Lactobacillus paracasei Strain ST11 Has No Effect on Rotavirus but Ameliorates the Outcome of Nonrotavirus Diarrhea in Children From Bangladesh

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ABSTRACT. *Background.* **Previous studies have shown that selected strains of lactobacilli that are administered orally result in a modest reduction of diarrhea duration. However, duration alone is not considered optimal for therapeutic evaluation of any agent in diarrhea.**

Objective. **To examine the effect of a new probiotic, *Lactobacillus paracasei* strain ST11 (ST11), in acute childhood diarrhea by using evaluation criteria recommended by the World Health Organization.**

Methods. **In a randomized, double-blind, placebo-controlled clinical trial, 230 male infants and young children, 4 to 24 months of age, presenting with diarrhea of <2 days' duration were admitted to the metabolic research ward of the International Centre for Diarrheal Disease Research, Bangladesh, and fed 10^{10} colony-forming units of lyophilized ST11 or placebo daily for 5 days. Stool output and frequency, oral rehydration solution intake, and excretion of rotavirus were monitored daily.**

Results. **No effect of ST11 treatment on severe rotavirus diarrhea was observed. However, the probiotic treatment did significantly reduce cumulative stool output (225 ± 218 vs 381 ± 240 mL/kg), stool frequency (27.9 ± 17 vs 42.5 ± 26), and oral rehydration solution intake (180 ± 207 vs 331 ± 236 mL/kg) in children with less-severe nonrotavirus diarrhea compared with those receiving placebo treatment. A significantly higher proportion of nonrotavirus children receiving ST11 had their diarrhea resolve within 6 days of therapy (ST11 versus placebo: 76% vs 49%).**

Conclusions. **ST11 has a clinically significant benefit in the management of children with nonrotavirus-induced diarrhea, but it is ineffective in those with rotavirus diarrhea. *Pediatrics* 2005;116:e221–e228. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2004-2334; *children, diarrhea, rotavirus, lactobacillus, probiotic.***

ABBREVIATIONS. ST11, *Lactobacillus paracasei* strain ST11; ICDDR,B, International Centre for Diarrheal Disease Research, Bangladesh; ORS, oral rehydration solution; ELISA, enzyme-linked immunosorbent assay.

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For more than a century researchers have suggested that live bacterial cultures, such as those found in yogurt, might be useful in the prevention and treatment of gastrointestinal disorders.[1] Lactobacilli belong to the normal commensal bacterial flora of the human intestine and are currently being investigated extensively as probiotics (health-promoting bacteria).[2,3] Their antidiarrheal properties have been investigated since the 1960s.[4] Several recent controlled clinical trials have shown that selected strains of lactobacilli such as *Lactobacillus casei* strain GG,[5–9] *Lactobacillus reuteri*,[10] and *Lactobacillus acidophilus*[11] exhibit both therapeutic and prophylactic effects in children with viral, but not bacterially induced, diarrhea.[12,13] A multicenter trial demonstrated an effect of lactobacilli on rotavirus but not bacterial diarrhea.[8] In addition, *Bifidobacterium bifidum* combined with *Streptococcus thermophilus* has shown prophylactic activity against rotavirus gastroenteritis.[14]

A meta-analysis[15] demonstrated a significant impact of probiotic lactobacilli on the duration of rotavirus gastroenteritis, but the analysis also revealed significant statistical, clinical, and methodologic heterogeneity within the trials. Duration of diarrhea has been used as the primary measure of outcome in all previous studies. However, this measure alone is not considered optimal. With few exceptions, the studies did not quantitatively evaluate stool output. Quantitative diarrhea criteria are recommended by the World Health Organization for the evaluation of therapeutic agents in the management of acute diarrhea.[16]

In developing as compared with industrialized countries, diarrhea in children often runs a more severe course, and a greater variety of bacterial pathogens is commonly observed.[17]

A new probiotic strain, *Lactobacillus paracasei* strain ST11 (ST11), which was isolated from stools of a 4-week-old breastfed healthy girl (her name was Stephanie, and it was the 11th colony isolated from her stool, hence the name ST11). This strain was selected for its industrial properties (yield, stability) and already-shown in vitro antimicrobial activity. The purpose of this study was to assess if this strain could significantly reduce overall disease severity and duration in Bangladeshi children hospitalized with acute diarrhea or if the effects would be limited

to subgroups of patients differentiated by rotavirus diagnosis in the stool.

