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Authors

Freedman, Stephen B
Finkelstein, Yaron
Pang, Xiao Li
et al.

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MAJOR ARTICLE

Pathogen-Specific Effects of Probiotics in Children With Acute Gastroenteritis Seeking Emergency Care: A Randomized Trial

Stephen B. Freedman,[1] Yaron Finkelstein,[2] Xiao-Li Pang,[3,4] Linda Chui,[3,4] Phillip I. Tarr,[5] John M. VanBuren,[6] Cody Olsen,[6] Bonita E. Lee,[7] Carla A. Hall-Moore,[5] Robert Sapien,[8] Karen O'Connell,[9] Adam C. Levine,[10] Naveen Poonai,[11] Cindy Roskind,[12] Suzanne Schuh,[13] Alexander Rogers,[14] Seema Bhatt,[15] Serge Gouin,[16] Prashant Mahajan,[17] Cheryl Vance,[18] Katrina Hurley,[19] Elizabeth C. Powell,[20] Ken J. Farion,[21] and David Schnadower[15]; on behalf of Pediatric Emergency Research Canada (PERC) and the Pediatric Emergency Care Applied Research Network (PECARN)

[1]Sections of Pediatric Emergency Medicine and Gastroenterology, Alberta Children's Hospital, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Canada; [2]Divisions of Pediatric Emergency Medicine and Clinical Pharmacology and Toxicology, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; [3]Alberta Precision Laboratories-Public Health Laboratory, Alberta, Canada; [4]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada; [5]Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA; [6]Department of Pediatrics, University of Utah, Salt Lake City, Utah, USA; [7]Department of Pediatrics, University of Alberta, Women and Children's Health Research Institute, Edmonton, Alberta, Canada; [8]Department of Emergency Medicine, University of New Mexico Health Sciences Center, Albuquerque, New Mexico, USA; [9]Departments of Pediatrics and Emergency Medicine, The George Washington University School of Medicine and Health Sciences, Division of Emergency Medicine, Children's National Hospital, Washington D.C., USA; [10]Department of Emergency Medicine, Rhode Island Hospital/ Hasbro Children's Hospital and Brown University, Providence, Rhode Island, USA; [11]Departments of Pediatrics, Internal Medicine, Epidemiology and Biostatistics, Schulich School of Medicine & Dentistry, London, Ontario, Canada; [12]Department of Emergency Medicine, Columbia University Medical Center, New York, New York, USA; [13]Division of Pediatric Emergency Medicine, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; [14]Departments of Emergency Medicine and Pediatrics. Michigan Medicine, University of Michigan, Ann Arbor, Michigan, USA; [15]Division of Emergency Medicine, Cincinnati Children's Hospital Medical Center and Department of Pediatrics University of Cincinnati College of Medicine, Cincinnati, Ohio, USA; [16]Departments of Pediatric Emergency Medicine & Pediatrics, Université de Montréal, Montréal, Quebec, Canada; [17]Department of Emergency Medicine and Pediatrics, University of Michigan, Ann Arbor, Michigan, USA; [18]Departments of Emergency Medicine and Pediatrics, UC Davis, School of Medicine, Sacramento, California, USA; [19]Department of Emergency Medicine, IWK Health, Dalhousie University, Halifax, Nova Scotia, Canada; [20]Department of Pediatrics, Division of Emergency Medicine, Ann & Robert H. Lurie Children's Hospital of Chicago, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA; and [21]Departments of Pediatrics and Emergency Medicine, University of Ottawa, Ottawa, Canada

Background. It is unknown if probiotics exert pathogen-specific effects in children with diarrhea secondary to acute gastroenteritis.

Methods. Analysis of patient-level data from 2 multicenter randomized, placebo controlled trials conducted in pediatric emergency departments in Canada and the United States. Participants were 3–48 months with >3 diarrheal episodes in the preceding 24 hours and were symptomatic for <72 hours and <7 days in the Canadian and US studies, respectively. Participants received either placebo or a probiotic preparation (Canada-*Lactobacillus rhamnosus* R0011/*Lactobacillus helveticus* R0052; US-*L. rhamnosus* GG). The primary outcome was post-intervention moderate-to-severe disease (ie, ≥9 on the Modified Vesikari Scale [MVS] score).

Results. Pathogens were identified in specimens from 59.3% of children (928/1565). No pathogen groups were less likely to experience an MVS score ≥9 based on treatment allocation (test for interaction = 0.35). No differences between groups were identified for adenovirus (adjusted relative risk [aRR]: 1.42; 95% confidence interval [CI]: .62, 3.23), norovirus (aRR: 0.98; 95% CI: .56, 1.74), rotavirus (aRR: 0.86; 95% CI: .43, 1.71) or bacteria (aRR: 1.19; 95% CI: .41, 3.43). At pathogen-group and among individual pathogens there were no differences in diarrhea duration or the total number of diarrheal stools between treatment groups, regardless of intervention allocation or among probiotic sub-groups. Among adenovirus-infected children, those administered the *L. rhamnosus* R0011/*L. helveticus* R0052 product experienced fewer diarrheal episodes (aRR: 0.65; 95% CI: .47, .90).

Conclusions. Neither probiotic product resulted in less severe disease compared to placebo across a range of the most common etiologic pathogens. The preponderance of evidence does not support the notion that there are pathogen specific benefits associated with probiotic use in children with acute gastroenteritis.

Clinical Trials Registration. NCT01773967 and NCT01853124.

Keywords. child; probiotic; gastroenteritis; diarrhea; emergency service; hospital.

