Efficacy of daily intake of *Lactobacillus casei* Shirota on respiratory symptoms and influenza vaccination immune response: a randomized, double-blind, placebo-controlled trial in healthy elderly nursing home residents[1–3]

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ABSTRACT

Background: Age is associated with immune dysregulation, which results in an increased infection rate and reduced effectiveness of vaccination.

Objective: We assessed whether an intervention with *Lactobacillus casei* Shirota (*Lc*S) in elderly nursing home residents reduced their susceptibility to respiratory symptoms and improved their immune response to influenza vaccination.

Design: Between October 2007 and April 2008, a randomized, double-blind, placebo-controlled trial was conducted in 737 healthy people aged ≥65 y in 53 nursing homes in Antwerp, Belgium. Volunteers were randomly assigned to receive a probiotic (n = 375; 2 bottles of fermented milk that contained $\geq 6.5 \times 10^9$ live *Lc*S/bottle) or a placebo (n = 362; similar drink with no bacteria) for 176 d. After 21 d, all subjects received an influenza vaccination. Primary outcome parameters were the number of days with respiratory symptoms, the probability of respiratory symptoms, and anti-influenza antibody titer by hemagglutination inhibition after vaccination.

Results: Univariate and multivariate modeling showed no effect of the probiotic on clinical outcome parameters. Generalized linear mixed modeling showed no effect of the probiotic itself on the probability of respiratory symptoms [OR of probiotic: 0.8715; 95% CI: 0.6168, 1.2887). No significant difference regarding the influenza-vaccination immune response was shown.

Conclusion: The results of this study show that daily consumption of a fermented milk drink that contains *Lc*S has no statistically or clinically significant effect on the protection against respiratory symptoms. This trial was registered at clinicaltrials.gov as NCT00849277. *Am J Clin Nutr* 2012;95:1165–71.

INTRODUCTION

The numbers of elderly people in Western countries continue to rise. Aging weakens the immune system and increases susceptibility to infections (1). Furthermore, the clinical presentation of a respiratory tract infection (RTI)[4] in elderly individuals is rather unspecific, whereby an RTI with limited symptoms can have serious consequences and often lead to hospitalization and death (2–4). Immunosenescence is also characterized by decreased antibody production and a shortened duration of protective immunity after vaccination and, in particular, by suboptimal functioning of the cell-mediated immune response (5).

To decrease the morbidity and mortality associated with annual seasonal influenza, the WHO recommends vaccination for certain at-risk population groups. One important risk group is people ≥65 y of age, particularly if they are living in nursing homes and other residential institutions. However, the ability of these people to respond to the trivalent influenza vaccine is low; studies have shown a decreased response to influenza vaccination in healthy elderly compared with that in young adults, which makes the vaccination much less effective (6).

Lactobacillus casei Shirota (*Lc*S) is a probiotic strain consumed in a fermented milk product that has been produced for >70 y and is now commercially available in many countries worldwide (eg, Japan, Taiwan, Philippines, Indonesia, Brazil, Mexico, and several European countries). Probiotics are believed to positively influence immune function, and several studies reported immunomodulatory activity associated with *Lc*S (7–12). Most of the studies have been in vitro studies or studies that focused on immunoregulatory pathways. The clinical effect is still unclear. The few studies conducted on the probiotic effect on RTIs have investigated healthy adults or children, except for 2 studies in free-living elderly (13, 14). All of these studies have given conflicting results (13–21), whereby some studies showed no effect (13, 16, 17), whereas other studies showed a lower incidence of RTIs in the probiotic group, but this was not always significant (14, 15, 18–21). To our

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[4] Abbreviations used: GMT, geometric mean titer; *Lc*S, *Lactobacillus casei* Shirota; RCT, randomized controlled trial; RTI, respiratory tract infection.

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knowledge, no studies on the effect of probiotics on RTIs have been conducted in a vulnerable population of institutionalized elderly individuals.

