British Journal of Nutrition (2010), **103**, 58–68
doi:10.1017/S0007114509991395

Consumption of a fermented dairy product containing the probiotic *Lactobacillus casei* DN-114 001 reduces the duration of respiratory infections in the elderly in a randomised controlled trial

E. Guillemard[1]*, F. Tondu[1], F. Lacoin[2] and J. Schrezenmeir[3]

[1]*Danone Research, Centre de Recherche Daniel Carasso, RD 128, 91767 Palaiseau Cedex, France*
[2]*Quintiles/MGRecherches, Levallois Perret, France*
[3]*Department of Physiology and Biochemistry of Nutrition, Max Rubner Institute, Karlsruhe, Germany*

(Received 18 March 2009 – Revised 16 June 2009 – Accepted 30 June 2009 – First published online 14 September 2009)

Common infectious diseases (CID) of the airways and the gastrointestinal tract are still a considerable cause of morbidity and mortality in elderly. The present study examined the beneficial effect of a dairy product containing the probiotic strain *Lactobacillus casei* DN-114 001 (fermented product) on the resistance of free-living elderly to CID. The study was multicentric, double blind and controlled, involving 1072 volunteers (median age = 76·0 years) randomised for consumption of either 200 g/d of fermented (*n* 537) or control (non-fermented) dairy product (*n* 535) for 3 months, followed by an additional 1 month's follow-up. The results showed that, when considering all CID, the fermented product significantly reduced the average duration per episode of CID (6·5 *v.* 8 d in control group; $P=0{\cdot}008$) and the cumulative duration of CID (7 *v.* 8 d in control group; $P=0{\cdot}009$). Reduction in both episode and cumulative durations was also significant for all upper respiratory tract infections (URTI; $P<0{\cdot}001$) and for rhinopharyngitis ($P<0{\cdot}001$). This was accompanied with an increase of *L. casei* species in stools throughout the fermented product consumption ($2–3{\cdot}8 \times 10^7$ equivalents of colony-forming unit/g of stools, $P<0{\cdot}001$). The cumulative number of CID (primary outcome) was not different between groups nor was the CID severity, fever, pathogens' occurrence, medication, immune blood parameters and quality of life. The fermented product was safe and well tolerated. In conclusion, consumption of a fermented dairy product containing the probiotic strain *L. casei* DN-114 001 in elderly was associated with a decreased duration of CID in comparison with the control group, especially for URTI such as rhinopharyngitis.

Probiotics: *Lactobacillus casei* DN-114 001: Respiratory and gastrointestinal infections: Elderly

Common infectious diseases (CID) remain a considerable cause of morbidity and mortality worldwide[1] particularly in the ageing population. The elderly experience more frequent and severe community-acquired respiratory and gastrointestinal infections[2]. Influenza and pneumonia are the fourth most common cause of death among the elderly[1,3]. Moreover, 77 % of deaths due to gastrointestinal infections[4] and 90 % of deaths due to respiratory infections[5] are reported to occur in patients over 65 years of age.

The increased susceptibility of the elderly has been ascribed to age-associated alterations of the immune system affecting both innate and adaptive immune responses. For example, the antibody and cell-mediated immune responses to influenza vaccine are reduced in the elderly[6–8], and the clinical effectiveness of annual influenza vaccination is 50–60 % in subjects over sixty-five as compared with 80–90 % in younger adults[9]. In addition, ageing-associated changes such as involution of the thymus, decline of naive T-cell numbers, reduction in T-cell repertoire diversity and accumulation of specific memory T cells result in disruption of T-cell population balance and decreases in protection against new pathogens[10]. Ageing is also associated with a reduction in the activity of different cell subsets from the innate immune system such as monocytes[11,12], polymorphonuclear neutrophils[13] and natural killer cells[14,15].