Acute gastroenteritis (AGE) remains a major cause of morbidity and mortality around the globe. Although treatment focuses on providing supportive care, some have advocated for probiotic administration to mitigate AGE severity [1–3]. However, the literature regarding probiotic use in children with AGE-related diarrhea is conflicting, with large negative trials [4, 5] challenging conclusions of meta-analyses [2]. Although recent guidelines no longer recommend probiotic administration to children with AGE [6], it remains a contentious issue [7].

Before the advent of rotavirus vaccines, rotavirus was the predominant etiology of severe AGE in children. However, by 2010, norovirus had become the dominant cause of

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Correspondence: S. Freedman, Alberta Children's Hospital Foundation Professor in Child Health and Wellness, Alberta Children's Hospital Research Institute, Cumming School of Medicine, University of Calgary, 28 Oki Dr NW, Calgary, Alberta T3B 6A8, Canada (Stephen.freedman@ahs.ca).

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medically-attended AGE in children [8]. This etiologic shift may explain why studies conducted before 2010 report reduced diarrhea duration associated with probiotic administration while more recent studies demonstrate no such benefit [9, 10]. In the most recent Cochrane review, the authors suggested that future studies identify etiologic pathogens to permit pathogen-specific analyses [10].

Although many effective therapies benefit only a minority of those treated, all treated patients are exposed to treatment-associated costs and harms [11]. Probiotic use in children with diarrhea is no exception, with decades of conflicting results in need of subgroup evaluations to provide clarity [12]. Recent pediatric AGE probiotics trials [4, 5] present an opportunity to evaluate potential explanations for divergent results as nucleic acid-based diagnostics permit the identification of pathogens in most children with AGE [13]. Here we offer a patient-level, etiology-informed analysis to confirm or refute the value of pathogen-specific use of probiotics in AGE.

METHODS

Design and Oversight

This was a planned secondary analysis of the Pediatric Emergency Research Canada (PERC) [4, 14] (6 sites) and Pediatric Emergency Care Applied Research Network (PECARN) [5, 15] (10 sites) multi-center, randomized, placebo-controlled probiotic trials in children with AGE-associated diarrhea, conducted between November 2013 and June 2017. Institutional Research Board approval was obtained at each study site (University of Calgary Research Ethics Board study no. 13-0045), and informed consent was obtained from each participant's family.

Participants

Eligible children were 3–48 months of age whose caregivers reported >3 watery stools in the preceding 24 hours and were diagnosed as having an acute intestinal infection in a participating emergency department (ED). Exclusion criteria included: hematochezia; bilious emesis; pancreatitis; chronic gastrointestinal disease; structural heart disease; indwelling vascular access line; immunotherapy or history of immunodeficiency; inability to be contacted for daily follow-up while symptomatic; previous enrolment in the trials; supplemental probiotic use in the preceding 14 days; critically ill; known allergy to the investigational product, placebo, or the antibiotics that would be employed to treat invasive infection by the probiotic; and presence of a household member with an indwelling vascular access line, or who is immunocompromised. The longest permissible duration of diarrhea prior to enrolment was 72 hours and 7 days in the PERC and PECARN trials, respectively. The decision to extend the eligibility window in the PECARN study was because an earlier study reported that benefits might be greatest in children with more prolonged diarrhea [16]. Complete study protocols are published [14, 15].

Outcomes

The primary outcome was moderate-to-severe gastroenteritis, defined as a post-index emergency department (ED) visit score ≥9 on the Modified Vesikari Scale (MVS) score stratified by pathogen-group and individual pathogens. MVS scores range from 0 to 20, with higher scores indicating more severe disease; Supplementary Table 1 [17, 18]. The score is calculated by adding the highest scores assigned to each of seven component variables, based on symptoms reported following enrolment through to symptom resolution or day 14, whichever occurred first.

Secondary outcomes, specified a priori, included: (1) repeat ED visits within 7 days of enrolment, reported by caregivers or identified during medical record review, due to AGE-related symptoms; (2) time to last diarrheal stool (ie, diarrhea duration) defined by the interval between administration of the first probiotic dose and the final liquid stool (reported as date-time variables); and (3) total number of diarrheal stools after randomization. For all outcomes, the illness was deemed to have ended after any 24-hour interval without any diarrhea or vomiting events.

Investigational Products

PERC participants received a 5-day course of a combined *Lactobacillus rhamnosus* R0011/*Lactobacillus helveticus* R0052 product (Lacidofil Strong®, Lallemand Health Solutions, Quebec, Canada), containing a total of 4.0×10^9 colony-forming units (CFU), administered twice daily. PECARN participants received a course of 5 days of twice daily *L. rhamnosus* GG (Culturelle®, i-Health Inc., Connecticut, United States), containing 1.0×10^{10} CFU or placebo. Both trials employed 1:1 allocation ratios and ensured that the sachets/capsules containing placebo and probiotics were identical in appearance, smell, texture and weight. In addition, investigational contents were tested intermittently throughout the trials to confirm product stability and CFU counts.

Randomization, Masking, and Intervention

As previously described [4, 5], random-number–generating software (www.randomize.net), which used permuted blocks with random block sizes, stratified according to site and symptom duration (PECARN trial only), was used to allocate children to probiotics or placebo. Assignment sequences were restricted to the research pharmacy at the coordinating center (PERC), the Data Coordinating Center (PECARN), 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. After assignment, participants received the first study

medication dose in the ED and caregivers received instructions for administering subsequent doses. To minimize recall bias, parents or guardians completed electronic or telephone follow-up surveys every 24 hours until both vomiting, and diarrhea had ceased for 24 hours.

