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Multicenter trial of a combination probiotic for children with gastroenteritis

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et al

ORIGINAL ARTICLE

Multicenter Trial of a Combination Probiotic for Children with Gastroenteritis

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ABSTRACT

BACKGROUND

Gastroenteritis accounts for approximately 1.7 million visits to the emergency department (ED) by children in the United States every year. Data to determine whether the use of probiotics improves outcomes in these children are lacking.

METHODS

We conducted a randomized, double-blind trial involving 886 children 3 to 48 months of age with gastroenteritis who presented to six pediatric EDs in Canada. Participants received a 5-day course of a combination probiotic product containing *Lactobacillus rhamnosus* R0011 and *L. helveticus* R0052, at a dose of 4.0×10^9 colony-forming units twice daily or placebo. The primary outcome was moderate-to-severe gastroenteritis, which was defined according to a post-enrollment modified Vesikari scale symptom score of 9 or higher (scores range from 0 to 20, with higher scores indicating more severe disease). Secondary outcomes included the duration of diarrhea and vomiting, the percentage of children who had unscheduled physician visits, and the presence or absence of adverse events.

RESULTS

Moderate-to-severe gastroenteritis within 14 days after enrollment occurred in 108 of 414 participants (26.1%) who were assigned to probiotics and 102 of 413 participants (24.7%) who were assigned to placebo (odds ratio, 1.06; 95% confidence interval [CI], 0.77 to 1.46; P=0.72). After adjustment for trial site, age, detection of rotavirus in stool, and frequency of diarrhea and vomiting before enrollment, trial-group assignment did not predict moderate-to-severe gastroenteritis (odds ratio, 1.06; 95% CI, 0.76 to 1.49; P=0.74). There were no significant differences between the probiotic group and the placebo group in the median duration of diarrhea (52.5 hours [interquartile range, 18.3 to 95.8] and 55.5 hours [interquartile range, 20.2 to 102.3], respectively; P=0.31) or vomiting (17.7 hours [interquartile range, 0 to 58.6] and 18.7 hours [interquartile range, 0 to 51.6], P=0.18), the percentages of participants with unscheduled visits to a health care provider (30.2% and 26.6%; odds ratio, 1.19; 95% CI, 0.87 to 1.62; P=0.27), and the percentage of participants who reported an adverse event (34.8% and 38.7%; odds ratio, 0.83; 95% CI, 0.62 to 1.11; P=0.21).

CONCLUSIONS

In children who presented to the emergency department with gastroenteritis, twice-daily administration of a combined *L. rhamnosus–L. helveticus* probiotic did not prevent the development of moderate-to-severe gastroenteritis within 14 days after enrollment. (Funded by the Canadian Institutes of Health Research and others; PROGUT ClinicalTrials.gov number, NCT01853124.)

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*A complete list of the members of the PERC PROGUT Trial Group is provided in the Supplementary Appendix, available at NEJM.org.

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A Quick Take is available at NEJM.org

Acute gastroenteritis accounts for approximately 1.7 million emergency department (ED) visits among children in the United States every year.[1] Although health care providers traditionally have had little to offer to modify the disease course,[2] probiotics are an expanding multibillion-dollar industry[3] with potential clinical benefits.[4] Consumers increasingly take probiotics to treat intestinal infections,[5,6] and 5 of 12 leading guidelines endorse the use of probiotics.[7] Most studies of probiotics with results that have been published have had methodologic limitations and small sample sizes, have included limited investigations of causative pathogens, and have not reported adverse events.[8] Numerous individual symptoms have been used as outcomes, but evaluations that incorporate both the duration and frequency of both diarrhea and vomiting are lacking.[9] Given the distressing symptoms of gastroenteritis[10,11] and the lack of benefit of probiotics shown in one North American study that enrolled children who received care in the ED,[12] the role of probiotics in outpatient management of acute gastroenteritis in children warrants clarification.

We conducted the Pediatric Emergency Research Canada (PERC) Probiotic Regimen for Outpatient Gastroenteritis Utility of Treatment (PROGUT) trial to evaluate the effectiveness of probiotics in children 3 to 48 months of age who present to the ED with acute gastroenteritis. We hypothesized that the percentage of children with moderate-to-severe gastroenteritis (defined according to a validated severity score[13,14]) within 14 days after enrollment would be significantly lower among those who received probiotics than among those who received placebo.

Methods

Trial Design and Oversight

In this multicenter, randomized, double-blind, placebo-controlled trial, participants with diarrhea were enrolled in six Canadian tertiary-care, university-affiliated, pediatric EDs. We sought to determine whether the administration of a two-strain, commercially available probiotic product (Lacidofil Strong, Lallemand Health Solutions) would be superior to placebo at reducing the severity of symptoms of acute gastroenteritis. Parents or guardians provided written informed consent for their children to participate. Participants received a 5-day course of combined *Lactobacillus rhamnosus* R0011 and *L. helveticus* R0052 at a dose of 4.0×10^9 colony-forming units (CFU) twice daily or placebo.

