PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Probiotics influencing response of antibodies over time in seniors after COVID-19 vaccine (PIRATES-COV): A randomized controlled trial protocol

Authors

Pasquier, Jean-Charles; Plourde, M; Ramanathan, Sheela; Chaillet, N.; Boivin, Guy; Laforest-Lapointe, Isabelle; Allard-Chamard, Hugues; Baron, Geneviève; Beaulieu, Jean-François; Fülöp, Tamas; Généreux, Mélissa; Mâsse, Benoît; Robitaille, Julie; Valiquette, Louis; Bilodeau, Sarah; H. Buch, Danielle; Piche, Alain

VERSION 1 - REVIEW

Reviewer	**1**
Name	**Mandal, Santi**
Affiliation	**University of California San Diego, Chemistry and Biochemistry**
Date	**11-Jul-2024**
COI	**no**

acceptable

Reviewer	**2**
Name	**Calder, Philip**
Affiliation	**University of Southampton, Institute of Human Nutrition**
Date	**21-Jul-2024**
COI	**I am an advisor to industry on probiotics and immunity.**

This manuscript describes the protocol for a trial of probiotics in older people with responsiveness to COVID-19 booster vaccine as the primary outcome. Secondary outcomes are immune markers and gut microbiota. The trial is ethically approved and registered. According to dates provided it may already have beer completed.

It is a DBRCT in 668 older individuals (age 65 to 89 years). Intervention is 15 days before and 15 days after vaccination. Follow up for the primary outcome is 6 months post vaccination. We are never told what the probiotic is.

Comments:

1. Abstract. Please name the probiotics to be used.

2. Introduction, end of first paragraph. The frequency -> A high frequency

3. Refs 22 and 23 are of meta-analyses of probiotics and flu vaccination. There is a new systematic review and partial meta-analysis restricted to trials in the elderly (PMID 38745493)

4. Methods. Please name the probiotics to be used and the dose. Please name the placebo.

5. How was the 33% reduction inn the primary outcome estimated?

6. Sample size calculation section. of 30% in -> in 30% of

Reviewer	**3**
Name	**DeSilva, Malini B.**
Affiliation	**HealthPartners Institute for Medical Education, Research**
Date	**14-Nov-2024**
COI	**None**

This manuscript describes plans for a double-blind randomized controlled trial taking part November 2022 - January 2024. It is assumed that at this time, the recruitment, intervention, vaccination, and follow-up has been completed. It is unclear why publishing a study protocol at this time is needed as it is assumed a full manuscript with at least partial results could be available in the not too distant future.

There are some specific modifications to the protocol that would improve clarity and help others in understanding the trial:

- Page 11, line 10 - how will the subset of 100 participants for advanced serological testing be chosen? Is this random sampling or some other method?

- Will all participants receive the same COVID-19 booster vaccine and how are differences in vaccine product accounted for in the analysis?

- The specific probiotics administered to study participants should be listed in the protocol.

- It is unclear whether antibody levels at inclusion be taken into account with results. If someone has a detectable antibody level at inclusion, and then becomes undetectable throughout, how is the dichotomous nature of the antibody level variable treated?

- Will current medications be included in the enrollment questionnaire? This is important as multiple medications such as steroids and biologics impact response to vaccines.

- The authors should include results of a meta analysis on the impact of probitoics on vaccine response - Arioz Tunc H, Childs CE, Swann JR, Calder PC. The effect of oral probiotics on response to vaccination in older adults: a systematic review of randomised controlled trials. Age Ageing. 2024 May 11;53(Suppl 2):ii70-ii79. doi: 10.1093/ageing/afae033. PMID: 38745493.

- The first strength listed does not seem related to the proposed study at all, unless the authors think of a clinical trial as a recruitment strategy for vaccines? This is not clear.

- There are no limitations listed, the authors should include limitations of the study either in the limitations or discussion section.

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1

Dr. Santi Mandal, University of California San Diego

Comments to the Author:

acceptable

Thank you for your revision.

Reviewer: 2

Prof. Philip Calder, University of Southampton

Comments to the Author:

This manuscript describes the protocol for a trial of probiotics in older people with responsiveness to COVID-19 booster vaccine as the primary outcome. Secondary outcomes are immune markers and gut microbiota. The trial is ethically approved and registered. According to dates provided it may already have beer completed.

It is a DBRCT in 668 older individuals (age 65 to 89 years). Intervention is 15 days before and 15 days after vaccination. Follow up for the primary outcome is 6 months post vaccination. We are never told what the probiotic is.

Comments:

1. Abstract. Please name the probiotics to be used.

We would like to thank you for your comment. The names of the strains cannot be disclosed at this stage of the study for intellectual property reasons. The strains will be revealed in the next article presenting the results of the study.

2. Introduction, end of first paragraph. The frequency -> A high frequency

We would like to thank you for pointing this out. See Main Document for the modification.

3. Refs 22 and 23 are of meta-analyses of probiotics and flu vaccination. There is a new systematic review and partial meta-analysis restricted to trials in the elderly (PMID 38745493)

Thank you for the suggestion. We will integrate this reference in the Main Document.

4. Methods. Please name the probiotics to be used and the dose. Please name the placebo.

As it was answered at comment no. 1, we cannot disclose the strains at this stage for proprietary reasons. The placebo is an inert preparation in a capsule identical to that of the probiotic.

