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Randomised Controlled Trial of Lactobacillus Rhamnosus (LGG) Versus Placebo in Children Presenting to the Emergency Department with Acute Gastroenteritis: the PECARN Probiotic Study Protocol.

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ABSTRACT

Introduction Acute gastroenteritis (AGE) is a common and burdensome condition that affects millions of children worldwide each year. Currently available strategies are limited to symptomatic management, treatment and prevention of dehydration and infection control; no disease-modifying interventions exist. Probiotics, defined as live microorganisms beneficial to the host, have shown promise in improving AGE outcomes, but existing studies have sufficient limitations such that the use of probiotics cannot currently be recommended with confidence. Here we present the methods of a large, rigorous, randomised, double-blind placebo-controlled study to assess the effectiveness and side effect profile of *Lactobacillus rhamnosus* GG (LGG) (ATCC 53103) in children with AGE.

Methods and analysis The study is being conducted in 10 US paediatric emergency departments (EDs) within the federally funded Pediatric Emergency Care Applied Research Network, in accordance with current SPIRIT and CONSORT statement recommendations. We will randomise 970 children presenting to participating EDs with AGE to either 5 days of treatment with LGG (10^{10}colony-forming unit twice a day) or placebo between July 2014 to December 2017. The main outcome is the occurrence of moderate-to-severe disease over time, as defined by the Modified Vesikari Scale. We also record adverse events and side effects related to the intervention. We will conduct intention-to-treat analyses and use an enrichment design to restore the statistical power in case the presence of a subpopulation with a substantially low treatment effect is identified.

Ethics and dissemination Institutional review board approval has been obtained at all sites, and data and material use agreements have been established between the participating sites. The results of the trial will be published in peer-reviewed journals. A deidentified public data set will be made available after the completion of all study procedures.

Trial registration number NCT01773967.

Strengths and limitations of this study

- This is a large multicentre randomised, double-blind, placebo-controlled trial in a diverse and geographically varied US population of children with gastroenteritis.
- We perform independent laboratory product testing to assess probiotic viability, dosing and purity.
- We use a statistical enrichment design to restore the statistical power if a subpopulation with a substantially low treatment effect is identified.
- Outcome is based on parental report of symptoms rather than direct observation
- Enrolment is limited to day and evening hours only, when research personnel is available.

INTRODUCTION

Acute gastroenteritis (AGE) is a leading cause of malnutrition and death worldwide.[1] Though rarely fatal in North America, ~48 million people in the USA contract AGE, and 128 000 are hospitalised annually.[2] Although the incidence of rotavirus infection in the USA has decreased since the introduction of the vaccine in 2006,[3] norovirus is now the leading cause of medically attended paediatric AGE in this country.[4] Unfortunately, current interventions are limited to rehydration, symptomatic management and supportive care and prevention of severe dehydration and secondary infections.[5–8]

Probiotics, defined as live microorganisms that when administered in adequate amounts are intended to confer health benefits on the recipients,[9 10] represent a novel approach to improved management of paediatric AGE.

Probiotics are generally considered to be safe, easily administered and hypothesised to modulate disease processes.[11] Meta-analyses of various probiotic products have reported reduced symptom durations in children with AGE who have been treated with these agents. However, the studies included in these analyses have had important methodological limitations such as small sample sizes, lack of probiotic quality control, outcomes that are of minimal relevance to patients and their families and unclear randomisation, allocation concealment and blinding and attrition biases.[12–15] Remarkably, few studies of probiotics have evaluated outpatients, a group that represents the preponderance of AGE episodes in the USA,[16–18] and only one small study has evaluated probiotics in children with AGE presenting to a US emergency department (ED), where no benefit was demonstrated.[19]

