PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Randomized Controlled Trial of Lactobacillus rhamnosus (LGG) versus Placebo in Children Presenting to the Emergency Department with Acute Gastroenteritis: The PECARN Probiotic Study Protocol
AUTHORS	Schnadower, David; Tarr, Phillip; Casper, T.; Gorelick, Marc; Dean, J; O'Connell, Karen; Mahajan, Prashant; Chun, Thomas; Bhatt, Seema; Roskind, Cindy; Powell, Elizabeth; Rogers, Alexander; Vance, Cheryl; Sapien, Robert; Gao, Feng; Freedman, Stephen

VERSION 1 – REVIEW

REVIEWER	Anna Petrova MD, PhD, MPH Rutgers Robert Wood Johnson Medical School, USA
REVIEW RETURNED	28-Jun-2017

GENERAL COMMENTS	To date, various studies have been designed to assess a role of probiotics in treatment acute diarrhea in pediatric patients. Meta-analysis published in 2007 showed decreased duration of rotavirus diarrhea and a small reduction in length of stay in children treated with Lactobacillus GG (LGG). 1. Randomized controlled trial from India demonstrated a lower frequency of repeated diarrheal episodes, impaired intestinal function, and higher level of IgG after four weeks of LGG administration to young children with acute diarrhea.2 Cochrane review identified a beneficial effect of probiotics in treatment of acute pediatric diarrhea, mainly due to reduction of diarrhea duration by a day, despite the significant heterogeneity of the reported effect size, participants' characteristics, diarrhea diagnosis, and type and dosage of probiotics.3 As a result, LGG has been recommended for treatment of acute rotavirus diarrhea in pediatric patients.4, 5 However, despite the existing reports, there is limited compelling evidence, in particular, concerning the initiation of LGG in children presenting with acute gastroenteritis to the Emergency Department (ED). The reviewed manuscript outlines the design of a placebo-controlled multicenter trial to identify the efficacy of inclusion of LGG (ATCC 53103) in the medical care of infants and young children presenting with acute gastroenteritis to the ED. Despite the similarity of this proposal to the previously published PROGUT protocol (except for the investigational probiotic), 6 the current review reveals several questions that need further clarification: 1. Efficacy versus effectiveness trial. It is important for clinicians to distinguish efficacy (exploratory) and effectiveness (pragmatic) of the intervention because of the clinical evidence related to the

external validity of the study results.7, 8 It appears that the proposed study is an effectiveness trial considering that the investigators will not be able to ensure full adherence to the LGG treatment post-discharge.

2. Age-based inclusion criteria. Because the majority of morbidity due to acute gastroenteritis in young children is related to rotavirus infection, the vaccination status could be a potential confounder variable. It would be more appropriate to use six months, as the age for the lower limit of the eligibility criteria.

3. Outcome measures. The Modified Vesikari Scale has significant limitations as the main outcome, including the feasibility of both, compliance (intervention and placebo) with treatment and the quality of parental assessment as well as the collection of clinical data post-discharge. Internal consistency of the Modified Vesikari Scale evaluated via Cronbach's alpha calculation is low.9 It is possible to use the Modified Vesikari Scale to measure the secondary outcome because instead of using single symptoms, it integrates all multidimensional symptoms into a composite score. 9, 10 For investigating the efficacy of LGG in children presenting with acute gastroenteritis to the ED, comparison of odds for re-visitation to the ED within 14 days after discharge, between the interventional and placebo groups, would be a clinically meaningful end-point (outcome) for the study.

Please do not consider safety as the “Primary Outcome” because the study is not appropriately powered for this question.

4. Sample size calculation. It would be logical to employ not only the statistical but also the clinical and cost-effectiveness for objective judgement of the 10% reduction of binary outcome (Modified Vesikari Scale <9 vs. >9) in the sample size calculation. Instead of the absolute risk reduction (difference between two proportions), Odd Ratio and 95% Confidence Interval should be used to calculate the sample size for the proposed trial.