Stool Testing

Rectal swabs, stool specimens, or both were obtained, as available, during the enrollment visit [13]. Bacterial culture was performed locally. A multiplex nucleic acid panel that detects 15 enteric viruses, bacteria, and parasites (Luminex xTAG Gastrointestinal Pathogen Panel, Luminex Corporation, Austin, Texas United States) was performed at the Alberta Precision Laboratories-Public Health Laboratory in Edmonton, Alberta, Canada and Washington University, in St. Louis, Missouri [19].

Statistical Analysis

All analyses were prespecified. We included data from all participants who underwent randomization and for whose stool was tested for an etiologic enteropathogen (ie, per protocol analysis). To find pathogen-specific effects, we included treatment and etiological agent in all analyses. To perform pathogen-level analyses with sufficient numbers of events in each strata, etiological agents were grouped as negative, bacteria (including bacteria/bacteria codetection), adenovirus serotypes 40/41, norovirus genogroups I/II, rotavirus group A, and "other" etiology. Isolated *Clostridioides difficile* detection in children <2 years of age was classified as negative [20]. The "other" group included virus/bacteria codetection, parasites, and parasite/virus or parasite/bacteria co-detection.

We fit modified Poisson regression models to test for a treatment-by-etiological agent interaction, and to estimate relative risks of post-enrolment MVS scores ≥9 comparing treatment and conditioned on etiological agent. To maximize power [21], after the number of diarrheal stools in the preceding 24-hours was removed due to colinearity, adjustment was performed for the following a priori identified baseline co-variates: symptom duration (<24 hours vs 24–<48 hours vs 48–96 hours vs >96 hours), ED ondansetron administration, pre-enrollment (ie, includes symptoms reported at the time of the ED enrolment visit) MVS scores as a continuous variable, and clinical dehydration scale [22] score at the enrollment ED visit, and age. Correlation within enrolling site was adjusted for using generalized estimating equation (GEE) methods and an exchangeable working correlation structure. The *P*-value for an interaction term was obtained from a type-3 test with 10 degrees of freedom, which tested for evidence of a differential effect of either investigational product among the infectious agents. Relative risks and 95% Bonferroni confidence intervals, averaging the effect of the two investigational products, were estimated for each of the six etiological agent strata. The Bonferroni method generated 6 separate 99.2% Confidence Intervals (CIs). To estimate etiological-agent-specific treatment effects for both treatments, separate relative risks and 95% Bonferroni CIs were estimated for the product/etiological agent combinations, resulting in 12 separate 99.6% CIs. This analysis was repeated to assess the dichotomous secondary outcome of ED revisits within 7 days. The effects of treatment and AGE pathogens and their interaction on the continuous outcomes of time until last diarrhea stool and number of diarrheal stools were assessed using negative binomial regression models fit with GEE and using the same covariates as the primary model. This approach was selected as the aforementioned outcome variables were treated as counts. Time-to-event models would have been selected if censoring was a concern; however, that is not the case with our data. Use of GEE allows us to account for clustering within sites, use of a logarithmic link function to account for the skewness of the outcomes, and provides *P*-values for the treatment/etiological agent interaction along with rate ratios and 95% Bonferroni CIs. We compared the fit of these negative binomial models to competing log-linear regression models using Quasi information criterion statistics.

Multiple imputation was performed separately on each of the two trial datasets to account for missing data as described in their primary analyses [4, 5]. Imputation models assumed that data were missing at random and included key baseline characteristics, trial group, and all efficacy outcomes. Results from 10 imputed datasets were combined using standard methods [23]. Two-tailed *P*-values < .05 were considered statistically significant. Analyses were performed using SAS/STAT software, version 9.4 (SAS Institute).

RESULTS

The trials enrolled 1857 participants of whom 1565 (84.3%) completed follow-up, provided a specimen for enteropathogen analysis, and constitute our study population (Figure 1). Of the 1565 study participants, 787 (50.3%) were allocated to placebo arms, 370 (23.6%) to *L. rhamnosus* GG, and 408 (26.1%) to the *L. rhamnosus* R0011/*L. helveticus* R0052 product (Table 1; Supplementary Table 2). An enteropathogen was identified in specimens from 928 (59.3%) children, most commonly norovirus (23.2%; 363/1565), rotavirus (21.0%; 328/1565), and adenovirus (10.4%; 162/1565).

Primary Outcome

The proportion of participants who most commonly experienced moderate-to-severe AGE were those infected by rotavirus (24.9%; 72/288) and "other" pathogens (26.8%; 22/81) (Table 2). Those least likely to experience moderate-to-severe AGE were children whose stools contained no detectable pathogen (16.7%; 106/637). In adjusted analyses, no differences between groups were identified for children with adenovirus (adjusted relative risk [ARR]: 1.42; 95% CI: .62, 3.23), norovirus