Given the lack of adequate efficacy and safety evidence, most guidelines do not endorse the use of probiotics in paediatric AGE.[12 15 20–23] However, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition has offered a 'strong' recommendation in support of specific probiotics to treat previously healthy children with AGE, despite their acknowledgement of the 'low quality of the evidence'.[24] Furthermore, probiotic manufacturers aggressively market probiotics citing health claims that have not been supported by rigorous research,[25–28] and the US market for digestive health enzymes, prebiotics and probiotics was estimated at US$495 million in 2015 and was expected to grow at an annual rate of 13%.[29] Despite these concerns about their value, and issues surrounding safety and regulatory oversight,[30–32] parents of patients with AGE often administer probiotics to their children without guidance from medical professionals.[9 22] We are therefore concerned that the consumption of probiotics is increasing without adequate evidence to support their use, which underscores the necessity of conducting a definitive trial. There is strong consensus that an adequately powered randomised controlled trial (RCT), using a well-defined probiotic agent and comprehensive and clinically sensible validated outcome measures in a clinically relevant patient population will provide much needed clarity to this field.[12 15 33–35]

Here, we report on the methodology of a double-blind placebo-controlled pragmatic RCT (ClinicalTrials.gov: NCT01773967), using *Lactobacillus rhamnosus* GG (LGG) (ATCC 53103), the most available and studied probiotic in the USA as the intervention.[36 37] The research is supported by funding from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD R01HD071915) and is conducted under the oversight of the Food and Drug Administration (FDA investigational new drug 12371), in 10 US EDs within the federally funded Pediatric Emergency Care Applied Research Network (PECARN). The objectives of this double-blind placebo-controlled RCT are to (1) determine if, compared with placebo, LGG reduces the severity of AGE episodes in children aged 3–48 months presenting to an ED with AGE and (2) determine the safety and side effect profile of LGG in children with AGE.

We hypothesise that (1) in children with AGE, LGG will be associated with a clinically meaningful decrease in the proportion of children suffering from a moderate-to-severe episode of AGE defined by a validated Modified Vesikari Scale (MVS) score of ≥9, compared with placebo, and (2) LGG will not be associated with serious adverse events and will have a similar rate of side effects (eg, bloating and fever) compared with placebo-treated children.

METHODS AND ANALYSIS

Overview

This is a double-blind, 10-centre, paediatric ED-based RCT conducted by the PECARN. Children aged 91 days to <48 months who present to a participating ED between July 2014 to December 2017 will be assessed for eligibility, approached for informed consent and randomised to receive 5 days of a probiotic (LGG 10^{10} colony-forming unit (CFU) twice a day) or placebo. Physicians, patients, study personnel and outcome assessors are blinded to the intervention. LGG and the placebo are administered twice daily. The study will be conducted and reported according to the most recent SPIRIT and CONSORT statement recommendations.[38–40]

Setting

Patients are being recruited at 10 US paediatric EDs in PECARN (St. Louis Children's Hospital (St. Louis, Missouri, lead site), Lurie Children's Hospital (Chicago, Illinois), Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio), Children's Hospital of New York-Presbyterian (New York City, New York), Hasbro Children's Hospital (Providence, Rhode Island), Children's Hospital of Michigan (Detroit, Michigan), UC Davis Medical Center (Sacramento, California), CS Mott Children's Hospital (Ann Arbor, Michigan) and the University of New Mexico Children's Hospital (Albuquerque, New Mexico). Each centre has a strong research infrastructure and successfully participated in multicentre ED-based trials. Together the sites serve a large and diverse patient population. PECARN, the umbrella collaborative network, is the first federally funded paediatric research network in the USA and has an extensive record of successful multicentre research[41] The PECARN Data Coordinating Center (DCC), based at the University of Utah, is responsible for data management and data analysis. An independent Data Safety Monitoring Board (DSMB) composed of specialists in paediatric infectious diseases, paediatric gastroenterology, paediatric emergency medicine and biostatistics was formed to review enrolment, study procedures, case report form completion, data quality, loss to follow-up, drop-in rate and interim safety and efficacy results.

Inclusion criteria and rationale

1. *Presence of diarrhoea:* defined as ≥3 watery stools in the 24 hours prior to assessment,[42] with or without vomiting (vomiting alone, which may be the sentinel sign of AGE, could also represent non-infectious illnesses and is therefore not a sufficient criterion to qualify for eligibility).
2. *Duration of vomiting or diarrhea ≤7 days:* as we are focusing on acute diarrhoea, which typically is of less than 7 days' duration.[18] It is unclear if probiotics are useful in the early or later stages of AGE,[19] our enrichment design will allow for adaptive randomisation if a particular group is more likely to benefit from treatment.
3. *Age 91 days–<48 months:* AGE severity and frequency are greatest among young children,[43] including those who visit North American EDs.[17 44 45]
4. *Symptoms consistent with AGE per treating physician*: this is to ensure that only children with a presumptive diagnosis of AGE are included in the study.