SUBJECTS AND METHODS

The study was conducted between February 2001 and December 2002. Male infants and young children, aged 4 to 24 months, with a history of acute watery diarrhea (≥4 liquid stools during 24 hours) of <48 hours' duration, hospitalized at the Dhaka Hospital of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B), were included in this study. Only male subjects were selected to ensure accurate stool measurements not mixed with urine. Those with severe malnutrition (≤65% weight for age by the standard of the National Centre for Health Statistics), systemic infection requiring antimicrobial therapy, bloody diarrhea, and those children whose spot sample of stool revealed *Vibrio cholerae* by dark-field microscopy or who had antibiotic treatment in the preceding 2 weeks were excluded from the study. Written consent was required from parents or guardians for enrollment of each child in the study. The ethical and research review committees of both the ICDDR,B and Karolinska Institute approved the study.

After enrollment, children were assigned randomly to receive either lyophilized ST11 (5×10^9 colony-forming units) or placebo (whey-protein/skim-milk powder blend) twice daily for 5 days in addition to standard therapy with oral rehydration solution (ORS) and continued feeding.

Case Management

The study was conducted in the metabolic research ward of the ICDDR,B, which has a total of 12 beds. Children were placed on a "cholera cot," and urine collection bags were applied for separate collections of stool and urine. Initial dehydration was corrected by ORS, and the same volume of ORS replaced ongoing stool loss. Intravenous solutions were used if ORS therapy failed to match ongoing stool losses (unscheduled intravenous therapy) because of either severe vomiting or high rates of purging with reappearance of the signs of dehydration as assessed by a physician using World Health Organization guidelines.[18] Unscheduled intravenous fluid was discontinued as soon as dehydration was corrected and administration of ORS was resumed for matching ongoing loss. Fluid-maintenance therapy was continued until cessation of diarrhea.

Exclusively breast fed infants continued to receive mother's milk, and partially breastfed children received a milk-based formula (294 kJ/100 mL) in addition to breast milk. Nonbreastfed children were offered 460 kJ/kg per day of milk formula or semisolid and solid foods appropriate for their age and food habits.

The output of stool, urine, and vomitus and the intake of ORS and food were measured by a nurse, who was assisted by a health assistant, using a balance with a sensitivity of 1 g. Foods were weighed before serving, and the amount left was weighed to determine the amount consumed. To count the stool frequency, mothers were provided with small rounded pieces of metal sheet and instructed to put one of these in a box for each bowel motion. Changes in the consistency of the stool (loose or watery, stool that can be poured from one container to another; soft stool that conforms to the anatomy of patient's rectum; or formed stool having its own shape) were also monitored and recorded. A case-report form was used to collect and record all information in accordance with the protocol.

Nurses measured and recorded vital signs every 8 hours, and body weight was recorded on admission, after correction of initial dehydration, and daily thereafter. A physician clinically evaluated all infants twice a day.

Laboratory studies included determination of serum concentration of sodium, potassium, and chloride, total carbon dioxide, serum specific gravity, total protein, and complete blood count, and stools were tested for *Salmonella*, *Shigella*, and *Campylobacter*. The presence of rotavirus was measured by enzyme-linked immunosorbent assay (ELISA) within 4 hours of admission; this test was repeated daily for the entire study period if the patients' admission samples were positive.

According to the protocol, we planned to compare the effect of probiotic versus placebo treatment successively for all children (step 1), rotavirus-infected patients (step 2), and non–rotavirus-infected patients (step 3).

End Points

The children remained in the metabolic research ward for 6 days or until cessation of diarrhea, which was defined as passage of the last watery or loose stool before passage of 2 consecutive soft or formed stools or no stool in >2 consecutive 8-hour periods.

Outcome Measures

The outcome measures were daily and cumulative stool output (g/kg of body weight), number of stools, ORS intake (mL/kg), duration of diarrhea from initiation of therapy (hours), and time to disappearance (days) of rotavirus from stools.

Randomization Schedule

A randomly permutated block design was used for allocation of patients into the treatment (ST11) or placebo group. All patients qualifying for entry into the study were assigned a sequential random number. The master randomization code and 2 sets of code envelopes detailing treatment allocation of a given patient were generated by an independent statistician not involved in the study.

