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Acidified Milk Formula Supplemented With Bifidobacterium lactis: Impact on Infant Diarrhea in Residential Care Settings

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ABSTRACT

Objectives: Probiotics may be useful in preventing acute infectious diarrhea. Bifidobacteria are particularly attractive as probiotics agent because they constitute the predominant colonic flora of breastfed infants and are thought to play a role in the decreased incidence of diarrhea in breastfed infants.

Methods: This was a multicenter, double-blind, controlled study to evaluate the efficacy of a milk formula supplemented with viable *Bifidobacterium lactis* strain Bb 12 (BbF) in the prevention of acute diarrhea in infants younger than 8 months living in residential nurseries or foster care centers.

Results: Ninety healthy children received either the BbF or a conventional formula (CF) daily. The mean duration of the stay in the residential center was similar (137 *v* 148 days). At enrollment, there were no differences between the two groups with respect to age (3.7 ± 2.1 months), gender, anthropometric data, history of allergy or gastrointestinal disease, frequency of breast-feeding in the neonatal period or timing of introduction of solid food. Altogether, 28.3% of the BbF infants had diarrhea during the study compared with 38.7% of controls (NS). There was a statistically insignificant trend for shorter episodes of diarrhea in the BbF group (5.1 ± 3.3 days v 7 ± 5.5 days, NS). The number of days with diarrhea was 1.15 ± 2.5 in the BbF group with a daily probability of diarrhea of 0.84 versus 2.3 ± 4.5 days and 1.55, respectively, in the CF group (P = 0.0002 and 0.0014). Feeding infants with the BbF reduced their risk of getting diarrhea by a factor of 1.9 (range, 1.33–2.6). Analysis of the cumulative incidence of diarrheal episodes showed a trend that the first onset of diarrhea occurred later in the BbF group.

Conclusion: These results provide some evidence that viable *Bifidobacterium lactis* strain Bb 12, added to an acidified infant formula, has some protective effect against acute diarrhea in healthy children. ***JPGN 38:288–292, 2004.*** **Key Words:** *Bifidobacterium lactis*—Diarrheal disease—Prevention of diarrhea—Probiotics. © 2004 Lippincott Williams & Wilkins, Inc.

Gastroenteritis is a significant cause of childhood morbidity even in the developed world. It represents 16% of all illnesses reported in children younger than 5 years in the United States of America (1). The incidence of acute diarrhea in children years has been estimated at 1.3 to 2.3 episodes per child per year (1), but is 2 to 5 times higher in children attending day care centers and residential facilities (2,3), where nosocomial acute diarrheal illness more common and can result in prolonged hospital stays and increased medical costs (2). Thus, the development of effective methods to prevent acute gastroenteritis is an important goal for infant health.

Breast-feeding is associated with a decreased incidence of gastrointestinal infections (4,5), possibly because it promotes the growth of bifidobacteria in the intestine creating an acidic environment inhospitable to infectious organisms (4,6,7). Formula and fermented milk containing various bacteria have been used for many years for their potential benefit on intestinal digestion and function (8,9). It seems reasonable that modifying the intestinal flora by providing exogenous nonpathogenic bacteria might also prevent or treat infectious diarrhea (8,9). Bifidobacteria are particularly attractive as potential probiotic agents because they are the predominant colonic flora of breast fed infants (6,7, 10) and are felt to contribute to the mechanisms by which breast-feeding protects against diarrhea (4). Bifidobacteria appear to delay the onset of symptoms in rotavirus infection of laboratory animals (11). The efficacy of supplemental nonpathogenic bacteria in preventing infantile gastroenteritis has been assessed in few controlled clinical trials (8,9,12–15), including only one study with an infant formula containing *Bifidobacterium lactis* Bb 12 (13). Thus, we conducted a multicenter, double-blind, controlled study to assess the efficacy and tolerability of an acidified milk formula containing this strain of bifidobacteria for the prevention of acute diarrhea in infants living in residential nurseries or foster care centers.