Exclusion criteria and rationale

1. *Presence of an indwelling vascular access line or structural heart disease*: potential bacteraemia risk with intervention.[46]
2. *Receiving immunosuppressive therapy, or history of immunodeficiency:* potential bacteraemia risk with intervention.[47]
3. *Haematochezia*: (studies show little efficacy of probiotics in children with bacterial AGE, and visible blood in the stool is a marker for such pathogens).[48 49]
4. *Chronic gastrointestinal problems (eg, short gut syndrome and inflammatory bowel disease):* diarrhoea in such children is more likely to be related to non-infectious causes.
5. *Critically ill patients or patients admitted to the intensive care unit:* these patients are at risk of invasive disease, and their ability to comply with an oral intervention might be limited.
6. *Household member with an indwelling vascular access line, on immunosuppressive therapy or with a known immunodeficiency:* risk for invasive disease if there is intrahousehold dissemination of the LGG (note that this exclusion does not extend to household contacts who use a short course (<7 days) of oral steroids or are using inhaled steroids).
7. *Bilious emesis:* might indicate a diagnosis other than AGE.
8. *Probiotic use (supplement) in the preceding 2 weeks:* confounding risk; consumption of foods containing probiotics will not result in exclusion as they are ubiquitous.
9. *Previously enrolled in this trial*: to ensure that the observations are independent.
10. *Daily telephone follow-up not possible while symptomatic:* avoid loss to follow-up because of travel plans or language barrier.
11. *Allergy to* Lactobacillus *or microcrystalline cellulose (MCC):* contents of capsule and placebo.
12. *Allergy to beta-lactam antibiotics, erythromycin and clindamycin:* these antibiotics might be used in the event of LGG extraintestinal dissemination.

Children taking antibiotics will *not* be excluded because probiotics remain viable when given concomitantly with antibiotics, and the survival of the active bacterial strains is not diminished.[50]

Participant allocation

Sequence generation

The PECARN DCC produced randomisation lists, stratified by study site and duration of symptoms, using random number-generating software. The lists were sent to the central pharmacy (Cincinnati Children's Hospital Medical Center) that prepares consecutively numbered study kits according to the randomisation schedule. These are sent by courier to the clinical sites where they are stored in the research support pharmacies.

Allocation concealment

Randomisation was performed at the DCC using random block sizes with a 1:1 allocation ratio. Stratifying by clinical site ensures that variations (eg, site-specific practice patterns and gastrointestinal pathogens) are comparably distributed across treatment arms. Only the DCC retains the randomisation code. Unblinding can be requested by treating medical personnel in case of an emergency requiring such information.

Implementation

Potentially eligible patients are identified by triage nurses at each site who contact the research assistant (RA). The RA then (1) screens patients for eligibility, (2) maintains a log of all screened patients, (3) discusses the details of the study with the caregivers of all eligible children, (4) obtains consent, (5) enrols children, (6) consecutively assigns a patient identification number, (7) randomises the patient (using a web-based system: www.randomize.net), (8) collects baseline demographic clinical variables and (9) in conjunction with the treating physician, completes data collection forms.