Both placebo and lyophilized *L paracasei* were packaged in identical sachets. Powders in both types of sachets were identical in texture and color, and both were mixed with ORS before dispensing. The codes were broken only at the end of data collection and after performing a blinded analysis.

Calculation of Sample Size

The sample size was calculated based on 3 outcome measures: duration of diarrhea, stool volume, and frequency of stool, which were reported earlier to be 74 hours,[19] 217 g/kg ever 48 hours,[20] and 13.75 per d,[21] respectively, in acute watery diarrhea. Assuming that the intervention would result in a ≥25% reduction of all the outcome measures, the sample size was determined to be 78, 102, and 100, respectively, at 5% significance and 80% power. The largest sample size of 102 patients in each group was therefore chosen as a desired sample size. To adjust for 10% dropouts for any reason, the final sample size was determined to be 115 patients in each group.

Data Analyses

Data were entered onto a personal computer and analyzed by using the Statistical Package for Social Science (Version 10.0 for Windows; SPSS, Chicago, IL). Baseline characteristics and outcome measures, expressed as mean ± SD, were compared between the 2 study groups by using the Student's t test. Stool output, ORS intake, and frequency of stools were also compared with the Student's t test for normally distributed data and with the Mann-Whitney test for comparison of data that were not normally distributed. The χ^2 or Fisher's exact test was used to compare dichotomous variables (eg, proportion of children with resolution of diarrhea). Differences having a P value of ≤.05 were considered significant.

RESULTS

In total, 272 children were screened for enrollment into the study. Of them, 42 children were not subsequently enrolled, because they had a positive test for dark-field detection of vibrios, suspected systemic infection, or electrolyte imbalance or their parents declined to participate in the study. Two hundred thirty children fulfilled the study criteria and were assigned to treatment regimens. Figure 1 shows the number of patients in both the treatment and placebo groups according to rotavirus-infection status. Of the 115 ST11-treated children, 78 (68%) were positive and 34 (30%) were negative for rotavirus infection as determined by ELISA. In 3 children, the parents withdrew consent after enrollment. In the placebo-treated group, 73 children (63%) were positive and