In this randomized controlled trial (RCT), we assessed whether probiotic treatment with *Lc*S *1*) improved protection against RTIs in healthy elderly nursing home residents and *2*) increased the antiinfluenza antibody titer (through hemagglutination inhibition) and maintained the immune response for a longer period after influenza vaccination.

SUBJECTS AND METHODS

Subjects

A randomized, double-blind, placebo-controlled trial was conducted over a period of 176 d during the winter of 2007–2008 in 53 different nursing homes in the province of Antwerp, Belgium. We recruited healthy men and women aged ≥65 y who were living in nursing homes and were willing and able to swallow the study drink twice a day during the study period. Exclusion criteria were as follows: any medical or practical condition that made the volunteer not suitable for participation in the study at the discretion of the investigator (assessment of cognitive deficits); any current relevant infectious disease; any current known disorder that had a negative repercussion on the immune system of the participant (such as autoimmune diseases, chronic obstructive pulmonary disease requiring the use of oxygen, cancer, or chronic inflammatory disease); allergy to influenza vaccine, eggs, neomycin, amphotericin B, erythromycin, or amantadine; ongoing treatment with immunosuppressive drugs, chemotherapeutic agents, or other antineoplastic medication; current use of antibiotics or use of antibiotics 6 wk before study entry; use of any investigative drug (other drugs that were also under investigation) within 90 d before study entry; and markedly abnormal results in any of the screening laboratory tests. Participants were asked to stop the intake of probiotic or prebiotic supplements 3 wk before the start of the study and during the entire treatment period. All volunteers gave informed consent. Ethical approval for the study was granted by the Medical Research Ethics Committee of the University Hospital of Antwerp. The trial was registered at clinicaltrials.gov as NCT00849277.

Random assignment and blinding

Participants were stratified for age, sex, and nursing home. Random assignment was performed by a third party before enrollment of the volunteers into the study. Participants, nursing home staff, and investigators were blinded to allocation for the probiotic or placebo. Study drinks were labeled A, B, C, or D, with 2 letters assigned for the probiotic drink and the other 2 letters assigned for the placebo. To maintain the double-blind condition and to avoid any bias, there was no difference in the packaging, appearance, and taste of the probiotic and placebo drinks.

Procedures

Twice daily, participants were given uniquely labeled bottles of 65 mL fermented milk product that contained $\geq 6.5 \times 10^9$ live *Lc*S (ie, a total daily dose of 1.3×10^{10} *Lc*S/d) or a matched placebo. The placebo milk drink, which was similar in taste and appearance to the test product, had not undergone fermentation and did not contain any bacteria. A baseline blood sample was collected (day 1) before intake of the first study drink. After a prevaccination period of 21 d, a trivalent influenza vaccine was administered (Influvac; Solvay Biologicals BV) (**Figure 1**). A second blood sample was collected at day 50 (4 wk after the influenza vaccination), and finally, a third blood sample was taken at the end of the study (day 176). The drinks were consumed throughout the entire study period and were usually taken during or after meals (breakfast and dinner).

The primary clinical outcome measurement of the study was the probability of acquiring RTI symptoms (ie, runny nose, sore throat, fever, or cough). Participants (and nursing home staff) were asked to make a daily record in a study diary of any of these RTI symptoms and their intake of the study drink. Every 3 wk, study investigators visited the participants. During the visits, information was gathered about the functional status (Katz score) of the participants as well as any report of influenza-like respiratory tract symptoms. The Katz scale is a scale used to assess the functional status as a measurement of the ability to perform activities of daily living independently. Six items (ie, bathing, dressing, use of the toilet, transfer, continence, and feeding) were scored from 1 (totally independent) to 4 (totally dependent) (22, 23). Nutritional assessment was made on the basis of the screening tool Mini Nutritional Assessment short-form, which is a validated screening tool used in geriatric health care (24), and BMI.