Intervention

LGG and placebo capsule contents

LGG, ATCC 53103 is supplied in a gelatin capsule containing 10^{10} CFU LGG. Each LGG capsule contains 75 mg of LGG and 250 mg of MCC, an inert ingredient. The placebo capsules contain only MCC (325 mg). Each capsule is wrapped in double foil to protect it against light, air and moisture. Blister packs are labelled with the lot number. LGG and placebo capsules and powder are identical in appearance, taste, texture and odour. LGG capsules and placebo capsules have active Drug Master Files at the FDA (BB-MF 213668 and MF2 13646, respectively). The dose and duration of therapy are based on the currently available evidence.[51–63]

ED intervention

The patient's nurse administers the first dose of either LGG (10^{10} CFU/dose) or placebo on site by sprinkling

the capsule's contents into 30 mL of room temperature, non-carbonated liquid. The RA provides caregivers with verbal and written instructions regarding (1) study drug administration; (2) completion of study forms; (3) what and how much fluid to drink; (4) criteria for seeing a healthcare practitioner or returning to the ED; and (5) standardised AGE discharge instructions and letter to their primary care provider explaining the study. All other aspects of medical care will be at the discretion of the treating physician.

Home intervention

All patients consume one capsule of LGG or placebo, based on randomisation, every 12 hours for 5 days (10^{10} CFU twice daily × 5 days, for a total of nine home doses). Patients receive the medication at meal time, mixed with 30 mL of a room temperature non-carbonated liquid and ingested immediately to optimise probiotic viability. Oral fluid therapy is encouraged according to established guidelines.[21] The study protocol is continued in the subset of children (estimated <5%) who are hospitalised.[18] Also, caregivers are provided with a letter to share with their primary care provider (in case they visit their provider during the course of the study). The letter describes the study and the care plan, and it includes site investigator's contact information and the importance of adhering to the study protocol. Patients may withdraw from the study at any time based on their or their physician's discretion; however, efforts will be made to proceed with safety follow-up, and the subjects will be included in the intention-to-treat (ITT) analysis.

Stool sample testing

Stool samples (swab or bulk stool, as available) from all enrolled children are collected, frozen and sent to Washington University – St. Louis Children's Hospital Virology Laboratory and tested with multiplex PCR using the Luminex xTag Gastrointestinal Pathogen Panel (Luminex, Austin, Texas, USA), which identifies the following organisms (and specific bacterial loci): viruses: norovirus (GI and GII), adenovirus F 40/41 and rotavirus (A); bacteria: *Escherichia coli* O157, enterotoxigenic *E. coli* (*lt/st*), Shiga toxin-producing *E. coli* (*stx1/stx2*), *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Clostridium difficile* (*tcd* A/B); and parasites: *Cryptosporidium* spp., *Giardia* spp. and *Entamoeba histolytica*.

In addition, St. Louis Children's Hospital and the New Mexico Children's Hospital collect and freeze bulk stool specimens in the acute phase (within 24 hours of presentation) and following resolution (14 days after presentation) using a home stool collection protocol. The protocol consists of providing the family with a specimen collection kit, gel packs and an insulated envelope at enrolment. When the specimen is ready for collection, a courier retrieves the specimen and cool pack at the patient's home and delivers it to a logistics collection centre. The stools are frozen on receipt in St. Louis and Albuquerque, and then stored in the Tarr Laboratory at Washington University at −80°C for future testing.

Data Collection

All caregivers receive discharge instructions that include information on tasks required following discharge along with a diary to record daily symptoms and all information requested during the telephone calls or electronic surveys, including side effects (see supplemental file: follow-up surveys). Follow-up occurs daily until symptoms resolve or 5 days, whichever occurs later, and again at 14 days and 1, 3, 6, 9 and 12 months following enrolment. Data collected daily and at day 14 follow-up are used to measure efficacy and short-term safety outcomes. Long-term follow-up data (1 month onwards) are used to assess long-term adverse events, unanticipated medical encounters and development of new chronic illnesses in accordance to FDA guidelines (Guidance for Industry and Investigators: Safety Reporting and Requirements for INDs and BA/BE Studies).[64] We use a standardised script and data collection forms to obtain follow-up information by telephone or via email survey. Follow-up procedures are centralised at the lead site. We also perform chart reviews to verify data regarding revisits, intravenous hydration, hospitalisation and microbiology testing using each centre's medical record database. Personal data will be handled in compliance of the Health Insurance Portability and Accountability Act. Data are entered in to encrypted and secure central databases managed by the DCC at the University of Utah, where state-of-the-art equipment and procedures ensure data quality and security.