Achieving optimal success in preventing and controlling CID among the elderly requires not only the development of more effective vaccines and antimicrobial drugs, but also new strategies to oppose to the age-associated alterations of the immune system. The latter includes improvement of the living conditions, promotion of physical activity and better nutrition including functional foods[16,17]. These foods include fermented dairy products such as yoghurt or fermented milk some of which contain probiotics that may improve the resistance of old people to infection[18–23]. Products containing probiotics were also shown to have an immunomodulatory effect in different classes of age[20,24–29], and in several studies, modifications of immune parameters have been correlated with a protective effect against CID of the gastrointestinal or respiratory tract[20,27–29]. The present study was

Abbreviations: AGGIR, Gerontological Autonomy Iso-resource Group; CID, common infectious diseases; eq. CFU, equivalent of colony-forming unit; GITI, gastrointestinal tract infections; ITT, intention to treat; LRTI, lower respiratory tract infections; URTI, upper respiratory tract infections.

* **Corresponding author:** E. Guillemard, fax +33 1 69 35 76 46, email eric.guillemard@danone.com

prompted by a pilot observation that the consumption of a fermented dairy product containing the probiotic strain *L. casei* DN-114 001 (Actimel®) in the elderly population is associated with a reduction in the duration of winter infections (gastrointestinal and respiratory infections taken together)[30]. In a higher sample size and a more extended observation period in the present study, we investigated the effect of daily consumption of the same product on the resistance to CID. To this end, 1072 elderly volunteers were included in a multicentre, randomised, double-blind, controlled study over a 3-month winter period.

Materials and methods

Design

A multicentre, randomised, double-blind, controlled parallel study was conducted to evaluate the effect of the consumption of a fermented dairy product containing a probiotic on the resistance to CID of the airways and gastrointestinal tract (Table 1) in free-living elderly volunteers. One group of volunteers was randomly allocated to the fermented product and the other to a control product. The study consisted of a 3-month (84 d) product consumption phase and a 1-month (28 d) follow-up phase without consumption of the study product. The product consumption phase was preceded by a 2-week period of dietary restriction that was maintained until the end of study. The trial was conducted between 2 November 2006 (first subject included) and 4 May 2007 (last subject completed the study) by general practitioners in 125 centres distributed in twenty-five departments in France.

The study received approval from the independent ethics committee (Independent Ethics Committee/Institutional Review Board) Lyon A (Hôpital Hôtel Dieu, Lyon, France) on 23 August 2006 and was conducted in line with the principles of the Declaration of Helsinki, and of Good Clinical Practice, and European regulatory requirements.

Subjects

Inclusion criteria were: male and female individuals of at least 70 years of age who were free-living (not residing in an institution), with a Gerontological Autonomy Iso-resource Group (AGGIR) score between 5 and 6 (AGGIR score assesses the individuals' physical and psychological independence on a six-point scale, where a score of 6 represents total autonomy and a score of 1 represents total dependence); vaccination against the influenza virus at least 14 d before inclusion; a mini-mental state score of at least 24; a BMI between 17 and 25 kg/m^2 (bounds included); compliance with a dietary restriction during the 2 weeks preceding the product consumption phase and throughout the study. The restriction implied the exclusion of fermented dairy products with probiotics other than those used in the study, yoghurts and over-the-counter medication containing probiotics, vitamins, minerals or other nutrients.

Written consent was obtained from all participants and all of them were registered with the national social system security or benefited from a similar programme.

Exclusion criteria included: residing in institutions; any current or past severe respiratory, gastrointestinal or metabolic pathology; chronic or iatrogenic immunodeficiency, any infection in the last 14 d, laxatives more than twice in the last week; food allergy or intolerance; any progressive or chronic disease such as unstable type 1 and 2 diabetes or cancer, cognitive, neurological, cardiac or renal diseases; major surgery with general anaesthesia during the last month or gastrointestinal surgery during the last 3 months; artificial nutrition within the last 2 months; special medicated diets; nutritional complements; eating or transit disorders; alcohol abuse; currently receiving or having received in the 4 last weeks; drugs likely to interfere with evaluation of the study parameters, including antibiotics, intestinal or respiratory antiseptics, antifungal (except topical), corticoids, vaccines (except influenza vaccine), anti-histaminic molecules, non-corticoid anti-inflammatory substances (except aspirin or equivalent at doses preventing from aggregation of platelets or blood clotting) and immunosuppressant treatment.

