OPEN

Fermented Milk Consumption and Common Infections in Children Attending Day-Care Centers: A Randomized Trial

*Andrey Prodeus, †Violeta Niborski, ‡Juergen Schrezenmeir, §Alexander Gorelov,
‖Anna Shcherbina, and ‖Alexander Rumyantsev

ABSTRACT

Objectives: This multicenter, double-blind, randomized, placebo-controlled clinical trial investigated the effect of a fermented milk product containing the *Lactobacillus casei* National Collection of Microorganisms and Cell Cultures (CNCM) I-1518 strain on respiratory and gastrointestinal common infectious diseases (CIDs) in children attending day-care centers in Russia.

Methods: Children ages 3 to 6 years received 100 g of a fermented milk product (n = 300) or a control product (n = 299) twice daily for 3 months, followed by a 1-month observation period. The primary outcome was the incidence of CIDs during the product consumption period.

Results: There was no significant difference in the incidence of CIDs between the groups (N = 98 with fermented milk product vs N = 93 with control product). The overall number of CIDs (and no severe cases at all) in both study groups and in all 12 centers, however, was unexpectedly low resulting in underpowering of the study. No differences were found between the groups in the duration or severity of disease, duration of sick leave from day-care centers, parental missed working days, or in quality-of-life dimensions on the PedsQL questionnaire (*P* > 0.05).

There was, however, a significantly lower incidence of the most frequently observed CID, rhinopharyngitis, in children consuming the fermented milk product compared with those consuming the control product (N = 81 vs N = 100, relative risk 0.82, 95% confidence interval 0.69–0.96, *P* = 0.017) when considering the entire study period.

Conclusions: Although no other significant differences were shown between the fermented milk and control product groups in this study, lower incidence of rhinopharyngitis may indicate a beneficial effect of this fermented milk product.

Key Words: Common infectious diseases, gastrointestinal infection, *Lactobacillus casei*, *Lactobacillus casei* CNCM I-1518, respiratory infection, rhinopharyngitis

---

What Is Known

- Effects of some probiotics on common infectious diseases in children were demonstrated in single studies.
- A fermented milk product containing *Lactobacillus casei* CNCM I-1518 reduced the incidence and duration of acute diarrhea in children in 4 studies and the incidence of upper respiratory tract infections in 1 study.

What Is New

- The incidence of rhinopharyngitis was lower during the entire study period in the active product group compared with the control product group.
- The incidence of common infectious diseases during the product consumption period did not differ between the groups, which might be because of an unexpectedly low overall incidence.

C ommon infectious diseases (CIDs) are endemic in the general population. They can be generally classified into 3 main types: upper respiratory tract infections (URTIs) such as rhinopharyngitis (the common cold), respiratory tract infections involving lower respiratory tract (LRTIs) such as influenza and influenza-like illness, and gastrointestinal tract infections (GITIs), typically gastroenteritis. Acute respiratory and GITIs are the most

common diseases in children. Common infections are responsible for millions of days of missed work or school and account for 27 million physician visits in the United States alone (1,2), and common cold recurrences affect children and parents' quality of life (3). A greater exposure to infective pathogens, for example, in day-care centers, increases the risk of infection (4–7). Indeed, day-care attendance is associated with the incidence of both respiratory and gastrointestinal infections (5,8). Children attending day-care centers have a greater number of physician visits, emergency room visits, and use of prescription medications than children who do not attend day-care centers (9). Moreover, the high rate of infections observed in day-care centers result in many missed days of both day-care attendance and parental work (4,10).

The economic impact of such infections is significant. Infections related to day-care centers are estimated to cost $1.8 billion annually in the United States, with $66 million to $90 million related to physician visits alone (5). In Canada, the average cost of common infections occurring in toddlers during a 6-month observation period was found to be $260.70 per child (11).

Probiotics are defined as ''A preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microbiota (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host'' (12). There is evidence that these products can be used either as preventive or as treatment in a range of diseases including respiratory and gastrointestinal tract infections (13–18). According to these meta-analyses (14–16), probiotics have been identified as effective in decreasing both the incidence and duration of common cold, and this is estimated to have substantial impact on public health and socioeconomics. For instance, in France, the estimated savings varied between €14.6 and €37.7 million per season for the general population with an even higher impact for children (19). Such probiotics can include bacteria (eg, lactobacilli and bifidobacteria, and lactococci and enterococci), fungi (eg, *Aspergillus, Saccharomyces cerevisiae,* and *boulardii*), and are administered either in the form of a fermented milk or as a lyophilized capsule.

As children attending day-care centers are particularly vulnerable to CIDs (5,6,8), preventive intervention within this population may be particularly beneficial, both for the individual and for society. There is increasing evidence to suggest that probiotics may have a favorable impact on health within this population in general and on gastrointestinal and respiratory tract infections, in particular (20–22). For the effects on gastrointestinal infections, evidence from meta-analyses is available for some strains such as *Lactobacillus rhamnosus* GG, *Saccharomyces boulardii,* and *Lactobacillus reuteri* ATCC 55730 (23–25). The product in the present study is fermented milk containing the strain *Lactobacillus casei* CNCM I-1518 (also named *Lactobacillus paracasei* subsp *paracasei* after the current nomenclature). In clinical studies, survival of the *L casei* through the gastrointestinal transit was demonstrated and supported (26–30), and this product was found to be effective in reducing both the incidence and duration of acute diarrhea in children compared with a control product not containing this strain (31–33) and was also found to confer therapeutic benefit on *Helicobacter pylori* eradication in children under triple therapy for gastritis (34). In adults, this product was reported to reduce the proportion of volunteers experiencing ≥1 CID, the cumulated duration of fever, and increased the time until the occurrence of the first CID episode (35). In the older people, it reduced the duration of CID, URTI, and rhinopharyngitis episodes (30). In firemen, it reduced the mean duration of illness episodes by 1 day (36).

Based on the above literature, the hypothesis of the present study was that the consumption of this fermented milk containing the probiotic strain *L casei* CNCM I -1518 may prevent CIDs in the population of children. To verify the hypothesis, the objective of the present study was to investigate the effect of regular consumption of this fermented milk product on the incidence of CIDs in 3- to 6-year-old children regularly attending a day-care center. It paralleled the study conducted by Merenstein et al (37) carried in the same winter season in the United States. In both studies, 3- to 6-year-old children attending day-care centers were investigated. The primary objective included the incidence of common infections and its categories, URTIs, LRTIs, and GITIs.

METHODS

Design

This multicenter, double-blind, randomized, placebo-controlled study with 2 parallel arms was conducted in healthy children attending 1 of the 12 day-care centers across Moscow. The study was conducted over 4 months, comprising a 3-month product consumption period and a 1-month follow-up period (Fig. 1). One group received the fermented milk containing *L casei* CNCM I-1518 (active product) and the other received the control product.