Compliance

We assess patient compliance with therapy on day 5 and collect final data on day 14. To maximise compliance, caregivers are reminded of the importance and method of administering the probiotic/placebo. A similar scheme has been used in our previous studies.[18 65–67]

Probiotic quality control/independent testing

We test samples of all batches of probiotic product at an independent laboratory twice a year until expiration date to ensure adequate bacterial counts. In order to maximise bacterial viability, probiotic products are kept refrigerated at research pharmacies between 0°C and 4°C. Shipping and storage logs are retained.

Study monitoring

The DCC coordinated site (in-person and remote) monitoring as well as pharmacy monitoring at the beginning and once during the study. The monitor has provided each site with a written report, and sites have been required to respond to and resolve deficiencies. Sponsoring and regulatory agency monitoring is at the discretion of such agencies.

Outcome measures

The primary outcome to measure efficacy is the presence of moderate-to-severe AGE, as defined by a total

Table 1 Modified Vesikari Score

Points	0	1	2	3
Diarrhoea duration	0	1–96 hours	97–120 hours	≥121 hours
Max no. of diarrhoeal stools/24 hours	0	1–3	4–5	≥6
Vomiting duration	0	1–24 hours	25–48 hours	≥49 hours
Max no. of vomiting episodes/24 hours	0	1	2–4	≥5
Max recorded fever	≤37°C	37.1–38.4°C	38.5–38.9°C	≥39°C
Unscheduled healthcare visit	0	–	Primary care	Emergency department
Treatment	None	Rehydration	Hospital admission	–

postenrolment MVS score ≥9 during the 2-week follow-up period (table 1). This scale has been validated in our patient populations.[18 45] Each of the seven items in the scale is tabulated individually (maximum of 20 points); the sum of these individual variables represents the total MVS score. At the time of randomisation (time 0), a pre-enrolment MVS score is assigned based on symptoms prior to presentation. This score serves as a covariate in a secondary analysis of the primary outcome. The postenrolment MVS score used to determine the presence/absence of the primary study outcome, is based only on symptoms that occur between time 0 (ie, randomisation) and the conclusion of the study period (ie, day 14). The postenrolment score is calculated only once, on day 14. At that time, each of the seven variables are assigned a score for the entire study period (time 0 to day 14). Each variable is scored in 1 of 3 methods: (1) worst 24 hours period—maximal number of episodes of vomiting in a 24-hour period, maximal number of episodes of diarrhoea in a 24-hour period and maximal temperature); (2) total duration of symptoms, including the number of days on which any gastroenteritis-related symptom occurred. For scoring purposes, the episode of AGE concludes after absence of symptoms for 24 hours; and (3) occurrence of an outcome—treatment and subsequent healthcare utilisation. A score of ≥9 defines moderate-to-severe disease because on the original score, severe disease was defined as ≥11[68–72] and moderate as ≥9.[73] In our derivation and validation pilot studies,[18 45] construct validity was demonstrated and validated by using scores of ≥9 to define moderate and ≥11 to define severe disease. These cut-points were associated with significant increases in other measures of disease severity such as degree of dehydration, likelihood of admission and daycare and parental work absenteeism.[18 45]

Main safety outcome

The main safety outcome is the occurrence of extraintestinal infection by the administered probiotic agent—LGG. Based on previous human experience with LGG in healthy volunteers, pregnant women, neonates and children with AGE, we do not anticipate that any extraintestinal infections will occur. Adverse event analysis will follow FDA guidelines for assessment of attribution, toxicity grading scale and criteria for patient withdrawal. Per FDA recommendations, we conducted an interim safety analysis after the first 80 patients, including 40 less than 1 year of age, had completed their 1-month follow-up.