Intervention

Volunteers were randomised to receive either the fermented product or the control product. The fermented product was a sweetened, flavoured, fermented dairy drink (Actimel®) containing at least 10^{10} colony-forming units (CFU)/100 g of

Table 1. Common infectious diseases (CID) and associated symptoms

CID	Symptoms
Upper respiratory tract infections	
Rhinopharyngitis (cold, coryza)	Nasal discharge, sneeze, headache, asthenia, ache, temperature (rarely)
Sore throat	High temperature (except in viral sore throat), pharyngitis, red tonsil, swelling (sometimes purulent), cervical adenopathy pain
Acute sinusitis	Headache, pain sinus, purulent nasal discharge, sore throat, cough, fever
Acute otitis	Auricular pain, transmission deafness, auricular discharge
Lower respiratory tract infections	
Acute bronchitis	Moderate temperature, cough, purulent expectorations
Pneumopathy	High temperature, shiver, cough with expectoration or purulent cough, thoracic pain, dyspnoea
Flu, flu-like syndrome	Shiver, high temperature, headache, ache, painful articulations, asthenia, anorexia, dry cough, pharyngitis
Gastrointestinal tract infections	
Gastro-enteritis	Temperature, headache, ache, abdominal pain, diarrhoea, vomiting

the probiotic strain *L. casei* DN-114 001 (international reference: CNCM I-1518, also named *Lactobacillus paracasei* subsp. *paracasei* following the current nomenclature), combined with a symbiosis of two cultures commonly used in yoghurt, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (at least 10^9 CFU/100 g for the whole symbiosis). The health effects are carried by the entire complex product containing not only the strains (*L. casei* DN-114 001 and yoghurt ferments), but also all of the metabolites resulting from the proprietary fermentation process. Therefore, a non-fermented, acidified, sweetened, flavoured dairy drink was chosen as an appropriate control to respect double blinding without the active components[31,32]. The nutritional composition, appearance, taste and packaging of the fermented product and control were identical throughout the study in order to maintain blinding. The study products were provided and manufactured by Danone (Bierun, Poland) and distributed by the investigators every 14 d.

During the phase of consumption, volunteers had to ingest two bottles of 100 g/d of fermented or control product (one bottle at breakfast and one at dinner, preferably with an interval of at least 8 h).

Procedure

The total duration of the study was 4·5 months (112 d). At the selection visit, 2 weeks before initiation of the study, volunteers underwent a clinical examination and started the dietary restriction that was maintained during the entire study. Two weeks after the selection visit, a certificate of eligibility was issued by the investigator, and the volunteers were randomly allocated to fermented product or control product group on day 0 of the study. Symmetric randomisation was carried out in blocks on a 1:1 ratio. Volunteers were assigned to study groups using an individual randomisation number (study product allocation concealed) and were included sequentially in accordance with the randomisation list, which was stratified by centre. Participants consumed the product for 3 months and were observed for a further 1 month. During the consumption phase, planned evaluation visits were conducted at the end of months 1, 2 and 3. A final evaluation was conducted at the end of the 1-month follow-up phase. At all the planned visits, a clinical examination was undertaken, including measurements of weight, BMI, blood pressure and heart rate. Dietary restriction compliance was also assessed, adverse events and concomitant medication were recorded and the SF-36® quality of life questionnaire was completed. Compliance was assessed by asking volunteers to record their daily consumption in a personal diary and to return any unused bottles at each visit. For the analysis of biological parameters, blood samples were also taken for a subset of included volunteers at day 0 just before the first product consumption (for analysis at baseline), at month 2 (following 2 months of product consumption) and at the end of the 1-month follow-up visit. Stool samples were also taken at each planned visit for a subset of included volunteers.

In addition, volunteers attended an additional evaluation visit each time they presented clinical symptoms related to the defined CID classified by category: upper respiratory tract infections (URTI, defined as the following types: rhinopharyngitis; sore throat; sinusitis; otitis), lower respiratory tract infections (LRTI, defined as the following types: bronchitis; pneumopathy; flu and flu-like syndromes) and gastrointestinal tract infections (GITI, defined as gastro-enteritis) (Table 1). These evaluation visits were conducted 3 d (± 1 d) after the initiation of the symptoms. At each additional visit, a clinical examination was performed, the type of CID was diagnosed and the start date (first day of symptoms), end date (last day of symptoms) and global severity (defined as mild, moderate or severe) of the disease were reported. A CID for which data were captured at the additional visits was termed a 'CID regular reported' and was thus diagnosed by a doctor during the CID. Occasionally, data were captured at the planned visits and the infection defined as a 'CID delayed reported', which refers to a CID diagnosed by a doctor on the basis of symptoms reported by the volunteers after the end of CID event. During each CID, in addition to their symptoms, the volunteers also recorded their temperature and their medication taken. At each of the additional visit, biological samples were taken for the identification of specific pathogens according to the type of CID. In addition, blood samples were also collected in a subset of volunteers to assess the same biological parameters as for planned visits. All the analytical dosages in the study were performed in blind.