The diaries were reviewed with the participants themselves and the nursing home staff for completeness, accuracy, and compliance with the study requirements. Compliance in consumption of the test product was also checked on the basis of the diary records, and participants were individually encouraged to continue their participation in the study. If the participant had a severe RTI (ie, an RTI that required a visit by the general practitioner), this was also recorded.

The serologic outcome parameter of the study was the antiinfluenza antibody titer. Blood samples were analyzed for antiinfluenza antibodies by using hemagglutination inhibition of A/H3N2 for each participant. The extent of the humoral response to the vaccine was assessed by analyzing the prevaccination and postvaccination geometric mean titers (GMTs) as well as the rates of seroconversion and seroprotection.

Sample-size calculation

First, a sample-size calculation was performed on the basis of published data. To determine the appropriate sample size, the most critical point to consider was the incidence of RTIs in people >65 y of age in Belgium, and this incidence was assessed by using data from the integrated computerized network project (25), which is a network of general practitioners that collects data since 1994 on diseases presented to practices of general practitioners. Integrated computerized network data for RTI in noninstitutionalized elderly people showed an incidence of ≥3%/mo during the period October–March. The overall incidence of RTI in institutionalized elderly persons was often higher at ≤7–10% RTI/mo (26, 27). However, influenza vaccination could reduce this by 30–60% (28). When all of these facts were taken into account, the incidence of RTI for vaccinated elderly people in nursing homes was estimated as 5%/mo during