5. How was the 33% reduction inn the primary outcome estimated?

In the emergency context of COVID-19, it was not feasible to perform a DELPHI method to estimate the clinically significant difference. An expert opinion from our research team was sought and was accepted the Canadian Institutes of Health Research (CIHR) committee. Upon reflection, we decided to remove the 33% from the objective to avoid ambiguity. We kept the 33% in the hypothesis and in the sample size calculation.

6. Sample size calculation section. of 30% in -> in 30% of

We would like to thank you for pointing out this typo. See Main Document for the modification.

Reviewer: 3

Dr. Malini B. DeSilva, HealthPartners Institute for Medical Education

Comments to the Author:

This manuscript describes plans for a double-blind randomized controlled trial taking part November 2022 - January 2024. It is assumed that at this time, the recruitment, intervention, vaccination, and follow-up has been completed. It is unclear why publishing a study protocol at this time is needed as it is assumed a full manuscript with at least partial results could be available in the not too distant future.

The submission of this article has been delayed due to scientific revision, partner revision and journal revision. We still think it is important to publish this protocol to support the upcoming results that

could modify subsequent vaccination campaigns for COVID-19 and other viruses such as avian influenza.

There are some specific modifications to the protocol that would improve clarity and help others in understanding the trial:

- Page 11, line 10 - how will the subset of 100 participants for advanced serological testing be chosen? Is this random sampling or some other method?

For the choice of 100 participants: When we proposed the study there was no precedent in the COVID-19 landscape. We used what is wasknown in the influenza domain. Many of the studies in the elderly included between 50 and 200 participants. Assuming 20% COVID-19 infections, 10-20% loss in paired samples, and the evolving need to analyze data in males and females separately we chose the proposed numbers.

For the recruitment strategy: At inclusion, participants are invited, if they are living nearby, to visit the research centre for blood tests. This was offered until 100 participants were reached. Participants were not selected, it was a convenience sampling.

- Will all participants receive the same COVID-19 booster vaccine and how are differences in vaccine product accounted for in the analysis?

All participants received either the Pfizer or Moderna mRNA vaccine. As they both vaccines act in a similar way, no differences will be accounted for in the analysis. See the Main Document for the modification

- The specific probiotics administered to study participants should be listed in the protocol.

We would like to thank you for your comment. The names of the strains cannot be disclosed at this stage of the study for intellectual property reasons. The strains will be revealed in the next article presenting the results of the study.

- It is unclear whether antibody levels at inclusion be taken into account with results. If someone has a detectable antibody level at inclusion, and then becomes undetectable throughout, how is the dichotomous nature of the antibody level variable treated?

We would like to thank you for your suggestion. Anti-N Antibodies will provide a better understanding of antibodies kinetics in relation to vaccine doses and symptomatic and asymptomatic COVID-19; these are now included in the analyses of this study. In regard to antibody levels, we modify our secondary objectives to integrate this analysis.

- Will current medications be included in the enrollment questionnaire? This is important as multiple medications such as steroids and biologics impact response to vaccines.

Yes they will be included. See the Main Document for precisions.

- The authors should include results of a meta analysis on the impact of probitoics on vaccine response - Arioz Tunc H, Childs CE, Swann JR, Calder PC. The effect of oral probiotics on response to vaccination in older adults: a systematic review of randomised controlled trials. Age Ageing. 2024 May 11;53(Suppl 2):ii70-ii79. doi: 10.1093/ageing/afae033. PMID: 38745493.

We would like to thank for the suggestion. We will integrate this reference in the Main Document

- The first strength listed does not seem related to the proposed study at all, unless the authors think of a clinical trial as a recruitment strategy for vaccines? This is not clear.

Your constructive feedback has helped us improve our manuscript. See Main Document for revised section Strengths and Limitations.

- There are no limitations listed, the authors should include limitations of the study either in the limitations or discussion section.

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VERSION 2 - REVIEW

Reviewer	**2**
Name	**Calder, Philip**
Affiliation	**University of Southampton, Institute of Human Nutrition**
Date	**21-Dec-2024**
COI	

This is the revised version of tis manuscript which describes a trial protocol. Revisions address the concerns raised previously, apart from the authors reluctance to name the active ingredient (i.e. the probiotic) and the placebo. I believe this reluctance needs to be explicitly provided to readers - the most obvious question to a reader is "what is the probiotic?". Given that the manuscript currently does not provide this information, this places the reviewers in a position of appearing to be inept in not noticing this and places the journal at risk of publishing the protocol for an intervention that does not name the intervention. Thus the authors must make the following statement at the end of the Introduction: "The probiotic being used in this study cannot be named at this time for commercial reasons".

VERSION 2 - AUTHOR RESPONSE

Reviewer: 2
Prof. Philip Calder, University of Southampton

Comments to the Author:
This is the revised version of tis manuscript which describes a trial protocol. Revisions address the concerns raised previously, apart from the authors reluctance to name the active ingredient (i.e. the probiotic) and the placebo. I believe this reluctance needs to be explicitly provided to readers - the most obvious question to a reader is "what is the probiotic?". Given that the manuscript currently does not provide this information, this places the reviewers in a position of appearing to be inept in not noticing this and places the journal at risk of publishing the protocol for an intervention that does not name the intervention. Thus the authors must make the following statement at the end of the Introduction: "The probiotic being used in this study cannot be named at this time for commercial reasons".

We integrated the species name and dose in the Main Document.

Reviewer: 2
Competing interests: Not applicable