The first volunteer was selected on November 9, 2006; the first subject inclusion was on November 24, 2006, and the last subject's last visit was on April 4, 2007. The study received approval from an independent ethics committee (Independent Interdisciplinary Committee on Ethical Expertise of Clinical Studies, Moscow) and was conducted in line with the principles of the Declaration of Helsinki, the requirements of Good Clinical Practice, and in accordance with European regulatory requirements.

Male and female children ages 3 to 6 years, who were attending day-care centers 5 days per week in the Moscow area, were included in the study. Inclusion depended upon a written, informed consent by the parents/legal guardians. Volunteers were medically healthy, in particular free from respiratory and gastrointestinal tract symptoms, and expressed appreciation for the consumption of multifruit flavor dairy products.

Volunteers having experienced an infectious disease 7 days before study entry were excluded. Furthermore, subjects were excluded from participation if: current or recent systemic or topical treatment likely to interfere with the study parameters (antibiotics, antiseptics, corticosteroids, vaccines, antifungal, or antihistaminic agents); having parents who did not read nor understand an informed consent; allergy or hypersensitivity to milk proteins or dairy food, any other allergy, severe evolutive or chronic pathology that may affect the study outcomes, current diarrhea or constipation, artificial nutrition, special medicated diet or eating disorder, any surgery requiring general anesthesia 2 months before study participation, and enrollment in another clinical study.

Intervention

Random Sequence Generation and Allocation Concealment

Volunteers were randomly allocated to either the active or control product. To avoid selection bias, the randomization scheme was generated by data managers in line with the Cochrane guidelines (38) (with even assignment at investigation sites ascertained by using symmetric [1:1 ratio] randomization) using Statistical Analysis Software (SAS Institute Inc, Cary, NC) version 8.02 for Windows New Technology. The randomization list was kept confidential at the sponsor's premises and remained confidential with the exception to those involved in the production of the study products and for biostatistical managers (after the first part of data locking was performed).
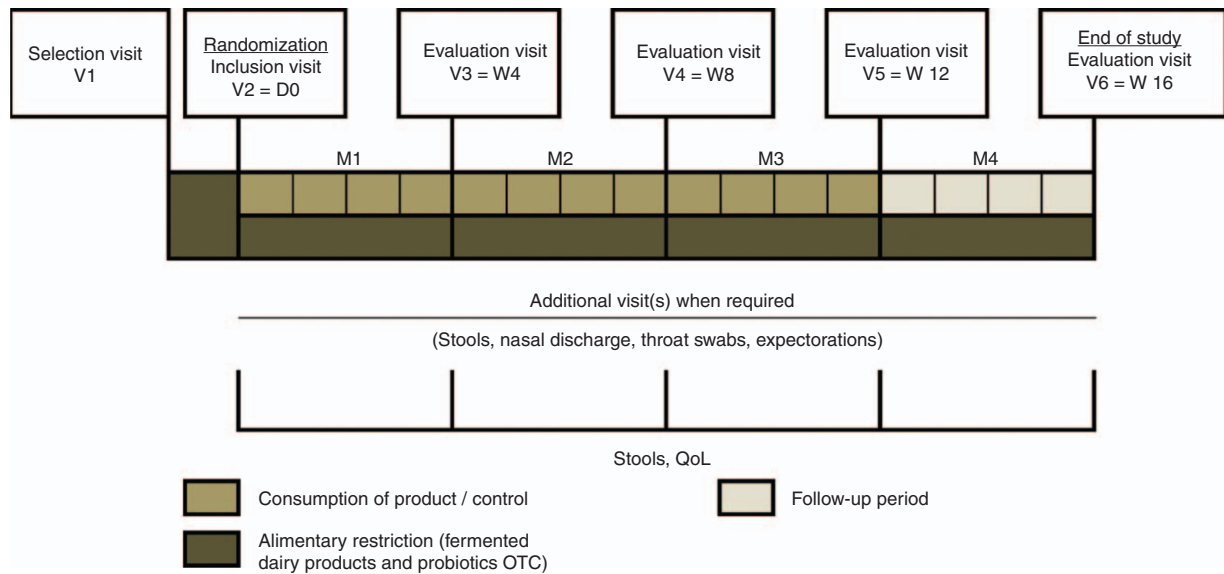
FIGURE 1. Design of the study. D = day; M = month; OTC = over the counter; QoL = Quality of life questionnaire; V = visit; W = week.

Test Products and Blinding of Participants and Personnel

The active product was a sweetened, flavored fermented dairy drink, Actimel (Danone, Warszawa, Poland), containing $\geq 10^{10}$ colony-forming units (CFU)/100 g of the probiotic strain *L casei* CNCM I-1518 (also named *L paracasei* subsp *paracasei* following the current nomenclature) combined with a symbiosis of 2 cultures commonly used in yogurt—*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* ($\geq 10^9$ CFU/100 g for the whole symbiosis). The control product was a sweetened, flavored nonfermented acidified dairy drink without lactobacilli and *Streptococcus thermophilus*. The appearance, taste, and packaging of the 2 products were identical throughout the study, to avoid performance bias (38) by ensuring the blinding of both study participants and key study personnel including the outcome assessors. Code-breaking systems were available in case of occurrence of a serious adverse event for which the medical personnel needed to be aware of what the participant received (active or control product). Raw data were also blinded during the blind review. The code was broken after the database was locked.

Two bottles of 100 g of the active or control product were ingested per day for 3 consecutive months (200 g per day in total). The 2 bottles were ingested separately, usually 1 in the morning and 1 in the afternoon.

Dietary Restriction

The parents/legal guardians were given clear instructions for dietary qualitative restriction during the study phase: subjects were asked to abstain from vitamin or probiotics supplementation and any product containing live ferments: probiotic drinks, kefir, and fresh yoghurts. Other dairy products were allowed that do not contain live bacteria (or low rate): milk, soft and hard cheeses, sour cream, cream, butter, ice cream, and pasteurized fermented dairies.

Tests and Apparatus

A personal diary was completed daily by the caregiver of each study participant. Caregivers were required to complete the diary by recording a yes/no answer for product intake compliance (took the product), occurrence of illness (if yes, record global severity with 1 mild, 2 moderate, and 3 severe), report of the category and type of infection such as URTI (rhinopharyngitis, sore throat, sinusitis, and otitis), LRTI (acute bronchitis, pneumonia, flu, or flu-like illness), and GITI (gastroenteritis), report of missed days at day-care center, missed parental work because of child's illness, any other event (other infections, injuries, or allergies), medications taken (if yes, by reporting the name and quantity ingested), and consumption of nonallowed products (if yes, by reporting the name and ingested amount). During a CID, the volunteer's temperature was also measured daily, preferably in the evening, and recorded in the personal diary.