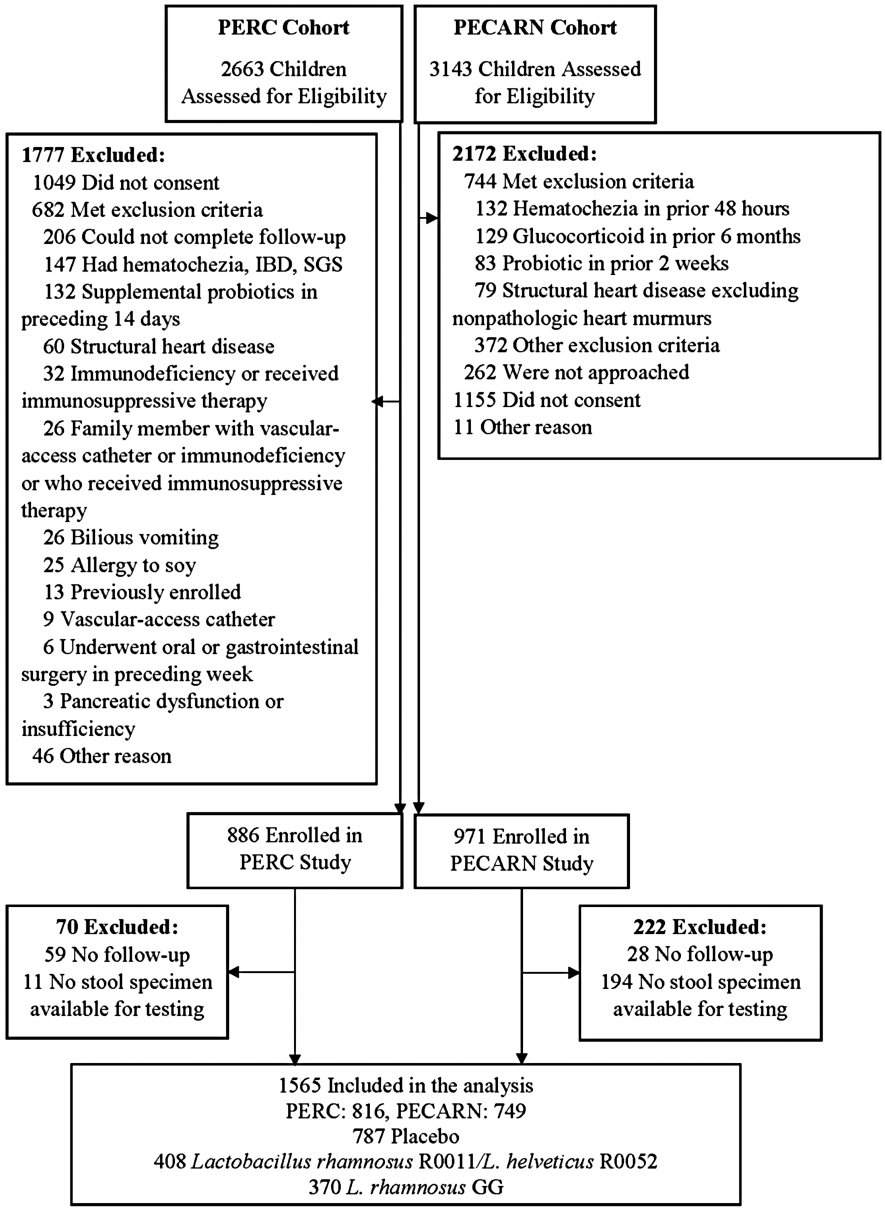

Figure 1. Patient flow diagram. Abbreviations: PERC, Pediatric Emergency Research Canada; PECARN, Pediatric Emergency Care Applied Research Network.

(ARR: 0.98; 95% CI: .56, 1.74), rotavirus (ARR: 0.86; 95% CI: .43, 1.71) or bacteria (ARR: 1.19; 95% CI: .41, 3.43). Although the test for interaction between treatment and organism was not significant ($P = .35$), those with no pathogen detected were less likely to experience the outcome of interest (ie, MVS ≥9) when treated with a probiotic (ARR: 0.73; 95% CI: .54, .99) (Figure 2). When the treatment effect was estimated for individual organisms, the only difference between treatment groups was a reduced risk of developing moderate-to-severe AGE among children with no enteropathogen identified who received *L. rhamnosus* GG compared with placebo (ARR: 0.56; 95% CI: .35, .90) (Figure 3). The effect was not seen with the *L. rhamnosus* R0011/*L. helveticus* R0052 product.

Secondary Outcomes

The proportion of participants who most commonly experienced a repeat ED visit were those infected by rotavirus (13.2%; 38/288); those least likely were infected by norovirus (6.5%; 21/322) (Table 2). Although there was no evidence of interaction ($P = .15$), in adjusted analyses of the treatment effect within individual enteropathogens, among those whose stool had no pathogen identified, children administered a probiotic were less

Table 1. Demographics, Clinical Characteristics and Infectious Etiologies, by Treatment Group