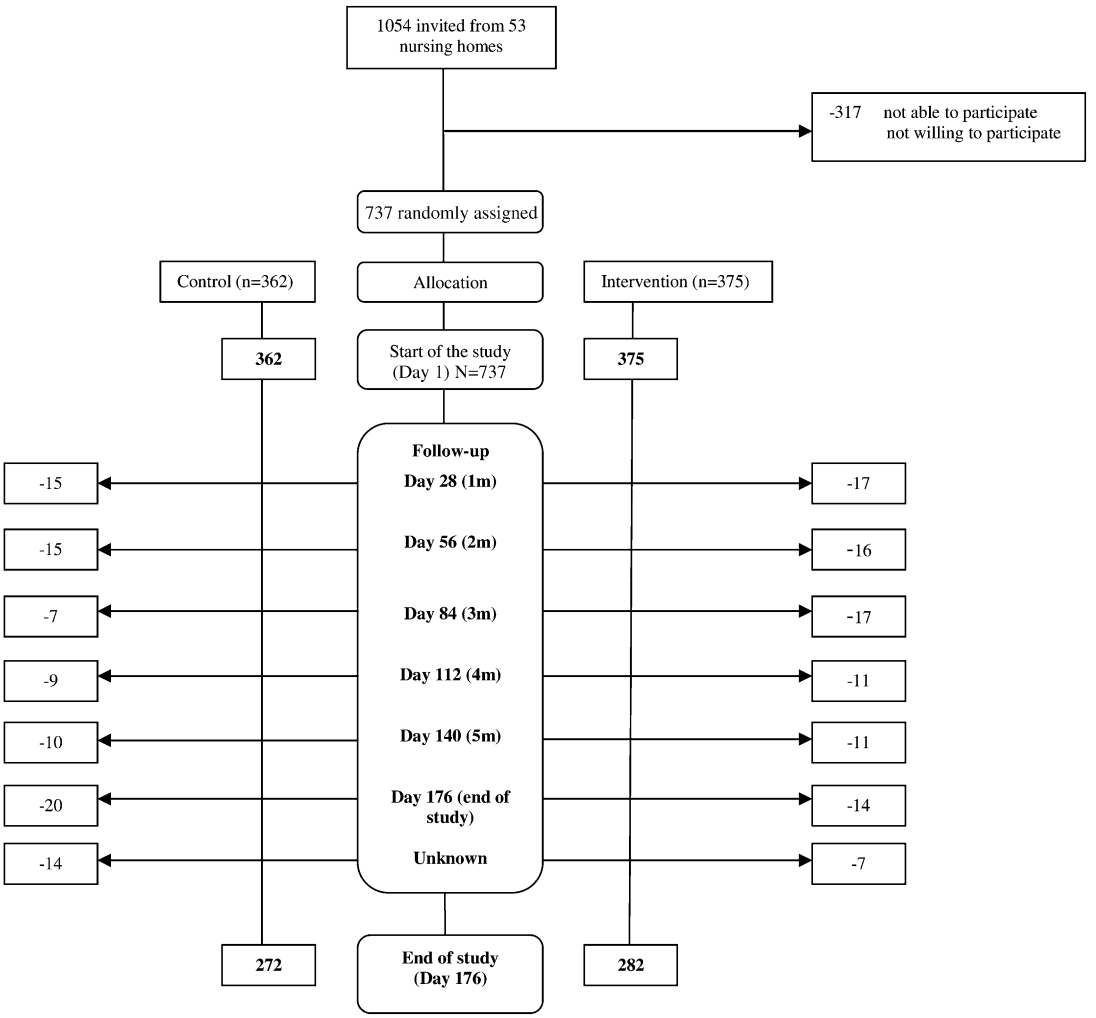

FIGURE 1. Participant flow. Values are expressed as the number of participants. m, month.

the October–March period (ie, 30% for the whole study period). The sample size was calculated to study the ability of the probiotic under study to reduce the (cumulative) incidence of RTIs in a period with a high burden of illness with ≥10% at a 5% significance level and with a power of 80%. It was shown that 370 people were needed per study group, which made a total of 740 volunteers for the complete study. Because it was anticipated that not every volunteer would complete the study according to the guidelines of the protocol, more volunteers had to be included to achieve the necessary 740 inclusions, as previously calculated. On the basis of an estimated 25% dropout rate, it was calculated that nearly 930 volunteers had to be recruited in the initial phase of the study.

Second, the results of an earlier feasibility study were taken into consideration to calculate the sample size. A pilot study had been carried out during the winter season of 2004–2005 in which 76 participants from 5 nursing homes in the Antwerp region were recruited, randomly assigned, and allocated to an intervention group (n = 38) or a control group (n = 38). The intervention consisted of consumption of a probiotic (2 bottles of fermented milk that contained $\geq 6.5 \times 10^9$ live *Lc*S per bottle) or placebo (similar drink but with no *Lc*S) for 176 d. The results of the pilot study (unpublished) showed a positive trend (NS) associated with probiotic consumption in lowering the incidence of respiratory symptoms in nursing home residents. This feasibility study showed that a minimum of 218 residents in each study group would be needed to achieve significant differences. With consideration of these 2 calculations, it was decided to base the protocol on the estimation of a larger group of participants (ie, 930 volunteers).

Statistical analysis

SPSS 16.0 (SPSS Inc) and SAS 9.2 (SAS Institute) statistical software were used to conduct an intention-to-treat analysis of all participants enrolled in the study according to allocation. Approximately 16% of the data were lost in the current study. Missing data did not allow the number of RTI episodes to be counted (with the consequence that the incidence could not be measured). Therefore, we used the number of days with respiratory symptoms as an outcome parameter in the analyses.

A multivariate linear regression analysis with the outcome parameter number of days with respiratory symptoms and a binary logistic regression analysis with an outcome parameter of ≥1 d with respiratory symptoms throughout the course of the study compared with no symptoms were performed to determine

which factors, other than the probiotic, influenced the outcome parameters. Covariates of interest included age (in y), sex, nursing home, Katz score, smoking status, pneumococcal vaccination, chronic illness, and postinfluenza vaccination titer. The covariates used in the analysis were based on univariate analysis (Pearson's chi-square test and ANOVA for discrete variables and Student's *t* test and linear regression analysis for continuous variables) with $P < 0.3$ (29).

