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

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Probiotics **i**nfluencing **r**esponse of **a**ntibodies over **t**ime in **s**eniors after **CO**VID-19 **v**accine (PIRATES-COV): A randomized controlled trial protocol.

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Protocol amendments since v3, March 9, 2022**:**
Participants: inclusion criteria – from “never had COVID” to “not had COVID in the past 3 months”, to account for COVID spread.
Recruitment strategies revised.
Updated inclusion/exclusion criteria to account for continued boosters, as recommended by public health agencies.
Blood spot samples limited to 3 timepoints instead of 5.
Modified telephone interview for cognitive status dropped from inclusion/exclusion criteria testing.

Any modifications to the protocol that may impact study procedures, outcomes and potential risks/benefits to participants require an amendment. All amendments must be agreed upon by the study team and approved by the Research Ethics Board of the CIUSSS de l’Estrie – CHUS prior to implementation, then disseminated to the rest of the research team as appropriate.

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H Allard-Chamard received some honoraria from Merck for long COVID conferences.
A Piché had grants from Canadian Institutes of Heatlh Research for his work on long COVID prevention, complications, and treatment. He also received honoraria as speaker from the Congrès Québécois en Santé Respiratoire 2023, the Journée de la pharmacologie 23 and at the Fédération des Médecins Spécialistes du Québec. He also participates to working group for the Canadian guidelines for long COVID and he's a member steering committee of BQC19 (Biobanque québécoise de la COVID-19).

Author Contributions:
Co-appliant to the funding grant application (supervised by JC Pasquier, principal appliant): M Plourde, S Ramanathan, N Chaillet, G Boivin, I Laforest-Lapointe, G Baron, T Fülöp, M Généreux, B Mâsse, J Robitaille, H Allard-Chamard, A Piché, JF Beaulieu.
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Critical revision of the protocol and manuscript for important intellectual content: All authors
Substantial contributions to interpretation:
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Data sharing statement: There are no plans at present to share Individual Participant Data (IPD).

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ABSTRACT

Introduction: The elderly are particularly vulnerable to morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Approximately 20% of elderly showed no antibodies 3-5 months post-2nd dose of the COVID-19 vaccine. As probiotics have been shown to increase influenza-specific antibody levels post-influenza vaccination, we aim to reduce by 33% participants without antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD) at 6 months post-vaccination.

Methods and analysis: Our study design is a double-blind randomized controlled trial, using intention-to-treat analysis. Eligible participants are a purposive sample of 668 adults aged 65-89 years, in Quebec, Canada, 2022-2024, not diagnosed with COVID-19 in the 3 months prior to recruitment and who wish to receive a government-recommended mRNA booster (Pfizer-BioNTech, Moderna) vaccine. The intervention consists of 1 capsule/day of a probiotic dietary supplement or a placebo, for 15 days pre- and post-booster vaccine. All participants provide dried blood spot samples at 3 timepoints (inclusion, 3- and 6-months post-vaccination) and a stool sample for microbiome analysis. A subgroup of 100 participants living near Sherbrooke, Quebec, is expected to volunteer for 2 onsite blood-test visits (at inclusion and 6 months post-vaccination). The primary outcome is participants without anti-S1-RBD antibodies at 6 months post-vaccination. Secondary outcomes include longitudinal analysis of anti-S1-RBD antibodies at 3 timepoints. In the subgroup, serum levels of neutralizing anti-RBD antibodies will be determined at 2 timepoints. Probiotic and vaccine side effects are

monitored. At the end of the study, we expect to identify the adjuvant effect of probiotic on vaccine-induced immune response.

Ethics and dissemination: The study was approved by institutional Research Ethics Boards as well as Health Canada. All participants will provide informed consent. Results will be disseminated to the scientific community and to all networks related in this research.

Trial registration number (ClinicalTrials.gov identifier): NCT05195151.

KEYWORDS: COVID-19, SARS-CoV-2, COVID vaccination, booster dose, mRNA vaccine, vaccine adjuvant, elderly, probiotics, microbiome, immunity.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- COVID-19 infection is now mostly self-resolving, not requiring hospitalization. To reach the elderly population prior to booster doses, new recruitment strategies must be developed.
- Remote study methods pave the way for future cost-effective research methodologies not requiring onsite visits.
- Probiotics are already available at low cost without prescription in Canada and other countries, suggesting a cost-effective and easily implementable option worldwide.
- Regardless of probiotic results, our longitudinal findings will inform public health for optimal spacing of COVID-19 booster vaccination in older adults.

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INTRODUCTION

The elderly population is particularly at risk of morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1–3] Vaccination against COVID-19 began in Canada in 2021, mainly using the mRNA vaccines by Pfizer-BioNTech and Moderna. However, due to waning immunity and subsequent large-scale infection by SARS-CoV-2 delta and omicron variants,[4] booster doses have proven necessary.[5] While two doses of vaccine induced robust activation of cell-mediated and humoral immune response,[6,7] the magnitude was less than that conferred by natural COVID-19 infection.[8–10] A recent meta-analysis showed that COVID-19 vaccines in the elderly were effective and tolerated, with a decrease in COVID-19-related deaths.[11] Nonetheless, 3 months after vaccination, antibody levels declined,[12] at a faster rate in the elderly than in the general population.[1,2] Three to five months after the 2nd dose, 20% of older adults showed no trace of anti-COVID antibodies.[1,13,14] Many countries, including Canada, have offered booster doses every 5 or 6 months. The frequency of booster administration is a strain on public health resources and increases societal and economic costs. COVID vaccine durability is thus a public health concern.

The human microbiota, defined as all microorganisms colonizing the human body in the gut and oral cavity, are known to play a role in host protection, from tight junction regulation in the intestinal mucosa to the production of short-chain fatty acids to increasing innate and acquired immunity.[15–18] Microbiota involvement has been reported in digestive, respiratory, auto-immune, and metabolic disease.[16] In the elderly, gut microbiota showed less diversity, lower levels of beneficial bacteria, and higher levels of

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pathogenic species.[19] Furthermore, the use of antibiotics reduced immune response to vaccine boosters in individuals with low titres of existing antibodies.[20] If gut microbial dysbiosis (i.e., a state of imbalance leading to a loss of diversity or a change in community composition) was to partially explain differential response to vaccination, intervention with probiotics might improve vaccination effectiveness.[21]

Two meta-analyses reported improved response to the influenza vaccine with probiotic co-administration,[22,23] as has a randomized controlled trial in the elderly.[24] Nevertheless, it remains difficult to draw clear conclusions on the benefits of probiotic co-administration with vaccination based on these studies, as they included different sample sizes, different strains and doses of probiotics, and different modes of administration and lengths of treatment.[25] A recent trial on probiotics and COVID-19 vaccination in nursing home residents showed conflicting results, with a trend toward positive effects.[26] Three trials on probiotic co-administration with COVID-19 vaccination are currently registered on clinicaltrials.gov.[27] We hypothesized that probiotics taken at the time of the COVID-19 booster dose would improve both humoral (antibodies to the SARS-CoV-2 receptor-binding domain) and cell-mediated (T and B cells) immune response in the elderly. This would increase immunity in the medium term and allow for longer spacing between booster doses. Further, increased memory cell immunity might prove protective against future variants.

To test this hypothesis, we will conduct a randomized controlled trial using a 2-strain probiotic dietary supplement as adjuvant to the COVID vaccine booster dose, with the aims of reducing by 33% the percentage of elderly without antibodies to the SARS-CoV-2 spike protein at 6 months post-vaccination. Our secondary objectives include a

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longitudinal analysis of antibodies at inclusion and at 3 and 6 months post-vaccination (dried blood spots), as well as a comparison of neutralizing antibodies and spike-specific T and B cell levels at inclusion and at 6 months post-vaccination in a subset of participants. We will also monitor local and systemic side effects.

METHODS

Study design and population

The study design is a remote- and hybrid-design randomized controlled trial, using intention-to-treat analysis. The intervention consists of a probiotic supplement, as compared to placebo, for enhancing immune response in the elderly for a period of up to 6 months following COVID-19 vaccination. The trial is to be conducted remotely throughout Quebec (Canada) from November 2022 to January 2024 and coordinated by our study site at the Sherbrooke Hospital Integrated University Health and Social Services Centre, Estrie (*Centre Intégré Universitaire en Santé et Services Sociaux*, CIUSSS de l'Estrie – CHUS). A subset of 100 participants living in the Eastern Townships (Estrie), Quebec, will provide onsite blood samples for additional serological testing. With the COVID-19 situation in constant flux, the targeted booster vaccination is defined as the next booster dose of the mRNA COVID-19 vaccine, as recommended by Quebec Public Health.

Inclusion/exclusion criteria

Adults aged 65 to 89 years, of either sex, living at home or in independent-living retirement homes in Quebec, Canada, are eligible for participation if they wish to receive the next government-recommended COVID-19 mRNA vaccine booster dose, if they have already received three vaccine doses (Pfizer-BioNTech or Moderna), and if the latest

dose was at least 5 months prior to inclusion. Other inclusion criteria are telephone and/or internet access and cognitive ability to provide informed consent. The latter will be assessed using the Functional Activities Questionnaire (FAQ)[28]; participants with scores ≥ 9 will be excluded. Other exclusion criteria are allergy (soy, lactose, yeast, maltodextrin), chronic immunodeficiency/immunosuppression (e.g., concurrent cancer chemotherapy or radiotherapy; concurrent active treatment for intestinal disorders such as duodenal ulcers, celiac disease, ulcerative colitis, and Crohn's), and use of probiotics and/or antibiotics at date of inclusion. Individuals with a serious condition precluding safe participation to the end of the study are excluded, as well as those having had COVID-19 in the past 3 months (confirmed clinically or, in the case of the 100-people subgroup, *a posteriori* by serological testing). Individuals who speak neither French nor English are excluded.

Recruitment, screening and consent

The study will be advertised at community centres and on social media, with a telephone number for callback, as well as through word of mouth from participants already registered. Different recruitment strategies will be used, including advertising in traditional media, information meetings in retirement homes, and contacting potential participants from professional community lists.

