

Responses to the reviewers' comments and the list of corrections

Dear Editor and Reviewers:

Thank you for your kind consideration of our manuscript entitled “*Lactobacillus rhamnosus* GG in the treatment of acute pediatric diarrhea: A systematic review with meta-analysis” (NO: 48679) for publication in *World Journal of Gastroenterology* and for your extensive help during the revision process. We also want to express our sincere gratitude to the reviewers for their comments and thoughtful suggestions, especially those providing optimal solutions to certain problems, which improved the organization and accuracy of this revision.

We reviewed the questions and suggestions very carefully and have tried our best to address every issue raised by the reviewers according to their suggestions. All the changes to the manuscript are highlighted in yellow. In addition, we tried our best to explain why these changes were applied and how these changes affected the results or conclusions of this manuscript in detail.

Thank you for your kind attention. Please do not hesitate to contact us if we can do anything else for you.

Our responses to the reviewers' comments and a list of the corrections are provided below.

Editors' comments:

Sincerely,

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Response to the editorial comments:

1. The requested audio core tip has been uploaded.
2. A short running title has been modified.
3. Background in abstract has been added. (Lines 52-55)
4. Abbreviations in abstract have been corrected in line 53.
5. References have been checked throughout the manuscript. The first page of papers numbered 28, 32 and 56 without PMID and DOI were provided in the last page of manuscript.
6. Regarding the figures: The decomposable figure of figures was provided, organized into a PowerPoint file, and submitted as “(13) 48679 -Figures.ppt” on the system.

Reviewer's Comments to Author:

Reviewer's code: 03260131

Dear authors, This is a well documented hard study that identified the effect of LGG in children with diarrhea.

Thanks a lot for your recognition.

Reviewer's code: 00503628

The study by Dr. Li and colleagues represents a meta-analysis on the use of *Lactobacillus rhamnosus* GG in the treatment of acute pediatric diarrhea. The study presented is well conceived, thorough and addresses an important question. The investigators searched the major publication databases as well as the Cochrane Central Register of Controlled trials and selected a total of 19 randomized controlled trials that describe LGG usage on acute diarrhea. Their exclusion criteria are well described and include antibiotic-associated and persistent diarrhea as well as studies with more than one strain or studies with incomplete data sets. After analysis of the findings in their study the authors reached the conclusions that better outcomes were noted in children receiving LGG $> 10^{10}$ CFU at early stages, particularly if they were rotavirus-positive diarrhea cases. Interestingly, the reduction in diarrhea was noted in studies conducted in Asia and Europe, but not in other regions. This intriguing observation was not addressed in the study. Overall, this exhaustive manuscript is well written and of interest.

Many thanks for your highly professional summaries. The reduction in diarrhea was found in Asian and European patients. In fact, most of the researches were conducted in Europe or Asia and only 4 studies in other regions respectively, Brazil, United States, Australia and a multicenter study located in Egypt, Africa, Europe and single site (Montevideo) in S. America (variable child and adult mortality). The related content was shown in Table 1 and described in lines 231, 285, 478 and 512.

Reviewer's code: 00002649

MS 48679: Li et al. *L. rhamnosus* GG (LGG) in the treatment of acute pediatric diarrhea: a systematic review with meta-analysis. This MS analyzes RCTs which evaluated LGG treatment in children with acute diarrhea. Nineteen were selected. These reportedly showed that LGG reduced the duration of diarrhea—but only when given at a dose $> 10^{10}$ CFUs daily. Asian and European studies found similar results. Efficacy pertained only to studies enrolling subjects with diarrhea that started < 3 days earlier; and children with rotaviral diarrhea responded the best.

Thank you for your kind consideration and professional advice. Your concerns and suggestions for improving the manuscript have been carefully studied and addressed.

We have provided point-by-point responses to the comments of the editors. The changes to the text are highlighted in yellow in the revised manuscript.

COMMENTS:

MAJOR:

1.The MS is poorly written, with many grammatical errors and a poor discussion. The authors need an English expert to attend to this MS paragraph by paragraph. Pages are not numbered, so I did it by hand.

Thank you for this thoughtful reminder. The manuscript has been thoroughly edited and proofread by native English speakers, and the pages have been numbered.

2.Nevertheless, the recent NEJM publication by Schnadower et al. involving > 900 patients has raised concern that this probiotic is not effective. Therefore, the publication is timely and relevant.

Thank you for your thoughtful comment.

3. Publication bias is a clear concern, yet the discussion of figure S8 is inadequate and the figures are tiny and cannot be read.