	Placebo (N = 787)	Probiotic (N = 778)	Overall (N = 1565)
Age in months, mean (SD)	18.4 (11.3)	19.1 (11.4)	18.8 (11.3)
Age in months, median (IQR)	15.9 (9.4–25.0)	16.0 (10.0–26.0)	16.0 (10.0–25.9)
Sex: male, N (%)	438 (55.7%)	420 (54.0%)	858 (54.8%)
Country/Study			
Canada PERC study, N (%)	408 (51.8%)	408 (52.4%)	816 (52.1%)
US PECARN study, N (%)	379 (48.2%)	370 (47.6%)	749 (47.9%)
Weight-for-age Z-score, median (IQR)	0.3 (−0.5, 1.1)	0.3 (−0.5, 1.0)	0.3 (−0.5, 1.0)
Has child received any antibiotics in the past 14 days, N (%)	90 (11.5%)	81 (10.4%)	171 (11.0%)
Has child received a vaccine against rotavirus, N (%)	430 (54.6%)	418 (53.7%)	848 (54.2%)
Symptom duration prior to randomization (days), median (IQR)	2.0 (1.2, 2.8)	2.1 (1.2, 2.9)	2.0 (1.2, 2.9)
Clinical dehydration scale score, [22] N (%)			
None (0)	482 (61.2%)	442 (56.8%)	924 (59.0%)
Mild to moderate (1–4)	288 (36.6%)	317 (40.8%)	605 (38.7%)
Severe (5–8)	17 (2.2%)	19 (2.4%)	36 (2.3%)
Baseline MVS score, [17, 18], mean (SD)	11.2 (2.89)	11.3 (2.79)	11.3 (2.84)
Vomiting at presentation, N (%)	597 (75.9%)	596 (76.6%)	1193 (76.2%)
Number of vomiting episodes in the 24 hours prior to randomization, median (IQR)	4.0 (2.0, 6.0)	4.0 (2.0, 6.0)	4.0 (2.0, 6.0)
Number of diarrheal episodes in the 24 hours prior to randomization, median (SD)	5.0 (4.0, 8.2)	5.0 (4.0, 8.0)	5.0 (4.0, 8.0)
Fever (measured or tactile), N (%)	402 (51.1%)	415 (53.3%)	817 (52.2%)
IV fluids administered during ED visit, N (%)	107 (13.6%)	102 (13.1%)	209 (13.4%)
Admitted to the hospital from the ED, N (%)	28 (3.6%)	32 (4.1%)	60 (3.8%)
Infectious etiology, N (%)			
Adenovirus 40/41	85 (10.8%)	77 (9.9%)	162 (10.4%)
Aeromonas spp.	4 (0.5%)	0 (0.0%)	4 (0.3%)
Campylobacter spp.	8 (1.0%)	13 (1.7%)	21 (1.3%)
Clostridioides difficile[a]	6 (0.8%)	3 (0.4%)	9 (0.6%)
Cryptosporidium	4 (0.5%)	8 (1.0%)	12 (0.8%)
Entamoeba histolytica	1 (0.1%)	1 (0.1%)	2 (0.1%)
Enterotoxigenic *E. coli* LT/ST	11 (1.4%)	4 (0.5%)	15 (1.0%)
Giardia	2 (0.3%)	3 (0.4%)	5 (0.3%)
Negative	338 (42.9%)	299 (38.4%)	637 (40.7%)
Norovirus GI/GII	187 (23.8%)	176 (22.6%)	363 (23.2%)
Rotavirus A	138 (17.5%)	190 (24.4%)	328 (21.0%)
Salmonella spp.	12 (1.5%)	15 (1.9%)	27 (1.7%)
Shiga toxin producing *E. coli*	4 (0.5%)	7 (0.9%)	11 (0.7%)
Shigella spp.	18 (2.3%)	25 (3.2%)	43 (2.7%)
Vibrio cholerae	1 (0.1%)	0 (0.0%)	1 (0.1%)

Abbreviations: ED, emergency department, IQR, interquartile range; IV, intravenous; MVS, Modified Vesikari Scale; PERC, Pediatric Emergency Research Canada; PECARN, Pediatric Emergency Care Applied Research Network.

[a]*Clostridioides difficile* detection in children <2 years of age was classified as negative (ie, reflecting colonization).

Table 2. Outcomes Following Enrollment by Identified Enteropathogen

Enteropathogen	N	Moderate-Severe Acute Gastroenteritis (MVS[2] Score ≥ 9) N (%)	Repeat ED Visit Within 7 Days N (%)	Diarrhea Duration (days) Median (IQR)	Number of Diarrhea Episodes Median (IQR)
Norovirus genogroups I/II	322	68 (21.2%)	21 (6.5%)	2.5 (0.9, 4.2)	7.0 (2.0, 15.1)
Rotavirus group A	288	72 (24.9%)	38 (13.2%)	2.2 (0.9, 3.5)	7.1 (3.0, 13.2)
Adenovirus serotypes 40/41	139	29 (20.5%)	13 (9.0%)	2.2 (1.0, 3.4)	7.0 (2.0, 12.3)
Bacteria[a]	98	21 (21.4%)	12 (12.2%)	2.9 (1.8, 4.9)	11.2 (5.0, 25.0)
Other[b]	81	22 (26.8%)	8 (9.9%)	2.4 (0.6, 4.1)	8.7 (2.0, 16.5)
No pathogen identified	637	106 (16.7%)	47 (7.3%)	2.0 (0.8, 3.9)	6.0 (2.0, 12.0)
All study participants	1565	318 (20.3%)	138 (8.8%)	2.2 (0.9, 3.9)	7.0 (2.0, 14.0)

Abbreviations: ED, emergency department; IQR, interquartile range; MVS, Modified Vesikari Scale.

[a]Includes isolated (single agent identified) *Aeromonas* spp., *Campylobacter* spp., Shiga toxin producing *E. coli* (STEC) Stx1/Stx2, *Clostridioides difficile* Toxin A/B, enterotoxigenic *E. coli* LT/ST, *Shigella* spp., *Salmonella* spp. and combinations of those.

[b]Includes virus/bacteria codetection, parasites, parasite/bacteria codetection, and parasite/virus codetection.

likely to experience an ED revisit (ARR: 0.46; 95% CI: 22, .94) (Figure 2). After adjusting for multiple analyses, in all cases, the treatment-pathogen interaction terms were not significant (ie, there is no evidence of pathogen-specific treatment effects) (Figure 3).

Diarrhea duration was longest among those with bacterial enteropathogens (median = 2.9 days; interquartile range [IQR]: 1.8, 4.9) (Table 2). In adjusted analyses, there were no difference between treatment groups, regardless of intervention allocation (Figure 2), or probiotic formulation (Figure 3).

The total number of diarrheal episodes following enrolment was greatest among those with bacterial enteropathogens (median = 11.2 episodes; IQR: 5.0, 25.0) (Table 2). In adjusted analyses, there were no difference between treatment groups overall (Figure 2); however, among those with adenovirus identified, those administered the *Lactobacillus rhamnosus* R0011/*L. helveticus* R0052 combination product experienced fewer diarrheal episode (ARR: 0.65; 95% CI: .47, .90) (Figure 3). Quasi information criterion statistics suggested that the negative binomial models for both diarrhea duration and number of diarrheal episodes were more appropriate than alternative log-linear models.