Preliminary eligibility will be determined online or by telephone. A research assistant will then call potential participants, to explain the study, answer questions, and confirm eligibility using the FAQ. Consent will be documented online or through telephone recordings.

Randomization

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Participants are randomized 1:1 into the placebo or intervention group, using permuted block randomization stratified by age (65-79 years, 80-89 years) and sex (male, female). Randomization will be programmed by the Applied Clinical Research Unit of the Sainte-Justine Research Centre and performed online using REDCap. The subset of 100 participants for advanced serological testing will be chosen and randomized as above.

The study will be double-blinded. Until unlocking of the database at study end, neither the participants nor the research team, partner, or affiliated laboratories will be informed of the participants' group allocation. Pharmacists at Sherbrooke Hospital will prepare study products (probiotics or placebo) as per REDCap blinding requirements, using investigational products provided by Lallemand Health Solutions Inc. (Mirabel, Quebec, Canada) prior to study start.

Intervention

The intervention consists of an oral probiotic dietary supplement containing 2 bacterial strains. Capsules for both probiotic and placebo are otherwise identical.

All participants take 1 capsule of the investigational product (probiotic or placebo) per day for 30 days: 15 days before and 15 days after receiving the COVID-19 vaccine booster shot. Should the vaccination appointment be delayed by more than 3 days once treatment has begun, treatment will continue until 15 days post-vaccination. An extra bottle of 35 capsules is kept for each participant at the hospital pharmacy, if needed.

Outcome Measures

Primary outcome measure

The primary outcome measure is the percentage of participants without detectable antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD

Ab) at 6 months following the vaccination booster dose. Anti-S1-RBD antibodies prevent binding of SARS-CoV-2 to the ACE-2 receptor and thus correlate well with neutralizing antibodies (NAb) and vaccine effectiveness.[29] Levels of anti-S1-RBD antibodies will be determined by colorimetric enzyme-linked immunosorbent assays (ELISA) on dried blood spot samples. As per manufacturer's guidelines, positivity threshold is set at 3 standard deviations above the mean of negative controls (optical density on colorimetric blotting paper). ELISA tests have 95% sensitivity and 100% specificity.[29]

Secondary outcome measures

The main secondary outcome measure is a longitudinal analysis of anti-S1-RBD antibodies at 3 different time points (inclusion; 3- and 6-months post-vaccination). The analysis will be performed as described above on dried blood spot samples.

Another secondary outcome is the level of neutralizing antibodies and spike-specific T and B cells, at inclusion and at 6 months post-vaccination, in the subset of 100 participants providing venous blood samples. Lymphocyte-binding to the spike protein will be measured by flow cytometry as described.[30,31] Absence of COVID-19 infection within the previous 3 months will be assessed by ELISA against the viral nucleocapsid.[32,33]

A third outcome is the analysis of the human gut microbiota to compare gut microbial communities in both groups at baseline, using stool samples provided by all participants at time of inclusion. DNA will be extracted from stool samples and then sequenced using shotgun metagenomic sequencing on a NovaSeq 6000 platform (Illumina, Genome Quebec, Montreal, Canada). The bioBakery workflow (Huttenhower lab, MA, USA) will be used for computer analysis. Shotgun sequencing data will go

through metagenomic phylogenetic analysis (MetaPhlAn v3.0) to obtain a profile of microbial communities (bacterial, archaeal, viral, eukaryotic).

Clinically, we will monitor side effects of the investigational product as well as those of the vaccine, both local (pain or redness at the injection site, swollen lymph nodes) and systemic (fever, fatigue, headache, muscle aches, digestive troubles, flu-like symptoms). We will also take note of COVID-19 infections (FLU-PRO questionnaire,[34] followed by COVID-19 rapid testing).

Contamination bias

To prevent potential contamination bias, as probiotics are widely available and potential benefits assumed by the general population, participants are asked not to use probiotics (other than the investigational product) for the duration of the study. Questionnaires on probiotic use are completed before and during the study. Participants found to have used over-the-counter probiotics will be documented but retained in study analyses.

Data and biological samples – collection and storage

For most participants, there are no in-person study visits. Biological samples are collected at home. These include one baseline stool sample and 3 dried blood spot samples: at inclusion, at 3 months post-vaccination, and at 6-months post-vaccination. Blood spot samples are done by finger-prick testing on blotter paper; stool samples are self-performed. All samples are done at home and sent in by courier. Should any participants require help with sampling, a research team member will be available by phone or in person, upon request.

For a subset of 100 participants in the Sherbrooke area, there will be 2 in-person visits, one at baseline prior starting any treatment and one 6 months after vaccination.

Venous blood collection will be done by a nurse at both visits, using the same brand of vacuum blood collection tubes with EDTA for anticoagulation. For each participant, five 4 mL tubes of blood will be collected. The final visit will include a satisfaction questionnaire regarding the research team, recruitment process, and acceptability of data and specimen collection. To decrease loss to follow-up, for those with mobility issues or if requested by participants, nurses will collect dried blood spot samples at the participant's home.

Biological samples

Dried blood spot samples will be couriered to the Sherbrooke study site, then sent to Guy Boivin's lab in Quebec City for analysis. Venous blood samples will be processed and stored at the Sherbrooke study site (S. Ramanathan). Stool samples will be couriered and stored in A. Piché's lab, sent to Genome Quebec for analysis, and interpreted at I. Laforest-Lapointe's lab in Sherbrooke.

Data collection

At the time of enrolment, questionnaires will be completed. These include questions on sociodemographic data, height and weight, ethnicity, vaccination status, medical history, and comorbidities such as cancer. A validated food frequency questionnaire[35] will assess dietary habits.

Information on investigational product intake will be collected every 2 weeks for the first 33 days, online or by telephone. This will help in ensuring adherence to treatment and will be used to monitor adverse effects, if any.

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Information on booster vaccination will be collected once a week, for 2 weeks. This includes local and systemic side effects, such as injection site pain/redness, fever, muscle aches, digestive troubles, and flu-like symptoms.[36]

Any changes to health will be collected once a month. Participants infected with COVID-19 will remain in the study and fill out questionnaires describing symptoms. In the case of hospitalization or death, data will be extracted from the participant's Quebec Health Record.

Data confidentiality and databank/biobank security

REDCap questionnaires are encrypted and kept at the Applied Clinical Research Centre (URCA) of the Sainte-Justine Research Centre. Data will be coded and the code kept by the principal investigator. Biological samples are collected in accordance with international standards.[37,38] Samples will be stored at the *Banque québécoise de la COVID-19* (BQC19),[39] under the responsibility of the co-investigator.

Adverse events and data monitoring

Other than bloating, intestinal irritation, or stool softer than usual, very few side effects are associated with probiotic use, although caution is warranted in critically ill patients.[40] Vaccine safety and tolerance are well established in the elderly. A 4-member monitoring committee chaired by Bruno Piedboeuf will be advised of all adverse events (serious adverse events, within 48 hours; others, every three months). The steering committee will meet every 2 weeks. A scientific committee will meet monthly for 4 months and bi-monthly thereafter. No interim analysis is planned.

COVID-19 infections

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In the event of COVID-19 infection during the trial and prior to administration of the booster shot (as determined by rapid COVID tests upon declaration of symptoms), participants will be asked whether they wish to delay the booster for several months or forego it altogether. In either case, participants will remain in the trial. Investigational product regimen and data/sample collection will begin anew 2 weeks prior to the new vaccination date, if applicable.

Statistical analysis

Analysis is by intention-to-treat. Intervention and placebo groups will be compared at baseline for sociodemographic and clinical characteristics, as well as for microbiota composition. Results for continuous variables will be presented as means ± standard deviations (SD) or as geometric means and 95% confidence intervals (CI); for categorical variables, as N (%). For the primary outcome, we will consider antibody level as a dichotomous variable (detectable, undetectable) and use a generalized method of moments (GMM) logistic regression model with time-dependent covariates for longitudinal binary data. The model will be stratified in subgroup analyses by age and sex at 3 different timepoints (inclusion; 3- and 6-months post-vaccination). The outcomes at the 3 timepoints are the interaction between group (intervention vs. control) and timepoint.[41] Secondary outcomes at inclusion and at 6 months post-vaccination will be analyzed on log-transformed original measures for continuous variables; at 6 months, groups will be compared using ANCOVA with inclusion data covariates. CD4 and CD4/CD8 ratios with log-transformed antibody levels will be estimated using Spearman's correlation and 95% CIs. *P* values < 0.05 will be considered statistically significant.

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Sample size calculation

We calculated the sample size to discern an effect size of 33% reduction in the number of participants presenting undetectable levels of anti-S1-RBD antibodies at 6 months post-vaccination. With an expected undetectable antibody level of 30% in the placebo group and therefore 20% in the intervention group, an estimated 584 participants would provide 80% power and a two-tailed alpha of 5%. With an estimated 15% attrition rate, the study would therefore require 668 participants.

Patient and public involvement statement

The study will involve the Patient-Partnership Strategic Committee (*Comité stratégique patient-partenaire*, CSPP) of the Sherbrooke Hospital Research Centre (CRCHUS), co-ordinator C. Wilhelmy and the Patient-Partnership Initiative (*Initiative patient-partenaire FMSS-UdeS et Réseau Universitaire Intégré de Sherbrooke*), M. Garriss. In collaboration with the CIUSS-Estrie living lab for geriatric research (*Laboratoire d'innovations par et pour les aînés*, LIPPA), seniors will have the opportunity to join focus groups, collaborate at various stages of the research, and participate in the pilot study. We are also grateful to the National COVID-19 Clinical Trials Network, led by R. Fowler, and the Quebec Research Network on Aging, led by P. Gaudreau, for their support.