Linear mixed models were used to account for repeated measures and to quantify the effect of *Lc*S on respiratory symptoms. Serum titers were converted to natural logarithms and analyzed by using Student's *t* tests (after testing for normality and equality of variances). Differences in seroprotection and seroconversion (binary variable) were assessed by using Pearson's chi-square tests.

A longitudinal analysis [generalized linear mixed model (30)] was used to assess whether the duration of probiotic intake influenced the probability of respiratory symptoms. Furthermore, this method could be used in the presence of missing data.

Many of the participants' diaries were incomplete, often with data missing for ≥1 d. On the basis the missing-data taxonomy of Rubin (31), it was assumed that the data were missing at random, which meant that the probability of an observation to be missing possibly depended on observed measurements but not on unobserved measurements. Different techniques can be used to deal with such missing data. The technique of multiple imputations was considered, but this technique was shown to be unsuccessful because of the presence of nonmonotone missingness patterns. An alternative technique was likelihood-based analysis, which could obtain valid inferences under the missing at random assumption and which had the additional advantage of not requiring a model for the dropout process. Therefore, a generalized linear mixed model was used, which was a likelihood-based random-effects model for repeated measures of a non-Gaussian type and which is a standard advanced statistical method for longitudinal analysis with missing data (32). The random-effects model is individual specific and therefore made it possible to measure whether there was any effect of *Lc*S on respiratory symptoms for each individual nursing home resident. Correction for clustering within nursing homes was done by using the nursing home as a covariate in the model. However, this model did not converge. Therefore, generalized linear mixed models were used for every single nursing home, and a meta-analysis was done to evaluate the effect of the probiotic intervention on the whole study population (*see* Supplemental material under "Supplemental data" in the online issue).

RESULTS

Participants in 53 nursing homes were recruited in the Antwerp region. The flow of participant involvement through the trial is shown in Figure 1. Between November 2006 and August 2007, 1054 elderly residents expressed interest in participating in the study. Of these residents, 317 individuals were subsequently unable to or decided not to participate in the study. In September 2007, 737 participants were randomly assigned to enter the trial.

During the course of the study, 183 participants dropped out (90 participants in the control group and 93 participants in the intervention group). The reasons for dropout and time course of dropouts were comparable in both groups (**Table 1**; Figure 1). The most frequent reason for dropout was gastrointestinal problems. Because the number of gastrointestinal problems was equal in placebo and probiotic groups, it was expected that this reason for dropout was not due to the intake of the probiotic and, therefore, was not an adverse effect. Eleven deaths in each group were recorded during the trial; however, no adverse events associated with the consumption of the probiotic or placebo were reported. The characteristics of the 2 study groups were similar at baseline (**Table 2**).

Comparison with weekly data registered by the Scientific Institute of Public Health in Belgium (33) showed that the profile of respiratory symptoms over the course of the study in the nursing home followed the national trend in Belgium for the same period (**Figure 2**).

TABLE 1
Reasons for dropout during the study (176 d)[1]

Reason for dropout	Placebo (n = 90)	Probiotic (n = 93)
	n (%)	*n (%)*
Gastrointestinal problems	22 (24)	24 (26)
Hyperglycemia	3 (3)	0 (0)
Lack of motivation (decided to stop)	9 (10)	7 (8)
Deterioration of health status	2 (2)	7 (8)
Taste	1 (1)	2 (2)
Hospital admission	15 (17)	16 (17)
Unable to comply with requirements of study	2 (2)	2 (2)
Death	11 (12)	11 (12)
Other	9 (10)	6 (6)
Unknown	16 (18)	18 (19)

[1] There were no significant differences between the placebo and probiotic groups (Pearson's chi-square test).