DISCUSSION

Our planned secondary analysis of children enrolled in 2 large probiotic randomized clinical trials found that when children were grouped based on etiologic enteropathogens identified in

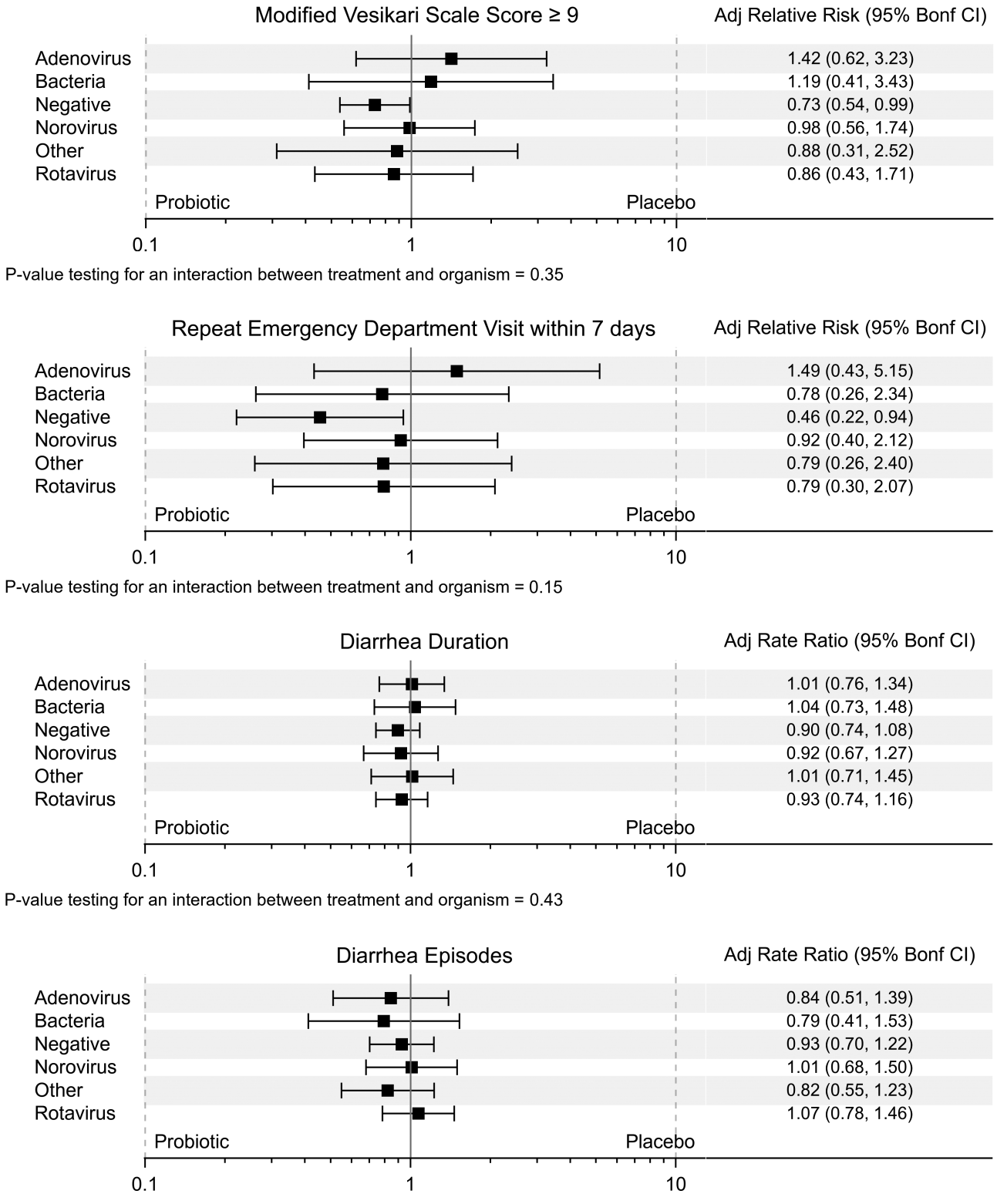

Figure 2. Primary (Modified Vesikari Scale score) and secondary outcomes (emergency department revisits, diarrhea duration and number of episodes) analyzed with probiotic groups combined. Average treatment effect of the 2 probiotic exposures compared to placebo is estimated with 95% Bonferroni confidence intervals. Abbreviation: CI, confidence interval.