Probiotic and placebo sachets were provided free of charge by Lallemand Health Solutions, which tested quantitative bacterial cultures obtained from unused sachets. None of the funders had any input into the design or conduct of the trial; the collection, management, analysis, or interpretation of the data; the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. Research ethics boards at the participating sites approved the trial (see the Supplementary Appendix, available with the full text of this article at NEJM.org). The full protocol and statistical analysis plan are available at NEJM.org.[15] All the authors vouch for the completeness and accuracy of the data and analyses presented and for the fidelity of the trial to the protocol.

Trial Participants

Children 3 to 48 months of age were eligible for participation if they presented to the ED, had three or more episodes of watery stools in a 24-hour period,[16] had vomiting or diarrhea for less than 72 hours, and had received a clinical diagnosis (i.e., by the responsible physician) of an acute intestinal infection. Children were excluded if they or a person living in their household had an indwelling vascular-access catheter or if they had structural heart disease,[17] were immunocompromised,[18] or were receiving immunosuppressive therapy. Additional exclusion criteria were hematochezia, bilious vomiting, a chronic gastrointestinal disorder (e.g., inflammatory bowel disease or the short gut syndrome), pancreatic dysfunction or insufficiency,[19] the use of probiotics during the preceding 14 days, an allergy to soy, and an inability to complete follow-up. Children who had undergone oral or gastrointestinal surgery within the preceding 7 days or had previously participated in the trial were also excluded. Concomitant use of antibiotics was permitted.

Randomization and Blinding

Random-number–generating software, accessed through a Web-based randomization system (www.randomize.net), which used random block sizes of 4 and 6 and a 1:1 trial-group assignment ratio stratified according to site, was used to sequentially assign children to probiotics or placebo. The assignment sequence was restricted to the

research pharmacy at the coordinating center and www.randomize.net until the databases were locked. Participants and their parents or guardians, trial and clinical staff, and specimen and data analysts were unaware of the trial-group assignments.

PROCEDURES

The probiotic preparation is a lyophilized powder containing 4.0×10^9 CFU of two bacterial strains — *L. rhamnosus* R0011 and *L. helveticus* R0052 — in a 95:5 ratio. Sachets containing placebo and probiotics were identical in appearance, smell, and weight. The contents of one sachet containing the probiotics or placebo, which had been maintained at a temperature between 0° and 25°C, were sprinkled into 30 ml of the child's preferred liquid twice daily.[20] Five extra sachets were included in each kit to enable repeat dosing if vomiting occurred within 15 minutes after administration. Quantitative bacterial culture of the investigational product was performed when the use of each batch of the probiotic preparation was completed (see the Supplementary Appendix).

Research assistants collected demographic data and data on clinical characteristics and completed trial interventions in the ED. To maximize accuracy and minimize recall bias, parents or guardians completed electronic or telephone follow-up surveys every 24 hours until both vomiting and diarrhea had ceased in the participant for 24 hours. Survey questions targeted clinical symptoms, health care utilization, and adverse events during the preceding 24-hour period. On day 5, parents or guardians reported the adherence to the trial regimen (i.e., the number of sachets received of the number prescribed) and were asked to return all unused sachets for enumeration. If the two approaches to documentation of adherence differed, we determined a priori that the sachet count would be used.

Rectal swabs, stool specimens, or both were obtained during the enrollment visit.[21] Bacterial culture was performed locally. A multiplex nucleic acid panel that detects 15 enteric viruses, bacteria, and parasites (Luminex xTAG Gastrointestinal Pathogen Panel) (see the Supplementary Appendix) was performed at the Provincial Laboratory for Public Health–Alberta Public Laboratories, in Edmonton, Alberta, Canada.[22]

OUTCOMES

The primary outcome was the occurrence of moderate-to-severe gastroenteritis, which was defined according to a total modified Vesikari scale symptom score of 9 or higher (scores range from 0 to 20, with higher scores indicating more severe disease) (Table 1, and the Supplementary Appendix).[13,24] The score was based on symptoms during the follow-up period and was calculated at the day 14 follow-up. The modified Vesikari scale quantifies severity over a broad range of symptoms and interventions,[25] has been designed for outpatients, and was validated at most of the participating hospitals.[13,14,26] The 14-day timeline was used to capture relationships between the use