Data analysis

The primary outcome measure was a comparison between groups of the cumulative number of all CID (Table 1) reported during the 3 months of study product consumption. Additionally, the occurrence of CID (defined as the number of subjects experiencing at least one CID) was investigated as a secondary outcome. Only CID occurring after the start date of product consumption were considered. A new CID occurring after a previous CID of the same type was considered as a separate event only if there was at least 2 d between the two events. Evolution from one type of disease to another was recorded as two CID. Multiple events of different infectious diseases occurring jointly (e.g. an URTI and a gastro-enteritis) were counted separately and added.

Another secondary objective of the study was to determine whether fermented product consumption changes the duration of CID (both cumulative and average duration per episode) calculated from the first to the last day of symptoms as defined in Table 1 for each CID. Additional secondary objectives were to assess the impact of fermented product consumption on the time span to the first occurrence of CID (time of event), on the severity of CID assessed with a three-point scale (mild–moderate–severe, based on the degree of interference with the subject's daily activity), on fever associated with CID (occurrence, duration and maximum, with fever defined as temperature $\geq 38°C$) and on the occurrence or duration of medications (prescribed and/or auto-administered). In addition, analyses of the micro-organisms responsible for CID were performed by measuring the occurrence and amount of specific pathogens in biological samples (laboratory: CDL Pharma, Marseille, France). For URTI viruses, identification and quantification in nasal fluid samples were performed by quantitative reverse transcription-PCR. For bacteria identification and quantification, in case of LRTI in expectorations or throat swabs (the latter in case of sore throat) or in case of GITI in stools samples, a microscopical

examination and Gram staining were performed and samples were cultured on enriched and specific media (API® gallery method).

In a subset of the included population, secondary objectives were to assess changes in biological (haemogram, serum C-reactive protein) and immunological parameters and the presence of *L. casei* species in the stools (laboratory: CDL Pharma). The immune system was assessed by analysis of blood parameters defined as oxidative burst activity in monocytes, cytolytic activity and count of blood natural killer cells and cytokines production in serum (IL-1 and IL-6, interferon-α, interferon-β, interferon-γ, IL-12, IL-10, TNF-α and IL-8). All biological analyses were performed by using standard procedures. The quantification of *L. casei* species in stools has been assessed by measuring total *L. paracasei* (which include *L. casei* DN-114 001) by quantitative PCR following the procedure described by Haarman & Knol[33] with some modifications. Briefly, total DNA was extracted from stools by using a commercial kit according to the manufacturer recommendations (QIAamp DNA Stool kit, Qiagen, Courtaboeuf, France). Purity and concentration of DNA were then evaluated by measuring absorbance at 260 and 280 nm in a spectrophotometer. *L. paracasei* group-specific real-time PCR (TaqMan universal master mix, Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) was then performed. For the selection of primer and probe sequences, the 16S–23S intergenic spacer regions of the different *Lactobacillus* species were retrieved from the GenBank, EMBL and DDBJ databases: *L. paracasei* AB035487, AF182724 and U32964. Sequences were aligned and the conserved regions were determined by using DNASIS for Windows version 2.5 (Hitachi Software Engineering Co., Ltd, Wembley, UK). Using Primer Express 1.5a (Applied Biosystems), specific sequences were identified to design primers and probes (primers: F_paca_IS 5′-ACA TCA GTG TAT TGC TTG TCA GTG AAT AC-3′ and R_paca_IS 5′-CCT GCG GGT ACT GAG ATG TTT C-3′; probe P: paca_IS 5′-TGC CGC CGG CCA G-3′). A standard curve has been included in the quantitative polymerase chain reaction by using a suspension of *L. paracasei* of known concentration allowing the quantification of *L. paracasei* in the samples in equivalent of CFU (eq. CFU)/gram of stools.