Thank you for this important question. We are also very concerned about the publication bias. No publication bias was evident ($P = 0.10$ and 95% CI: -11.33–1.14) for the duration of diarrhea according to a funnel plot (Supporting Information Figure S8) in line 370-372. For the sub-analysis based on ‘the duration of diarrhea before enrollment’, no significant publication bias was found ($P = 0.33$ and 95% CI: -8.89–3.58). In addition, the publication bias assessment in this sub-analysis was informal as fewer than 10 trials were included.^[1] Furthermore, we are sorry that you felt that the figures were too small and could not be read. All the figures have been modified.

4. Adverse effects: How many studies effectively evaluated safety of LGG, for example with diary cards, scheduled phone calls, or daily email access?

In our study, eight studies effectively evaluated the safety of LGG. Adverse effects were reported on a coded reporting form or during daily telephone calls^[2, 3]. In Schnadower D’s study, the caregivers completed a daily diary that was collected by

telephone or through email.^[4] However, the reporting methods were unclear in 5 articles.^[5-9] In general, no adverse effects or similar rates of side effects were documented in the experimental and control groups (Line 347-352).

5. Risk of bias: Of 18 trials

Thank you for your thoughtful reminder. The relevant data are reported in the Results section.

Nineteen RCTs were found in the literature. The two experimental arms in one article from Basu et al are listed separately to show the different doses of probiotics, which are marked as Basu 2009a and Basu 2009b. For further clarification, we modified the phrase to “in 18 articles”.

6 were not strictly blinded. Does this weaken the conclusions of the authors?

Thank you for your important question. Although the inclusion of articles without a strictly blinded design weakens the conclusions, the data in these articles are consistent with the conclusions overall. Studies with larger sample sizes and a strictly blinded

design are needed for further validation. The limitations associated with the conclusions are thoroughly discussed in the manuscript (line 457-461).

MINOR:

1. Abstract: Results: “Nineteen RCTs ... indicated that LGG notably ameliorated diarrheal duration.” I would replace “indicated” with “showed.”

The word “indicated” has been changed to “showed” in line 67.

2. I do not understand why 2 significant digits or more are reported, e.g. “mean difference = 24.02 hours” and 95% CI 36.58.

The number of digits following the decimal point throughout the manuscript was unified, as 2 significant digits after the decimal point, which was consistent with most of the meta-analysis.^[1, 10]

3. Needs a period after 14.79.

A period has been added after 14.79.

4. “The obvious elimination of duration...” is not correct. I think they mean the “shortening of duration of diarrhea.” “Clearly” should be removed. In “reducing rotavirus diarrheal duration.”

The phrase “The obvious elimination of duration...” has been replaced by “A reduced duration of diarrhea” in line 73.

“Clearly” has been removed.

The sentence has been rewritten as “High-dose LGG effectively reduced the duration of rotavirus-induced diarrhea” in line 75-76.

5. Results: line 7: “participator” should be “participants.”

The word was changed to “participants”.

6. Core tip: Again there are serious grammar problems. “LGG was confirmed to be effective in reducing ...number of stools per day. LGG was particularly efficacious in the subsets of patients treated with $> 10^{10}$ CFU/day, treated at an early stage of illness, or diagnosed with rotavirus diarrhea.”

This sentence in lines 90-92 has been rewritten. LGG was particularly efficacious in patients treated with $> 10^{10}$ CFU/day, those treated at an early stage of illness, and those diagnosed with rotavirus-positive diarrhea.

7. Similar grammar problems are seen throughout the MS. On p.3, 3rd paragraph:

“shortening the duration of diarrhea and” Next sentence needs a comma after inhibitor; and “substantial” needs to be replaced with “the incidence of...”

This sentences have been corrected.

8. I am not aware that the WHO feels that a gut motility inhibitor is underused.

The referenced literature was published in 2006. The phrase “a gut motility inhibitor” has been deleted.

9. What is meant by “SCFA modulate the AMP-independent cycle.” Next sentence about rotavirus diarrhea is incorrect. “Leading guidelines” refers to “leading experts.”

“Schnadower et al. found no evidence...”

For clarification, we have rewritten the sentence as follows: “such as short-chain fatty acids increase colonic Na and fluid absorption through a cyclic adenosine monophosphate-independent mechanism”^[11]. (Lines 120-121)

Both “leading experts” and “Schnadower et al. found no evidence...” have been corrected.

10. Study selection: The first 3 sentences need changes.

The sentences have been changed as follows:

“Nineteen randomized controlled trials describing LGG interventions for acute diarrhea were included. The PRISMA statement and the guidelines from the Cochrane Collaboration were followed for this evidence-based medicine study.” (Line 146-148)

11. Page 7: After “The Cochrane Review Manager...” the sentence beginning with “The inverse of variance contributes... sounds like a lecture.

The sentence has been deleted because of insufficient information and inappropriate phrasing.