TABLE 2
Baseline characteristics of study participants[1]

Characteristic	Placebo (n = 362)	Probiotic (n = 375)
Age (y)[2]	84.17 (55–101)[3]	83.95 (64–101)
<80 y [*n* (%)]	83 (23)	92 (25)
Men [*n* (%)]	85 (23)	99 (26)
Influenza vaccination in 2006 [*n* (%)]	309 (85)	337 (90)
Pneumococcal vaccination [*n* (%)]	45 (12)	58 (15)
Katz score[4]	11.65 (6–24)	11.78 (6–24)
Current smoker [*n* (%)]	23 (6)	33 (9)
BMI (kg/m^2)	28.2 (16–52)	28.1 (18–56)
Mini Nutritional Assessment Short-Form	11.3 (5–14)	11.1 (6–14)

[1] There were no significant differences between the placebo and probiotic group (Mann-Whitney *U* and Pearson's chi-square tests).

[2] Three participants were <65 y of age because some of the participants were sharing a room or apartment in the nursing home with their partner in life, who in these 3 cases were <65 y of age and asked to participate in the study (2 partners were 64 y old at the time of inclusion and became 65 y old during the study period).

[3] Mean; range in parentheses (all such values).

[4] Score to assess functional status as a measurement of the ability to independently perform activities of daily living; 6 items were scored from 1 (totally independent) to 4 (totally dependent).

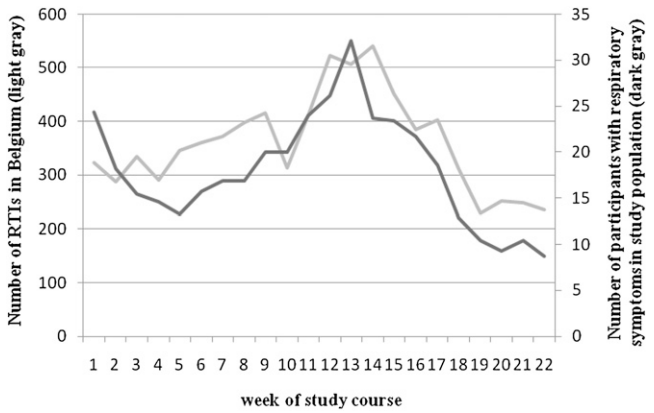

FIGURE 2. Number of RTIs per 100 consultations during the winter of 2007–2008 in Belgium [data from the Scientific Institute of Public Health (33)] compared with the number of participants with respiratory symptoms in the study population. RTIs, respiratory tract infections.

Respiratory symptoms

Univariate analysis

As shown in **Table 3**, there was no significant difference between probiotic and placebo groups for the number of days with respiratory symptoms (P = 0.342) or for the number of participants with respiratory symptoms (P = 0.325).

Multivariate regression analysis

On the basis of univariate analyses, the following covariates were used in the linear multivariate model with the number of days with respiratory symptoms as the outcome parameter: probiotic, pneumococcal vaccination, Katz score, age, post-influenza vaccination titer, and nursing home. Sex had a P value >0.3 but was used in the model as confounder. The only determinant in the final regression model was pneumococcal vaccination (B = 1.908; P = 0.076).

The logistic regression model with an outcome parameter of one or more respiratory symptoms was based on the following covariates: probiotic, sex, chronic illness, smoking status, Katz score, and nursing home. Age had a P value >0.3 but was added in the model as confounder. The significant factors of the final logistic regression model were nursing home and smoking status (OR of smoking status: 2.2; 95% CI: 1.09, 4.09; P = 0.027).

TABLE 3
Number of days with respiratory symptoms and number of participants with respiratory symptoms in the placebo and probiotic groups

Characteristic	Placebo (n = 362)	Probiotic (n = 375)	P
RD/ED-factor[1]	3.76 ± 9.48[2]	4.51 ± 10.99	0.342
Participants with at least one day of respiratory symptoms[3] [n (%)]	153 (42)	172 (46)	0.325

[1] RD/ED-factor, number of days with respiratory symptoms divided by the effective number of days of participation multiplied by 100. There was no significant difference in the RD/ED-factor (Student's t test).
[2] Mean ± SD (all such values).
[3] There was no significant difference in the number of participants with at least one day of respiratory symptoms (Pearson's chi-square test).