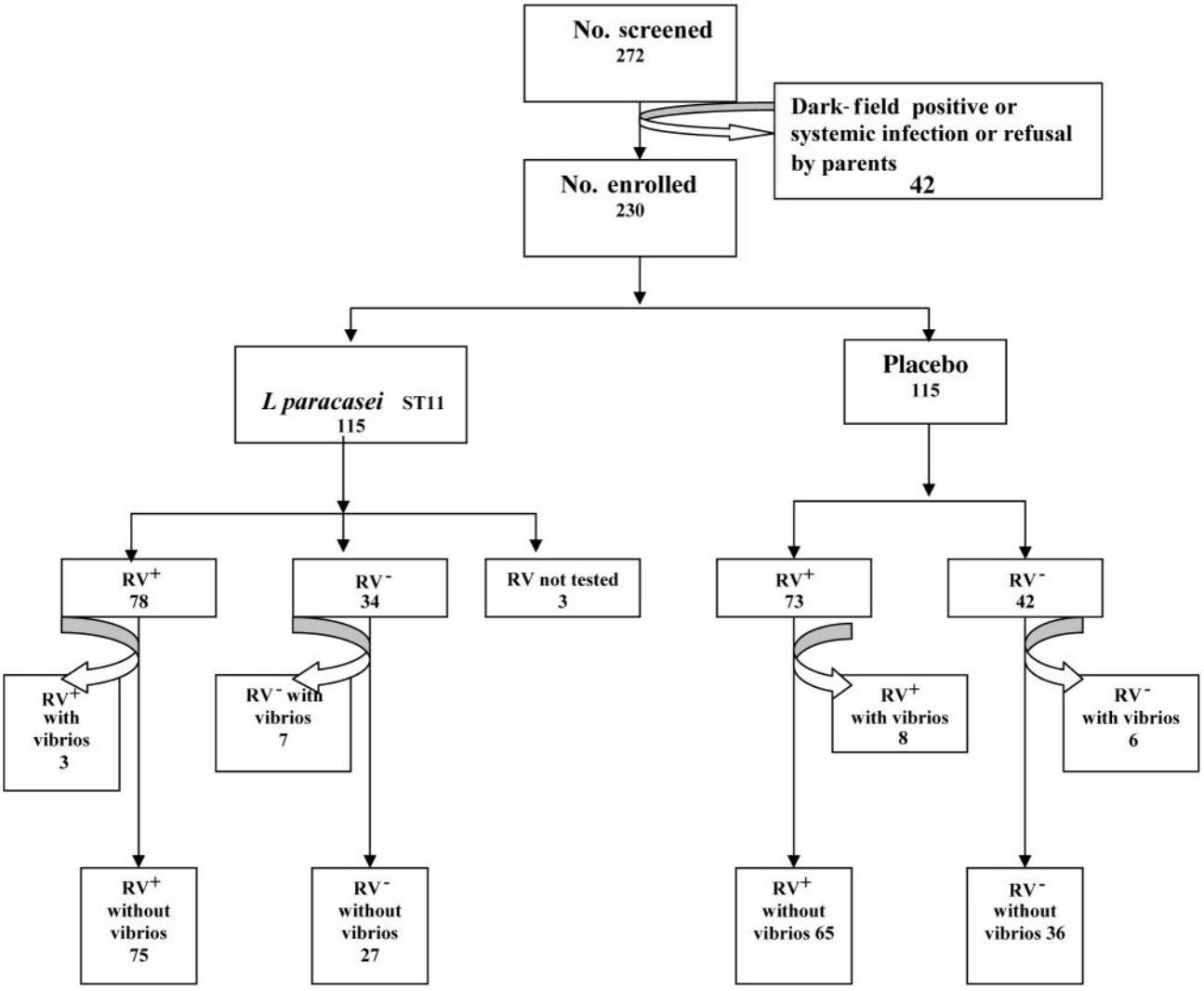

Fig 1. Consort diagram showing the distribution of the patients enrolled into the trial into a treatment and placebo arm and according to rotavirus and bacterial pathogen detection in the stool samples. RV indicates rotavirus.

42 (37%) were negative for rotavirus by ELISA. Ten children in the ST11-treated group (3 rotavirus-positive and 7 rotavirus-negative children) and 14 children in the placebo-treated group (8 rotavirus-positive and 6 rotavirus-negative children) also yielded vibrios in stool culture and were excluded from the analysis according to the protocol subsequently.

The admission characteristics of the 115 children in each arm of the trial were comparable, and there were no differences in clinical features, breastfeeding status, or biochemical, hematologic, or microbiologic data between the groups (Table 1). The non–rotavirus-infected patients in the treatment group also did not differ from those of the placebo group with respect to admission characteristics (Table 2). On the basis of undifferentiated diarrhea, ST11 treatment did not result in a significant reduction of daily or cumulative (days 1–6) stool output, ORS intake, or stool frequency as compared with the placebo treatment (Table 3).

In rotavirus-infected patients, excluding those with vibrios ($n = 75$ in the ST11 group and $n = 65$ in the placebo group; Fig 1), there were no differences in the clinical outcomes between the treatment and placebo groups. That is, the amounts of ORS and the duration of diarrhea, stool frequency, and time to recovery from illness were similar. Also, the daily or cumulative (days 1–6) stool outputs between the groups were similar (Table 4).

After excluding those infected with rotavirus (27 in the treatment group and 36 in the placebo group), the cumulative ORS intake, cumulative stool output, and stool frequency were all reduced in the ST11-treated group when compared with the placebo group. These reductions were clinically significant, and all differences were statistically significant at $P < .05$, indicating amelioration of clinical symptoms in the ST11-treated group. The mean duration of diarrhea was also reduced in the treated children, but the difference did not reach statistical significance (Table 5). Resolution of diarrhea by the sixth day of the study occurred in a significantly higher proportion of children in the intervention group than the controls (Table 5; Fig 2).