DISCUSSION

This double-blind randomized controlled trial will test a probiotic supplement as an adjuvant for enhancing COVID-vaccine immune response in the elderly population. Improved immune response and extended spacing of vaccine booster doses in high-risk populations are both major public health issues. If successful, probiotic adjuvants could

rapidly be implemented worldwide. They have few side effects, are currently available without prescription, and are relatively affordable. Furthermore, if a probiotic supplement can act as a COVID-19-vaccine adjuvant to enhance immunity, this low-cost intervention could lead to important benefits such as hospitalization reductions, longer time between booster shots, and higher immunity against new variants. This intervention would then be clinically, socially, and economically beneficial.

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PIRATES-COV

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

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

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Protocol amendments since v3, March 9, 2022**:**
Participants: inclusion criteria – from "never had COVID" to "not had COVID in the past 3 months", to account for COVID spread.
Recruitment strategies revised.
Updated inclusion/exclusion criteria to account for continued boosters, as recommended by public health agencies.
Blood spot samples limited to 3 timepoints instead of 5.
Modified telephone interview for cognitive status dropped from inclusion/exclusion criteria testing.

Any modifications to the protocol that may impact study procedures, outcomes and potential risks/benefits to participants require an amendment. All amendments must be agreed upon by the study team and approved by the Research Ethics Board of the *CIUSSS de l'Estrie – CHUS* prior to implementation, then disseminated to the rest of the research team as appropriate.

PIRATES-COV

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A Piché had grants from the Canadian Institutes of Health Research for his work on long COVID prevention, complications, and treatment. He also received honoraria as speaker from the *Congrès Québécois en Santé Respiratoire 2023*, the *Journée de la pharmacologie 23* and at *the Fédération des Médecins Spécialistes du Québec*. He also participates in a working group for the Canadian guidelines for Post COVID condition and he is a member of the steering committee of the BQC19 (*Biobanque québécoise de la COVID-19*).

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Drafting of the manuscript: Danielle Buch
Critical revision of the protocol and manuscript for important intellectual content: All authors
Substantial contributions to interpretation:
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ABSTRACT

Introduction: The elderly are particularly vulnerable to morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Approximately 20% of elderly showed no antibodies 3-5 months post-2nd dose of the COVID-19 vaccine. As probiotics have been shown to increase influenza-specific antibody levels post-influenza vaccination, we aim to reduce the percentage of participants without antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD) at 6 months post-vaccination.

Methods and analysis: Our study design is a double-blind randomized controlled trial, using intention-to-treat analysis. Eligible participants are a purposive sample of 688 adults aged 65-89 years, in Quebec, Canada, 2022-2024, not diagnosed with COVID-19 in the 3 months prior to recruitment and who wish to receive a government-recommended mRNA booster (Pfizer-BioNTech, Moderna) vaccine. The intervention consists of 1 capsule/day of a probiotic dietary supplement or a placebo, for 15 days pre- and post-booster vaccine. All participants provide dried blood spot samples at 3 timepoints (inclusion, 3- and 6-months post-vaccination) and a stool sample for microbiome analysis. A subgroup of 100 participants living near Sherbrooke, Quebec, is expected to volunteer for 2 onsite blood-test visits (at inclusion and 6 months post-vaccination). The primary outcome is participants without anti-S1-RBD antibodies at 6 months post-vaccination. Secondary outcomes include longitudinal analysis of anti-S1-RBD and anti-N antibodies at 3 timepoints. In the subgroup, serum levels of neutralizing antibodies will be determined at inclusion and 6 months post-vaccination. Probiotic and vaccine side

effects are monitored. At the end of the study, we expect to identify the adjuvant effect of probiotic on vaccine-induced immune response.

Ethics and dissemination: The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MEO-31-2022-6278* as well as Health Canada. All participants will provide informed consent. Results will be disseminated to the scientific community and to all networks related in this research.

Trial registration number (ClinicalTrials.gov identifier): NCT05195151.

KEYWORDS: COVID-19, SARS-CoV-2, COVID vaccination, booster dose, mRNA vaccine, vaccine adjuvant, elderly, probiotics, microbiome, immunity.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first RCT to evaluate the interaction between oral probiotics and on the durability of COVID-19 vaccine protection in the elderly.
- Probiotics are already available at low cost without prescription in Canada and other countries, suggesting a cost-effective and easily implementable option worldwide.
- Recruiting to target sample size will be a challenge mainly because participants have to be contacted 3-4 weeks before their booster dose appointment (for 2 weeks of probiotic/placebo intake before vaccination)
- Maintaining adherence during a 6-month-study with multiple sample collection is a challenge as well.

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INTRODUCTION

The elderly population is particularly at risk of morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1–3] Vaccination against COVID-19 began in Canada in 2021, mainly using the mRNA vaccines by Pfizer-BioNTech and Moderna. However, due to waning immunity and subsequent large-scale infection by SARS-CoV-2 delta and omicron variants,[4] booster doses have proven necessary.[5] While two doses of vaccine induced robust activation of cell-mediated and humoral immune response,[6,7] the magnitude was less than that conferred by natural COVID-19 infection.[8–10] A recent meta-analysis showed that COVID-19 vaccines in the elderly were effective and tolerated, with a decrease in COVID-19-related deaths.[11] Nonetheless, 3 months after vaccination, antibody levels declined,[12] at a faster rate in the elderly than in the general population.[1,2] Three to five months after the 2nd dose, 20% of older adults showed no trace of anti-COVID antibodies.[1,13,14] Many countries, including Canada, have offered booster doses every 5 or 6 months. A high frequency of booster administration is a strain on public health resources and increases societal and economic costs. COVID vaccine durability is thus a public health concern.

The human microbiota, defined as all microorganisms colonizing the human body in the gut and oral cavity, are known to play a role in host protection, from tight junction regulation in the intestinal mucosa to the production of short-chain fatty acids to increasing innate and acquired immunity.[15–18] Microbiota involvement has been reported in digestive, respiratory, auto-immune, and metabolic disease.[16] In the elderly, gut microbiota showed less diversity, lower levels of beneficial bacteria, and higher levels of

pathogenic species.[19] Furthermore, the use of antibiotics reduced immune response to vaccine boosters in individuals with low titres of existing antibodies.[20] If gut microbial dysbiosis (i.e., a state of imbalance leading to a loss of diversity or a change in community composition) was to partially explain differential response to vaccination, intervention with probiotics might improve vaccination effectiveness.[21]

Two meta-analyses reported improved response to the influenza vaccine with probiotic co-administration,[22,23] as has a randomized controlled trial in the elderly.[24] Nevertheless, it remains difficult to draw clear conclusions on the benefits of probiotic co-administration with vaccination based on these studies, as they included different sample sizes, different strains and doses of probiotics, and different modes of administration and lengths of treatment.[25] A recent trial on probiotics and COVID-19 vaccination in nursing home residents showed conflicting results, with a trend toward positive effects.[26] Three trials on probiotic co-administration with COVID-19 vaccination are currently registered on clinicaltrials.gov.[27] We hypothesized that probiotics taken at the time of the COVID-19 booster dose would improve both humoral (antibodies to the SARS-CoV-2 receptor-binding domain) and cell-mediated (T and B cells) immune response in the elderly. This would increase immunity in the medium term and allow for longer spacing between booster doses. Further, increased memory cell immunity might prove protective against future variants.

To test this hypothesis, we will conduct a randomized controlled trial using a 2-strain probiotic dietary supplement as adjuvant to the COVID vaccine booster dose, with the aims of reducing the percentage of elderly without antibodies to the SARS-CoV-2 spike protein at 6 months post-vaccination. Our secondary objectives include a

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longitudinal analysis of antibodies at inclusion and at 3- and 6-months post-vaccination (dried blood spots), as well as a comparison of antibodies and spike-specific T and B cell levels at inclusion and at 6 months post-vaccination in a subset of participants. Further, we will also compare the proportion confirmed cases of COVID-19 (by rapid antigen testing or polymerase chain reaction (PCR) with symptoms and the proportion of COVID-19 asymptomatic cases in each study group. We will also monitor local and systemic side effects.

METHODS

Study design and population

The study design is a remote- and hybrid-design randomized controlled trial, using intention-to-treat analysis. The intervention consists of a probiotic supplement, as compared to an inert placebo, for enhancing immune response in the elderly for a period of up to 6 months following COVID-19 vaccination. The trial is to be conducted throughout Quebec (Canada) from November 2022 to January 2024 and coordinated by our study site at the *Centre de recherche du Centre hospitalier universitaire de Sherbrooke* (CR-CHUS). A convenience sample of 100 participants living in the Eastern Townships (Estrie), Quebec, in addition to taking part in the proposed study, will provide onsite blood samples for additional serological testing as part of the proposed study i.e.if participants are able to travel to the CR-CHUS, they will be invited for two in-person blood tests, until 100 subgroup participants is reached. With the COVID-19 situation in constant flux, the targeted booster vaccination is defined as the next booster dose of the mRNA COVID-19 vaccine (Moderna or Pfizer), as recommended by Quebec Public Health.

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Inclusion/exclusion criteria

Adults aged 65 to 89 years, of either sex, living at home or in independent-living retirement homes in Quebec, Canada, are eligible for participation if they wish to receive the next government-recommended COVID-19 mRNA vaccine booster dose, if they have already received three vaccine doses (Pfizer-BioNTech or Moderna), and if the latest dose was at least 5 months prior to inclusion. Other inclusion criteria are telephone and/or internet access and cognitive ability to provide informed consent. The latter will be assessed using the Functional Activities Questionnaire (FAQ)[28]; participants with scores ≥ 9 will be excluded. Other exclusion criteria are allergy (soy, lactose, yeast, maltodextrin), chronic immunodeficiency/immunosuppression (e.g., concurrent cancer chemotherapy or radiotherapy; concurrent active treatment for intestinal disorders such as duodenal ulcers, celiac disease, ulcerative colitis, and Crohn's), and use of probiotics and/or antibiotics at date of inclusion. Individuals with a serious condition precluding safe participation to the end of the study are excluded, as well as those having had COVID-19 in the past 3 months (confirmed clinically or, in the case of the 100-people subgroup, *a posteriori* by serological testing). Individuals who speak neither French nor English are excluded.