Longitudinal analysis

As previously stated, longitudinal analysis was used to assess the effect of time and to overcome the problem of missing data.

Generalized linear mixed modeling (individual specific) with the outcome parameter of one or more respiratory symptoms showed no effect of the probiotic itself (OR of probiotic: 0.8715; 95% CI: 0.6168, 1.2887) or when the effect of time was taken into account (*see* Supplemental material under "Supplemental data" in the online issue for additional information on the analyses). The model without interaction gave similar results and therefore was omitted.

As stated in **Table 4**, a nonsignificant decreasing trend throughout the course of the study (from an OR of 0.89 in month 1 to an OR of 0.74 in month 5) was seen; however, because the time × probiotic interaction was not significant, these results need to be interpreted with caution.

Severe RTI

A multiple logistic regression analysis indicated that the probiotic under study had no significant influence on risk of the development of a severe RTI (OR: 0.592; 95% CI: 0.335, 1.049; P = 0.073), but the sample size of this study was not based on this outcome (ie, severe RTI) and was too small to make a conclusion about the effect of *Lc*S on severe RTI.

Influenza vaccination immune response

At baseline, 172 participants were shown to be seroprotected (antiinfluenza antibody titer >40) and therefore were excluded from the serologic analysis. There was no statistical difference in baseline seroprotection rates between probiotic and control groups. To evaluate the effect of the probiotic drink on the effectiveness of the influenza vaccination, GMTs and seroconversion and seroprotection rates of the 2 groups were compared at days 50 and 176 of the study. This comparison showed no significant difference.

DISCUSSION

This randomized, double-blind, placebo-controlled study showed that, over the course of a study that lasted 176 d, risk of the development of one or more respiratory symptoms was not significantly influenced by the consumption of the probiotic *Lc*S. After exclusion of data for participants who were seroprotected against influenza at baseline, serologic analysis showed no significant effect on GMTs and seroconversion and seroprotection rates.

TABLE 4
ORs and 95% CIs per month of one or more respiratory symptoms

Month	OR	95% CI
0	0.93	0.58, 1.53
1	0.89	0.47, 1.73
2	0.85	0.37, 1.95
3	0.81	0.30, 2.20
4	0.78	0.24, 2.49
5	0.74	0.19, 2.80

The problem of lost or missing data is unavoidable in large clinical trials. Despite all efforts made to overcome this problem, ~16% of data was lost in the current study, which is an acceptable number, especially in this population (32). Missing data do not allow the number of RTI episodes to be counted (with the consequence that the incidence could not be measured). Therefore, to draw a conclusion of this study, we had to change the outcome parameter into the probability of acquiring one or more RTI symptoms and the number of days with respiratory symptoms, which was a limitation of this study. Currently, there is no gold standard (clinical or laboratorial) for the diagnosis of an upper RTI or common cold, especially in the elderly. The diagnosis is made clinically and on the basis of respiratory symptoms (34). In this study, we made use of individual respiratory symptoms because of the lack of a valid definition of an upper RTI in this specific population.

To the best of our knowledge, this was the first large RCT in which the effect of probiotics on respiratory symptoms in institutionalized elderly people over a 6-mo period was studied. Because of the intensive follow-up of participants, compliance was very good. There was no evidence of registration fatigue, which is a frequent problem in self-reporting studies, because the profile of the probability of respiratory symptoms for the study population was similar to the RTI incidence recorded for the general population in Belgium over this period.