12. P. 7, bottom: In addition, a larger dose was suggested—actually one is in *C. difficile* prevention, not relevant.

The article related to *C. difficile* prevention has been deleted.

13. Subgroup analyses: The titles should be underlined or put in italics.

The titles have been formatted in italics.

14. P. 8, top paragraph. Again, serious grammar issues related to parallel structure.

After Gieruszk-Bialek concluded that...” the next title should be Site of treatment (inpatient vs. outpatient); then Vaccination status. Early administration. Publication date.

The titles have been corrected according to your request.

15. Bottom of p.8: A large number of trials were conducted in Europe and Asia.

The sentence has been corrected according to your request. (Line 231)

16. P. 9, sentence beginning with “Inconsistency” does not make sense; and the next sentence probably means “Different criteria were used to define diarrhea among all the included studies.”

The sentence has been corrected according your request. (Line 234)

17. P. 9, bottom para: The authors are referring to duration of diarrhea before entry, I believe. Also, “Fortunately” should be replaced with “However.”

The word “enrollment” has been revised to “study enrollment”, and “Fortunately” has been replaced by “However”. (Line 252, 255)

18. P. 10, 3rd line from the bottom and throughout the MS: “no less than” should be replaced with “>”.

The phrase “no less than” has been replaced by “>”. (Line 275)

19. Same page, bottom 2 lines: add “although there were only 3 studies with the lower dosages.”

This text has been added in line 276.

20. P. 11: The authors say that “No studies evaluated the effectiveness of LGG in children vaccinated against rotavirus” but what about the Schnadower study? Also, this sentence should go after “(Figure 2)”

In Schnadower’s study, the coverage rates of the rotavirus vaccine were approximatively equal in the LGG group and placebo group. However, the effectiveness of LGG in vaccinated and non-vaccinated populations was not reported.

In addition, the sentence was placed after “(Figure 2)” following your advice.

21. Please reword the last sentence on p. 11.

The sentence has been reworded as follows: “In addition, similar frequencies were observed in the two groups on day 3, with no differences between them.” (Lines 318-320)

22. Diarrhea > 4d: 3C and 3D are not described.

We are confused about Figure 3C and 3D because Figure 3 consists of 2 parts that are separately marked as A and B. In addition, diarrhea > 4 d is described in lines 305-307.

23. P. 13: “David” does not need to be there; also “However” should be removed from the next sentence.

“David” and “However” have been removed.

24. The Discussion is very difficult to read. Perhaps the authors should make subsections with different points to be made. One reasonable conclusion is that the original meta-analysis by Szajewska made the correct conclusions. I do not understand the top paragraph on p.16 that starts with “The guidelines” and goes to the end of the paragraph.

The discussion has been divided into subsections, and the referenced paragraph has been reworded. (Lines 404-408)

25. “Vomiting is one of the most common symptoms of diarrhea”-incorrect, authors mean “associated with diarrhea.”

The sentence has been reworded as requested.

26. P. 17: I am aware that probiotics benefit the immune response to viruses, but I am not aware that the “viral cycle” is impacted. This sounds like an interference with viral DNA or RNA replication.

Probiotics (e.g., *Bifidobacterium*, *Lactobacillus*) were proven to sequester the newly synthesized viral proteins and reduce RV replication. The phrase “viral cycle” has been replaced by “viral replication”. The original articles are listed as follows:

“*Bifidobacterium infantis* MCC12 and *Bifidobacterium breve* MCC1274 have been associated with a significant reduction in RV titers in infected porcine intestinal epithelial (PIE) cells, and the beneficial effects of both bifidobacteria were associated with improvements in MxA and RNase L expression. Upregulation of MxA inhibits viruses by sequestering the newly synthesized viral proteins. Thus, probiotics and prebiotics are associated with a generalized antiviral effect and specific anti-RV activity.”^[12]

“Lactobacillus ruminis SPM0211 and *Bifidobacterium longum* SPM1205 and SPM1206 inhibited rotavirus replication in a rotavirus-infected neonatal mouse model.”

[13]

27. P. 18, 2nd to last paragraph: I would add “Overall, our study support the previous systematic reviews which concluded that LGG if an effective treatment for children with acute diarrhea.”

The sentence has been added in lines 452-454.

28. Figure 4: What is meant by “stool times”? What is the x-axis? It is not stool number.

The word “times” has been replaced by “number”.

The x-axis shows the mean difference in stool number between the two groups.

29. Figure 4: “frequency on day 3.”

The word has been corrected.

30. Figure 5: A is duration of hospital stay? Why are hours 149, 223 reported. That is 6-10 days. Is this correct?

The unit for “the duration of hospital stay” is hours; 149 and 223 hours are equivalent to 6.2 and 9.2 days, respectively.