In rotavirus-infected children, 30 children (12 in the ST11 group and 18 in the placebo group) had additional pathogens other than vibrios such as *Salmonella*, *Shigella*, and *Campylobacter*, as indicated in

TABLE 1. Clinical Characteristics of the Study Children on Admission

	Treatment Group (n = 115)	Control Group (n = 115)	P
Characteristic			
Age, mo, mean ± SD	10.2 ± 3.5	9.8 ± 3.5	.35
Weight, kg, mean ± SD	7.7 ± 0.9	7.7 ± 1.2	.94
Height, cm, mean ± SD	70.2 ± 3.8	69.8 ± 4.3	.41
Duration of diarrhea before admission, h, mean ± SD	34 ± 9.8	32 ± 10.8	.10
Dehydration status on screening, no sign/some, *n*	61/54	50/65	.15
Currently breastfed, yes/no, *n*	105/10	98/17	.11
Reported stool frequency, no. in previous 24 h, mean ± SD	13.5 ± 6.5	14.3 ± 6.1	.10
Serum electrolytes, mmol/L			
Sodium			
<135.0	3	3	1.0
135.01–145.0	108	108	1.0
>145.0	4	4	1.0
Potassium			
<3.50	24	15	.11
3.51–4.50	57	65	.29
>4.50	34	35	.89
Total carbon dioxide			
<10.0	1	2	.56
10.01–15.0	38	39	.89
>15.0	76	74	.78
Rectal temperature, °C, mean ± SD	37.3 ± 0.7	37.3 ± 0.7	1.0
Pathogen isolated			
Rotavirus alone, *n* (%)	66 (57)	55 (48)	.14
Bacteria alone, *n*	11	16	.41
Cholera, *n*	4	2	.67
Shigella sp., *n*	3	2	1.0
Salmonella sp., *n*	0	1	1.0
C jejuni, *n*	1	7	.05
Other vibrios, *n*	3	4	1.0
Rotavirus with vibrios, *n*	10	14	.38
None of the above, *n*	23	26	.62
ELISA not done, *n*	3	0	.24

TABLE 2. Admission Characteristics in Non–Rotavirus-Infected Children

Characteristic	Treatment Group (n = 27)	Control Group (n = 36)	P
Age, mo, mean ± SD	10.6 ± 3.5	9.9 ± 4.3	.45
Weight, kg, mean ± SD	7.7 ± 1.1	7.3 ± 1.3	.23
Height, cm, mean ± SD	71.0 ± 4.0	69.0 ± 4.7	.25
Duration of diarrhea before admission, h, mean ± SD	32.0 ± 9.0	33.0 ± 10.8	.82
Dehydration status on screening, no sign/some, *n*	13/14	15/21	.61
Currently breastfed, yes/no, *n*	24/3	32/4	.11
Reported stool frequency, number in previous 24 h, mean ± SD	12.0 ± 6.5	13.9 ± 6.0	.16
Serum electrolytes, mmol/L, mean ± SD			
Sodium	136.0 ± 4.0	136.0 ± 3.0	.66
Potassium	4.1 ± 0.7	4.2 ± 0.7	.75
Chloride	110 ± 5.0	110 ± 4.6	.51
Total carbon dioxide	18.3 ± 3.3	17.2 ± 4.1	.27
Rectal temperature, °C	37.1 ± 0.7	37.2 ± 0.6	.45

Table 1. We therefore additionally compared the effect of probiotic versus placebo treatment in children excluding those with additional copathogens (exclusively infected with rotavirus: n = 66 in the ST11 group and n = 55 in the placebo group). There were no significant reductions whatsoever in cumulative stool output, duration of diarrhea, stool frequency, or recovery from illness between the groups (data not shown).

DISCUSSION

In this study we found that ST11 feeding had no overall effect on the clinical course of acute nonvibrio diarrhea in children hospitalized with acute disease in a large city hospital of a poor, developing country. Diarrhea is a multifaceted disease with respect to etiology, and some intervention might only be effective with diarrhea caused by specific pathogens. To explore this possibility, we separated the patients into 2 groups: rotavirus-infected patients and non–rotavirus-infected patients. The latter group in our study is mainly defined negatively, because we only did a partial bacteriologic analysis. However, according to previous epidemiologic surveys in the same hospital, bacteria, especially enterotoxigenic and enteropathogenic *Escherichia coli*, figure prominently as the agents of acute childhood diarrhea that lead to hospitalization. We encountered an increased pro-

TABLE 3. Comparison of Outcome Measures of All Children Treated With ST11 or Placebo