Recruitment, screening and consent

The study will be advertised at community centres and on social media, with a telephone number for callback, as well as through word of mouth from participants already registered. Different recruitment strategies will be used, including advertising in traditional media, information meetings in retirement homes, and contacting potential participants from professional community lists.

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Preliminary eligibility will be determined online or by telephone. A research assistant will then call potential participants, to explain the study, answer questions, and blind telephone recordings.

Randomization

Participants are randomized 1:1 into the placebo or intervention group, using permuted block randomization stratified by age (65-79 years, 80-89 years) and sex (male, female). Randomization will be programmed by the Applied Clinical Research Unit of the Sainte-Justine Research Centre and performed online using REDCap.

The study will be double-blinded. Until unlocking of the database at study end, neither the participants nor the research team, partner, or affiliated laboratories will be informed of the participants' group allocation. Pharmacists at Sherbrooke Hospital will prepare study products (probiotics or placebo) as per REDCap blinding requirements, using investigational products provided by Lallemand Health Solutions Inc. (Mirabel, Quebec, Canada) prior to study start.

Intervention

The intervention consists of an oral probiotic dietary supplement containing 2 bacterial strains. Capsules for both probiotic and placebo are otherwise identical.

All participants take 1 capsule of the investigational product (probiotic or placebo) per day for 30 days: 15 days before and 15 days after receiving the COVID-19 vaccine booster shot. Should the vaccination appointment be delayed by more than 3 days once treatment has begun, treatment will continue until 15 days post-vaccination. An extra bottle of 35 capsules is kept for each participant at the hospital pharmacy, if needed.

Outcome Measures

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Primary outcome measure

The primary outcome measure is the percentage of participants without detectable antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD Ab) at 6 months following the vaccination booster dose. Anti-S1-RBD antibodies prevent binding of SARS-CoV-2 to the ACE-2 receptor and thus correlate well with neutralizing antibodies (NAb) and vaccine effectiveness.[29] Levels of anti-S1-RBD antibodies will be determined by colorimetric enzyme-linked immunosorbent assays (ELISA) on dried blood spot samples. As per manufacturer's guidelines, positivity threshold is set at 3 standard deviations above the mean of negative controls (optical density on colorimetric blotting paper). ELISA tests have 95% sensitivity and 100% specificity.[29]

Secondary outcome measures

Secondary outcomes measures are a longitudinal analysis of anti-S1-RBD and Anti-Nucleocapsid (Anti-N) antibodies at 3 different time points (inclusion; 3- and 6-months post-vaccination). Possibly, a quantitative variable will also be available. The analysis will be performed as described above on dried blood spot samples.

We will also compare the percentage of confirmed cases of COVID-19 with and without symptoms in each group (COVID-19 confirmed by rapid antigen testing or PCR). The analysis will be done using the reported adverse events and presence of Antibodies Anti-N.

Another secondary outcome is the level of neutralizing antibodies and spike-specific T and B cells, at inclusion and at 6 months post-vaccination, in the subset of 100 participants providing venous blood samples. Lymphocyte-binding to the spike protein will be measured by flow cytometry as described.[30,31] Absence of COVID-19 infection

within the previous 3 months will be assessed by ELISA against the viral nucleocapsid.[32,33]

Subsequent analyses will include the comparison of gut microbial communities in both study groups at baseline, using stool samples provided by all participants at time of inclusion. DNA will be extracted from stool samples and then sequenced using shotgun metagenomic sequencing on a NovaSeq 6000 platform (Illumina, Genome Quebec, Montreal, Canada). The bioBakery workflow (Huttenhower lab, MA, USA) will be used for computer analysis. Shotgun sequencing data will go through metagenomic phylogenetic analysis (MetaPhlAn v3.0) to obtain a profile of microbial communities (bacterial, archaeal, viral, eukaryotic).

Clinically, we will monitor side effects of the investigational product as well as those of the vaccine, both local (pain or redness at the injection site, swollen lymph nodes) and systemic (fever, fatigue, headache, muscle aches, digestive troubles, flu-like symptoms). We will also take note of COVID-19 infections (FLU-PRO questionnaire,[34] followed by COVID-19 rapid testing).

Changes in outcome measures

Secondary outcomes have been modified to add an Anti-N antibody analysis. This type of analysis was not available when the protocol was first written and seems to be essential to giving more answers about the proportion of COVID-19 infections.

Contamination bias

To prevent potential contamination bias, as probiotics are widely available and potential benefits assumed by the general population, participants are asked not to use probiotics (other than the investigational product) for the duration of the study. Questionnaires on

probiotic use are completed before and during the study. Participants found to have used over-the-counter probiotics will be documented but retained in study analyses.

Data and biological samples – collection and storage

For most participants, there are no in-person study visits. Biological samples are collected at home. These include one baseline stool sample and 3 dried blood spot samples: at inclusion, at 3 months post-vaccination, and at 6-months post-vaccination. Blood spot samples are done by finger-prick testing on blotter paper; stool samples are self-performed. All samples are done at home and sent in by courier. Should any participants require help with sampling, a research team member will be available by phone or in person, upon request.

For a subset of 100 participants in the Sherbrooke area, there will be 2 in-person visits, one at baseline prior starting any treatment and one 6 months after vaccination. Venous blood collection will be done by a nurse at both visits, using the same brand of vacuum blood collection tubes with EDTA for anticoagulation. For each participant, five 4 mL tubes of blood will be collected. The final visit will include a satisfaction questionnaire regarding the research team, recruitment process, and acceptability of data and specimen collection. To decrease loss to follow-up, for those with mobility issues or if requested by participants, nurses will collect dried blood spot samples at the participant's home.

Biological samples

Dried blood spot samples will be couriered to the Sherbrooke study site, then sent to Guy Boivin's lab in Quebec City for analysis. Venous blood samples will be processed and stored at the Sherbrooke study site (S. Ramanathan). Stool samples will be couriered and

stored in A. Piché's lab, sent to Genome Quebec for analysis, and interpreted at I. Laforest-Lapointe's lab in Sherbrooke.

Data collection

At the time of enrolment, questionnaires will be completed. These include questions on sociodemographic data, height and weight, ethnicity, vaccination status, current medications, medical history, and comorbidities such as cancer. A validated food frequency questionnaire[35] will assess dietary habits.

Information on investigational product intake will be collected every 2 weeks for the first 33 days, online or by telephone. This will help in ensuring adherence to treatment and will be used to monitor adverse effects, if any.

Information on booster vaccination will be collected once a week, for 2 weeks. This includes local and systemic side effects, such as injection site pain/redness, fever, muscle aches, digestive troubles, and flu-like symptoms.[36]

Any changes to health will be collected once a month. Participants infected with COVID-19 will remain in the study and fill out questionnaires describing symptoms. In the case of hospitalization or death, data will be extracted from the participant's Quebec Health Record.

Data confidentiality and databank/biobank security

REDCap questionnaires are encrypted and kept at the Applied Clinical Research Centre (URCA) of the Sainte-Justine Research Centre. Data will be coded and the code kept by the principal investigator. Biological samples are collected in accordance with international standards.[37,38] Samples will be stored at the *Banque québécoise de la COVID-19* (BQC19),[39] under the responsibility of the co-investigator.

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Adverse events and data monitoring

Other than bloating, intestinal irritation, or stool softer than usual, very few side effects are associated with probiotic use, although caution is warranted in critically ill patients.[40] Vaccine safety and tolerance are well established in the elderly. A 4-member monitoring committee chaired by Bruno Piedboeuf will be advised of all adverse events (serious adverse events, within 48 hours; others, every three months). The steering committee will meet every 2 weeks. A scientific committee will meet monthly for 4 months and bi-monthly thereafter. No interim analysis is planned.

COVID-19 infections

In the event of COVID-19 infection during the trial and prior to administration of the booster shot (as determined by rapid COVID tests upon declaration of symptoms), participants will be asked whether they wish to delay the booster for several months or forego it altogether. In either case, participants will remain in the trial. Investigational product regimen and data/sample collection will begin anew 2 weeks prior to the new vaccination date, if applicable.

Statistical analysis

Analysis is by intention-to-treat. Intervention and placebo groups will be compared at baseline for sociodemographic and clinical characteristics, as well as for microbiota composition. Results for continuous variables will be presented as means ± standard deviations (SD) or as geometric means and 95% confidence intervals (CI); for categorical variables, as N (%). For the primary outcome, we will consider antibody level as a dichotomous variable (detectable, undetectable) and use a generalized method of moments (GMM) logistic regression model with time-dependent covariates for

longitudinal binary data. The model will be stratified in subgroup analyses by age and sex at 3 different timepoints (inclusion; 3- and 6-months post-vaccination). If Ac RBD as a quantitative variable is available, a GEE regression model with appropriate link function for continuous outcome will be used. Generalized linear models will be used to assess the effect of the group at specific time points (3 months and 6 months). The outcomes at the 3 timepoints are the interaction between group (intervention vs. control) and timepoint.[41] Secondary outcomes at inclusion and at 6 months post-vaccination will be analyzed on log-transformed original measures for continuous variables; at 6 months, groups will be compared using ANCOVA with inclusion data covariates. CD4 and CD4/CD8 ratios with log-transformed antibody levels will be estimated using Spearman's correlation and 95% CIs. *P* values < 0.05 will be considered statistically significant. For COVID-19 confirmed cases, a chi-squared test of independence will be used to compare the proportion of symptomatic and asymptomatic participants between the study groups. Cox proportional hazard models will be used to compare the risk of a COVID-19 outcome between the study groups, measured at six different time points.

Sample size calculation

We calculated the sample size to discern an effect size of 33% reduction in the number of participants presenting undetectable levels of anti-S1-RBD antibodies at 6 months post-vaccination. With an expected undetectable antibody level in 30% of the placebo group and therefore 20% in the intervention group, an estimated 584 participants would provide 80% power and a two-tailed alpha of 5%. With an estimated 15% attrition rate, the study would therefore require 688 participants.