The 3 main categories of common infections were defined as follows:

- URTI: Rhinopharyngitis (cold, acute coryza) symptoms including rhinorrhea, sneezing, nasal congestion, headache, asthenia, sore/stiff muscles, and fever (rare); sore throat including fever, burning throat, painful, red, swollen, bubbling tonsils, and adenopathy with neck pain; sinusitis including headache, sinus pain, stuffy nose, purulent rhinorrhea, sore throat, cough, and fever; otitis including ear pain, sensation that ear is full, hearing impairment, and discharge.
- LRTI: Acute bronchitis symptoms including fever, cough, and mucous or purulent expectoration; flu and flu-like illness including discomfort, intense shivers, high fever, headaches, sore/stiff muscles, arthralgia, asthenia, anorexia, dry, painful cough, and red throat; pneumonia including fever, shivers, chest pain, wheezing, and dyspnea.
- GITI (Gastroenteritis): symptoms including fever, headache, sore/stiff muscles, abdominal pain, diarrhea, and vomiting.

Quality of life was assessed by using the PedsQL (Pediatric Quality of Life inventory) questionnaire, composed of 21 items for children ages 2 to 4 years old, and 23 items for children ages 5 to 7 years old. Both sets of items enable the evaluation of 4 dimensions: physical functioning (PF), emotional functioning (EF), social functioning (SoF), and school functioning (ScF). These dimensions are evaluated on a scale of 0 to 100. For all dimensions, a low score

corresponds to a poor quality of life. Two summary scores can also be calculated: the psychosocial health summary score (PsyHSS), comprising the emotional, social, and school functioning dimensions, and the physical health summary score (PhyHSS), which is similar to the physical functioning score. The total score takes into account all 4 dimensions.

Compliance was recorded at each visit and was based on information reported in the personal subject's diary. Vital signs including systolic and diastolic blood pressure, heart rate, and body weight were assessed during the clinical examinations conducted at monthly intervals and at additional visits. Spontaneously reported adverse events were also recorded. Adverse events were defined as any unwanted effect occurring during the clinical study, whether related or not to the study product. The severity of an adverse event was recorded as mild, moderate, or severe.

Procedure

Screening for study participation occurred 15 days before inclusion in the study, and was conducted by the physician investigator at each study site (visit 1). Volunteers meeting the inclusion and exclusion criteria were randomized at day 0 (visit 2), at which point they received their first intake of the active or control product. Subjects were administered the drink twice daily at the day-care center for 5 consecutive days per week under a nurse or physician supervision. Parents received 4 bottles of the study products for consumption during the weekend. Evaluation visits were conducted at week 4 (visit 3), week 8 (visit 4), and week 12 (visit 5). A final evaluation visit occurred at week 16 (visit 6), 1 month after the final intake of the active or control product. Additional visits were conducted when any CID occurred. Baseline data and demographic characteristics for each volunteer were collected at visit 1, and a clinical examination was conducted by physicians. At visit 2, the PedsQL quality of life questionnaire was completed and the personal diary was initiated. The personal diary was then completed daily for the duration of the study period. At each evaluation visit, the PedsQL quality of life questionnaire was completed, the volunteer responses were recorded within the personal diary, and a clinical examination was conducted.

Other visits resulting from the occurrence of clinical symptoms related to CIDs were conducted 2 to 4 days following the start of such symptoms. The diagnosis of CID was assessed by the physician, and data on the type and severity of the infection, prescribed medication, and subject's temperature were recorded. For each CID, the start date, end date, and global severity were recorded, and the number of days of sick leave from day-care and of parental absence from work was noted from the personal diary. A clinical examination was also performed at each additional visit, and biological samples were collected for pathogens analysis according to the CID type (nasal discharge, and/or throat swabs, and/or stools).

Data Analysis

The primary outcome measure was the number of all CIDs reported during the 3 months of the study product's consumption. The secondary outcome measures were the number of all CIDs reported during the 1-month follow-up period and during the entire study period, and the number of URTIs, LRTIs, or GITIs or each type of CID during the product consumption, follow-up period or entire study period. Other secondary outcome measures were the time to event (beginning of a CID episode), the global severity of infection, the duration of infection, the occurrence of fever, medication intake and pathogens associated to CID, the duration of sick

leave from day-care and parental missed days at work, and the impact on quality of life. Safety was further evaluated through vital signs (blood pressure and heart rate), body weight, and spontaneously reported adverse events.

Statistics

The hypothesis was dedicated to evaluating whether there was a statistically significant difference between both groups for the cumulated number of CIDs over the 3 months of study product consumption using a Poisson regression with a 2-sided test at the 5% α-level assuming moderate overdispersion.

For the sample size estimation before the study, an average number of 3 CID events over a 3-month period were assumed in the control group, and a 15% relative decrease was expected in the active group (ie, the expected average number of events was 2.55 in the active group). Using a Poisson regression with a 2-sided test at the 5% α-level assuming moderate overdispersion (scale parameter of 1.1), around 230 evaluable children were estimated to be needed in each arm with $\geq$80% power. Furthermore, a 15% dropout rate was taken into account. As products were randomized to families (each eligible child of a family was given the same product), the sample size had to be adjusted to account for the family cluster, that is, the possible dependence of the occurrence of an infectious episode within the eligible children of the same family. Assuming an intraclass correlation coefficient of 0.1, and an average number of eligible children per family of 1.2, a total of 276 children per arm were needed. In the same way, the sample size had to be adjusted to account for the dependence of the occurrence of an infectious episode among eligible children in the same day-care centers or preschools. Assuming an intraclass correlation coefficient of 0.01, and an average number of eligible children per day-care of 10, around 300 children were needed in each arm. Therefore, a total of 600 children were planned to be included in the study.

To meet the recommendations of the Cochrane Collaboration for preventing detection bias (38), blinding of outcome assessment was ensured by a blind review of raw data and by unblinding after the database was locked, and by conducting statistical analysis in compliance with the statistical analysis plan. All volunteers who were randomized and received the study products were included in the intent-to-treat (ITT) population and were used for all statistical analyses. The per protocol (PP) population was built-up with all volunteers from the ITT population without any major protocol deviations during the intervention phase (product consumption period). As the number of subjects with $\geq$1 major protocol deviation was low, the ITT and PP populations were close. Therefore, analysis on the PP was conducted on the primary main outcome only.

To avoid attrition bias according to the recommendations of the Cochrane Collaboration (38), the distribution of the missing data across the intervention groups and the magnitude compared with the effect size were assessed. Missing data were replaced by the last observation carried forward (LOCF) method using the last post-baseline value for 1 subject at the previous time.