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PATIENTS AND METHODS

Infants

The study protocol and consent procedure were approved by the ethical committee of Grenoble Medical University (CCPPRB-1996/01/10). The study was performed from February 1996 to February 1997 in residential child care centers throughout France. All infants <8 months admitted to one of the selected centers and expected to remain for at least 4 months were evaluated for inclusion in the study. Children were excluded if, at enrollment, they were being breast fed or if they had a history of gastrointestinal disease or suspected malabsorptive disorder. They were also excluded if they were receiving antibiotics or, for any reason, a lactose-free, protein hydrolysate or soy-based formula. Infants meeting these criteria and for whom informed consent was obtained from both parents or legal surrogates formed the subjects of this trial. The sample size was based on the study of Saavedra et al. (13). With an alpha statistic of 0.05 and a beta risk of 0.20, 90 infants were required. The study was continued until this number was reached.

Feeding Protocol

Subjects were assigned to the supplemented or control formula by a block randomization procedure in each center and received the assigned formula for the duration of their stay in the center. Thus, they were fed with either a biologically acidified infant formula supplemented with viable bifidobacteria strain Bb 12 (BbF) or a commercial, conventional, nonacidified formula (CF). The supplemented formula (BbF) was acidified by fermentation using a mixture of L(+) lactic acid producing bacteria, i.e. *Streptococcus thermophilus* and *Lactobacillus helveticus*, and then dried. Subsequently, *Bifidobacterium lactis* strain Bb 12, was added to guarantee a level of living bifidobacteria at 10^6 CFU per gram of powder, which resulted in 1.5×10^8 CFU/L. Cultures of these organisms were from Christian Hansen Biosystems A/S, Hoersholm, Denmark. The concentration of bifidobacteria was verified in samples of the study formula before and after completion of the study. The infants received at least 10^8 CFU of bifidobacteria per day, depending on the volume injested. The pH of the reconstituted formula was around 4.8. Two kinds of formula were used in each group depending on infant age and diet: infants < 4 months received a starter formula, and older infants (ready for solid foods) received a follow-up formula. The four study formulas were prepared and supplied in powdered form by Nestlé-France and were freshly prepared by dilution with mineral water according to the manufacturer's recommendations. The composition of the formulas is listed in Table 1. The attending physician, nurses, and study monitors were unaware of which formula was being fed.

Follow-up

Patients were evaluated daily for stool number and consistency, body weight, and formula intake. Number of episodes of regurgitation or vomiting were recorded weekly for each infant. Any change in clinical status was noted. Crown-heel length was recorded each month.

TABLE 1. *Nutrient composition of the formulae (100 mL)*

	Age < 5 months		Age > 5 months	
	BbF1	CF1	BbF2	CF2
Energy (kcal)	67	67	73	72
Protein (g)	1.7	1.8	2.2	2.4
Casein	50%	77%	77%	77%
Carbohydrates (g)	7.7	6.8	8.6	8.5
Lactose (g)	5.74	4.7	3.4	3.5
Fat (g)	4.22	3.6	3.3	3.2
Minerals (g)	0.25	0.4	0.5	0.54
Phosphorus (mg)	21	56	68	74
Calcium (mg)	43	66	80	87
Iron (mg)	0.8	0.8	1.3	1.3
pH	4.8	6.8	4.8	6.8

BbF = supplemented formula with viable bifidobacteria, strain Bb 12; CF = conventional nonsupplemented formula; 1 = starter formula; 2 = follow-up formula.

Acute gastroenteritis was defined as diarrheal disease of rapid onset. Diarrhea was defined as the passage of > three unusually loose stools per day for more than 24 hours, or as an increment of more than 50% in the daily number of stools if previously more than two. A diarrheal episode was defined as diarrhea lasting 7 days or less.

Episodes of diarrhea were treated according to the recommendations of the American Academy of Pediatrics (16) and more recently by the ESPGAN working group on acute diarrhea (17). After oral rehydration with a standard oral rehydration solution (ORS) for infants during the first 4 to 6 hours, formula was restarted together with ORS during the next 24 to 36 hours. Use of the ORS was then discontinued depending on the clinical condition of the child. When possible, stools were tested for pathogenic bacteria (*Escherichia coli, Salmonella, Shigella, Campylobacter,* and *Yersinia*), and for rotavirus by standard methods. For that purpose, fecal samples obtained within 3 days of an episode of diarrhea.