Table 1. Modified Vesikari Scale.*

Scale component	Score on the Vesikari Scale			
	0 Points	1 Point	2 Points	3 Points
Duration of diarrhea (hr)	0	1–96	97–120	≥121
Maximum no. of watery stools per 24 hr	0	1–3	4–5	≥6
Duration of vomiting (hr)	0	1–24	25–48	≥49
Maximum no. of vomiting episodes per 24 hr	0	1	2–4	≥5
Maximum recorded rectal temperature (°C)†	<37.0	37.1–38.4	38.5–38.9	≥39.0
Unscheduled health care visit	None	NA	Primary care	Emergency department
Treatment	None	Rehydration with intravenous fluids	Hospitalization	NA

* In the modified Vesikari scale score, one variable (percent dehydration) in the original score was replaced with the variable of unscheduled health care visits to better measure the effect of acute gastroenteritis in outpatients, given that the ability to perform frequent in-person assessments in an outpatient cohort of children can be challenging. Scores range from 0 to 20, with higher scores indicating more severe disease. Children with a score of 9 or more were considered to have moderate-to-severe gastroenteritis.[13,14] NA denotes not applicable.

† Temperatures were adjusted for the location of measurement: 1.1°C was added to axillary temperatures and 0.6°C was added to oral temperatures.[23]

of probiotics and differences from placebo in the percentages of children who had prolonged diarrhea.[27] Baseline symptoms that occurred before the visit to the ED were not included in the outcome measure.

Secondary outcomes specified a priori included the duration of diarrhea and the duration of vomiting after enrollment; unscheduled visits to a health care provider for vomiting, diarrhea, dehydration, fever, or because the participant declined to drink fluids within 14 days after enrollment; and adverse events, which were coded with the use of definitions from the *Medical Dictionary for Regulatory Activities*, version 19.0. Additional outcomes specified a priori included the number of repeat visits to the ED, intravenous rehydration, hospitalization, the number of days of work missed by parents or guardians, and the number of days of day care missed by participants.

STATISTICAL ANALYSIS

We assumed that moderate-to-severe gastroenteritis would occur in 25% of the children who received placebo.[13,14] At a significance level of 5%, we calculated that a sample of 670 participants would provide the trial with 90% power to detect an absolute between-group difference of 10 percentage points in the outcome. We intended to recruit 886 participants to allow for a rate of loss to follow-up of 10%, a dropout rate of 5%, and a crossover rate of 2.5%, with adjustment for O'Brien–Fleming monitoring boundaries. Conservative boundaries, implemented with the use of the Lan–DeMets alpha-spending function, guided the early stopping boundary for safety or efficacy. All statistical tests of hypotheses were two-sided. The data and safety monitoring committee (see the Supplementary Appendix) met after 200 and 500 participants were recruited.

All analyses were specified a priori.[15] We included data from all participants who underwent randomization, according to the intention-to-treat principle. Multiple imputation was used to account for missing data. The model assumed that data were missing at random and included key baseline characteristics, trial group, and all efficacy outcomes. The overall significance level for statistical tests of secondary and tertiary outcomes was set at 0.05. The Holm method was used to adjust for multiple comparisons.[28] Analyses were performed with SPSS software, version 24.0.0.1 (IBM), and Stata software, version 15.0 (StataCorp).

Baseline variables were summarized with the use of standard descriptive statistics. Logistic regression, stratified according to trial site, was used to estimate odds ratios and 95% confidence intervals for the risk of moderate-to-severe gastroenteritis associated with probiotics as compared with placebo. Secondary analysis of the primary outcome included adjustment for other covariates identified a priori as being prognostic of the outcomes.[29] These covariates were age, frequency of vomiting and diarrhea in the 24-hour period before enrollment, trial site, and rotavirus infection. We compared the percentage of participants with modified Vesikari scores of 9 or higher after randomization, accounting for the interaction with the intervention, in subgroups according to age (<1 year vs. ≥1 year), whether the child had been exclusively breast-fed, use of oral antibiotics in the 14 days before enrollment, and adherence to the trial regimen (receipt of >70% of doses prescribed). The effect of rotavirus infection was evaluated in a logistic-regression model through the addition of an interaction term combining detection of rotavirus infection and trial group. The modified Vesikari score was also analyzed as a continuous variable with the use of a linear-regression model with adjustment for site.