Reporting bias by selective outcome reporting (38) was prevented by the availability of the study protocol and prespecification of (primary and secondary) outcomes, and by adhering to these specifications. The baseline demographic characteristics of the 2 groups were compared using analysis of variance (ANOVA), χ^2 test, or Wilcoxon test as appropriate. The primary outcome measure was subjected to a Poisson regression analysis, followed by a sensitivity analysis with negative binomial regression and a Cochran Mantel Haenzel (CMH) test. Secondary outcome measures were assessed using statistical methods including Poisson

regression analysis for the number of CIDs reported in total and by category, logistic regression analysis for global severity, nonparametric CMH tests based on ranks for CID duration and sick leave duration, and a mixed ANCOVA linear model (for repeated measures) for PedsQL scores. Comparisons between the active group and the control group were performed using 2-sided statistical tests with a significance level of 5% ($P < 0.05$).

RESULTS

Volunteers' Demographics

As shown in the flow diagram (supplementary Fig. 1, *http://links.lww.com/MPG/A680*), of the 626 screened children, 602 were considered suitable for inclusion and 600 continued to randomization at visit 2. Of the 600 randomized children, 599 received the study products with 300 children in the active group and 299 children in the control group, and thus comprise the ITT population.

Among the 599 randomized subjects, 21 subjects reported major deviations (3.2%) among which, 15 subjects (2.5%) withdrew prematurely (2% in the active group, 3% in the control group). One subject in the active group withdrew because of an adverse event that led to a permanent discontinuation of the active product and withdrawal from the study, this subject had 2 major deviations, whereas 14 subjects discontinued because of consent withdrawal (5 in the active group, 9 in the control group). There were only few missing data because of premature withdrawal, and they were balanced in numbers across the 2 groups with similar reasons for missing data. This makes reasons for missing data unlikely to be related to true outcome and unlikely to have a clinically relevant impact on the intervention effect estimate indicating low risk of attrition bias according to the Cochrane Collaboration (35). The 3 remaining subjects with major protocol deviations had problems with compliance; these subjects withdrew prematurely from the study: 1 subject presented with a clinically significant abnormality at the clinical examination at V1, 1 subject had a serious adverse event during product consumption, and 1 subject did not respect the visit window between V4 and V5.

The baseline characteristics for this ITT population, originating from the 12 different centers, are shown in Table 1. The randomization by site and day-care center was well-balanced, and there was no relevant difference between these 2 groups at baseline in terms of demographics, medical history, or current disease. Furthermore, there were no differences in total score,

summary scores, or dimension scores on the PedsQL questionnaire at baseline. Compliance in taking the study products was excellent, 98.6% in the active group and 98.0% in the control group.

Primary Outcome Measure: Number of Common Infectious Diseases Reported During Product Consumption

The number of reported CIDs experienced during the 3-month product consumption period was 98 in the active group compared with 93 CIDs reported in the control group. The raw mean number of CIDs (adjusted for covariates site, age, presence/absence of a CID episode in the month before inclusion) per subject during the 3 months of study consumption was 0.33 in the active group and 0.31 in the control group. There was no significant difference between the groups for the cumulated number of CIDs over the 3 months of the study product consumption (relative risk [RR] 1.06, 95% confidence interval 0.84–1.33, Poisson regression). Similar results were obtained with the PP population.

The majority of children did not experience any CID, regardless of the study group allocation. The number of CIDs in the control group was 90% lower compared with the initial assumption made for the sample size calculation (ie, 3 episodes expected during the 3 months of intervention period). All CIDs were medically diagnosed during the episodes as planned except for 2 subjects who reported rhinopharyngitis after the end of the infections. The percentage of subjects experiencing 2 CID episodes was 6.3% in the active group compared with 4.0% in the control group, and the percentage experiencing 3 CID episodes was 0.0% in the active compared with 1.0% in the control group.

Secondary Outcome Measures

Number of Common Infectious Diseases Reported During the Follow-up Period and the Total Study Period

During the 1-month follow-up period, a lower number of CIDs (N = 25) were observed in the active group compared with the number in the control group (N = 35); the difference was however not significant. During the entire study period, the raw (adjusted) mean number of CIDs was 0.41 (0.32) per subject for the active group and 0.43 (0.33) in the control group.

TABLE 1. Baseline characteristics of the study volunteers in the ITT population

	Active product N = 300	Control product N = 299
Age, mean (SD) years	4 (1)	4 (1)
Sex, male (%)	173 (57.7%)	152 (50.8%)
BMI, mean (SD) kg/m^2	15.6 (1.7)	15.8 (1.8)
Vaccination during the previous year, number (%)	90 (30%)	99 (33.1%)
Delay of flu vaccination before selection time, mean (SD) days	69.2 (105.7) [N = 74]	55.4 (93.3) [N = 76]
Any infectious disease during the previous year, number (%)	232 (77.3%)	243 (81.3%)
Any infectious disease during the previous month, number (%)	54 (18.0%)	59 (19.7%)
Current disease or medical symptoms, number (%)	160 (53.3%)	155 (51.8%)
Physical functioning/physical health summary score, mean (SD)	85.8 (14.7) [N = 232]	85.2 (14.5) [N = 258]
Emotional functioning, mean (SD)	74.9 (17.0) [N = 228]	75.6 (15.3) [N = 258]
Social functioning, mean (SD)	84.0 (13.6) [N = 232]	82.4 (15.3) [N = 256]
School functioning, mean (SD)	76.6 (17.7) [N = 231]	77.9 (17.9) [N = 256]
Psychosocial health summary score, mean (SD)	78.5 (13.6) [N = 232]	78.5 (13.7) [N = 258]
Total scale score, mean (SD)	81.3 (13.0) [N = 232]	80.9 (13.1) [N = 258]

(A) Analysis of variance, (C) Chi-square test, (W) Wilcoxon test. ITT = intent-to-treat.

Number of Common Infectious Diseases by Category

Overall, the majority (206 episodes, 82%) of CIDs were URTIs. In contrast, only 39 (16%) and 6 (2%) CIDs were classified as LRTIs and GITIs, respectively. Out of the reported URTIs, most of these (181 episodes, 72% of all CIDs) were identified as rhinopharyngitis. Sore throat (16 episodes, 6% of all CIDs) and otitis (9 episodes, 4% of all CIDs) accounted for the remaining URTIs. The most common LRTI was flu (27 episodes, 11% of all CIDs), with acute bronchitis occurring in 12 cases. All GITIs were identified as gastroenteritis (2% of all CIDs).

Within the active group, 97 (79%) of all infections during the 4-month study period were URTIs. Similarly, 109 (85%) of all CIDs within the control group were URTIs. The number of URTIs did not differ significantly between the active and control groups during the 3 months of study product consumption, nor during the 1-month follow-up or the entire study period. The number of both LRTIs (24 active vs 15 in control groups) and GITIs (2 in active vs 4 in the control groups) was too small to perform meaningful similar statistical analysis, and the differences were deemed statistically nonsignificant.