Patient and public involvement statement

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The study will involve the Patient-Partnership Strategic Committee (*Comité stratégique patient-partenaire*, CSPP) of the Sherbrooke Hospital Research Centre (CRCHUS), co-ordinator C. Wilhelmy and the Patient-Partnership Initiative (*Initiative patient-partenaire FMSS-UdeS et Réseau Universitaire Intégré de Sherbrooke*), M. Garriss. In collaboration with the CIUSS-Estrie living lab for geriatric research (*Laboratoire d'innovations par et pour les aînés*, LIPPA), seniors will have the opportunity to join focus groups, collaborate at various stages of the research, and participate in the pilot study. We are also grateful to the National COVID-19 Clinical Trials Network, led by R. Fowler, and the Quebec Research Network on Aging, led by P. Gaudreau, for their support.

Ethics and Dissemination

The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MEO-31-2022-6278*. Written informed consent of all participants will be obtained for participation in the study and publication of results. National and international regulations on participant privacy and rights will be followed.

Participants will receive compensation for their participation in the study. One payment at mid-study and another one at the end. Participants who travel to the clinical research center *CR-CHUS* for venous blood samples or to obtain help from the research team for dried blood spot samples will receive a compensation supplement.

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The results of the present study will be published in peer-reviewed journals, and presented at international meetings/committees. We will also prepare communications for professionals and the general public.

DISCUSSION

This double-blind randomized controlled trial will test a probiotic supplement as an adjuvant for enhancing COVID-vaccine immune response in the elderly population. Improved immune response and extended spacing of vaccine booster doses in high-risk populations are both major public health issues. If successful, probiotic adjuvants could rapidly be implemented worldwide. They have few side effects, are currently available without prescription, and are relatively affordable. Furthermore, if a probiotic supplement can act as a COVID-19-vaccine adjuvant to enhance immunity, this low-cost intervention could lead to important benefits such as hospitalization reductions, longer time between booster shots, and higher immunity against new variants. This intervention would then be clinically, socially, and economically beneficial.

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

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

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Protocol amendments since v3, March 9, 2022**:**
Participants: inclusion criteria – from "never had COVID" to "not had COVID in the past 3 months", to account for COVID spread.
Recruitment strategies revised.
Updated inclusion/exclusion criteria to account for continued boosters, as recommended by public health agencies.
Blood spot samples limited to 3 timepoints instead of 5.
Modified telephone interview for cognitive status dropped from inclusion/exclusion criteria testing.

Any modifications to the protocol that may impact study procedures, outcomes and potential risks/benefits to participants require an amendment. All amendments must be agreed upon by the study team and approved by the Research Ethics Board of the *CIUSSS de l'Estrie – CHUS* prior to implementation, then disseminated to the rest of the research team as appropriate.

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ABSTRACT

Introduction: The elderly are particularly vulnerable to morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Approximately 20% of elderly showed no antibodies 3-5 months post-2nd dose of the COVID-19 vaccine. As probiotics have been shown to increase influenza-specific antibody levels post-influenza vaccination, we aim to reduce the percentage of participants without antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD) at 6 months post-vaccination.

Methods and analysis: Our study design is a double-blind randomized controlled trial, using intention-to-treat analysis. Eligible participants are a purposive sample of 688 adults aged 65-89 years, in Quebec, Canada, 2022-2024, not diagnosed with COVID-19 in the 3 months prior to recruitment and who wish to receive a government-recommended mRNA booster (Pfizer-BioNTech, Moderna) vaccine. The intervention consists of 1 capsule/day of a probiotic dietary supplement of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei 6x10^9 CFU/capsule* or a placebo, for 15 days pre- and post-booster vaccine. All participants provide dried blood spot samples at 3 timepoints (inclusion, 3- and 6-months post-vaccination) and a stool sample for microbiome analysis. A subgroup of 100 participants living near Sherbrooke, Quebec, is expected to volunteer for 2 onsite blood-test visits (at inclusion and 6 months post-vaccination). The primary outcome is participants without anti-S1-RBD antibodies at 6 months post-vaccination. Secondary outcomes include longitudinal analysis of anti-S1-RBD and anti-N antibodies at 3 timepoints. In the subgroup, serum levels of neutralizing antibodies will be determined at inclusion and 6 months post-vaccination. Probiotic and vaccine side

effects are monitored. At the end of the study, we expect to identify the adjuvant effect of probiotic on vaccine-induced immune response.

Ethics and dissemination: The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MEO-31-2022-6278* as well as Health Canada. All participants will provide informed consent. Results will be disseminated to the scientific community and to all networks related in this research.

Trial registration number (ClinicalTrials.gov identifier): NCT05195151.

KEYWORDS: COVID-19, SARS-CoV-2, COVID vaccination, booster dose, mRNA vaccine, vaccine adjuvant, elderly, probiotics, microbiome, immunity.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first RCT to evaluate the interaction between oral probiotics and on the durability of COVID-19 vaccine protection in the elderly.
- Probiotics are already available at low cost without prescription in Canada and other countries, suggesting a cost-effective and easily implementable option worldwide.
- Recruiting to target sample size will be a challenge mainly because participants have to be contacted 3-4 weeks before their booster dose appointment (for 2 weeks of probiotic/placebo intake before vaccination)
- Maintaining adherence during a 6-month-study with multiple sample collection is a challenge as well.

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INTRODUCTION

The elderly population is particularly at risk of morbidity and mortality from COVID-19, the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1–3] Vaccination against COVID-19 began in Canada in 2021, mainly using the mRNA vaccines by Pfizer-BioNTech and Moderna. However, due to waning immunity and subsequent large-scale infection by SARS-CoV-2 delta and omicron variants,[4] booster doses have proven necessary.[5] While two doses of vaccine induced robust activation of cell-mediated and humoral immune response,[6,7] the magnitude was less than that conferred by natural COVID-19 infection.[8–10] A recent meta-analysis showed that COVID-19 vaccines in the elderly were effective and tolerated, with a decrease in COVID-19-related deaths.[11] Nonetheless, 3 months after vaccination, antibody levels declined,[12] at a faster rate in the elderly than in the general population.[1,2] Three to five months after the 2nd dose, 20% of older adults showed no trace of anti-COVID antibodies.[1,13,14] Many countries, including Canada, have offered booster doses every 5 or 6 months. A high frequency of booster administration is a strain on public health resources and increases societal and economic costs. COVID vaccine durability is thus a public health concern.

The human microbiota, defined as all microorganisms colonizing the human body in the gut and oral cavity, are known to play a role in host protection, from tight junction regulation in the intestinal mucosa to the production of short-chain fatty acids to increasing innate and acquired immunity.[15–18] Microbiota involvement has been reported in digestive, respiratory, auto-immune, and metabolic disease.[16] In the elderly, gut microbiota showed less diversity, lower levels of beneficial bacteria, and higher levels of

pathogenic species.[19] Furthermore, the use of antibiotics reduced immune response to vaccine boosters in individuals with low titres of existing antibodies.[20] If gut microbial dysbiosis (i.e., a state of imbalance leading to a loss of diversity or a change in community composition) was to partially explain differential response to vaccination, intervention with probiotics might improve vaccination effectiveness.[21]

Two meta-analyses reported improved response to the influenza vaccine with probiotic co-administration,[22,23] as has a randomized controlled trial in the elderly.[24] Nevertheless, it remains difficult to draw clear conclusions on the benefits of probiotic co-administration with vaccination based on these studies, as they included different sample sizes, different strains and doses of probiotics, and different modes of administration and lengths of treatment.[25] A recent trial on probiotics and COVID-19 vaccination in nursing home residents showed conflicting results, with a trend toward positive effects.[26] Three trials on probiotic co-administration with COVID-19 vaccination are currently registered on clinicaltrials.gov.[27] We hypothesized that probiotics taken at the time of the COVID-19 booster dose would improve both humoral (antibodies to the SARS-CoV-2 receptor-binding domain) and cell-mediated (T and B cells) immune response in the elderly. This would increase immunity in the medium term and allow for longer spacing between booster doses. Further, increased memory cell immunity might prove protective against future variants.

To test this hypothesis, we will conduct a randomized controlled trial using a 2-strain probiotic dietary supplement as adjuvant to the COVID vaccine booster dose, with the aims of reducing the percentage of elderly without antibodies to the SARS-CoV-2 spike protein at 6 months post-vaccination. Our secondary objectives include a

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longitudinal analysis of antibodies at inclusion and at 3- and 6-months post-vaccination (dried blood spots), as well as a comparison of antibodies and spike-specific T and B cell levels at inclusion and at 6 months post-vaccination in a subset of participants. Further, we will also compare the proportion confirmed cases of COVID-19 (by rapid antigen testing or polymerase chain reaction (PCR) with symptoms and the proportion of COVID-19 asymptomatic cases in each study group. We will also monitor local and systemic side effects.

METHODS

Study design and population

The study design is a remote- and hybrid-design randomized controlled trial, using intention-to-treat analysis. The intervention consists of a probiotic supplement, as compared to an inert placebo, for enhancing immune response in the elderly for a period of up to 6 months following COVID-19 vaccination. The trial is to be conducted throughout Quebec (Canada) from November 2022 to January 2024 and coordinated by our study site at the *Centre de recherche du Centre hospitalier universitaire de Sherbrooke* (CR-CHUS). A convenience sample of 100 participants living in the Eastern Townships (Estrie), Quebec, in addition to taking part in the proposed study, will provide onsite blood samples for additional serological testing as part of the proposed study i.e. if participants are able to travel to the CR-CHUS, they will be invited for two in-person blood tests, until 100 subgroup participants is reached. With the COVID-19 situation in constant flux, the targeted booster vaccination is defined as the next booster dose of the

mRNA COVID-19 vaccine (Moderna or Pfizer), as recommended by Quebec Public Health.