Secondary outcomes were adjusted for trial site with the use of the appropriate regression models. The durations of diarrhea and vomiting were measured in hours and compared between groups with the use of a linear-regression model. The outcome of duration of vomiting included only participants with three or more episodes of vomiting in the 24-hour period before enrollment. Incidence rate ratios were analyzed to compare the number of episodes of diarrhea and vomiting after enrollment with the use of a negative binomial model that included terms for trial group, trial site, and the number of episodes of diarrhea, vomiting, or both, in the 24 hours before enrollment. The percentages of children who had unscheduled health care visits and any adverse event were compared with the use of logistic-regression models. The subgroups of children who attended day care and gainfully employed parents or guardians were evaluated for absenteeism with the use of the van Elteren

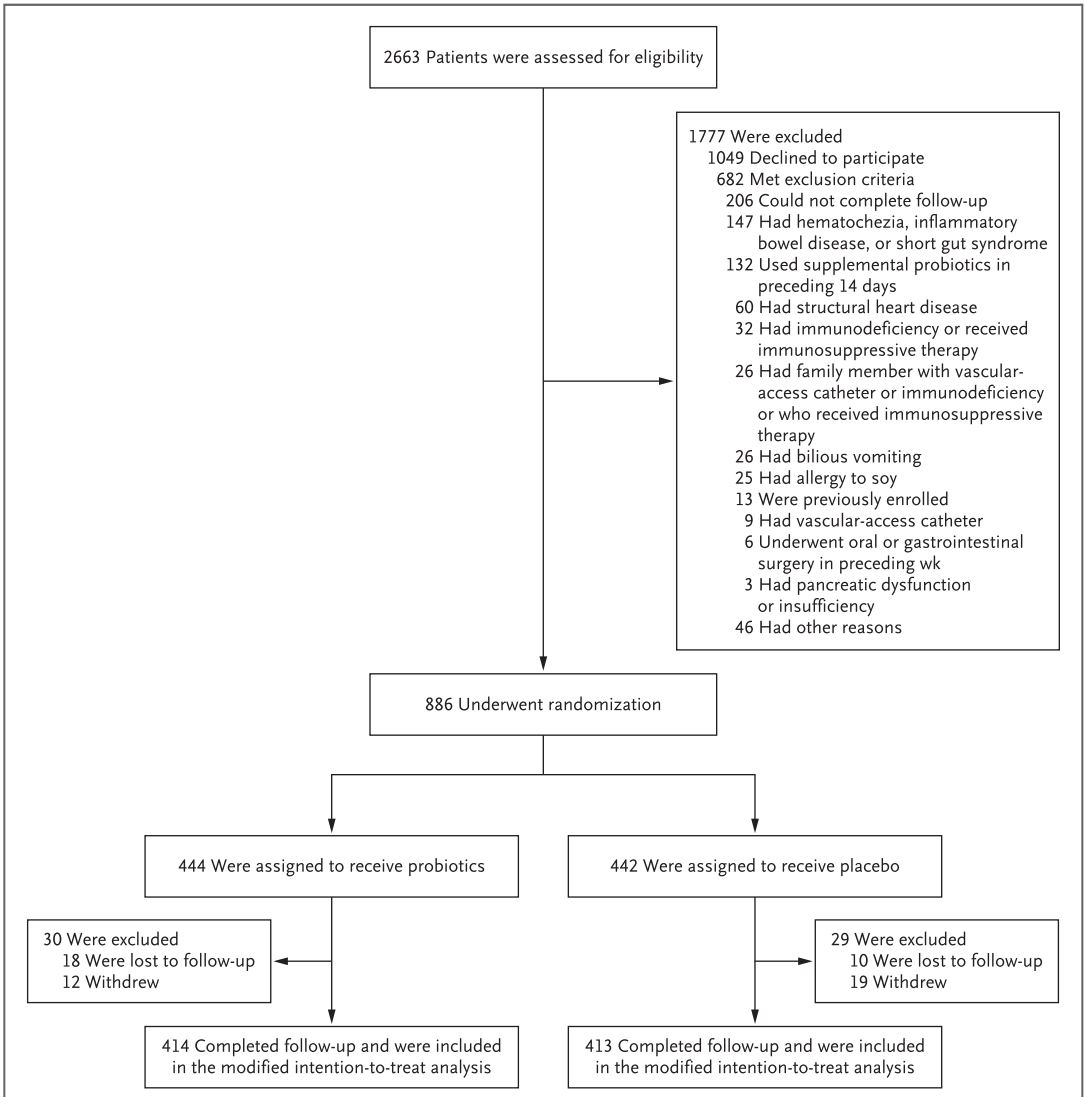

Figure 1. Enrollment, Randomization, and Outcomes.
Some participants met more than one criterion for exclusion.

test.[30] Exploratory analyses are described in the Supplementary Appendix.

