions for proliferation of intestinal lactobacilli and discourage growth of pathogens such as E. coli, Salmonella, and Shigella. In Mediterranean countries and The Balkans, yogurt, with a reduced fat content, has been consumed for years as a remedy for infantile diarrhea. Additionally, many shepherds in the regions of the Near and Middle East use yogurt diluted with water for protection against diarrhea as well as other intestinal disorders (91). Niv et al. (82) studied the beneficial effects of yogurt in 45 hospitalized children (most of whom were under 1 year of age) with diarrhea. The experimental group of children was fed 100 ml of yogurt, three times a day, and the control group received one teaspoon of Neomycin-kapectate, three times a day, until recovery. Both groups of children received identical dietary treatment. The mean time to recovery was 2.76 d for children fed yogurt and 4.80 d for those fed Neomycin-kapectate.

Recently, Alm (2) examined the ability of fermented milk products in the presence of human gastric juice to inhibit growth of Salmonella and Shigella during a 7-10-h incubation. Addition of human gastric juice to yogurt greatly decreased the growth rate of Salmonella and Shigella. Addition of gastric juice to kefir inhibited Salmonella. For acidophilus milk with human gastric juice, inhibition of Salmonella and Shigella was not as effective.

PROBIOTICS

L. acidophilus, L. bulgaricus, and Streptococcus faecium were used in human and animal studies to produce a healthy microbial flora in the intestine and to protect against colonization by enteropathogens such as E. coli (10,51,90,102,110,113). Several possible mechanisms have been suggested to explain the observed protective effect obtained by colonization with lactobacilli and streptococci: (a) lowering of intestinal pH, (b) adhesion to the intestinal wall preventing colonization by pathogens, (c) competition for nutrients, (d) production of antibacterial substances, and (e) production of antitoxins (antiacterotoxins). A commercially available preparation called Lactinex (containing L. acidophilus and L. bulgaricus) is used in the treatment and prevention of diarrhea.

Robins-Browne and Levine (95) examined the ability of lactobacillus preparations to survive in the human digestive tract. Lactobacilli survived passage through the stomach and remained in the upper small bowel for up to 6 h, when taken with milk. Both in vitro and in vivo experiments indicated that lactobacilli prevented colonization of enteropathogenic E. coli on the intestinal walls and inhibited E. coli-induced enterotoxin (37,48,55,77,81,97). However, some controversy exists concerning the efficacy of lactobacillus preparations in the treatment or prevention of diarrhea. Most claims related to the beneficial effects of the lactobacilli come from limited studies (7,77). In some controlled experiments, such as in rabbit ileal loops, lactobacillus preparations (containing L. acidophilus and L. bulgaricus) reduce intestinal secretion induced by enterotoxigenic E. coli heat-labile enterotoxin (37,55). In contrast, Pearce and Hamilton (89) found no beneficial effects of a lactobacillus preparation (containing 50-60% S. thermophilus, 35-45% L. acidophilus, and 5% L. bulgaricus) on short-term or long-term cases of infantile diarrhea. Clements et al. (19) found that prophylactic administration of Lactinex to humans before and after experimental challenge with enterotoxigenic E. coli had no effect on diarrhea. Clements et al. (20) conducted clinical and microbiological studies to evaluate the ability of exogenous lactobacilli to col-
onize in the upper small intestine and prevent enterotoxigenic E. coli diarrhea in man. Studies on jejunal aspirates indicated that orally ingested L. acidophilus and L. bulgaricus (from Lactinex) and L. acidophilus (from Infloran Berna) survived passage through the stomach and remained viable in the proximal small bowel for 3 to 6 h in most individuals. Challenge studies with enterotoxigenic E. coli revealed that despite the ability of lactobacilli to persist in the upper small intestine for several hours, these preparations did not prevent or alter the course of enterotoxigenic E. coli diarrhea in humans. Additional studies should be conducted to determine if lactobacilli produce an inhibitory effect on growth of enterotoxigenic E. coli, Vibrio, Salmonella, and Shigella in infants. Most studies have been conducted with adults and little information is available on infants.

S. faecium is a normal inhabitant of the small intestinal microflora in the early life of humans as well as monkeys and rats. Several mechanisms have been proposed for the ability of S. faecium to prevent growth of intestinal bacterial pathogens: (a) adhesion to the epithelial lining of the intestine in direct competition with E. coli for binding sites, (b) lowering intestinal pH, (c) production of antibacterial substances, (d) changing redox potential, (e) production of antienterotoxins, and (f) promotion of a more favorable enteric bacterial balance (69,90,110,112).

Rafstedt et al. (90) conducted double-blind studies of S. faecium M-74 with 40 healthy children (2-14 years of age). They found that S. faecium M-74 is well tolerated at high dosages (400 million freeze-dried organisms in capsules) over a 14-d period. In vitro, S. faecium M-74 inhibited some strains of enterotoxigenic E. coli, Salmonella, Shigella, and non-cholera Vibrio. Rafstedt et al. (90) suggested that S. faecium M-74 may be a good prophylaxis against invasion of pathogenic bacteria. Wadstrom (113) evaluated S. faecium M-74 as a prophylacticum for enterotoxigenic E. coli with CFA/I and CFA/II surface fimbrial hemagglutinins (adhesions) in a rabbit model. Young rabbits (3-4 d old) were given S. faecium M-74 organisms at a titer of 5 \times 10^7 15 min before (group A), 6 h before (group B), and 12 h after (group C) challenge with enterotoxigenic E. coli. Only 4 of 26 rabbits in group A, 6 of 21 in group B, and 7 of 23 in group C developed diarrhea. However, S. faecium M-74 at a lower dosage (10^7 or 10^8) gave poor protection against diarrhea in groups A, B, and C. Wadstrom (113) suggested that S. faecium M-74 should be considered as a prophylacticum for infantile diarrhea.

S. faecium C-68 has also been reported to protect gnotobiatic piglets against enterotoxigenic E. coli (porcine ETEC) induced diarrhea (110,111). S. faecium C-68 prevented invasive action and neutralized the toxic effect of porcine ETEC. S. faecium appears to offer some promise in prevention of diarrheal diseases. This organism could possibly be used as an oral intervention in preventing diarrhea in infants. Additional research needs to be conducted to evaluate the efficacy of S. faecium towards diarrhea causing organisms in infants.

CONCLUSION

It can be concluded that any foods or food ingredients found effective in preventing diarrheal illness should be provided to infants in addition to, and not instead of, breast-feeding. Foods, which include yogurt, acidophilus milk, and other fermented products, and food ingredients such as human milk components and probiotics, offer protection against diarrheal causing organisms under in vitro conditions. The effectiveness of these foods and food ingredients under in vivo conditions remains to be determined before these can be included in foods in a similar manner to food fortification programs which help combat diarrhea in children of developing countries. More comprehensive studies need to be undertaken to evaluate the potential foods and food ingredients to identify additional substances which may be beneficial (useful) in helping to prevent diarrheal disease in children of developing countries.

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