No significant difference between active and control groups was observed in the number of rhinopharyngitis during the 3 months of study product consumption (RR [95% CI] 0.86 [0.69, 1.07], $P = 0.176$) nor during the 1-month follow-up period (RR [95% CI] 0.65 [0.38, 1.10], $P = 0.107$). Over the entire study period, there were 81 cases of rhinopharyngitis in the active group and 100 cases in the control group, and the adjusted mean rate of rhinopharyngitis was significantly lower in the active group (0.217) compared with the control group (0.266): RR [95% CI] was 0.82 [0.69, 0.96], $P = 0.017$ (Fig. 2) corresponding to a risk reduction of 18.45% in favor of the active group.

The occurrence, as assessed by the number of subjects with ≥ 1 rhinopharyngitis, tended to be lower in the active group during the 3 months product consumption period (OR [95% CI] 0.78 [0.58, 1.04], $P = 0.087$) and was significantly lower during the entire study period (OR [95% CI] 0.77 [0.62, 0.96], $P = 0.021$).

Time to Event

Median duration without CID was similar in both groups, 52.0 versus 51.0 days during the 3 months of study product consumption, 11.0 vs 12.0 days during the 1-month follow-up, and 56.0 vs 54.0 days duration for the entire study period.

Global Severity

There were only 2 cases of severe infection observed during the entire study period with both cases occurring in the active group during the 1-month follow up period, hence unlikely related to active product consumption. Of the reported CIDs, 99 events were defined as mild (48 in the active group and 51 in the control group; no significant difference), and 55 events were defined as moderate (31 in the active group and 24 in the control group; no significant difference). No significant differences in the severity of episodes were observed at the level of CID category (URTI, LRTI, and GITI) whatever the study period. Neither occurrence of fever (number of subjects with fever), nor cumulated days with fever or maximum temperature differed significantly between the groups.

Medication Use

There was no significant difference between the groups for the number of subjects who took medications for CID, neither for the number of medications nor the duration of intake, whatever the study period being investigated.

Common Infectious Disease Duration

For the ITT population, the mean (SD) cumulative duration of infection was 2.60 (5.13) days in the active group versus 2.43 (5.25) days in the control group (no significant difference, $P = 0.607$ in nonparametric CMH test based on ranks). In subjects experiencing a CID episode, the cumulated duration of infection was 9.89 (5.28) versus 9.67 (6.32) days ($P = 0.946$), the mean duration per episode was 7.92 (2.76) days in the active group and 7.57 (2.69) days in the control group ($P = 0.649$).

Sick Leave Duration

The mean (SD) cumulative number of days of sick leave from day-care centers during the products consumption period was 1.64 (3.76) in the active group compared with 1.61 (3.96) in the control group. Overall, there was no statistically significant
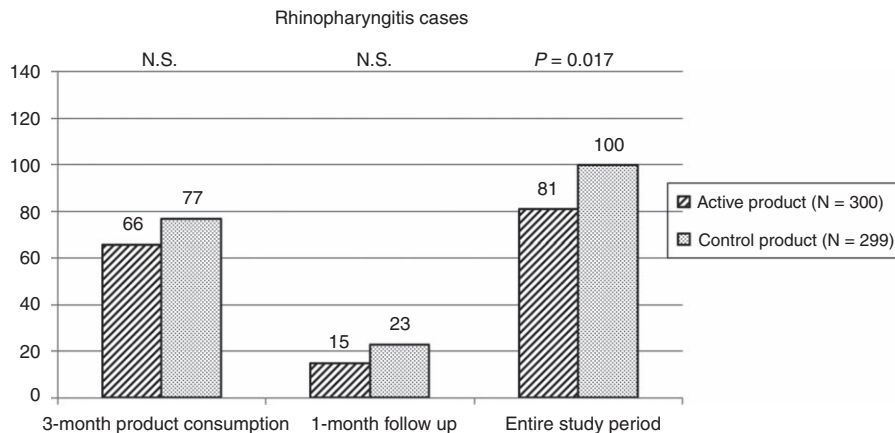
FIGURE 2. Absolute number of cases with rhinopharyngitis experienced during different study periods in which the active (fermented milk) product group is indicated by diagonal lines. Statistically significant difference between active and control, as indicated, was found in the number of rhinopharyngitis cases (these being the major part of CIDs observed) during the total study period (including 3 months of products consumption period and 1 month of follow-up). CID = common infectious disease.

difference between the 2 groups (nonparametric CMH test based on ranks, $P = 0.969$). Similarly, the mean (SD) cumulated parental missed days at work were 1.26 (3.38) days in the active group compared with 1.16 (3.64) days in the control group ($P = 0.69$) during the product consumption period and 1.59 (3.75) versus 1.71 (4.30) days ($P = 0.855$) during the entire study period.

Quality of Life

Since the questions within the PedsQL questionnaire differed between the children age groups (2–4 years group and 5–7 years group) the scores could not be calculated for all age groups altogether. Therefore, a total of 76 children were excluded from the analysis of quality of life because of switching PedsQL questionnaires during the study period by attaining a higher age group (50 in the active group and 26 in the control group). The results for the included children are shown in Table 2. There were no statistically significant differences between the active and control groups on any of the scales.

Pathogens Associated With Common Infectious Disease

Pathogens were detected in few samples without significant differences between the groups whatever the type of samples.

Safety

Overall safety was good, with <5% of the children experiencing ≥1 adverse events. In total, 29 children reported 31 adverse

events (Table 3). Seventeen children in the active group reported ≥1 adverse event compared with 12 children in the control group. The number of children reporting adverse events was not significantly different between the groups (χ^2 test, $P = 0.346$). The majority of adverse events were considered as mild, and none of the events was classified as severe. The most frequently reported adverse event in both groups was varicella.

Only 1 volunteer reported a serious adverse event (pneumonia in the active group). This was considered to be of moderate intensity but was not thought to be related to the study product, and the volunteer continued to receive the study product throughout the duration of the study. Only 1 volunteer reported an adverse event leading to withdrawal from the study (atopic dermatitis in the active group). It was judged to be of moderate intensity and probably related to consumption of the study product. No clinically significant changes of any measure of vital signs (systolic and diastolic blood pressure, heart rate), and weight were observed.

DISCUSSION

Assessment of the study according to the recommendation of Cochrane collaboration (38) indicated a low risk of either selection bias (random sequence generation and allocation concealment), performance bias (blinding of participants and personnel), detection bias (blinding of outcome assessment), attrition and exclusion bias (incomplete outcome data), bias because of selective reporting, bias because of baseline imbalance, and finally design-specific risks of bias. The rate of major protocol deviations was low and was well balanced between the active and control groups.

The extended use of 2 doses of active and the taste-matched control product resulted in no significant difference in the occurrence of adverse effects. The study products were judged to be palatable and pleasant, altogether resulting in very high compliance rates in both groups (98%).