The study was based on the notion that probiotics are believed to influence immunoregulatory pathways, mainly by stimulating regulatory T cell responses. Human studies have shown that certain probiotic bacteria can influence a range of different components of the immune response to infection (eg, phagocytic activity, natural killer cell activity, and mucosal immunoglobulin A production). These effects seem to be strain specific (35). With regard to *Lc*S, research has mainly focused on natural killer cells, which are known to be a first-line defense mechanism against viral pathogens (36). However, the clinical effect is still unclear. Despite this, in commercial advertising, probiotics are often presented as a product to boost the immune system on the basis of results of biological studies rather than clinical evidence. From this perspective, we have developed this study to investigate the clinical relevant effect of probiotics on RTIs in a specific vulnerable population. Most previous studies on probiotics and RTIs have investigated the duration, incidence, and severity of infections in healthy, free-living adults or children. These trials have given conflicting results. Studies that showed no probiotic effect on RTI incidence either were small or had intervention periods of <6 mo (13, 14, 37, 38). An exception was a study by de Vrese et al (17) that investigated a probiotic and multivitamin product and combined results from 2 intervention periods of 3 and 5 mo. The product had no effect on the incidence of RTI but was associated with a shorter duration of episodes and less severe symptoms. In contrast, other RCTs conducted in adults and children have reported a risk reduction in RTIs associated with probiotic intake, although not all data were significant (18–21, 39, 40). A nonsignificant 25% risk reduction in RTI was reported after a 7-mo probiotic treatment in a study by Hatakka et al (19) in children who attended day care centers.

Only a few studies have been published on the effect of prebiotic and probiotics on influenza vaccination. Two studies reported a positive effect on the antibody response (41, 42), but both of these studies were rather small or had some limitations in their design (analyses based on a small number of participants). The current study showed no significant probiotic effect on influenza vaccination. Influenza vaccination, which is recommended in all elderly individuals >65 y of age, showed a risk reduction in RTIs of 22% (43).

In this study, no significant benefit of probiotics in helping to reduce the incidence of respiratory symptoms of healthy elderly living in nursing homes was shown. In this population, we showed a nonsignificant trend with a relative risk reduction of 26% in month 5 of the study. However, in terms of absolute risk reduction, this only resulted in a reduction of the probability of an RTI from 5% to 3.7%, which was clinically irrelevant, in addition to the fact that this trend needs to be interpreted with caution because the time factor was not significant.

Furthermore, the sample-size calculation was based on an ability of the probiotic under study to reduce the incidence of RTIs by 10%, which is already a rather small effect. If there was a significant effect of the probiotic under study, it would have reduced the incidence of RTIs by <10%.

There are some possible factors that could have contributed to these negative results in this population. First, the study was conducted in elderly individuals with a mean age >80 y. One could question whether the immune system in the very elderly is still sensitive for stimulation. Second, the winter of 2007–2008 was a very mild influenza season, although in our study population we showed more RTIs than expected.

In general, as previously mentioned, the effect of probiotics is still controversial (44) and has limited evidence, whereas in most studies, a trend can be seen. Publication bias (ie, studies that showed no effect may not have been published) can partly explain that a trend is almost always present in studies. In contrast, other yet unknown factors might affect the efficacy of probiotics in stimulating the immune system (eg, genetic factors or the individual composition of gut microbiota) (45). Therefore it would be worthwhile to do additional research on better defining target subgroups that might benefit from probiotic treatment. Furthermore, a focus on hard outcomes such as biomarkers would be beneficial to measure probiotic effects in an unambiguous way.

In conclusion, these data indicate no significant protective effect of *Lc*S on the probability of the development of respiratory symptoms in elderly people living in nursing homes. A probiotic effect on the immune response to influenza vaccination could not be detected in this population.

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The authors' responsibilities were as follows—KVP, SC, BM, VV, and PVR: design of the experiment; KVP and VV: data collection; KVP, CB, NH, GM, and VV: analysis of data; KVP: drafting of the manuscript; VV, SC, BM, PVR, NH, CB, and GM: critical review of the manuscript; VV: study supervision; and KVP: management of all data in the study and final responsibility for decisions regarding publication. The sponsor of the study had no role in the study design, data collection, data analysis, data interpretation, or report writing. None of the authors had a conflict of interest.

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