Outcome Measures	Treatment Group (n = 115)	Placebo (n = 115)	P
Cumulative ORS intake, mL/kg, mean ± SD			
0–24 h	73 ± 46	84 ± 50	.10
0–48 h	144 ± 96	161 ± 100	.20
0–72 h	208 ± 144	225 ± 139	.37
0–96 h	265 ± 197	278 ± 171	.62
0–120 h	309 ± 246	316 ± 200	.82
0–144 h	334 ± 280	343 ± 230	.81
No. of children required unscheduled intravenous fluid	1	4	.18
Cumulative stool frequency, n, mean ± SD			
0–24 h	8.9 ± 4.7	10.0 ± 5.4	.11
0–48 h	16.5 ± 8.6	18.9 ± 10.4	.06
0–72 h	23.7 ± 11.7	26.8 ± 13.7	.07
0–96 h	29.9 ± 14.9	33.3 ± 16.9	.12
0–120 h	34.2 ± 17.8	37.6 ± 19.4	.18
0–144 h	37.2 ± 20.6	41.0 ± 21.8	.19
Cumulative stool output, g/kg, mean ± SD			
0–24 h	80 ± 56	90 ± 64	.21
0–48 h	158 ± 113	172 ± 116	.37
0–72 h	231 ± 170	245 ± 157	.53
0–96 h	296 ± 229	304 ± 191	.78
0–120 h	341 ± 277	370 ± 237	.91
0–144 h	385 ± 330	389 ± 259	.93
Duration of diarrhea after the first dose of therapy, h, mean ± SD	90.4 ± 45.0	94.2 ± 43.3	.52
Total duration (before and after first dose), h, mean ± SD	123.8 ± 46.0	126.1 ± 44.8	.71
Cessation of diarrhea			
Yes/no, n	81/28	73/34	.40
Not known, n*	6	8	

* Patients left against medical advice during the study.

TABLE 4. Comparison of Outcome Measures in Rotavirus-Infected Children Treated With ST11 or Placebo

Outcome Measures	Treatment Group (n = 75)	Placebo (n = 65)	P
Cumulative ORS intake, mL/kg, mean ± SD			
0–24 h	82 ± 46	91 ± 54	.29
0–48 h	163 ± 101	184 ± 105	.23
0–72 h	235 ± 148	256 ± 142	.40
0–96 h	297 ± 203	315 ± 176	.59
0–120 h	339 ± 253	350 ± 206	.80
0–144 h	370 ± 288	366 ± 229	.94
Cumulative stool frequency, n, mean ± SD			
0–24 h	9.8 ± 4.7	10.6 ± 5.4	.33
0–48 h	17.8 ± 8.9	20.6 ± 10.1	.09
0–72 h	25.5 ± 12.2	29.0 ± 12.7	.10
0–96 h	32.0 ± 15.6	35.8 ± 15.6	.16
0–120 h	36.3 ± 18.6	39.2 ± 17.4	.36
0–144 h	39.0 ± 21.3	41.8 ± 19.5	.45
Cumulative stool output, g/kg, mean ± SD			
0–24 h	93 ± 59	106 ± 73	.28
0–48 h	183 ± 120	203 ± 125	.34
0–72 h	265 ± 182	287 ± 166	.47
0–96 h	335 ± 242	352 ± 202	.67
0–120 h	378 ± 292	380 ± 235	.97
0–144 h	421 ± 345	417 ± 273	.94
Duration of diarrhea after the first dose of therapy, h, mean ± SD	94.0 ± 43.0	95.0 ± 37.9	.94
Total duration (before plus after first dose), h, mean ± SD	127.1 ± 43.4	126.5 ± 37.0	.94
Cessation of diarrhea			
Yes/no, n	56/18	45/15	.88
Not known, n*	1	5	

* Patients left against medical advice during the study.

portion (65%) of rotavirus-infected subjects in this study. The factors for increased proportion of rotavirus-infected subjects might be related to the stringent enrollment criteria used in this study, which favored severe diarrhea and, hence, perhaps rotavirus infections. The first factor is that the age of the children, which was in the 4- to 24-month category, is an age at which the rate of rotavirus infection is rather high (~50%) in the study setting (ICDDR,B, surveillance report, 2002). The second factor is the nutritional status: we enrolled relatively nourished children, among whom the rates of rotavirus infection are at large more common than those with malnutrition.[22] The third factor is the exclusion of children suspected to have been infected with cholera and invasive diarrhea on enrollment by using clinical

TABLE 5. Comparison of Outcome Measures in Non–Rotavirus-Infected Children Treated With ST11 or Placebo