Secondary outcomes (efficacy)

Secondary outcomes include the following: (1) diarrhoea duration: time from treatment initiation until the appearance of the last watery stool as reported during daily phone conversations, (2) vomiting duration, (3) return visits for unscheduled care to a healthcare provider related to vomiting, diarrhoea, dehydration, fever or fluid refusal, within 2 weeks of the index visit. We will not include scheduled visits (eg, reassessment, vaccinations and unrelated issues). This outcome is important because >50% of children with AGE have a follow-up office visit,[16] 8%–18% require an ED visit and 5%–8% are hospitalised.[16] (4) Days of daycare missed by subjects, (5) days of work missed by caregivers and (6) household transmission rate: a household census is obtained at the time of enrolment, and we obtain information about incident household symptoms during the telephone follow-up calls. Secondary transmission is an integral feature of AGE, and households are relevant and well-established study units.[74–76]

Secondary outcomes (safety)

The secondary safety outcome is the presence of potential side effects such bloating, gas, intestinal rumbling, diarrhoea, blood in stool, abdominal pain, abdominal cramps, nausea, vomiting, loss of appetite, abnormal taste, heartburn, constipation, skin rash, fever, nasal congestion, sore throat, cough, headache, malaise, muscle aches and chills. We acknowledge, however, that some toxicities will be difficult to distinguish from abdominal symptoms related to the AGE, and only at the time of data analysis will we be able to determine if these signs and symptoms differ between the groups (ie, by comparing the differences in occurrence between the active and placebo groups). The study physicians complete the appropriate form for all adverse events identified during the scheduled or unscheduled phone calls. During long-term follow-up telephone calls (ie, those occurring after

14 days postenrolment), we inquire about unexpected events obligating medical attention and new onset of chronic disorders, especially those involving the digestive system.

Data analysis and sample size

All analyses will be undertaken by the ITT principle, except for side effects, which will use the 'as-treated' principle (compare the subjects based on the treatment regimen that they received). Patients who withdraw, drop out or crossover will be followed and included in the ITT analysis. All statistical tests of hypotheses will be two sided. For cases where information needed to derive the primary outcome is incomplete, we will use multiple imputation methods. The proportion of children with moderate-to-severe disease (ie, MVS ≥9), the primary outcome will be analysed by comparing proportions using a Mantel-Haenszel test, stratified by participating centre and duration of symptoms prior to presentation. Significance for this primary outcome measure will be set at 0.05. Secondary analyses of the primary outcome will use logistic regression methods to adjust for covariates (eg, age, pre-enrolment MVS, hydration assessment and need for hospitalisation at index visit). We will also analyse the outcome using MVS as a continuous variable through a stratified Wilcoxon rank-sum test and compare the results with the primary analysis.

The overall significance level for statistical tests on the secondary outcomes will be set at 0.05. Holm's method will be used to adjust for multiple comparisons.[77] The continuous variables of durations of (1) diarrhoea and (2) vomiting will be measured in hours and analysed with a Van Elteren test[78] and stratified by clinical centre and duration of symptoms. Similarly, the number of days (3) the child is absent from daycare and (4) the caregiver is absent from work will be analysed with a Van Elteren test, stratified by clinical centre and duration of symptoms. Dichotomous outcomes to be evaluated include ED AGE-related revisits, intravenous rehydration and hospitalisation. These six outcomes will be jointly assessed for significance using Holm's method. Additional analyses involving these outcomes will include linear and logistic regression models that adjust for possible effects of baseline characteristics. The proportions of children experiencing (5) an unscheduled healthcare visit or (6) any potential adverse effect, as reported by the caregivers, will be compared between groups using the Mantel-Haenszel test, stratified by site and duration of symptoms. The analysis will evaluate the presence/absence of prespecified side effects, as an aggregate outcome variable. A per-protocol analysis will be conducted to provide additional insight as non-compliance may result in an underestimation of the benefits of probiotics in the ITT analysis.[79]