- 1 **Szajewska H**, Skorka A, Ruszczynski M, Gieruszczak-Bialek D. Meta-analysis: Lactobacillus GG for treating acute gastroenteritis in children--updated analysis of randomised controlled trials. *Alimentary pharmacology & therapeutics* 2013; **38**(5): 467-476 [PMID: 23841880 DOI: 10.1111/apt.12403]
- 2 **Canani RB**, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, Albano F, Passariello A, De Marco G, Manguso F, Guarino A. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *BMJ* 2007; **335**(7615): 340 [PMID: 17690340 DOI: 10.1136/bmj.39272.581736.55]
- 3 **Nixon AF**, Cunningham SJ, Cohen HW, Crain EF. The effect of Lactobacillus GG on acute diarrheal illness in the pediatric emergency department. *Pediatric emergency care* 2012; **28**(10): 1048-1051 [PMID: 23023475 DOI: 10.1097/PEC.0b013e31826cad9f]
- 4 **Schnadower D**, Tarr PI, Casper TC, Gorelick MH, Dean JM, O'Connell KJ, Mahajan P, Levine AC, Bhatt SR, Roskind CG, Powell EC, Rogers AJ, Vance C, Sapien RE, Olsen CS, Metheney M, Dickey VP, Hall-Moore C, Freedman SB. Lactobacillus rhamnosus GG versus Placebo for Acute Gastroenteritis in Children. *The New England journal of medicine* 2018; **379**(21): 2002-2014 [PMID: 30462938 DOI: 10.1056/NEJMoa1802598]
- 5 **Basu S**, Paul DK, Ganguly S, Chatterjee M, Chandra PK. Efficacy of high-dose Lactobacillus rhamnosus GG in controlling acute watery diarrhea in Indian children: a randomized controlled trial. *Journal of clinical gastroenterology* 2009; **43**(3): 208-213 [PMID: 18813028 DOI: 10.1097/MCG.0b013e31815a5780]
- 6 **Aggarwal S**, Upadhyay A, Shah D, Teotia N, Agarwal A, Jaiswal V. Lactobacillus GG for treatment of acute childhood diarrhoea: an open labelled, randomized controlled trial. *The Indian journal of medical research* 2014; **139**(3): 379-385 [PMID: 24820831]
- 7 **Sindhu KN**, Sowmyanarayanan TV, Paul A, Babji S, Ajjampur SS, Priyadarshini S, Sarkar R, Balasubramanian KA, Wanke CA, Ward HD, Kang G. Immune response and intestinal permeability in children with acute gastroenteritis treated with Lactobacillus rhamnosus GG: a randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2014; **58**(8): 1107-1115 [PMID: 24501384 DOI: 10.1093/cid/ciu065]
- 8 **Salazar-Lindo E**, Miranda-Langschwager P, Campos-Sanchez M, Chea-Woo E, Sack RB. Lactobacillus casei strain GG in the treatment of infants with acute watery

diarrhea: a randomized, double-blind, placebo controlled clinical trial [ISRCTN67363048]. *BMC pediatrics* 2004; **4**: 18 [PMID: 15345099 DOI: 10.1186/1471-2431-4-18]

9 **Ritchie BK**, Brewster DR, Tran CD, Davidson GP, McNeil Y, Butler RN. Efficacy of Lactobacillus GG in aboriginal children with acute diarrhoeal disease: a randomised clinical trial. *J Pediatr Gastroenterol Nutr* 2010; **50**(6): 619-624 [PMID: 20400916 DOI: 10.1097/MPG.0b013e3181bbf53d]

10 **Allen SJ**, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. *The Cochrane database of systematic reviews* 2010(11): CD003048 [PMID: 21069673 DOI: 10.1002/14651858.CD003048.pub3]

11 **Binder HJ**, Brown I, Ramakrishna BS, Young GP. Oral rehydration therapy in the second decade of the twenty-first century. *Curr Gastroenterol Rep* 2014; **16**(3): 376 [PMID: 24562469 DOI: 10.1007/s11894-014-0376-2]

12 **Gonzalez-Ochoa G**, Flores-Mendoza LK, Icedo-Garcia R, Gomez-Flores R, Tamez-Guerra P. Modulation of rotavirus severe gastroenteritis by the combination of probiotics and prebiotics. *Arch Microbiol* 2017; **199**(7): 953-961 [PMID: 28634691 DOI: 10.1007/s00203-017-1400-3]

13 **Kang JY**, Lee DK, Ha NJ, Shin HS. Antiviral effects of Lactobacillus ruminis SPM0211 and Bifidobacterium longum SPM1205 and SPM1206 on rotavirus-infected Caco-2 cells and a neonatal mouse model. *J Microbiol* 2015; **53**(11): 796-803 [PMID: 26502964 DOI: 10.1007/s12275-015-5302-2]