	Treatment Group (n = 27)	Placebo (n = 36)	P
Cumulative ORS intake, mL/kg, mean ± SD			
0–24 h	45 ± 35	73 ± 45	.01
0–48 h	81 ± 64	129 ± 91	.02
0–72 h	111 ± 97	183 ± 129	.02
0–96 h	140 ± 132	232 ± 156	.02
0–120 h	179 ± 181	285 ± 189	.03
0–144 h	180 ± 207	331 ± 236	.02
Cumulative stool output, g/kg, mean ± SD			
0–24 h	40 ± 26	68 ± 43	.01
0–48 h	79 ± 53	130 ± 91	.01
0–72 h	118 ± 82	189 ± 127	.01
0–96 h	151 ± 118	248 ± 155	.01
0–120 h	188 ± 167	318 ± 193	.01
0–144 h	225 ± 218	381 ± 240	.01
Cumulative stool frequency, n, mean ± SD			
0–24 h	6.2 ± 3.8	8.9 ± 5.6	.04
0–48 h	11.7 ± 6.4	16.8 ± 11.3	.04
0–72 h	17.1 ± 8.5	24.2 ± 15.4	.04
0–96 h	21.5 ± 10.7	31.0 ± 19.5	.03
0–120 h	25.5 ± 13.6	37.3 ± 23.1	.02
0–144 h	27.9 ± 17.0	42.5 ± 26.0	.02
Cessation of diarrhea, yes/no, n	19/6	17/18	.03
Not known, n*	2	1	
Duration of diarrhea after first dose, h, mean ± SD	77 ± 48	99 ± 51	.09
Total duration (before plus after first dose), h, mean ± SD	109 ± 49	131 ± 54	.10

* Patients left against medical advice during the study.

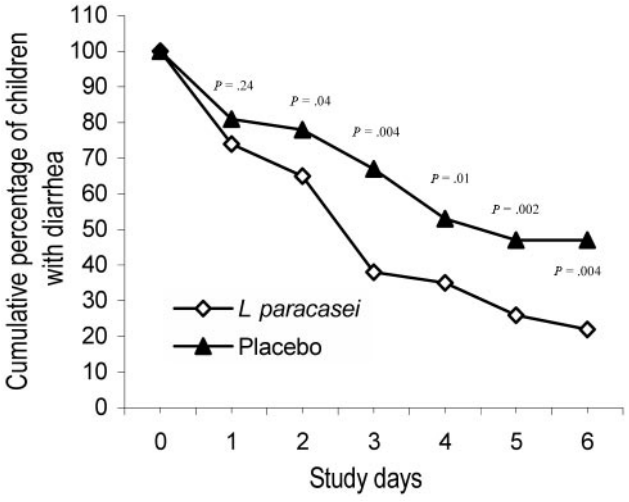

Fig 2. Percentage of nonrotavirus children with diarrhea at the indicated day of hospitalization in the treatment and placebo arms.

judgment, which further increased the proportion of enrollment of children with rotavirus infection.

ST11 also had no effect on rotavirus diarrhea. This negative outcome was not the result of a too-low lactobacillus dose or a titer decrease because of improper storage. In fact, the dose was as high as in previous studies.[2,3,6–9] In addition, we maintained the cold chain for storage, and analysis of the leftover sachets at the end of the study showed the same viable bacterial count as that at the beginning of the study. What factors could explain the discrepancy between our findings and those of previous studies in rotavirus-induced diarrhea? One difference might relate to the severity of the illness. In the previously published studies, the children were affected less severely than those in our study. The duration of illness after intervention in 5 previous studies was 1.6 to 3.8 days compared with 4.0 days in the control patients of the present study. Similarly, the stool frequency at the second day of hospitalization was 2.5, 3, 3.8, and 7 in the previous studies compared with 10 in the controls of the current study. In fact, the only other study investigating the affect of probiotics in severe acute dehydrating diarrhea was conducted in Brazil,[23] which also revealed a lack of efficiency of *Lactobacillus* GG in pediatric patients hospitalized with rotavirus gastroenteritis. It is therefore likely that neither *Lactobacillus* GG nor ST11 is of clinical benefit in the management of severe rotavirus gastroenteritis that leads to hospitalization in developing countries. It is possible that lactobacilli only have a beneficial effect in the treatment of mild forms of rotavirus gastroenteritis in developed countries. There is another difference that might explain the discrepancy. Previous studies, with the notable exception of the Brazilian trial,[23] used less-stringent criteria of diarrhea by counting stools and judging the form of the stool, raising methodologic questions for the former studies.