RESULTS

PARTICIPANTS

From November 5, 2013, through April 7, 2017, a total of 886 participants were enrolled and underwent randomization (Fig. 1). A total of 414 of the 444 participants who were assigned to receive probiotics (93.2%) and 413 of the 442 participants who were assigned to receive placebo (93.4%) completed follow-up. Among the participants for whom data on the number of doses administered could be evaluated, the percentage of participants who received more than 70% of the doses prescribed did not differ significantly between the groups (295 of 383 participants [77.0%] in the probiotic group and 303 of 378 participants [80.2%] in the placebo group). Rotavirus A infection was identified more often in participants in the probiotic group than in the placebo group; otherwise, the trial groups were well matched with respect to baseline character-

Table 2. Baseline Characteristics of the Enrolled Participants.*

Characteristic	Probiotic Group (N=440)	Placebo Group (N=437)
Median age (IQR) — mo	16.0 (10.0–24.8)	15.0 (9.5–24.0)
Male sex — no. (%)	243 (55.2)	252 (57.7)
Median weight (IQR) — kg	10.6 (9.0–13.0)	10.7 (8.8–12.6)
Exclusively breast-fed — no. (%)	23 (5.2)	32 (7.3)
Received antibiotics in previous 14 days — no. (%)	56 (12.7)	63 (14.4)
Received rotavirus vaccine — no. (%)	214 (48.6)	213 (48.7)
Median duration of illness (IQR) — hr†	42.5 (26.7–58.1)	43.8 (27.7–58.8)
Median modified Vesikari score (IQR)‡	10 (9–12)	10 (8–12)
Vomiting — no. (%)	345 (78.4)	327 (74.8)
Median no. of vomiting episodes in preceding 24 hr (IQR)§	5 (3–8)	5 (2–8)
Median no. of diarrhea episodes in preceding 24 hr (IQR)	6 (4–8)	6 (4–9)
Febrile — no. (%)¶	198 (45.0)	196 (44.9)
Median clinical dehydration scale score (IQR)‖	1 (0–2)	0 (0–2)
Received ondansetron at index visit — no./total no. (%)	100/440 (22.7)	91/437 (20.8)
Received intravenous rehydration at index visit — no./total no. (%)	40/440 (9.1)	33/437 (7.6)
Admitted to hospital at index visit — no./total no. (%)	11/439 (2.5)	11/437 (2.5)
Stool testing results — no./total no. (%)**		
Norovirus GI or GII	102/432 (23.6)	124/428 (29.0)
Rotavirus A	124/432 (28.7)	85/428 (19.9)
Clostridium difficile toxin A or B	51/432 (11.8)	61/428 (14.3)
Adenovirus 40 or 41	50/432 (11.6)	45/428 (10.5)
Salmonella	11/432 (2.6)	9/428 (2.1)

* No significant differences were observed between the groups in any of the baseline characteristics, with the exception of positivity for rotavirus infection (P=0.003). However, after adjustment for pairwise comparisons of 12 pathogens, none of the differences remained significant. For variables for which data were missing, summary data are based on the adjusted number. Four participants in the probiotic group and five in the placebo group withdrew from the trial before they provided baseline demographic information. Additional data are provided in Table S9 in the Supplementary Appendix. IQR denotes interquartile range.

† This variable was defined according to the duration of vomiting or the duration of diarrhea before enrollment, whichever was greater.

‡ Scores on the modified Vesikari scale range from 0 to 20, with higher scores indicating greater disease severity.[13,14]

§ The denominator for this variable was the number of children who had vomiting.

¶ Febrile was defined as a documented adjusted rectal temperature of at least 38.0°C.

‖ Scores on the clinical dehydration scale range from 0 to 8, with higher scores indicating more severe dehydration.[31,32]

** A participant may have tested positive for more than one pathogen; all detected pathogens are reported. Results are reported for the children from whom submitted specimens were obtained for analysis. Only pathogens identified in more than 10 participants per trial group are listed.

istics (Table 2), discharge diagnoses (Table S1 in the Supplementary Appendix), and coadministered medications (Table S2 in the Supplementary Appendix).

PRIMARY OUTCOME

The percentage of participants who had a modified Vesikari score of 9 or higher after enrollment was similar in the two groups (26.1% in the probiotic group [108 of 414 participants] and 24.7% in the placebo group [102 of 413 participants]; difference, 1.4 percentage points; 95% confidence interval [CI], −4.5 to 7.3; P=0.65). Regression analysis with adjustment for trial site showed no benefit of probiotic use (odds ratio, 1.06; 95% CI, 0.77 to 1.46; P=0.72) (Table 3). In a multivariable analysis, trial-group assignment did not predict moderate-to-severe gastroenteri-