Calculations and Statistics

Data are expressed as mean or frequency ± the standard deviation (SD), with the 95% confidence interval between brackets. The mean number of days with diarrhea was calculated for each group by dividing the total of the number of days each infant had diarrhea by the number of infants in the group. The incidence density of diarrhea was calculated in each group by plotting the number of days of diarrhea per child against the total of days of follow-up and multiplying the result by 365 days (diarrhea days per child-year).

Statistical analyses were accomplished using the software SAS 6.12. Analyses of quantitative data were performed using Student *t* test. Comparisons of the distributions of patient characteristics were performed using χ^2 test or Fisher exact test, as appropriate. The logistic regression was used to compare the number of days with diarrhea. The cumulative incidence of diarrheal episodes was estimated using the Kaplan-Meier method, and univariate tests of significance were performed using the log-rank test. All tests were performed with an alpha level of 5%. *P* values smaller than 0.05 were considered significant.

RESULTS

Study Population

Ninety infants (sex ratio 1:1) were studied. Forty-six infants were randomized to receive the *Bifidobacterium lactis* supplemented formula. Eleven centers participated in the study, with a mean of 8.2 ± 5 (range, 5–11) infants per center. At enrollment, there was no significant difference between the two groups with respect to age, weight, height, head circumference, family history of allergy, or gastrointestinal disease (Table 2).

Forty-six percent of infants receiving BbF were breastfed during the early neonatal period versus 32% of those receiving CF ($P = 0.16$). Fifty-seven percent of infants receiving BbF versus 46% of those receiving CF were already receiving solid foods at enrollment ($P = 0.29$). Seventy percent of the infants did so between 3 and 4 months of age. The age at introduction of solid food was similar in the two groups using the life test procedure. There was no difference in the mean duration of monitoring of in the mean number of days each formula was ingested per subject: 137.1 ± 60.1 days (range, 119.5–154.7 days) in the BbF group versus 148.1 ± 64.9 days (range, 129.1–167.1 days) in the CF group. There was no difference in the volume of formula injested.

Effect of *Bifidobacterium lactis*-supplemented Formula on Diarrheal Disease

None of the centers experienced any diarrheal epidemics during the study. Fewer infants (28.3%) receiving BbF than those receiving CF (38.6%) experienced acute diarrheal disease during the study (Table 3), but the difference was not statistically significant ($P = 0.3$). More than 300 subjects in each group would have been needed to show any difference according to the Casagrande and Pike method for a two-way test with $\alpha = 0.05$ and $\beta = 0.2$. Logistic regression analysis failed to show any difference related to previous treatment, age at enrollment, or number of bowel movements before inclusion.

The cumulative incidence of diarrhea in infants receiving BbF was not significantly different from that in infants receiving CF ($P = 0.325$). The first episode of diarrhea tended to occur earlier in the control group but this difference was also not statistically significant (122.3 ± 5.8 days *v* 132.1 ± 9.5 days; $P = 0.33$) (Fig. 1). The severity of diarrhea, as judged by the number of stools per day, was similar in the two groups (Table 3). The episodes tended to be slightly shorter in the BbF group but this difference was not statistically significant (Table 3) ($P = 0.273$). None of the episodes lasted more than 7 days, and none of the infants experienced dehydration greater than 5%. None of the infants were switched to soy or hydrolysate formula, so none were excluded from the study. The groups were similar with respect to the number of diarrheal episodes per subject.

The mean number of days with diarrhea, calculated as specified in Patients and Methods, was lower in the infants receiving BbF, even when adjusted according to age ($P = 0.0002$) using a logistic procedure. The number of days with diarrhea for each infant plotted against the number of days of follow-up gave a significantly lower daily probability of diarrhea in infants receiving BbF (Table 3) ($P = 0.0014$). Feeding infants with the control formula consequently increased their risk of developing diarrhea during the study by a factor of 1.9 times (range, 1.33–2.6) relative to the acidified formula containing *Bifidobacterium lactis* strain Bb 12. Depicting the data per child-year gives an incidence density of 3.06 days/child-year in the BbF group versus 5.67 days/child-year in the control group ($P = .0002$). By this method, the relative risk of diarrhea in the BbF group is 0.54 (95% CI = 0.39–0.75, $P < 0.001$).