5. Stratification versus adjustment. Stratification of the data with respect to the duration of diarrhea prior to the ED visit and settings is debatable. The study should be specifically powered for subgroup analysis prior to the implementation of the trial; otherwise, the trustfulness of the subgroup analysis will be problematic. It is unrealistic to perform an analysis of the data after stratification by 10 settings. Moreover, the duration of symptoms prior to admission to the ED might be comparable between interventional and placebo groups due to the internal validity insured by randomization. Multivariate regression models would be a better approach for controlling for the duration of diarrhea (and/or other variables) in case of dissimilarity between the interventional and placebo groups.

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REVIEWER	Geurts Erasmus Medical Centre Rotterdam Sophia Children's Hospital
REVIEW RETURNED	10-Jul-2017

GENERAL COMMENTS	I read the manuscript 'Randomized Controlled Trial of Lactobacillus rhamnosus (LGG) versus Placebo in Children Presenting to the Emergency Department with Acute Gastroenteritis: The PECARN Probiotic Study Protocol' with great interest. It is an important study on a common pediatric illness. I would like to complement the authors on a very thorough design and description of the study and on the organization of a very large multi-center double-blind randomized controlled trial, which possibly is the only way to answer this research question(s). It also arises some important issues, which I hope the authors are able to address. I consider this manuscript suitable for publication after addressing the following comments. Abstract The abstract is well- written, and according to the CONSORT criteria for abstracts, except for the Trial Register, which should be mentioned at the end of the abstract. Introduction The rationale is clear. The study concerns a common pediatric illness, with a large burden of disease all over the world with the need for including a large multi-center population in order to be able to answer the research questions, as evidence in smaller study was limited.1 2 Methods The outline is clear. Primary and secondary outcome measures are well defined. The Modified Vesikari Score is well chosen with an

	appropriate cut-off value. All items according the CONSORT checklist are present, except for a clear description of interim analysis and stopping criteria. These should be included, as the remark that the intervention is identical to the placebo in size, color and taste, this is probably the case, but can only be assumed from the method section. It is described in the discussion, however. Furthermore, I would like to be more informed on the characteristics of the ten different centers, population-wise, but also important differences in AGE (acute gastro-enteritis) - treatment guidelines are important to describe, perhaps in an appendix. Third, I would like to know more on the follow-up moments; 'what' is measured 'when' and more important 'why'? One could speculate one two primary measurement time points, for example at day 5 and day 14, knowing the (normal) disease course of AGE? And what is the rationale on measuring 'everything' at 9 and 12 months? One can speculate it has to do with prolonged diarrhea, etc, however, it is important information for readers of the manuscript. Fourth, a preview of the tables would help. Last, the paragraph on 'data-analysis and sample size' is difficult to read because of the large amount of technical details, however I do not have a suggestion on improvement. 1. Guarino A, Guandalini S, Lo Vecchio A. Probiotics for Prevention and Treatment of Diarrhea. J Clin Gastroenterol 2015;49 Suppl 1:S37-45. 2. Szajewska H, Ruszczynski M, Kolacek S. Meta-analysis shows limited evidence for using Lactobacillus acidophilus LB to treat acute gastroenteritis in children. Acta Paediatr 2014;103(3):249-55.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1

1. Efficacy versus effectiveness trial. It is important for clinicians to distinguish efficacy (exploratory) and effectiveness (pragmatic) of the intervention because of the clinical evidence related to the external validity of the study results. It appears that the proposed study is an effectiveness trial considering that the investigators will not be able to ensure full adherence to the LGG treatment post-discharge.