TABLE 2. Change in PedsQL scores for volunteers in the ITT population at 3 months of products consumption period

	Active product N = 300	Control product N = 299
Physical functioning score/PhyHSS		
N	227	251
Mean (SD) change from baseline $P = 0.60$	−2.85 (17.5)	−2.71 (17.85)
Emotional functioning score		
N	223	251
Mean (SD) change from baseline $P = 0.54$	1.22 (20.43)	1.56 (17.68)
Social functioning score		
N	226	249
Mean (SD) change from baseline $P = 0.75$	−2.74 (17.03)	−1.97 (19.27)
School functioning score		
N	225	249
Mean (SD) change from baseline $P = 0.36$	4.37 (19.84)	2.90 (20.71)
Psychosocial health summary score (PsyHSS)		
N	226	251
Mean (SD) change from baseline $P = 0.85$	0.62 (15.66)	0.76 (15.84)
Total scale score		
N	227	251
Mean (SD) change from baseline $P = 0.84$	−0.80 (14.74)	−0.52 (15.22)

P values indicated show no statistically significant differences (Mixed ANCOVA linear models for repeated measures).

TABLE 3. Incidence of treatment because of emergent adverse events by system organ class for the entire 4 months of study duration

	Active product N = 300		Control product N = 299	
	AEs	Volunteers (%)	AEs	Volunteers (%)
Gastrointestinal disorders	2	1 (0.3)	0	0 (0.0)
Gingival disorder	2	1 (0.3)	0	0 (0.0)
Infections and Infestations	13	13 (4.3)	12	11 (3.7)
Herpes simplex	1	1 (0.3)	0	0 (0.0)
Pneumonia	1	1 (0.3)	0	0 (0.0)
Pyoderma streptococcal	1	1 (0.3	1	1 (0.3)
Varicella	10	10 (3.3)	11	11 (3.7)
Respiratory, thoracic, and mediastinal disorders	0	0 (0.0)	1	1 (0.3)
Tonsillar hypertrophy	0	0 (0.0)	1	1 (0.3)
Skin and subcutaneous tissue disorders	2	2 (0.7)	0	0 (0.0)
Alopecia areata	1	1 (0.3)	0	0 (0.0)
Dermatitis atopic	1	1 (0.3)	0	0 (0.0)
Vascular disorders	1	1 (0.3)	0	0 (0.0)
Hypertension	1	1 (0.3)	0	0 (0.0)

AE = adverse event.

Results from the entire study period showed that 3 months consumption of the active product resulted in a markedly lower incidence of rhinopharyngitis (RR 0.82 [0.69, 0.96], $P = 0.017$) in the 3- to 6-year-old children attending day-care centers compared with those consuming the control product. However, there were no significant differences between the groups in the incidence of CIDs during the 3-month intervention period considered as the primary outcome measure of this study. These results are in contrast to those reported by Merenstein et al (37) who observed a 19% lower rate of CIDs in the active group compared with the control group ($P = 0.046$) in a randomized controlled trial (RCT) with a similar design and sample size (N = 636). In Merenstein's study, CIDs and in particular GITIs, however, occurred more frequently than in the present study, and the incidence rate of both GITIs and URTIs was lower in the active group when compared with the control group ($P = 0.042$ and $P = 0.036$, respectively).

The results on the primary parameters of the present study might have been affected by insufficient compliance to the dietary restriction of fermented products and by other factors known to influence the effects of probiotics on infections, such as the administered dose (39) and the length of intervention (15). A reduced compliance to dietary restriction is however unlikely since the reporting performed all along the study indicated that only 3 subjects took occasionally some nonauthorized product. Regarding a possible influence of the conditions of product administration, Merenstein et al, found however an effect on CIDs by administering an identical dose of the study product and for the same duration as in this study (38). Therefore, a more probable explanation for the results may be seen in the unexpectedly low number of CIDs (0.31 episodes per individual in the control group) and the very low number of gastroenteritis, when compared with Merenstein's study (in which 5.5 episodes of CIDs per individual were observed in the control group) and other similar studies in the same population. CID occurrence was particularly low compared with what was assumed for the sample size calculation, 0.31 episodes observed compared with the anticipated 3.0 episodes. In fact, only 10 % of the assumed number of episodes occurred. Furthermore, inclusion into the study was performed during a short-period winter season, in late November/early December, when the CID rate was expected to be at its highest. It is important to note that in the present study, ~3-quarters of all volunteers, in both study groups, did not experience any CID during the product consumption period. This is in contrast to what has been found in previous studies in children showing much higher rates of common infections. For example, in 1 study, 39% of children attending a day-care center were reported to experience a respiratory illness within only a 2-week period (40), whereas in another study respiratory tract infections occurred at a rate of 47% in 1- to 6-year-old children given a control product for a duration of 7 months (41). Overall, URTIs alone have been reported to occur in a third (32%) of all children attending day-care centers (4). Although in the present study the most frequently reported CID was URTI, its incidence was low, being only 24% in the control group and 22% in the active group during the 3-month product consumption period. Because of this low incidence of CIDs, the calculated power of the study and hence the likelihood of demonstrating a significant beneficial effect was considerably reduced. Furthermore, in a study led by Turchet et al (42), there was a beneficial product effect observed on the severity of infection as assessed by episode duration and level of fever. However, in the present study, the number of severe CIDs was very low (N = 2), which may have impeded the detection of a potential effect of the active product in decreasing the severity. As noted above, the inclusion period in this study may have resulted in overall fewer CIDs when compared with the previous year (77%–81% retrospectively reported; Table 1) indicating a ''study-placebo-effect''

for both the active and control groups (dropping to 31%–32%). More intensive hygienic, dietary, and behavioral instructions were given by the physicians and nurses in charge of the involved day-care centers than usually applied in the general population. Recommendations about hand washing, healthy diet, and behavioral measures are known to reduce the incidence of CIDs (43–46). The results in the present study are in line with what Kang et al (15) in 2013 found in a meta-analysis of existing RCTs in which the RR for common cold by administration of probiotics for ≤3 months was 0.82 (95% CI 0.70–0.97), whereas administration for >3 months was 1.00 (95% CI 0.92–1.0). Despite the low incidence of infections observed in our study, the active product showed a beneficial effect ($P = 0.017$) on the rate of rhinopharyngitis in these young children during the entire study duration. Rhinopharyngitis, as expected, was the most common experienced CID and is thus recognized as a primary cause of illness in children (47) and adolescents (48). Furthermore, rhinopharyngitis results in a considerable number of physician visits and thus consumes a significant proportion of healthcare resources (49,50). Van Cauwenberge et al (51) reported that children ages 6 months to 4 years requiring a physician visit in France and Italy had an average of 4 episodes of rhinopharyngitis the previous year, and experienced a further 4 infections during the study (average duration of study participation being 123 days).