TABLE 2. *Features of the 90 patients at enrollment and duration of follow-up*

	BbF	CF	*P*
Number	46	44	
Age (months)	3.9 ± 2.2	3.5 ± 2.1	NS
Weight (kg)	5.6 ± 2	5.1 ± 1.8	NS
Height (cm)	59.3 ± 5.4	56.6 ± 7.1	NS
Head circumference (cm)	39.4 ± 3.7	38.7 ± 3.6	NS
Family history of allergy	30%	41%	NS
Neonatal breastfeeding	46%	32%	NS
Solid food	57%	46%	NS
Follow-up (days)	137.1 ± 60.1	148.1 ± 64.9	NS

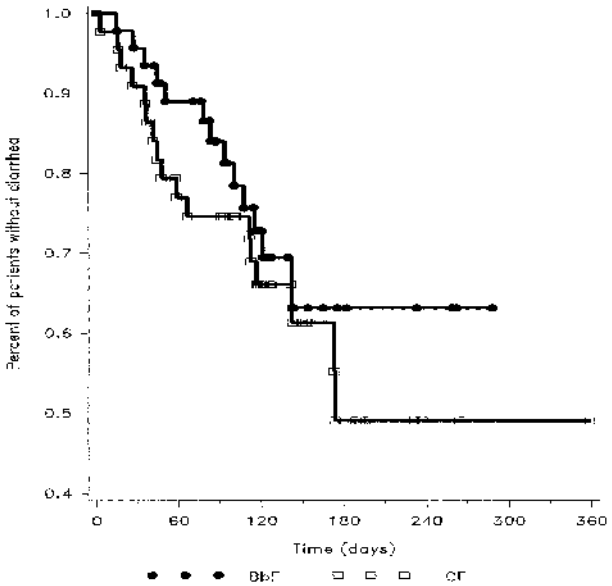

FIG. 1. Cumulative incidence of episodes of acute diarrhea in infants receiving formula supplemented with *Bifidobacterium lactis* strain Bb 12 (BbF, n = 46) (black square) and the conventional formula in the control group (CF, n = 44) (open square); the difference between the two groups is not significant.

TABLE 3. *Efficacy of the formula supplemented with viable bifidobacteria strain Bb 12 (BbF) on diarrheal disease*

	BbF (46)	CF (44)	*P*
Number of infants with diarrhea	13 (28.3%)	17 (38.7%)	NS
Number of episodes/infant	0.4 ± 0.9	0.5 ± 0.8	NS
Mean number of stools per day	4 ± 1.6	3.9 ± 1.3	NS
Mean cumulative duration of episodes of diarrhea (days)	5.1 ± 3.3	7 ± 5.5	0.273
Mean number of days with diarrhea/infant included	1.15 ± 2.5	2.3 ± 4.5	0.0002
Daily probability of diarrhea	0.84	1.55	0.0014
Number of days with diarrhea per child-year	3.06	5.67	0.0002
Relative risk of diarrhea	0.54	1	< 0.001

CF = conventional nonsupplemented formula.

A microbiologic evaluation of fecal samples was performed in only 18 cases. One of the 6 samples from children receiving BbF and 5 of the 12 samples from those on CF were positive for rotavirus ($P = 0.6$).

Tolerability

All infants accepted and seemed to be satisfied with all of the formulas. Adequate growth was recorded in all infants, with no difference between groups. There were no serious adverse effects associated either formula. The only clinical problems noted were spitting and regurgitation, which occurred in 11% of the infants receiving BbF and 13% of those receiving CF ($P = 0.49$).

DISCUSSION

Our study agrees with previous studies (13,15) that *Bifidobacterium lactis* may provide some protective effect against acute enteritis (4–7). We found a lower daily probability of diarrhea in the BbF group, with fewer days of diarrhea and a relative risk about half that of the CF group. Feeding with BbF also tended to postpone the onset of diarrheal disease. This is noteworthy, even if not statistically significant, because a report from Bangladesh suggested that breast feeding might merely postpone, rather than prevent, diarrheal disease caused by rotavirus (18).