Power analysis

The primary analysis will be performed on a binary outcome: development of moderate-to-severe disease. The power of this analysis depends on the proportion of patients with moderate-to-severe disease in each group considered. Data collected as part of our pilot evaluations of the MVS in 729 children aged 3–48 months demonstrated that when using the ED visit as time 0, 25% of eligible children had scores consistent with moderate-to-severe disease following discharge.[18 45] This is a lower rate than previous reports of diarrhoea in paediatric EDs[69 70] and in the community[68 71 73] but is attributed to our exclusion of symptoms that existed prior to the visit. Because both the populations and method of MVS calculation in the MVS derivation and validation studies and the current proposal are identical, 25% is supported by data from our pilot study and is likely to be accurate. To determine the minimal clinically important difference that we should aim to detect, 10 content experts were surveyed. Absolute risk differences ranging from 7.5% to 15% were suggested. We selected a conservative estimate of 10% for the primary outcome (ie, number needed to treat of 10). For the current study, our sample size calculation assumed a 25% event rate in the control group, and we desire to detect an absolute beneficial treatment effect of 10% with 90% power. Using a two-sided type I error (α) of 0.05 and the hypothesised proportions yields a required total sample size of 670 patients.[80] Our expected power, if true event rates in our two groups differ from those expected, is presented in table 2. Based on prior work by our group,[18 45 65 81 82] we assumed 10% loss to follow-up (adjustment: 670/0.90=744), 5% drop out and 3% drop in rate (caregivers who buy a probiotic agent to administer to their child) (adjustment: $744/(0.92)^2=879$). Adjustment for O'Brien-Fleming monitoring boundaries requires a further 2% increase. Thus, the total number randomised (final sample size) is therefore 900. In the fall of 2015, however, 36 patients were potentially exposed to a batch of LGG that was later found to contain inadequate bacterial counts on independent testing. We assumed that approximately 18 of these were exposed and having the same effect as drop-outs. In order to maintain study power under this worst-case scenario, we would have to increase the sample size to 970 patients $(900/(0.963)^2$, where 0.963 is 1 minus

Table 2 Power analysis.

Outcome control	Outcome intervention	% Difference	Power
0.30	0.21	9	0.76
0.30	0.20	10	0.85
0.25	0.15	10	0.90
0.25	0.16	9	0.82
0.25	0.17	8	0.72
0.20	0.10	10	0.95
0.20	0.12	8	0.81
0.20	0.13	7	0.69

Highlighted area corresponds to stated assumptions in the text.

the drop-out rate of 18/485=0.037). Based on preliminary surveys, we believe that achieving this sample size is feasible at our sites.

(1) Formal subgroup analyses will be based on (a) age <1 year, (b) antibiotic usage, (c) infectious agent (virus, bacteria, parasite or other). Treatment effect will be summarised across subgroups. A subgroup effect will be declared to be significant only if the interaction between treatment and the subgroup factor is significant in an appropriate statistical model (including multivariate regression analyses), using a significance level of <0.05/3=0.017 for each. (2) Duration of vomiting will be analysed only in those subjects reporting ≥3 episodes of vomiting in the 24 hours preceding enrolment. (3) Daycare and work absenteeism will only be analysed for those subjects who attend daycare and/or whose caregivers work outside of the home.

Enrichment design

The above study design and power analysis are based on the assumption of homogeneous treatment effect. We incorporated an enrichment design[83 84] to restore the statistical power if a subpopulation with a substantially low treatment effect is identified. We are particularly interested in two potential subpopulations: participants with <2 days of symptoms and those with ≥2 days of symptoms. Based on our pilot data, each subpopulation accounts for approximately 50% of the total population. The decision for enrolment modification was made at the first interim analysis for efficacy (350 enrolled patients). Specifically, three statistics (based on a normal approximation of binomial distribution or z-statistics) were calculated to compare the primary endpoint between treatment and control groups for subjects in the total population and the two subpopulations, respectively. If the z-statistic from a subpopulation is <0.3 and also smaller than that in the total population, subjects from this subpopulation are no longer to be considered in the subsequent enrolment. All subjects, regardless of symptom duration are to be included in the final analyses. Our simulation studies have showed that such an enrichment design can increase the power considerably when the treatment effects are different across subpopulations, while it will have little impact on power when the treatment effects are similar. Following these analyses performed after 350 patients were enrolled and recommendations by the DSMB, the decision not to modify enrolment was made.

Frequency of analysis

The DSMB met after 80 (safety at 1 month), 350 and 650 subjects had completed their 1-month follow-up assessments to review enrolment, study procedures, case report form completion, data quality, loss to follow-up, drop-in rate and interim safety and efficacy results. The analyses tested the hypothesis that the probability of developing moderate-to-severe AGE in the probiotic arm is equal to that in the placebo arm. Conservative O'Brien-Fleming monitoring boundaries, implemented using the Lan-DeMets alpha spending function approach, will be used as guidelines for early stopping for efficacy. At each step, the DSMB recommended that the study continue without modifications (table 3).