Inclusion/exclusion criteria

Adults aged 65 to 89 years, of either sex, living at home or in independent-living retirement homes in Quebec, Canada, are eligible for participation if they wish to receive the next government-recommended COVID-19 mRNA vaccine booster dose, if they have already received three vaccine doses (Pfizer-BioNTech or Moderna), and if the latest dose was at least 5 months prior to inclusion. Other inclusion criteria are telephone and/or internet access and cognitive ability to provide informed consent. The latter will be assessed using the Functional Activities Questionnaire (FAQ)[28]; participants with scores ≥ 9 will be excluded. Other exclusion criteria are allergy (soy, lactose, yeast, maltodextrin), chronic immunodeficiency/immunosuppression (e.g., concurrent cancer chemotherapy or radiotherapy; concurrent active treatment for intestinal disorders such as duodenal ulcers, celiac disease, ulcerative colitis, and Crohn's), and use of probiotics and/or antibiotics at date of inclusion. Individuals with a serious condition precluding safe participation to the end of the study are excluded, as well as those having had COVID-19 in the past 3 months (confirmed clinically or, in the case of the 100-people subgroup, *a posteriori* by serological testing). Individuals who speak neither French nor English are excluded.

Recruitment, screening and consent

The study will be advertised at community centres and on social media, with a telephone number for callback, as well as through word of mouth from participants already registered. Different recruitment strategies will be used, including advertising in

traditional media, information meetings in retirement homes, and contacting potential participants from professional community lists.

Preliminary eligibility will be determined online or by telephone. A research assistant will then call potential participants, to explain the study, answer questions, and blind telephone recordings.

Randomization

Participants are randomized 1:1 into the placebo or intervention group, using permuted block randomization stratified by age (65-79 years, 80-89 years) and sex (male, female). Randomization will be programmed by the Applied Clinical Research Unit of the Sainte-Justine Research Centre and performed online using REDCap.

The study will be double-blinded. Until unlocking of the database at study end, neither the participants nor the research team, partner, or affiliated laboratories will be informed of the participants' group allocation. Pharmacists at Sherbrooke Hospital will prepare study products (probiotics or placebo) as per REDCap blinding requirements, using investigational products provided by Lallemand Health Solutions Inc. (Mirabel, Quebec, Canada) prior to study start.

Intervention

The intervention consists of an oral probiotic dietary supplement containing 2 bacterial strains of *Lacticaseibacillus rhamnosus* and *Lacticaseibacillus casei 6x10^9 CFU/capsule or a placebo*. Capsules for both probiotic and placebo are otherwise identical.

All participants take 1 capsule of the investigational product (probiotic or placebo) per day for 30 days: 15 days before and 15 days after receiving the COVID-19 vaccine booster shot. Should the vaccination appointment be delayed by more than 3 days

once treatment has begun, treatment will continue until 15 days post-vaccination. An extra bottle of 35 capsules is kept for each participant at the hospital pharmacy, if needed.

Outcome Measures

Primary outcome measure

The primary outcome measure is the percentage of participants without detectable antibodies against the SARS-CoV-2 spike protein receptor-binding domain (anti-S1-RBD Ab) at 6 months following the vaccination booster dose. Anti-S1-RBD antibodies prevent binding of SARS-CoV-2 to the ACE-2 receptor and thus correlate well with neutralizing antibodies (NAb) and vaccine effectiveness.[29] Levels of anti-S1-RBD antibodies will be determined by colorimetric enzyme-linked immunosorbent assays (ELISA) on dried blood spot samples. As per manufacturer's guidelines, positivity threshold is set at 3 standard deviations above the mean of negative controls (optical density on colorimetric blotting paper). ELISA tests have 95% sensitivity and 100% specificity.[29]

Secondary outcome measures

Secondary outcomes measures are a longitudinal analysis of anti-S1-RBD and Anti-Nucleocapsid (Anti-N) antibodies at 3 different time points (inclusion; 3- and 6-months post-vaccination). Possibly, a quantitative variable will also be available. The analysis will be performed as described above on dried blood spot samples.

We will also compare the percentage of confirmed cases of COVID-19 with and without symptoms in each group (COVID-19 confirmed by rapid antigen testing or PCR). The analysis will be done using the reported adverse events and presence of Antibodies Anti-N.

PIRATES-COV

Another secondary outcome is the level of neutralizing antibodies and spike-specific T and B cells, at inclusion and at 6 months post-vaccination, in the subset of 100 participants providing venous blood samples. Lymphocyte-binding to the spike protein will be measured by flow cytometry as described.[30,31] Absence of COVID-19 infection within the previous 3 months will be assessed by ELISA against the viral nucleocapsid.[32,33]

Subsequent analyses will include the comparison of gut microbial communities in both study groups at baseline, using stool samples provided by all participants at time of inclusion. DNA will be extracted from stool samples and then sequenced using shotgun metagenomic sequencing on a NovaSeq 6000 platform (Illumina, Genome Quebec, Montreal, Canada). The bioBakery workflow (Huttenhower lab, MA, USA) will be used for computer analysis. Shotgun sequencing data will go through metagenomic phylogenetic analysis (MetaPhlAn v3.0) to obtain a profile of microbial communities (bacterial, archaeal, viral, eukaryotic).

Clinically, we will monitor side effects of the investigational product as well as those of the vaccine, both local (pain or redness at the injection site, swollen lymph nodes) and systemic (fever, fatigue, headache, muscle aches, digestive troubles, flu-like symptoms). We will also take note of COVID-19 infections (FLU-PRO questionnaire,[34] followed by COVID-19 rapid testing).

Changes in outcome measures

Secondary outcomes have been modified to add an Anti-N antibody analysis. This type of analysis was not available when the protocol was first written and seems to be essential to giving more answers about the proportion of COVID-19 infections.

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Contamination bias

To prevent potential contamination bias, as probiotics are widely available and potential benefits assumed by the general population, participants are asked not to use probiotics (other than the investigational product) for the duration of the study. Questionnaires on probiotic use are completed before and during the study. Participants found to have used over-the-counter probiotics will be documented but retained in study analyses.

Data and biological samples – collection and storage

For most participants, there are no in-person study visits. Biological samples are collected at home. These include one baseline stool sample and 3 dried blood spot samples: at inclusion, at 3 months post-vaccination, and at 6-months post-vaccination. Blood spot samples are done by finger-prick testing on blotter paper; stool samples are self-performed. All samples are done at home and sent in by courier. Should any participants require help with sampling, a research team member will be available by phone or in person, upon request.

For a subset of 100 participants in the Sherbrooke area, there will be 2 in-person visits, one at baseline prior starting any treatment and one 6 months after vaccination. Venous blood collection will be done by a nurse at both visits, using the same brand of vacuum blood collection tubes with EDTA for anticoagulation. For each participant, five 4 mL tubes of blood will be collected. The final visit will include a satisfaction questionnaire regarding the research team, recruitment process, and acceptability of data and specimen collection. To decrease loss to follow-up, for those with mobility issues or if requested by participants, nurses will collect dried blood spot samples at the participant's home.

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Biological samples

Dried blood spot samples will be couriered to the Sherbrooke study site, then sent to Guy Boivin's lab in Quebec City for analysis. Venous blood samples will be processed and stored at the Sherbrooke study site (S. Ramanathan). Stool samples will be couriered and stored in A. Piché's lab, sent to Genome Quebec for analysis, and interpreted at I. Laforest-Lapointe's lab in Sherbrooke.

Data collection

At the time of enrolment, questionnaires will be completed. These include questions on sociodemographic data, height and weight, ethnicity, vaccination status, current medications, medical history, and comorbidities such as cancer. A validated food frequency questionnaire[35] will assess dietary habits.

Information on investigational product intake will be collected every 2 weeks for the first 33 days, online or by telephone. This will help in ensuring adherence to treatment and will be used to monitor adverse effects, if any.

Information on booster vaccination will be collected once a week, for 2 weeks. This includes local and systemic side effects, such as injection site pain/redness, fever, muscle aches, digestive troubles, and flu-like symptoms.[36]

Any changes to health will be collected once a month. Participants infected with COVID-19 will remain in the study and fill out questionnaires describing symptoms. In the case of hospitalization or death, data will be extracted from the participant's Quebec Health Record.

Data confidentiality and databank/biobank security

PIRATES-COV

REDCap questionnaires are encrypted and kept at the Applied Clinical Research Centre (URCA) of the Sainte-Justine Research Centre. Data will be coded and the code kept by the principal investigator. Biological samples are collected in accordance with international standards.[37,38] Samples will be stored at the *Banque québécoise de la COVID-19* (BQC19),[39] under the responsibility of the co-investigator.

Adverse events and data monitoring

Other than bloating, intestinal irritation, or stool softer than usual, very few side effects are associated with probiotic use, although caution is warranted in critically ill patients.[40] Vaccine safety and tolerance are well established in the elderly. A 4-member monitoring committee chaired by Bruno Piedboeuf will be advised of all adverse events (serious adverse events, within 48 hours; others, every three months). The steering committee will meet every 2 weeks. A scientific committee will meet monthly for 4 months and bi-monthly thereafter. No interim analysis is planned.

COVID-19 infections

In the event of COVID-19 infection during the trial and prior to administration of the booster shot (as determined by rapid COVID tests upon declaration of symptoms), participants will be asked whether they wish to delay the booster for several months or forego it altogether. In either case, participants will remain in the trial. Investigational product regimen and data/sample collection will begin anew 2 weeks prior to the new vaccination date, if applicable.

Statistical analysis

Analysis is by intention-to-treat. Intervention and placebo groups will be compared at baseline for sociodemographic and clinical characteristics, as well as for microbiota

composition. Results for continuous variables will be presented as means ± standard deviations (SD) or as geometric means and 95% confidence intervals (CI); for categorical variables, as N (%). For the primary outcome, we will consider antibody level as a dichotomous variable (detectable, undetectable) and use a generalized method of moments (GMM) logistic regression model with time-dependent covariates for longitudinal binary data. The model will be stratified in subgroup analyses by age and sex at 3 different timepoints (inclusion; 3- and 6-months post-vaccination). If Ac RBD as a quantitative variable is available, a GEE regression model with appropriate link function for continuous outcome will be used. Generalized linear models will be used to assess the effect of the group at specific time points (3 months and 6 months). The outcomes at the 3 timepoints are the interaction between group (intervention vs. control) and timepoint.[41] Secondary outcomes at inclusion and at 6 months post-vaccination will be analyzed on log-transformed original measures for continuous variables; at 6 months, groups will be compared using ANCOVA with inclusion data covariates. CD4 and CD4/CD8 ratios with log-transformed antibody levels will be estimated using Spearman's correlation and 95% CIs. *P* values < 0.05 will be considered statistically significant. For COVID-19 confirmed cases, a chi-squared test of independence will be used to compare the proportion of symptomatic and asymptomatic participants between the study groups. Cox proportional hazard models will be used to compare the risk of a COVID-19 outcome between the study groups, measured at six different time points.