Table 3. Trial Outcomes and Subgroups.*

Outcome and Subgroup	Probiotic Group	Placebo Group	Odds Ratio (95% CI)	P Value
Primary efficacy outcome: modified Vesikari score of ≥9†‡				
All participants — no./total no. (%)	108/414 (26.1)	102/413 (24.7)	1.06 (0.77–1.46)	0.72
Age <1 yr — no./total no. (%)	45/134 (33.6)	48/150 (32.0)	1.01 (0.60–1.71)	0.97
Exclusively breast-fed — no./total no. (%)	7/22 (31.8)	10/31 (32.3)	0.82 (0.18–3.61)	0.79§
Receipt of antibiotics within 14 days before index visit — no./total no. (%)	12/51 (23.5)	17/59 (28.8)	0.86 (0.35–2.11)	0.74¶
Adherence to trial regimen, defined as having received >70% of doses prescribed — no./total no. (%)	72/295 (24.4)	66/303 (21.8)	1.16 (0.79–1.71)	0.45
Secondary efficacy outcomes				
Median duration of diarrhea in 827 participants (IQR) — hr	52.5 (18.3–95.8)	55.5 (20.2–102.3)		0.31
Median duration of vomiting in 409 participants (IQR) — hr‖	17.7 (0–58.6)	18.7 (0–51.6)		0.18
Visit to health care provider — no./total no. (%)†	125/414 (30.2)	110/413 (26.6)	1.19 (0.87–1.62)	0.27
Any adverse event — no./total no. (%)**	136/414 (32.9)	152/413 (36.8)	0.83 (0.62–1.11)	0.21
Tertiary efficacy outcomes				
Median no. of days of day care missed in 331 participants (IQR)††	1.0 (0–2.0)	1.0 (0–2.0)		0.55
Median no. of hours of work missed by parent or guardian of 653 participants (IQR)‡‡	0 (0–8.0)	0 (0–8.8)		0.18
Repeat visit to ED				
No. of participants/total no. (%)†	83/414 (20.0)	76/413 (18.4)	1.11 (0.77–1.60)	0.56
With administration of intravenous fluid — no./total no. (%)†	36/414 (8.7)	26/413 (6.3)	1.57 (0.75–3.28)§§	0.23
With hospitalization — no./total no. (%)†	33/414 (8.0)	22/413 (5.3)	1.65 (0.66–4.12)¶¶	0.28

* CI denotes confidence interval, and ED emergency department.
† This outcome was analyzed with the use of logistic regression, and the model was adjusted for the enrollment site.
‡ Scores on the modified Vesikari scale range from 0 to 20, with higher scores indicating greater disease severity.
§ The regression analysis excluded participants from three sites, since all exclusively breast-fed children at these sites were either in one group (either probiotic or placebo) or had the outcome of interest.
¶ The regression analysis excluded five participants from one site, since they all had the outcome of interest.
‖ Data include only participants with three or more episodes of vomiting in the 24 hours before enrollment.
** This outcome was analyzed with the use of logistic regression, and the model was adjusted for enrollment site; however, no imputation was performed for participants with missing data on adverse events.
†† These data, which include only children who attended day care, were analyzed with the use of the van Elteren test stratified according to enrollment site. No imputation was performed for participants with missing data on day-care absenteeism. Attendance in day care was defined as being cared for at least 2 half days (2.5 hours per day) per week by a relative or nonrelative, in a child care center, at home, or in someone else's home where there were, on average, a minimum of three children, including the index child.
‡‡ These data, which include only parents or guardians who worked, were analyzed with the use of the van Elteren test stratified according to enrollment site. No imputation was performed for participants with missing data on work absenteeism.
§§ The regression analysis excluded participants from a single site, since none of the 11 children who had a repeat visit to the ED at this site received intravenous fluids.
¶¶ The regression analysis excluded participants from three sites, since none of the 23 children who had a repeat visit to the ED at these sites were admitted to the hospital.

tis (odds ratio, 1.06, 95% CI, 0.76 to 1.49; P=0.74) (Table S3 in the Supplementary Appendix).

No significant difference was observed in the percentage of participants with a modified Vesikari score of 9 or higher in any of the subgroups defined a priori (Table S4 in the Supplementary Appendix). There was no interaction between trial-group assignment and the age of the participants (P=0.72), antibiotic use in the preceding 14 days (P=0.80), exclusive breast-feeding (P=0.57), and receipt of more than 70% of the doses prescribed (P=0.59). The interaction between detection of rotavirus in stool and trial group was not significant (P=0.99) (Table S5 in the Supplementary Appendix). When the modified Vesikari score was analyzed as a continuous variable, there was