The exact physiological mechanism of such effects on rhinopharyngitis is still largely undefined. Probiotics may influence the incidence of infection with rhinopharyngitis via stimulation of nonspecific immunity and/or enhancement of humoral and cellular immunity by various mechanisms such as pattern recognition receptors (52). Indeed, an association has been observed between the improvement of disease outcome in respiratory tract infections with probiotics and the upregulation of all leucocytes of the innate and adaptive immune system (53). When compared with the control product, the active product has been previously shown to increase both the cell count and activity of leukocyte, neutrophil, and natural killer (NK) during episodes of CIDs and rhinopharyngitis in shift workers (32). Furthermore, oral intake of probiotics has been found to decrease the occurrence of potentially pathogenic bacteria in the nasal cavity that are known to be responsible for respiratory disease (54). A new area of research investigated also the role of a gut-airways axis in the defense against respiratory infections. The function of the gut microbiota in the protection against *Streptococcus pneumoniae* or *Influenza* virus infection has been described in animal models (55,56) and many studies support the assumption that probiotics intervention could target the intestinal microbiota for the prevention of respiratory infectious diseases (57). Whether such a mechanism could be involved in the observed product effect on RTI in the present study is still a hypothesis that could be investigated in future trials.

In the present study, there was no statistically significant difference between the groups on the duration of the experienced CIDs. This is in contrast to previous studies noted above, and as also assessed in a recent meta-analysis on probiotics that demonstrated a reduction in the duration of infection (14,16). It is also in contrast with 2 studies in the older population and 1 study in firemen in which the same product used in the present study reduced the duration of winter infections (30,36,42). Similarly, there was no difference between the groups in the duration of sick leave from day-care, in contrast to previous studies, as assessed by a meta-analysis, reporting decreases in short-term sick leave from day-care, school or work by *Lactobacillus* and/or *Bifidobacterium* consumption (16). Again, this lack of effect may have resulted from both the low incidence and severity of CIDs observed in both groups.

There was no difference between the 2 groups in quality of life as measured by the PedsQL questionnaire, neither in the

individual dimensions nor the summary scores. The score, however, was rather high at baseline that may have limited the sensitivity for detecting alterations when compared with other studies with the same product tested in adult stressed populations (35,36). The safety profile of the active product was good, with <5% of volunteers experiencing only moderate to mild adverse events. Furthermore, the occurrence of adverse events did not differ between the 2 groups.

In conclusion, the fermented milk containing the probiotic *L casei* CNCM I-1518 strain was well tolerated when consumed by 3- to 6-year-old children attending day-care centers. The results may indicate a beneficial effect on CIDs classified as rhinopharyngitis, which is in line with a reduction of URTIs by this probiotic product found by Merenstein et al (37) in a randomized clinical trial with a similar design, sample size, and the same active product.

REFERENCES

1. Feeney A, North F, Head J, et al. Socioeconomic and sex differentials in reason for sickness absence from the Whitehall II Study. *Occup Environ Med* 1998;55:91–8.
2. McCutcheon H, Fitzgerald M. The public health problem of acute respiratory illness in childcare. *J Clin Nurs* 2001;10:305–10.
3. Berdeaux G, Hervié C, Smajda C, et al. Parental quality of life and recurrent ENT infections in their children: development of a questionnaire. Rhinitis Survey Group. *Qual Life Res* 1998;6:501–12.
4. Fleming DW, Cochi SL, Hightower AW, et al. Childhood upper respiratory tract infections: to what degree is incidence affected by day-care attendance? *Pediatrics* 1987;79:55–60.
5. Haskins R. Acute illness in day-care: how much does it cost? *Bull N Y Acad Med* 1989;65:319–43.
6. Benetictsdottir B. Upper airway infections in preschool children—frequency and risk factors. *Scand J Prim Health Care* 1993;11:197–201.
7. Louhiala PJ, Jaakkola N, Ruotsalainen R, et al. Form of day care and respiratory infections among Finnish children. *Am J Public Health* 1995;85:1109–12.
8. Zutavern A, Rzehak P, Brockow I, et al. Day care in relation to respiratory-tract and gastrointestinal infections in a German birth cohort study. *Acta Paediatrica* 2007;96:1494–9.
9. Silverstein M, Sales AE, Koepsell TD. Health care utilization and expenditures associated with child care attendance: a nationally representative sample. *Pediatrics* 2003;111:e371–5.
10. Dales RE, Cakmak S, Brand K, et al. Respiratory illness in children attending daycare. *Pediatr Pulmonol* 2004;38:64–9.
11. Carabin H, Gyorkos TW, Soto JC, et al. Estimation of direct and indirect costs because of common infections in toddlers attending day care centers. *Pediatrics* 1999;103:556–64.
12. Schrezenmeir J, de Vrese M. Probiotics, prebiotics, and synbiotics—approaching a definition. *Am J Clin Nutr* 2001;73(suppl):361S–4S.
13. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. *PLoS ONE* 2012;7:e34938.
14. Hao Q, Dong BR, Wu T. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev* 2015;CD006895.
15. Kang E-J, Kim SY, Hwang I-H, et al. The effect of probiotics on prevention of common cold: a meta-analysis of randomized controlled trial studies. *Korean J Fam Med* 2013;34:2–10.
16. King S, Glanville J, Sanders ME, et al. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. *Brit J Nutr* 2014;112:41–54.
17. Goldenberg JZ, Ma SSY, Saxton JD, et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst Rev* 2013;CD006095.
18. Johnston BC, Ma SSY, Goldenberg JZ, et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea—a systematic review and meta-analysis. *Ann Intern Med* 2012;157:878–88.
19. Lenoir-Wijnkoop I, Gerlier L, Bresson J-L, et al. Public health and budget impact of probiotics on common respiratory tract infections: a modelling study. *PLoS ONE* 2015;10:e0122765.
20. Aponte BG, Mancilla BCA, Pariasca CNY, et al. Probiotics for treating persistent diarrhea in children. *Cochrane Database Syst Rev* 2013CD007401.
21. Salari P, Nikfar S, Abdollahi M. A meta-analysis and systematic review on the effect of probiotics in acute diarrhea. *Inflamm Allergy Drug Targets* 2012;11:3–14.
22. Weizman Z. The role of probiotics and prebiotics in the prevention of infections in child day-care centres. *Benef Microbes* 2015;6:181–3.
23. H. Szajewska H, Skorka A, Dylag M. Meta-analysis: *Saccharomyces boulardii* for treating acute diarrhoea in children. *Aliment Pharmacol Ther* 2007;25:257–64.
24. Szajewska H, Skorka A, M. Ruszczynski M, et al. Meta-analysis: *Lactobacillus* GG for treating acute diarrhoea in children. *Aliment Pharmacol Ther* 2007;25:871–81.
25. Szajewska H, Urbańska M, Chmielewska A, et al. Meta-analysis: *Lactobacillus reuteri* strain DSM 17938 (and the original strain ATCC 55730) for treating acute gastroenteritis in children. *Benef Microbes* 2014;5:285–93.
26. Guerin-Danan C, Chabanet C, Pedone C, et al. Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: influence on intestinal microflora in healthy infants. *Am J Clin Nutr* 1998;67:111–7.
27. Oozeer R, Goupil-Feuillerat N, Alpert CA, et al. *Lactobacillus casei* is able to survive and initiate protein synthesis during its transit in the digestive tract of human flora-associated mice. *Appl Environ Microbiol* 2002;68:3570–4.
28. Oozeer R, Leplingard A, Mater DD, et al. Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol* 2006;72:5615–7.
29. Rochet V, Rigottier-Gois L, Levenez F, et al. Modulation of *Lactobacillus casei* in ileal and fecal samples from healthy volunteers after consumption of a fermented milk containing *Lactobacillus casei* DN-114 001Rif. *Can J Microbiol* 2008;54:660–7.
30. Guillemard E, Tondu F, Lacoin F, et al. Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114001 reduces the duration of respiratory infections in the elderly in a randomized controlled trial. *Br J Nutr* 2010;103:58–68.
31. Pedone CA, Bernabeu AO, Postaire ER, et al. The effect of supplementation with milk fermented by *Lactobacillus casei* (strain DN-114 001) on acute diarrhoea in children attending day care centres. *Int J Clin Pract* 1999;53:179–84.
32. Pedone CA, Arnaud CC, Postaire ER, et al. Multicentric study of the effect of milk fermented by *Lactobacillus casei* on the incidence of diarrhoea. *Int J Clin Pract* 2000;54:568–71.
33. Agarwal KN, Bhasin SK, Faridi MM, et al. *Lactobacillus casei* in the control of acute diarrhea—a pilot study. *Indian Pediatr* 2001;38:905–10.
34. Sykora J, Valeckova K, Amlerova J, et al. Effects of a specially designed fermented milk product containing probiotic *Lactobacillus casei* DN-114 001 and the eradication of *H. pylori* in children: a prospective randomized double-blind study. *J Clin Gastroenterol* 2005;39:692–8.
35. Guillemard E, Tanguy J, Flavigny A, et al. Effects of consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-110001 on common respiratory and gastrointestinal infections in shift workers in a randomized controlled trial. *J Am Coll Nutr* 2010;29:455–68.
36. Niborski V, Tanguy J, Ernouf C, et al. Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* CNCM I-1518 improves clinical outcome of common infections and quality of life in adults under stress (firemen students) in a randomized controlled trial. 7th EFSMA–European Congress of Sports Medicine, 3rd Central European Congress of Physical Medicine and Rehabilitation. Salzburg, 26.-29.10.2011. *Düsseldorf: German Medical Science GMS Publishing House* 2011Available at: *http://www.egms.de/static/en/meetings/esm2011/11esm067.shtml.*