Finally, when non–rotavirus-infected patients were considered, ST11 feeding had a positive impact on quantitative diarrhea outcome parameters when compared with placebo recipients. Here we should stress that the analysis of rotavirus-infected and non–rotavirus-infected patients was predetermined in the clinical protocol and not a post hoc analysis. The difference in cumulative ORS intake, cumulative stool output and stool frequency, and resolution of diarrhea was statistically significant despite the relatively small number of non–rotavirus-infected patients in our study. It was surprising that a decreased

need of ORS, a decreased stool output, and decreased stool frequency was already seen after a single day of ST11 application. However, a similarly rapid effect was also previously observed with rotavirus-specific bovine colostrums in rotavirus-infected acutely diarrheal children. These results were obtained in a trial conducted in the same setting with a comparable study design.[24] Although it is difficult to explain the mechanisms of rapid action of these 2 therapeutic applications on 2 different forms of pediatric diarrhea, we must conclude that successful antidiarrheal interventions can rapidly ameliorate the clinical symptoms of the patients. Before prematurely concluding that the probiotic bacterium was effective against nonrotaviral, probably bacterial diarrhea but inefficient against rotaviral diarrhea, we should consider 3 limitations of the study. One limitation concerns the difference in severity between rotavirus and nonrotavirus diarrhea as evident from separate analysis comparing the day-wise and cumulative stools of 2 placebo groups as shown in Tables 4 and 5 (rotavirus-infected children versus non–rotavirus-infected children; $P < .05$). This difference could suggest that the severity of disease as measured by stool output in the first 3 or 4 days after enrollment may be an as good or even better predictor of efficacy of the probiotic as is the etiology of the diarrhea. This interpretation is consistent with prior studies, in which a multi-European study of children with very mild rotavirus disease demonstrated probiotic efficacy[8] but a study of Brazilian children with severe disease showed no probiotic efficacy.[23] Another limitation concerns the diagnostic procedures: we did not screen for other viral etiologies (eg, norovirus), nor were some of the most common bacterial causes of diarrhea (eg, *E coli*) tested for. Already with the limited analysis we realized that some rotavirus-infected patients showed also a bacterial copathogen. In a comparative analysis of children with nonrotaviral infection and those with rotaviral infection along with coinfection with bacteria, a significant difference in cumulative stool outputs, ORS intake, and stool frequency was observed between the 2 groups (data not shown). One therefore might question whether our decision to separate the patients according to rotavirus detection in the stool neatly split the patients into 2 distinct diarrhea pathophysiologies. A third limitation concerns the number of patients. Previous epidemiologic surveys conducted in the same hospital diagnosed rotavirus infection in ~30% of the children, which is as much as enterotoxigenic and enteropathogenic *E coli* infections combined. However, the current study demonstrated a much higher rate of rotavirus infections. This higher figure probably reflects our exclusion criteria that apparently decreased the number of bacterial infections in the enrolled patients. This exclusion led to a relatively low number of non–rotavirus-infected patients in our study. Despite being backed by statistical significance, the results should therefore be interpreted with caution, because they are based on a small and perhaps selected patient collective.

We previously tested other therapeutic agents (bovine milk antibodies) for their antidiarrhea properties at the same hospital. It is interesting to note that oral administration of specific antibodies showed an impressive clinical efficiency against rotavirus diarrhea[24] but no impact on *E coli*–induced diarrhea.[25] Because these 3 studies were conducted with a similar protocol and the patients showed comparable admission characteristics, the divergent effects are interesting and suggest an antiviral effect of oral antibodies and a possible antibacterial effect of an oral probiotic bacterium. Both results are biologically plausible. However, there are also parallels: the beneficial effects of both biological agents are manifested rapidly (first day of treatment), excluding in both cases action mechanisms that take some time to develop.

CONCLUSIONS

The current trial suggests an effect of ST11 on nonrotavirus diarrhea, but a confirmation of this result with a greater number of children and a better definition of the etiology is clearly warranted. This might allow the targeted feeding of ST11 to patients who are likely to profit from this treatment.

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