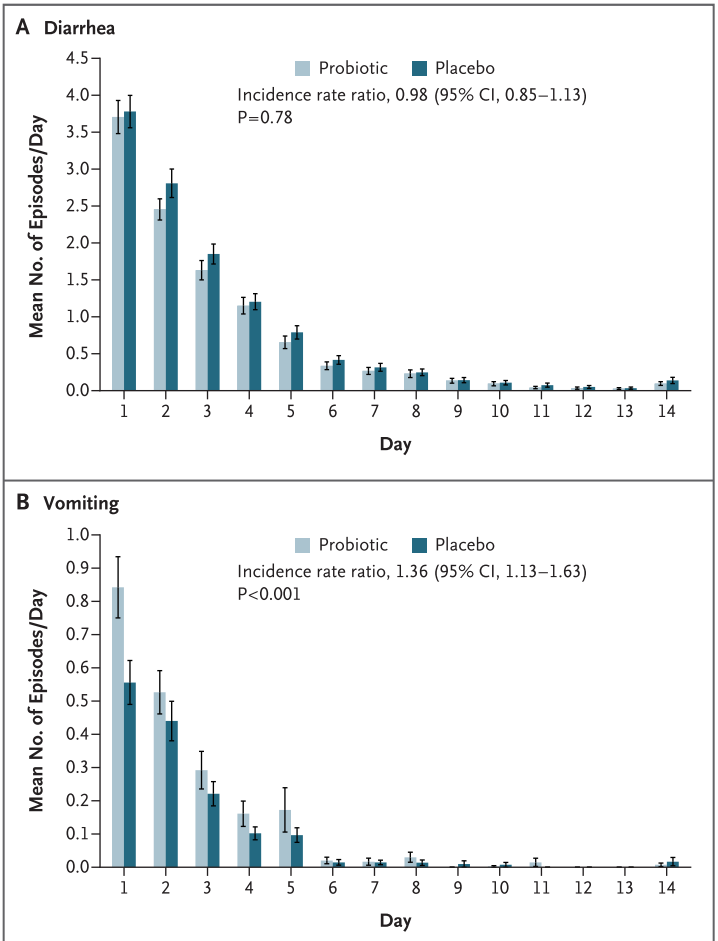

Figure 2. Episodes of Diarrhea and Vomiting, According to Trial Group.

Shown are the mean numbers of episodes of diarrhea (Panel A) and vomiting (Panel B) per 24-hour period after enrollment. Data on all participants are included, irrespective of the number of vomiting episodes that occurred before enrollment. I bars denote the standard error.

no significant difference between the trial groups (mean [±SD], 6.0±4.6 in the probiotic group and 5.8±4.4 in the placebo group; P=0.44). There was no evidence of benefit of probiotics according to the pathogen identified (Table S6 in the Supplementary Appendix).

SECONDARY OUTCOMES

No significant difference between the groups was found with regard to the median duration of diarrhea (52.5 hours [interquartile range, 18.3 to 95.8] in the probiotic group and 55.5 hours [interquartile range, 20.2 to 102.3] in the placebo group) and vomiting (17.7 hours [interquartile range, 0 to 58.6] in the probiotic group and 18.7 hours [interquartile range, 0 to 51.6] in the placebo group) (P=0.31 and P=0.18, respectively) (Table 3). Although the total number of episodes of diarrhea did not differ significantly between the groups (incidence rate ratio, 0.98; 95% CI, 0.85 to 1.13; P=0.78) (Fig. 2A), the number of episodes of vomiting was significantly higher in the probiotic group than in the placebo group (incidence rate ratio, 1.36; 95% CI, 1.13 to 1.63; P<0.001) (Fig. 2B). The percentage of children who had unscheduled health care visits did not differ significantly between the groups (30.2% [125 of 414 children] in the probiotic group and 26.6% [110 of 413 children] in the placebo group; odds ratio, 1.19; 95% CI, 0.87 to 1.62; P=0.27).

An adverse event was reported in 34.8% (144 of 414) of the participants who received probiotics and 38.7% (160 of 413) of the participants who received placebo (odds ratio, 0.83; 95% CI, 0.62 to 1.11; P=0.21) (Table S7 in the Supplementary Appendix). Two children in the placebo group had serious adverse events. One had a febrile seizure 6 hours after receiving the first dose of the trial agent and 1 received a diagnosis of Kawasaki disease 3 days after enrollment.

EXPLORATORY ANALYSES

There was no evidence of an interaction between trial group and the duration of symptoms at enrollment (P=0.54), detection of bacteria in the stool (P=0.86), or modified Vesikari score before enrollment (P=0.86). However, the modified Vesikari scale score before enrollment was associated with the primary outcome in the regression model (odds ratio, 1.14; 95% CI, 1.06 to 1.23; P=0.001). None of the results were significantly altered when the outcome of severe disease (i.e., a modified Vesikari score of ≥11) was considered (Table S8 in the Supplementary Appendix).