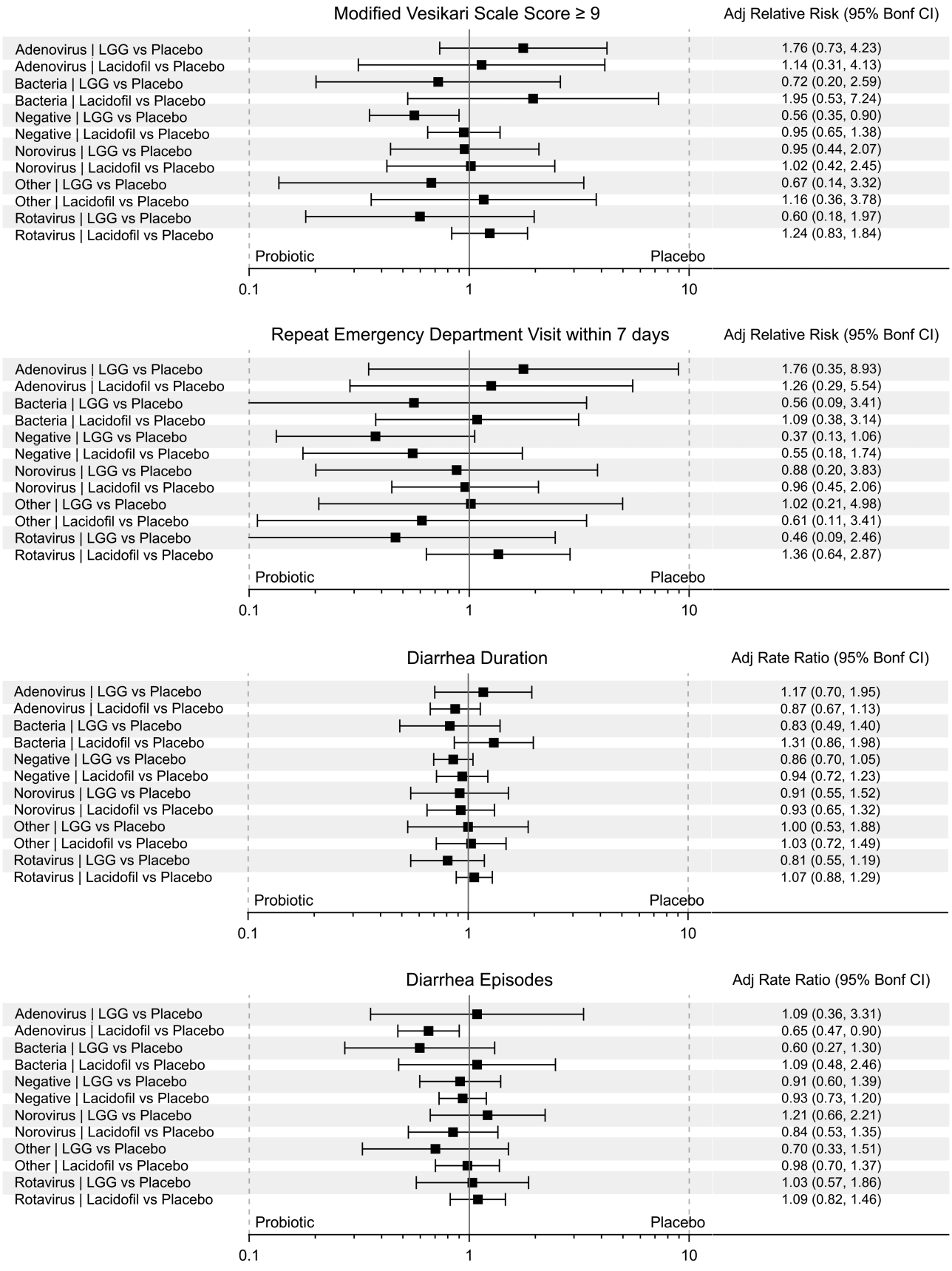

Figure 3. Primary (Modified Vesikari Scale score) and secondary outcomes (emergency department revisits, diarrhea duration and number of episodes) analyzed separately for each of the probiotic treatment groups. Treatment effect of each probiotic product compared to placebo is estimated with 95% Bonferroni CIs. Abbreviations: CI, confidence interval; LGG, *Lactobacillus* GG.

stool, when compared to placebo, no groups of children with identifiable pathogens were more likely to experience reduced AGE severity (ie, MVS score <9). Moreover, we found no interaction between treatment and identified microbial agents. These results indicate that the use of a probiotic in children with AGE, regardless of the type of enteropathogen (ie, bacteria, virus, other), did not reduce symptom severity following initiation compared with placebo.

In the most recent Cochrane review of the effectiveness of probiotics in acute diarrheal illness, rotavirus was the only enteropathogen for which sufficient studies reported outcomes to permit the conduct of a meta-analysis [10]. Their meta-analysis of 1414 children with rotavirus infection, found probiotics reduced diarrhea duration by 22 hours (95% CI: 14, 30), however the studies analyzed had marked heterogeneity (I^2 = 84%) and high risk of biases. Other meta-analyses have reached similar conclusions, calling for further research given the small sample sizes, unclear and inconsistent methodologic quality, and possible reporting bias [24, 25]. Although 54% of study participants were vaccinated against rotavirus, our study still included 328 children with rotavirus infection and found no benefits associated with probiotic use, thereby filling this knowledge gap.

Few studies have investigated the therapeutic potential of probiotics in norovirus infections. A double-blind, placebo-controlled RCT of children hospitalized with acute diarrhea in Vietnam studied *L. acidophilus* versus placebo. Among the 68 norovirus-infected children [26], there were no difference in stool viral load reduction between the intervention and placebo groups. Our study, which includes 363 norovirus-infected participants, extends these findings by adding that no clinical benefit was associated with probiotic administration in this population. Notably, 816 children from the Canadian study underwent viral load analyses, and among those infected with rotavirus or norovirus, there was no evidence of accelerated clearance of stool viral nucleic acid associated with probiotic use up to 28 days after enrolment [27]. Furthermore, fecal IgA concentrations did not differ among children infected with rotavirus or norovirus based on treatment allocation (ie, probiotic vs placebo) [28].

In our study, 41% (N = 637) of participants had no enteropathogen identified. This detection rate is in keeping with use of this diagnostic platform in children seeking ED care in AGE [29]. Although we employed a broad testing algorithm, recently identified candidate enteropathogens in stool (ie, astroviruses, human bocaviruses, polyomaviruses, sapovirus) [30–32] were not sought, and some of these children likely were infected by such pathogens. Our analysis however included a test for interaction term to reduce the probability of falsely claiming a spurious finding truly exists by adjusting for the fact that multiple comparisons were made. However, as the test for interaction was not significant, we can conclude there was no benefit associated with probiotic administration to this group of children.