Response: The reviewer is correct, this is an effectiveness trial. Indeed, our abstract states: "Here we present the methods of a large, rigorous, randomized, double blind placebo controlled study to assess the effectiveness and side effect profile of Lactobacillus rhamnosus GG (LGG) (ATCC 53103) in children with AGE."
We now add the descriptor "pragmatic" (page 4), in the Introduction: "Here, we report on the methodology of a double-blind placebo-controlled pragmatic RCT (clinical trials.gov NCT01773967), using Lactobacillus rhamnosus GG (LGG) (ATCC 53103), the most available and studied probiotic in the US as the intervention"

2. Age-based inclusion criteria. Because the majority of morbidity due to acute gastroenteritis in young children is related to rotavirus infection, the vaccination status could be a potential confounder variable. It would be more appropriate to use six months, as the age for the lower limit of the eligibility criteria.

Response: Rotavirus continues to be an important pathogen, but since the introduction of the rotavirus vaccine in the US in 2006,1 norovirus is now the leading cause of medically attended AGE in this country. 2 We added these data in the first paragraph of the introduction (page 4). Furthermore we are collecting rotavirus vaccine vaccination status in all participants, which will allow us to assess for potential biases. We are also collecting and testing stool on our subject, which will further clarify the issue. Finally, we believe the effectiveness of probiotics deserves to be studied in younger infants, who are potentially more susceptible to complications from AGE. Our microbial diagnostic efforts are detailed on page 7.

3. Outcome measures. The Modified Vesikari Scale has significant limitations as the main outcome, including the feasibility of both, compliance (intervention and placebo) with treatment and the quality of parental assessment as well as the collection of clinical data post-discharge. Internal consistency of the Modified Vesikari Scale evaluated via Cronbach's alpha calculation is low.9 It is possible to use the Modified Vesikari Scale to measure the secondary outcome because instead of using single symptoms, it integrates all multidimensional symptoms into a composite score. 9, 10 For investigating the efficacy of LGG in children presenting with acute gastroenteritis to the ED, comparison of odds for re-visitation to the ED within 14 days after discharge, between the interventional and placebo groups, would be a clinically meaningful end-point (outcome) for the study.Please do not consider safety as the “Primary Outcome” because the study is not appropriately powered for this question.

Response: Thank you for these comments. Indeed, because the AGE is a disease characterized by a constellation of symptoms (i.e. vomiting, diarrhea, fever) and is subject to potentially significant medical interventions dependent on health care systems and populations (i.e. ED or PMD visits, IV rehydration, hospitalization) measuring outcomes in AGE using a single data point for all populations is problematic and has been recognized as a major limitation in prior trials.3 4 Therefore, in preparation for this and our parallel trial in Canada (Progut) trial we conducted two separate pilot studies to evaluate the MVS in our populations.5 6 In these papers we detailed the validation process of this score and its limitations. While it is not perfect, we believe that this is the best validated composite, patient-centered, outcome measure that focuses on global disease severity. While revisits can be important, the decision to revisit is at the discretion of the caregiver and does not incorporate any measures of disease severity and thus, during the preparation of this study protocol, many experts in the field suggested that revisits not be employed for this clinical trial. Given the importance of the individual elements of the MVS, as suggested by the reviewer, they have been included as secondary outcome measures (including ED revisits).

We appreciate that the reviewer corrected our designation of safety as an outcome. Nonetheless, since safety is a priority, we have replaced the term “primary outcome” from the heading on page 8 with “Main Safety Outcome: The main safety outcome is the avoidance of extra-intestinal infection by the administered LGG…”

4. Sample size calculation. It would be logical to employ not only the statistical but also the clinical and cost-effectiveness for objective judgement of the 10% reduction of binary outcome (Modified Vesikari Scale <9 vs. >9) in the sample size calculation. Instead of the absolute risk reduction (difference between two proportions), Odd Ratio and 95% Confidence Interval should be used to calculate the sample size for the proposed trial.

Response: We refrained from using cost-effectiveness considerations in calculating the sample size given the lack of disease specific economic data in our population as well as the interdependency with the effectiveness objective.