Sample size calculation

We calculated the sample size to discern an effect size of 33% reduction in the number of participants presenting undetectable levels of anti-S1-RBD antibodies at 6 months post-

vaccination. With an expected undetectable antibody level in 30% of the placebo group and therefore 20% in the intervention group, an estimated 584 participants would provide 80% power and a two-tailed alpha of 5%. With an estimated 15% attrition rate, the study would therefore require 688 participants.

Patient and public involvement statement

The study will involve the Patient-Partnership Strategic Committee (*Comité stratégique patient-partenaire*, CSPP) of the Sherbrooke Hospital Research Centre (CRCHUS), co-ordinator C. Wilhelmy and the Patient-Partnership Initiative (*Initiative patient-partenaire FMSS-UdeS et Réseau Universitaire Intégré de Sherbrooke*), M. Garriss. In collaboration with the CIUSS-Estrie living lab for geriatric research (*Laboratoire d'innovations par et pour les aînés*, LIPPA), seniors will have the opportunity to join focus groups, collaborate at various stages of the research, and participate in the pilot study. We are also grateful to the National COVID-19 Clinical Trials Network, led by R. Fowler, and the Quebec Research Network on Aging, led by P. Gaudreau, for their support.

Ethics and Dissemination

The study was approved by Research Ethics Board of the *Centre Intégré Universitaire de Santé et des Services Sociaux de l'Estrie- Centre Hospitalier Universitaire de Sherbrooke* (*CIUSSS de l'Estrie-CHUS*) and the *CHU de Québec-Université Laval # MEO-31-2022-6278*. Written informed consent of all participants will be obtained for participation in the study and publication of results (see supplementary material). National and international regulations on participant privacy and rights will be followed.

Participants will receive compensation for their participation in the study. One payment at mid-study and another one at the end. Participants who travel to the clinical

PIRATES-COV

research center *CR-CHUS* for venous blood samples or to obtain help from the research team for dried blood spot samples will receive a compensation supplement.

The results of the present study will be published in peer-reviewed journals, and presented at international meetings/committees. We will also prepare communications for professionals and the general public.

DISCUSSION

This double-blind randomized controlled trial will test a probiotic supplement as an adjuvant for enhancing COVID-vaccine immune response in the elderly population. Improved immune response and extended spacing of vaccine booster doses in high-risk populations are both major public health issues. If successful, probiotic adjuvants could rapidly be implemented worldwide. They have few side effects, are currently available without prescription, and are relatively affordable. Furthermore, if a probiotic supplement can act as a COVID-19-vaccine adjuvant to enhance immunity, this low-cost intervention could lead to important benefits such as hospitalization reductions, longer time between booster shots, and higher immunity against new variants. This intervention would then be clinically, socially, and economically beneficial.

PIRATES-COV

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CENTRE DE RECHERCHE | CHUS

INFORMATION AND CONSENT FORM

Research study title:	Modulation of immune responses to COVID-19 vaccination by an intervention on the gut microbiota: a randomized controlled trial
Project number:	MP-31-2022-4426
Project funding:	IRSC (décembre 2021)
Principal investigator	Jean-Charles Pasquier, MD
Co-researchers **Université de Sherbrooke**	Jean-François Beaulieu, PhD, immunologie et biologie cellulaire Sheela Ramanathan, PhD, immunologie et biologie cellulaire Hugues Allard-Chamard, PhD, immunologie et biologie cellulaire Mélissa Généreux, MD, MSc, santé publique Isabelle Laforest-Lapointe, PhD, biologie Mélanie Plourde, MD, PhD, gériatrie Alain Piché, MD, MSc, microbiologie et infectiologie Geneviève Baron, MD, santé communautaire Fülöp, Tamas, MD, gériatrie Valiquette, Louis, MD, microbiologie et infectiologie
Université Laval	Nils Chaillet, PhD, méthodologie Guy Boivin, MD, PhD, infectiologie Julie Robitaille, PhD, nutrition
Université de Montréal	Benoît Mâsse, PhD, statistiques

FOR INFORMATION

From Monday to Sunday 8 a.m. to 4 p.m., please contact:

Sarah Bilodeau

Research assistant Tel.: 819-346-1110 extension 12836

Outside of these office hours, please go to the nearest emergency room and mention that you are taking part in a research study.

APPROUVÉ LE 30 MARS 2023
CÉR du CIUSSS de l'Estrie - CHUS

We are inviting you to take part in this research study because you are an adult between 65 and 89 years old, eligible for a booster dose of vaccine against COVID-19. However, before agreeing to participate in this project, please take the time to read and understand the following information carefully.

If you agree to participate in the research project, you will be asked to sign the consent form at the end of this document. We will provide you with a copy for your records.

This information and consent form explains the purpose of this research project, the procedures, the risk, harms and benefits, and who to contact if necessary. It may contain words that you do not understand. We encourage you to ask any questions you may have to the researcher in charge of the project or other people involved in the research project and ask them to explain any words or information that is not clear.

NATURE AND OBJECTIVES OF THE RESEARCH STUDY

Vaccination against COVID-19 appears to be essential to control the pandemic. Vaccination response and duration of immunity vary greatly among individuals and age appears to be a major factor.

We hypothesize that taking probiotics around the vaccination period may influence the vaccine response. The aim of this study is to compare the antibody level of patients after vaccination according to the intake or not of selected probiotics.

If you wish to participate, we offer you to take a product (probiotics or placebo) during 30 days, to answer questionnaires about your health status and to collect some samples. We will also invite you for two visits at the *Centre de recherche*, if you agree, to collect blood samples (optional).

The results of this study could lead to widespread the recommendations for the use of probiotics when elderly people receive a dose of COVID-19 vaccine.

DESCRIPTION OF THE RESEARCH PROCEDURES

Research Project Process:

Study Inclusion.

During a 30-45 minutes phone call, we will ask you various questions to determine if you are eligible for the project. If you live in the Eastern Townships, we will ask you if you would like to make two visits to the CRC as part of this study (optional component) in order to have your blood drawn.

Product intake

If you are eligible for the project and choose to participate, you will be randomly assigned to one of two products:

Probiotics: live bacteria suspected to be beneficial to health (similar to what is found in yogurt).

Placebo: a sugar-like substance.

The nature of the product to be tested (probiotics or placebo) will be determined at random. Neither you nor the research team will know which product will be assigned to you. However, in case of emergency and if justified, the researcher in charge of the research project will be able to obtain this information quickly from the CHUS Research Centre's pharmacy.

Study product: The probiotics and the placebo are provided by Lallemand Health Solutions. We will inform you by email or by phone when you should start taking the study product. You will have to take it once a day for 30 days with a cold liquid or a snack.

If you miss a capsule, it should not be taken on another day. You can indicate the omissions in the logbook provided for this purpose.

If there is a change in the scheduled vaccination date of more than three (3) days following the start of the product (no-show, change in the date of the appointment), the product will be extended for 30 days. For this purpose, we will send you a second bottle containing the additional capsules and ask you to complete the logbook suring this additional period.

Samples

The kit containing the investigative product, the material and the instructions for taking the samples will be sent to your home.

You will need to take a self-sample of stool and dried blood spot collection at home. The stool sample will be collected at your home by a delivery company. The dried blood samples will have to be sent by mail (postage paid envelope).

The research team provides you some ways to help in the realisation of your samples (instructions, tutorial, etc.). If necessary, a nurse can come at home to help you doing your dried blood spot collection or you can come to the CHUS research center if required.

Optional Component If you have agreed to come to the clinic, you will be invited to have a blood sample taken at CRC. You must not be fasting for this blood draw.

If you are infected to COVID-19 between inclusion in the study and your booster shot appointment, the study will be temporarily stopped. You will be asked if you intend to be vaccinated after this infection. If so, three weeks before the new booster shot appointment, we will ask you:

- If there have been any changes in your health in the past few months;

- To do an additional dried blood spot collection;

-To do an additional blood sample (*optional component*).

Questionnaires

In the following days, you will be asked anthropometric, identity, socio-cultural, socio-demographic, vaccination status, medical history and co-morbidities questions. You will be asked to complete a questionnaire on your dietary habits.

Vaccination

15 days after you start taking the product, you will be vaccinated. The appointment will have been booked by yourself in a vaccination center via the Clic Santé platform.

Study's Follow-up

During the 6 months following the inclusion in the study, you will have to fill in questionnaires online or by phone according to the frequency:

- Side effects regarding probiotic intake will be collected (product compliance, medication intake, adverse events) once/2 weeks and for 33 days;

  All adverse events, such as local reactions (injection site or arm pain, local erythema, adenopathy) and systemic reactions (fever, fatigue, headache, arthralgia, digestive disorders, flu-like symptoms...) will be recorded once/week for 2 weeks following the booster dose;

- Each month, data on any change in health status (ex. COVID-19) will be collected. Data will also be collected from your hospital medical records to confirm information about hospitalizations if applicable.

You will be asked to take a dried blood spot collection during this period and the sample will be sent by mail (at 3 months).

Final Visit

This is the conclusion of your participation in the study. You will be asked to take a dried blood spot collection and the sample will be mailed to us. You will be asked to complete a satisfaction questionnaire about your participation in the study.

Optional Component If you have agreed to travel, you will be invited to take a blood sample at CRC. You do not have to fast for this blood test.

Please refer to the schedule at the end of this document for an overview of the procedures performed during the research project.

PARTICIPANT'S COOPERATION

As a participant in this study, we ask that you follow these instructions:

- Do not take any other probiotics than those being tested as part of the research study (In any of these forms: enriched yogurt, kombucha, kefir, pills, capsules, powder…).