The potential mechanisms by which bifidobacteria might exert a protective effect against acute gastroenteritis are numerous (8,9). Bifidobacteria are the predominant micro-organisms in the stools of breast fed infants (6,10). They are anaerobic, nonmobile, Gram-positive rods. (19). In healthy adults and children, *B. lactis* species, ingested in fermented milk, survive transit through the gastrointestinal tract and proliferate in the intestine (19–23). Langhendries et al. (24) have found a similar incidence and degree of colonization with bifidobacteria in infants receiving a whey-adapted acidified formula containing 10^6 CFU of viable bifidobacteria/gm powdered formula similar to our study formula. Our study provides evidence that the number of bifidobacteria in infant stools can be increased by oral supplementation and that the potential beneficial effect of these bacteria may be a direct result of their presence in the gut, rather than changes they may bring about in the existing aerobic or anaerobic population (8,22–24).

Saavedra et al. (13) showed that hospitalized infants receiving a milk formula containing *B. lactis* and *Streptococcus thermophilus* had a decrease in incidence of diarrhea compared to infants receiving standard formula (7% vs 31%). Moreover, the rate of rotavirus-associated diarrhea was also lower than controls (7% vs 19%) and the duration of viral shedding was decreased. Administration of yogurt containing *B. lactis* to children receiving antibiotic therapy for intractable diarrhea resulted in normalization of enteric flora, in which bifidobacteria predominate, and a rapid improvement in clinical symptoms (25). In our study, the lack of a significant difference in the incidence of acute diarrhea between the two groups may be attributable to the lower *Bifidobacterium* content (10^6 CFU *v* 10^8 CFU per gram of powder) achieved. In addition, our trial enrolled healthy children in resident centers, whereas the study by Saavedra et al. was performed in hospitalized, sick children. The number of microbiologic evaluations of stools in our study was too low to permit any conclusions about duration of viral shedding.

Bifidobacteria are able to ferment glucose, lactose, and fructose (20), thereby lowering fecal pH (22). Luminal acidity derived from fermentation of lactose inhibits the development of putrefactive bacteria and may in part explain the resistance of breast fed infants to infectious gastroenteritis (5,8). The potential immunostimulating properties of *B. lactis* have been documented (8). The ingestion of a formula containing the same strain of Bifidobacteria Bb 12 as we used stimulated the production of IgA in the gastrointestinal tract of healthy children and thus might enhance mucosal resistance against gastrointestinal infections (23). This strain also has been shown in adults to induce a nonspecific activation of phagocytic cells and elevation of serum IgA (20). The increase in phagocytosis coincided with fecal colonization and persisted for 6 weeks after ingestion of the fermented product (21).

B. lactis fulfills the criteria for a probiotic agent (26,27) and is safe for human use (28). Moreover, the lower casein and phosphate content of the BbF, together with its higher lactose content and lower pH, are likely to favor the persistence of *B. lactis* in the intestine as has been suggested for breast milk, and thus the formula may have prebiotic properties (4,9). In fact, ingestion of a formula similar to the BbF used in this study led to an increase in resident bifidobacteria (bifidogenic effect) (22). A formula acidified by the addition of *Lactobacil-*

lus helveticus and *S. thermophilus* has been shown to exert a protective effect against diarrheal disease (12). Twice daily prophylactic administration of 6×10^9 CFU of *Lactobacillus* GG reduced the risk of nosocomial diarrhea in hospitalized children compared to (6.7% *v* 33.3%) (29). To date *Lactobacillus* GG has never been incorporated in a milk formula but has always been given as a supplement to feeding. When given during acute episodes of diarrhea the major impact is a consistent decrease in the duration of diarrhea (15).

The prophylactic use of an acidified formula with low casein and phosphate, high lactose content, and living *Bifidobacterium lactis* Bb 12 has a protective impact on infantile acute gastroenteritis and nosocomial infections in residential infant care centers. The formula itself is inexpensive and the decrease in diarrhea are both economic benefits. The preventive effect of the formula studied may be explained by the prebiotic influence of its composition and the increase in bifidobacteria of the strain Bb 12 in the infant intestine.

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