Ethics and dissemination

This trial is being conducted under an Investigational New Drug application approved by the FDA (Investigational New Drug application 15371). Institutional review board (IRB) approval has been obtained at all sites. Financial compensation is provided to compensate for parents' time completing follow-up. This compensation was approved by each site's IRB. All important modifications will be communicated to the pertinent parties. Data use agreements have been obtained between all sites, and the DCC and Material use agreements have been obtained between all sites and the lead site. The results of the study will be published in peer-reviewed journals. A deidentified public data set will be made available after the completion of all study procedures. The study investigators will have access to the final trial data set. Authorship will be conferred per the International Committee of Medical Journal Editors.

DISCUSSION

This is the largest RCT of probiotics in children presenting with AGE to an ED to date. We propose to improve outcomes in children affected by AGE by modifying the disease process through biologically plausible mechanisms. Translating this knowledge into a disease-modifying clinical intervention would represent a major change in the approach to this burdensome illness and provide clarity to clinical practice that has been hindered by aggressive marketing in the absence of valid data. Critical elements incorporated into our design that were absent in earlier studies are: (1) evaluating a specific regimen in a large number of participants in a

Table 3 Interim analyses stopping rules

Analysis	Two-sided p Value	Probability of stopping (80% power) (%)	Probability of stopping (90% power) (%)
First (350 patients)	<0.0007	4.9	8.5
Second (620 patients)	<0.014	40.2	51.2
Final	<0.046	34.9	30.4

geographically diverse network in the USA, (2) using a meaningful and validated outcome in our population, (3) identifying infectious causes, (4) using adaptive randomisation to target specific subgroups and (5) accounting for pre-evaluation administration of probiotics. We attempt to minimise bias by adhering to the 2013 SPIRIT guidelines and the 2010 CONSORT Statement recommendations including the use of 'third-party' assignment.[38] Placebo capsules and active drug are provided by I-Health Inc. The probiotic and placebo capsules and powder are identical in appearance, taste, texture and odour. Participants, families, healthcare providers, data collectors, outcome adjudicators and data analysts are blinded as to intervention arm, thereby preventing bias in outcome assessment. An ITT analysis will be performed to minimise bias associated with poor compliance and non-random loss of participants.[85] Cointerventions (eg, antiemetic administration and intravenous rehydration) and other potential sources of confounding are recorded. Our use of a published validated score as an outcome measure protects against the introduction of bias in the assessment of treatment effects.[86]

Of note, a similar study using a different probiotic product containing *Lactobacillus rhamnosus* and *L. helveticus* (Lacidofil) is being conducted in Canada with funding from the Canadian Institutes of Health Research.[87] This parallel study provides opportunities to enhance our knowledge about the effect of probiotics in children with AGE.

CONCLUSION

This double-blind, placebo-controlled RCT will quantify the benefits and potential side effects associated with probiotic administration in ambulatory children presenting to the ED with AGE. This will provide the first definitive evidence in the USA for or against using probiotic therapy for this condition and establish the safety of the intervention. The results of this multicentre study will guide the standard of care: if probiotic administration is associated with benefit, it offers a relatively inexpensive and safe to administer treatment to reduce morbidity from AGE. If the trial does not demonstrate probiotic efficacy, healthcare and family and societal resources may be refocused on different interventions.

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Contributors DS, MHG, PIT and SBF conceptualised the study, obtained funding, obtained an IND for LGG, obtained endorsement by PECARN and wrote the protocol. MJD provided study implementation and regulatory expertise. CTC and FG provided statistical expertise. KJO, PM, THC, SRB, CGR, ECP, AJR, CV and RES provided critical review of the protocol and built enrolment capacity at their sites. All authors read and approved the final manuscript and agree to be accountable for all aspects of the work.

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Randomised controlled trial of *Lactobacillus rhamnosus* (LGG) versus placebo in children presenting to the emergency department with acute gastroenteritis: the PECARN probiotic study protocol

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