DISCUSSION

In this trial involving children who had had symptoms of gastroenteritis for up to 72 hours and presented to the ED, a 5-day course of twice-daily administration of a combined probiotic formulation (4.0×10^9 CFU of a combination *L. rhamnosus* and *L. helveticus*) did not prevent the development of moderate-to-severe gastroenteritis. Among these children with predominantly viral infection, probiotics did not result in benefits

related to secondary outcomes. Adjustment for potential risk factors did not alter the findings.

Although the quality of evidence has been deemed by Szajewska et al. to be "low" or "very low,"[33] many experts consider acute infectious diarrhea to be the main indication for probiotic use.[7] Guideline recommendations vary from "not recommended" by the Centers for Disease Control and Prevention and the National Institute for Health and Care Excellence[34,35] to "strongly recommended" by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition.[36] These recommendations are largely based on meta-analyses such as a 2010 Cochrane review.[37] Although the authors of this review identified 63 eligible studies, they deemed only 10 to be methodologically adequate. The main finding of the review was a reduction of 25 hours in the mean duration of diarrhea; however, there was significant heterogeneity (assessed with I^2 values) that may have been due to differences in the trial populations. Seven of the included studies recruited outpatients, and none of the studies were performed in the United States or Canada. A previous North American, ED-based study that included 155 participants[12] showed no significant effect of another probiotic, *L. rhamnosus* GG, on any outcomes identified a priori.

The aforementioned Cochrane review showed a reduction of 29 hours in the duration of diarrhea among children with rotavirus infection.[37] In vitro and in vivo studies have revealed potential mechanisms of probiotic action against rotavirus, including the production of antimicrobial substances, stimulation of antimicrobial peptides and local adaptive and innate immune responses, and epithelial-cell mucin production.[38] Nonetheless, no beneficial effect of probiotics was observed in this subgroup in our trial.

Rather than emphasizing a single symptom,[39] we focused on the overall severity of a constellation of symptoms associated with gastroenteritis, quantified with the use of the modified Vesikari scale score. This approach quantitatively balances the frequency against the duration of symptoms. Analysis of a composite severity measure and of individual symptoms and subgroups of participants who were adherent to the trial regimens, as well as other measures of effectiveness, showed no significant difference between the groups and consistently showed no benefit of the probiotic strains at the dose evaluated. Although we studied only a single titer, the amount selected was higher than that recommended by the manufacturer,[40] and at the end of their shelf life, we confirmed that organism counts were in the target range (6.13×10^9 to 9.36×10^9 CFU per sachet); thus, underdosing was unlikely.

Differences in preparations of probiotics may account for differences in outcomes across studies. The strain ratio (95:5) and dose of the *L. rhamnosus* R0011 and *L. helveticus* R0052 used in our trial were based on data from previous studies that showed them to be the most economical means to achieve the maximum benefit. This combination was selected for this trial because the strains have been evaluated for safety[41] and efficacy in animal models and in small clinical trials involving humans.[42] In vitro, the strains have been shown to have benefits, including exopolysaccharide production,[43] adhesion and barrier function,[44] pathogen inhibition,[45] and immune response modulation.[46] In humans, the R0011 strain survives gut transit,[47] and it is approved by Health Canada to treat antibiotic-associated and acute infectious diarrhea. The R0011 strain contains the *L. rhamnosus* GG genes encoding the soluble proteins p75 and p40, which promote signaling pathways that are specific to intestinal epithelial homeostasis.[48] However, R0011 differs from GG in pilus gene clusters; thus, the two strains produce different functional pili.[48]

Our trial has several limitations. Although we performed daily follow-up to maximize accuracy in the ascertainment of outcomes, we cannot rule out recall bias. The use of composite outcome measures has been questioned, since they may be subject to inconsistent and selective reporting and post hoc modifications.[49] To overcome these concerns, we selected the modified Vesikari scale, which has face, content, and construct validity and is externally validated.[13,14] Analysis of all individual score elements supported the conclusions based on our primary outcome. Since we used a specific probiotic product and dose, the conclusions cannot be generalized to all products on the market. However, large, well-conducted clinical trials[50-52] have aroused similar concerns regarding the effectiveness of probiotics for other conditions. Nonetheless, there may be specific indications and populations that will benefit from alternative probiotic agents.[53] Since our trial was conducted in Canadian EDs, the findings should be interpreted in that context

with consideration given to local host microbiomes and infectious pathogens. Our findings cannot be extrapolated to longer-term use of probiotics or other outcomes such as stunting in regions where bacterial and protozoal infections are more common.[54]

In conclusion, we found that a twice-daily, 5-day course of 4.0×10^9 CFU of a combined *L. rhamnosus* and *L. helveticus* probiotic did not prevent the development of moderate-to-severe gastroenteritis within 14 days after enrollment in infants and young children who had had symptoms of gastroenteritis for up to 72 hours and had been brought for care in the emergency department.

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APPENDIX

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