- Do not take part in another research study at the same time as this one (randomized clinical trial).
- Notify the research team if you experience any discomfort, difficulty or side effect related to the product.
- Report all medication (including non-prescription medication and antibiotics) and natural products that you take (they are allowed during the study but they must be reported).
- Should you be taking antibiotics during the study, the investigational product must be taken at least 2 hours before or after the antibiotic.

RISKS AND INCONVENIENCES ASSOCIATED WITH THE RESEARCH STUDY

The **risks** associated with the study are low. There is no Health Canada warning of potential risk from taking probiotics and many studies suggest that they are beneficial to the immune system. However, there may be currently unknown risks associated with taking the study product.

Other than taking probiotics, the inconveniences associated with the study are essentially related to sample collection (possible discomfort, embarrassment and time dedicated to performing the collection), but they are generally well tolerated. In order to ensure safe sample collection, please follow the instructions that we send you.

Some people who have taken probiotics have experienced one or more side effects. You may not experience any of these side effects, or you may experience some of them: bloating (uncommon), gas (uncommon), loose stools (uncommon). Infection may also occur, but this side effect is very rare and occurs mostly in people with weakened immune systems. In all cases, it is important to notify the research team of any unusual and significant symptoms that you may experience during the study.

Regarding the collection of dried blood spot, it is possible that you feel some pain during the prick. It is possible that a hematoma will be formed. In general, this procedure is well tolerated and even performed for clinical studies in children. In order to ensure safe sampling, please follow the instructions that will be given to you.

For the optional component, two blood tests will be performed during the study (venipunctures). During the blood tests, you may experience weakness, fainting, local pain, bruising, discomfort, irritation, redness or bleeding at the needle entry site. In rare cases, infection may occur. Every precaution is taken to avoid these complications. The amount of blood drawn is 20-25 mL (less than two tablespoons). By comparison, during a blood donation to Héma-Québec, approximately 450mL of blood is collected.

BENEFITS ASSOCIATED WITH THE RESEARCH STUDY

You may benefit from your participation in this research study, but we can't guarantee it. However, the study results may contribute to the advancement of knowledge about vaccine's immunity to COVID-19.

VOLUNTARY PARTICIPATION AND RIGHT TO WITHDRAW

Your participation in this research study is voluntary. Therefore, you may refuse to participate. You may also withdraw from the study at any time, without giving any reasons, by informing the doctor in charge of this research study or a member of the research team.

Your decision not to participate in the study, or to withdraw from it, will have no impact on the quality of care and services to which you are otherwise entitled, or on your relationship with the teams providing them.

The doctor in charge of this research study or the Research Ethics Board may put an end to your participation without your consent. This may happen if new findings or information indicate that participation in this research study is no longer in your best interests, if you do not follow study instructions, or if there are administrative reasons to terminate thestudy.

Any new findings acquired during the course of the study that could influence your decision to continue your participation will be shared with you quickly. If you withdraw or are withdrawn from the study, no further data or samples will be collected. However, the information and biological material, blood and tissue samples, already collected for the study will be stored, analyzed and used to ensure the integrity of the study, as described in this document. If you wish to have one or more samples provided for the study destroyed, you must notify the principal investigator of the study (or a member of their team). The principal investigator is responsible for the destruction of the samples. If you sample has already been tested and if the results are already included in an analysis or a publication, it will not be possible to remove this information. However, the remainder of your sample will be destroyed and no further analysis will be performed on your sample.

CONFIDENTIALITY

Collection— Who? Reasons for which personal information is requested

During your participation in this study, the doctor in charge of the study and the research team will collect in a study file, the information needed to meet the scientific objectives of the study.

Collection—What?

The study file may include information from your medical charts [including your identity, such as your name, gender, date of birth, ethnicity], past and present health status, lifestyle, and the results of all tests, exams, and procedures that will be performed.

Storage of data/samples – Protection

All study data collected during this study (including personal information and samples) will remain confidential to the extent provided by law. You will be identified by a code number only. The key to the code linking your name to your study file will be kept by the doctor in charge of this research study.

Use of data and samples

The samples will be sent to the research teams collaborating on the project to by carrying out the study-related analyses. The samples as well as some data will be shared with them in a coded form to preserve your identity. Following the analysis, residual samples will be returned to the principal investigator and may be used by the investigator for technical optimization. Otherwise, the samples will be destroyed after the storage period.

Storage of data– Duration

Study data will be stored for 15 years following the end of the study by the doctor in charge of this research study and by the collaborator responsible for the analyses. The samples will be stored for 10 years by the study investigator. The coded data and samples will be stored at the CIUSSS de l'Estrie – CHUS, in Sherbrooke, ensuring controlled and secured access.

To ensure your safety, a document indicating your participation in this study **[**a data information sheet**]** is included in your medical chart. As a result, any person or company to whom you give access to your medical chart will have access to this information. Results from tests, procedures and medical examinations that you will undergo during this study will not appear in your medical chart.

Dissemination of overall results

The study data may be published or shared at scientific meetings, but it will not be possible to identify you. In addition, the research data may be used to obtain the marketing assessment of the natural health product (probiotic) under study by the authorized regulatory organizations.

Right of access for control and safety, including "Measure 9"

For monitoring, control, safety, security, and approval of the study drug by regulatory agencies, your study file as well as your medical charts may be examined by a person mandated by Canadian or international regulatory authorities, such as Health Canada, the institution, or the Research Ethics Board. All these individuals and organizations will have access to your personal data, but they adhere to a confidentiality policy.

You have the right to consult your study file in order to verify the information gathered, and to have it corrected if necessary.

You have the right to consult your research file to verify the information collected and have it corrected if necessary. On the other hand, access to certain information before the end of the project may require that you be removed from the project in order to preserve its integrity.

COMMERCIALIZATION OPPORTUNITIES / WAIVERS

The results of the research resulting from your participation in this project could lead to the creation of commercial products. Lallemand Health Solutions will provide the probiotics and the placebo. No data from this study will be shared with the company.

COMPENSATION

During the study, the product is offer to you, you don't have to pay any fee. You will receive an amount $145 as compensation for the inconveniences incurred during your participation in this research study. A check of 70$ will be mailed to you at the mid project and 75$ at the end. If you withdraw, the compensation will be proportional to the length of your participation.

In case of visits to the CRC (optional component), we will compensate your time and travel ($50 per visit).
If you need support for the dried blood spot collection and that you travel to the CHUS research center for additional support, we will add an amount of $20 per visit (for a maximum of three visits).

SHOULD YOU SUFFER ANY HARM

Should you suffer harm of any kind following administration of the product or any other procedure related to this research study, you will receive all the care and services required by your state of health.

By agreeing to participate in this research study, you are not waiving any of your rights nor discharging the doctor in charge of the study, or the institution of their civil and professional responsibilities.

CONFLICT OF INTEREST DECLARATION

The researcher has no financial relationship with the private partner contributing to the study, Lallemand Health Solutions.

CONTACT INFORMATION

If you have any questions or if you have a problem you think might be related to your participation in this research study, or if you would like to withdraw, you may communicate with the doctor in charge of this research study or with someone on the research team Please refer to the box on page 1.

For any questions regarding your rights as a research participant in this study, or if you have comments or wish to file a complaint, you may communicate with: Bureau des plaintes et de la qualité des services du CIUSSS de l'Estrie – CHUS via plaintes.ciussse-chus@ssss.gouv.qc.ca or at the following phone number: 1-866-917-7903.

The CIUSSS de l'Estrie – CHUS Research Ethics Board has given ethics approval to this research study and is responsible for monitoring the study at all participating institutions in the health and social services network in Quebec.

If you wish to communicate with a member of this board, you may communicate with: Bureau d'autorisation des projets de recherche du CIUSSS de l'Estrie – CHUS via ethique.recherche.ciussse-chus@ssss.gouv.qc.ca or at the following phone number: 819-346-1110, ext. 12856.

SIGNATURE

I have reviewed the Information and Consent Form. Both the research study and the Information and Consent Form were explained to me. My questions were answered, and I was given sufficient time to make a decision. After reflection, I consent to participate in the research study in accordance with the conditions stated above, including the use of all personal data and samples collected.
By signing this form, I authorize the research team to have access to my medical record for the purpose of this study.

Name of participant *(block letters)*	Signature of participant	Date

Name of a contact person (in case of emergency)

Telephone number: _____

*Link with the participant:*_____

I agree to provide stool and blood samples at this time, but I may refuse to do so at any time. This will allow us to analyze your microbiota and give important information about the efficacy of probiotics.

☐ **YES** ☐ **NO**

I also authorize that my data and samples will be used in future analyses related to this project, but which have not yet been defined. These analyses will occur during the allowed sample's conservation period.

☐ **YES** ☐ **NO**

I authorize the researcher in charge of this study to communicate with me to see if I am interested in participating in other research studies related to probiotics or COVID-19. For example, your health condition in a year.

☐ **YES** ☐ **NO**

I have explained the research study and the terms of this Information and Consent Form to the participant, and I answered all questions asked.

Name of the person obtaining consent *(block letters)*	Signature	Date

Steps	When	Description	How
Information call (30-45min)	At inclusion	Verify eligibility and projet explanations	By phone
Inclusion (15 min)	When the participant is ready to begin	Consent form signature	By phone
Questionnaires (1h30)	3 days following inclusion	Socio-demographic informations, health status vaccin status, food habits and other information.	By phone or online
Logbook (10 min)	During the product intake	Informations about product intake and side effects, modifications about your medication.	By phone or online
Product intake (5 min/jour)	1 time a day for 30 days	The research team will indicate you when to begin the product intake.	At home
Stool sample (20 min)	When you receive the study pak	Stool sample	At home
Dried blood spot collection	<u>3 times :</u> -When you receive the study pak -3 month after the booster shot -6 month after the booster shot	Dried blood spot collection	At home
Blood test (optional)	*Initial visit* *Final visit*	A nurse will collect a blood sample (20-25mL)	To the centre de recherche