We used absolute risk reduction in our sample size calculations rather than odds ratios because absolute risk reduction is the most basic and simplest measure used by clinicians to interpret the effectiveness and clinical meaningfulness of an intervention.7 The interpretation of odds ratios is more complex and could potentially lead to inaccurate estimations of the treatment effect.8

5. Stratification versus adjustment. Stratification of the data with respect to the duration of diarrhea prior to the ED visit and settings is debatable. The study should be specifically powered for subgroup analysis prior to the implementation of the trial; otherwise, the trustfulness of the subgroup analysis will be problematic. It is unrealistic to perform an analysis of the data after stratification by 10 settings. Moreover, the duration of symptoms prior to admission to the ED might be comparable between interventional and placebo groups due to the internal validity insured by randomization. Multivariate regression models would be a better approach for controlling for the duration of diarrhea (and/or other variables) in case of dissimilarity between the interventional and placebo groups.

Response: We appreciate these comments as they mirror discussions amongst the investigative team. Because the only prior study using LGG in a similar population demonstrated a potential benefit in patients who presented with more than 2 days of symptoms,9 we developed an enrichment design that would allow us to achieve statistical power if a subpopulation with a substantially low treatment effect is identified. We believe this is a more efficient and feasible strategy than powering the study for both populations,10 and we describe this in detail on pages 10 and 11 of the manuscript. Also, as indicated by the reviewer and as explained in our subgroup analysis paragraph (page 10), we will conduct analyses to assess the role of varied patient presenting symptoms/characteristics. We added “including multivariate regression analyses” to that section. The paragraph now reads: “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.

Reviewer 2
1. Abstract: The abstract is well- written, and according to the CONSORT criteria for abstracts, except for the Trial Register, which should be mentioned at the end of the abstract.

Response: The trial registration is now provided at the end of the abstract.

2. Introduction
The rationale is clear. The study concerns a common pediatric illness, with a large burden of disease all over the world with the need for including a large multi-center population in order to be able to answer the research questions, as evidence in smaller study was limited.

Response: Thank you

3. Methods: The outline is clear. Primary and secondary outcome measures are well defined. The Modified Vesikari Score is well chosen with an appropriate cut-off value. All items according the CONSORT checklist are present, except for a clear description of interim analysis and stopping criteria. These should be included, as the remark that the intervention is identical to the placebo in size, color and taste, this is probably the case, but can only be assumed from the method section. It is described in the discussion, however.

Response: We added a table (Table 3) to describe the interim analyses stopping criteria.

4. Methods: Furthermore, I would like to be more informed on the characteristics of the ten different centers, population-wise, but also important differences in AGE (acute gastro-enteritis) - treatment guidelines are important to describe, perhaps in an appendix.

Response: As part of the ongoing study, we are collecting population data and we plan to present them in the results paper. There are no nationally implemented AGE guidelines in the US, nor do all institutions have standardized protocols. We quite agree that such data, if available, would be interesting. This pragmatic trial does not dictate management of these children in an effort to assess the effectiveness of the intervention in the most generalizable manner.

5. Methods: I would like to know more on the follow-up moments; 'what' is measured 'when' and more important 'why'? One could speculate one two primary measurement time points, for example at day 5 and day 14, knowing the (normal) disease course of AGE? And what is the rationale on measuring 'everything' at 9 and 12 months? One can speculate it has to do with prolonged diarrhea, etc, however, it is important information for readers of the manuscript.

Response: thank you for these comments. In response, we now state on page 7:
"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. Follow-up occurs daily until symptoms resolve or five days, whichever occurs later, and again at 14 days and 1, 3, 6, 9 and 12 months following enrollment. 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 onward) 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)..11"

Also, we submitted our follow-up surveys as a supplemental file.

6. A preview of the tables would help.

Response: We now placed the tables where cited.

Thank you again for the opportunity to publish our manuscript. We are grateful for the review and we hope that you will find the answers and revisions responsive to the feedback.

Best regards

David Schnadower, MD MPH
On behalf of the PECARN Probiotic Study team.

References

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