However, we cannot exclude a role for probiotic therapy against these and other yet to be identified diarrheal pathogens. Pathogen-specific therapy will rely on the near-real time performance of molecular stool analyses. Given the lack of evidence of interaction, the absence of any clinically significant benefit in any of the subgroups analyzed, and the limited access to and the cost of point-of-care testing, such an approach is hard to justify.

Antibiotic use before enrolment was reported in 171 (11%) participants, and in such children, it is challenging to differentiate infectious AGE from antibiotic-associated diarrhea. However, as some of the most robust evidence in support of probiotic use is in regards to antibiotic-associated diarrhea [33], we do not believe the inclusion of children who did receive antibiotics alters our findings in any significant fashion.

In keeping with illness durations routinely seen in clinical practice, study participants had a median duration of diarrhea of 2.0 days. Based on limited evidence, some experts assert that probiotics are more effective when given early in the course of illness [34, 35]. However, more recently it has been shown that the lack of effect of probiotics is not explained by the duration of symptoms before probiotic initiation [36]. Although diarrhea frequency was analyzed as total number of episodes per participant, and not by day of illness, given the effect this would have had on power due to the large number of analyses, the original RCTs did report and analyze diarrhea frequency by day and found no benefit associated with probiotic use [4, 5].

Our study has several limitations. As this was a secondary analysis, we did not conduct formal sample size calculations. Although these two studies employed different probiotics, to maximize study power, we combined the results to produce the largest AGE probiotic RCT database to date, which enhanced the ability to detect a signal should one be present. We limited analyses to groupings with sufficient events in each pathogen group to support the construction of multivariable models that could sustain the pre-specified independent variables. As we also only evaluated two probiotic agents, our findings cannot be generalizable to other probiotics given the unique effects of every candidate agent nor to all individual enteropathogens [12]. We also cannot exclude the possibility that a subset of study participants might not have had a non-infectious etiology for their diarrhea. Finally, approximately 38% of children assessed for participation declined to consent and 16% did not complete follow-up or provide a specimen for enteropathogen analysis. Although we cannot rule out the possibility that this may have led to selection bias, given the blinded, randomized study design, this would be unlikely to have influenced our findings.

In summary, among children 3–48 months of age with AGE presenting to an ED, neither *L. rhamnosus* R0011/*L. helveticus* R0052 nor *L. rhamnosus* GG probiotic treatment resulted in less severe disease compared to placebo across of range of the most common etiologic pathogens. The preponderance of the

evidence does not support the notion that there are pathogen-specific benefits associated with probiotic use in children with AGE.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Contributors statement.

Conceptualization: S. Freedman, X.-L. Pang, L. Chui, P. Tarr, J. VanBuren, Y. Finkelstein, C. Olsen, B. Lee, and D. Schnadower.

Formal analysis: J. VanBuren and C. Olsen.

Data curation: S. Freedman, X.-L. Pang, L. Chui, P. Tarr,

K. Hurley, B. Lee, R. Sapien, C. Hall-Moore, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Funding acquisition: S. Freedman, X.-L. Pang, L. Chui, P. Tarr, Y. Finkelstein, B. Lee, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Investigation: S. Freedman, X.-L.Pang, L. Chui, P. Tarr, K. Hurley, Y. Finkelstein, B. Lee, R. Sapien, C. Hall-Moore, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Methodology: S. Freedman, X.-L. Pang, L. Chui, P. Tarr, J. VanBuren, K. Hurley, Y. Finkelstein, C. Olsen, B. Lee, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Project administration: S. Freedman, X.-L. Pang, L. Chui, P. Tarr, J. VanBuren, K. Hurley, Y. Finkelstein, C. Olsen, B. Lee, R. Sapien, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Resources: S. Freedman, X.-L. Pang, L. Chui, P. Tarr, J. VanBuren, K. Hurley, C. Olsen, B. Lee, R. Sapien, C. Hall-Moore, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, and K. Farion.

Software: J. VanBuren, C. Olsen, and D. Schnadower.

Supervision: S. Freedman, P. Tarr, J. VanBuren, Y. Finkelstein, and D. Schnadower.

Validation: J. VanBuren and C. Olsen.

Writing original draft: S. Freedman.

Writing review and editing: X.-L. Pang, L. Chui, P. Tarr, J. VanBuren, K. Hurley, Y. Finkelstein, C. Olsen, B. Lee, R. Sapien, K. O'Connell, A. Levine, N. Poonai, C. Roskind, S. Schuh, A. Rogers, S. Bhatt, S. Gouin, P. Mahajan, C. Vance, E. Powell, K. Farion, and D. Schnadower.

Data sharing. Individual de-identified participant data that underlie the results reported in this paper (text, tables, figures, and appendices) and the study protocol will be shared if requested. PECARN data will be made publicly available in July 2021 at https://www.pecarn.org/studyDatasets/Default. PERC data will be available beginning 12 months and ending 5 years after publication of this paper. Data will be available for researchers who provide a methodologically sound scientific proposal, which has been approved by an ethical committee. Proof of the latter should be provided. Analyses should achieve the aims reported in the approved proposal. Proposals for data should be directed to the corresponding author (stephen.freedman@ahs.ca).

Transparency statement: S. B. F. affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data verification: S. B. F., D. S., J. M. V.B., and C. O. confirm that they have verified the underlying data.

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Potential conflicts of interest. P. I. T. reports serving as member of the Data Safety Monitoring Board of Inmunova, which is developing immunotherapy to prevent Shiga toxin-producing infections from progressing to the hemolytic uremic syndrome (no compensation was provided, only expense reimbursement), outside the submitted work and Probiotic and placebo agents were provided in-kind by iHealth and Lallemand Health Solutions Inc. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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