37. Merenstein D, Murphy M, Fokar A, et al. Use of a fermented dairy probiotic drink containing *Lactobacillus casei* (DN-114 001) to decrease the rate of illness in kids: the DRINK study—a patient-oriented, double-blind, cluster-randomized, placebo-controlled, clinical trial. *Eur J Clin Nutr* 2010;64:669–77.

38. Cochrane Handbook for Systemic Reviews of Intervention, Version 5.1.0, updated March 2011, chapter 8. Available at: *http://handbook. cochrane.org/*.

39. Van Niel CW, Feudtner C, Garrison MM, et al. *Lactobacillus* therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002;109:678–84.

40. Presser HB. Place of child care and medicated respiratory illness among young American children. *J Marriage Fam* 1988;50:995–1005.

41. Hatakka K, Savilahti E, Ponka A, et al. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomized trial. *BMJ* 2001;322:1–5.

42. Turchet P, Laurenzano M, Auboiron S, et al. Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114001 on winter infections in free-living elderly subjects: a randomized, controlled pilot study. *J Nutr Health Aging* 2003;7:75–7.

43. Sandora TJ, Taveras EM, Shih M-C, et al. A randomized, controlled trial of a multifaceted intervention including alcohol-based hand sanitizer and hand-hygiene education to reduce illness transmission in the home. *Pediatrics* 2005;116:587–94.

44. Cowling BJ, Fung ROP, Cheng CKY, et al. Preliminary findings of a randomized trial of non-pharmaceutical interventions to prevent influenza transmission in households. *PLoS ONE* 2008;3:e2101.

45. Jefferson T, Del Mar CB, Dooley L, et al. Physical interventions to interrupt or reduce the spread of respiratory viruses. *Cochrane Database Syst Rev* 2011;CD006207.

46. Savolainen-Kopra C, Haapakoski J, Peltola PA, et al. Hand washing with soap and water together with behavioural recommendations prevents infections in common work environment: an open cluster-randomized trial. *Trials* 2012;13:10–20.

47. Benson V, Marano MA. Current estimates from the National Health Interview Survey, *1992. *Vital Health Stat* 1994;10:1–269.

48. Cardozo DM, Nascimento-Carvalho CM, Andrade AL, et al. The burden of acute nasopharyngitis among adolescents. *Arch Dis Child* 2006;91:1045.

49. Cypress BK. Patterns of ambulatory care in pediatrics: The National Ambulatory Medical Care Survey. *Vital Health Stat* 1983;13:1–60.

50. Hollinghurst S, Gorst C, Fahey T, et al. Measuring the financial burden of acute cough in pre-school children: a cost of illness study. *BMC Family Practice* 2008;9:10–5.

51. Van Cauwenberge P, Berdeaux G, Morineau A, et al. Use of diagnostic clusters to assess the economic consequences of rhinopharyngitis in children in Italy and France during the winter. Rhinitis Survey Group. *Clin Ther* 1999;21:404–21.

52. Winkler P, Ghadimi D, Schrezenmeir J, et al. Molecular and cellular basis of microflora-host interactions. *J Nutr* 2007;137: 756S–72S.

53. de Vrese M, Winkler P, Rautenberg P, et al. Effect of *Lactobacillus gasseri* PA 16/8, *Bifidobacterium longum* SP 07/3, *B. bifidum* MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. *Clin Nutr* 2005;24:481–91.

54. Gluck U, Gebbers JO. Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus, Streptococcus pneumoniae*, and beta-hemolytic streptococci). *Am J Clin Nutr* 2003;77:517–20.

55. Schuijt TJ, Lankelma JM, Scicluna BP, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut* 2015;0:1–9.

56. Ichinohe T, Pang IK, Kumamoto Y, et al. Microbiota regulates immune defense against respiratory tract influenza A virus infection. *Proc Natl Acad Sci USA* 2011;108:5354–9.

57. Samuelson DR, Welsh DA, Shellito JE. Regulation of lung immunity and host defense by the intestinal microbiota. *Front Microbiol* 2015;6:1085–98.