The impact of the fermented product on subjects' quality of life was also assessed using the SF-36® questionnaire[34]. The SF-36® questionnaire comprises thirty-six items used to construct scores across eight dimensions (physical functioning, role physical, bodily pain, social functioning, mental health, role emotional, vitality and general health) and two summary scores: the physical component score; the mental component score.

For all parameters, analyses were performed during the 3 months of study product consumption, during the 1-month follow-up and during the whole-study phases, for all CID (any type), or each category (URTI, LRTI, GITI) or each type of CID.

Throughout the study, tolerability and safety were assessed by recording spontaneously reported adverse events and by measuring evolution of vital signs, i.e. systolic and diastolic blood pressures, heart rate and weight during the 3-month product consumption phase, during the 1-month follow-up phase and during the whole-study overall.

The severity of adverse events was defined as mild (no interference with the subject's daily activity), moderate (moderate but acceptable interference with the subject's daily activity) or severe (marked and unacceptable interference with the subject's daily activity). A serious adverse event was defined as an adverse event that: resulted in death; was life threatening; was likely to result in disability or permanent invalidity; resulted in hospitalisation or prolonged hospitalisation; was medically significant. Any serious adverse events had to be reported up to a maximum of 24 h after the investigator became aware of it. CID were not reported as adverse events, except if considered serious.

Statistics

The study design planned to include 1000 volunteers. This sample size was determined based on the primary outcome of the study: the cumulative number of CID episodes occurring within the 3 months of product consumption. According to several publications[35,36], it was assumed that an average number of 1·5 events would be observed in the control group with an estimated 15 % relative decrease in the fermented product group. Also it was presumed that the winter period during which the study took place allowed the expectation of the maximum number of CID episodes. The distribution of the cumulative number of infectious events was assumed to be an overdispersed Poisson distribution, with overdispersion expected because the occurrences of CID events within a volunteer are not independent. With an expected rate of 1·5 events over 3 months in the control group and using a Poisson regression with a two-sided test at the 5 % α-level and assuming moderate overdispersion, about 450 evaluable volunteers in each arm were expected to be needed to detect a 15 % reduction rate with at least 80 % power. A 5 % dropout rate of volunteers was assumed and as such inclusion of approximately 500 volunteers in each arm was necessary.

For the assessment of biological parameters, blood was collected from only a certain number of subjects. According to available literature[37–39], a total of about 100 evaluable subjects were necessary to allow analysis of differences in immunomodulatory effects between groups. For the quantification of *L. casei* DN-114 001 in the stools, the number of subjects performing stool collection was fixed at forty-eight.

Data were treated to build up a cleaned and locked database, following data validation, including checking and plausibility assessments. Global database, including derivate parameters, has been locked and used for statistical analysis. Analyses were performed on the intention to treat (ITT) population, which comprised all volunteers who were included, randomised to the groups and having received the study product. Analyses were also performed on the per protocol population. The primary assessment was at 3 months, at the end of study product consumption, with additional assessments conducted for the follow-up phase and for the whole-study overall. Comparisons between the fermented product group and the control group were performed using two-sided statistical tests with a significance level of 5 % ($P<0·05$). Appropriate parametric and non-parametric methods were employed according to the distribution of the data. Baseline characteristics were compared using a *t* test or a Mann–Whitney test.

British Journal of Nutrition

For the outcome parameters, comparisons of continuous data were made using a mixed ANOVA model (or appropriate non-parametric analysis). For comparison of qualitative data, a χ^2/Fisher exact test, a logistic regression analysis with a binary response or a Cochran–Mantel–Haenzel test was used. Time to event analyses were conducted using a log-rank test and/or a Cox regression. For comparison between groups of count data, a Poisson regression model was used, taking into account the study duration.

Confounders, including centre, age, sex and AGGIR score, have been taken into account as appropriate. For the primary criteria, history of CID during the last month and study duration were also taken into account. Data were analysed using SAS® software (version 8.2; SAS Institute Inc., Cary, NC, USA).

Results

Volunteer demographics

The flow of the volunteers through the studies is displayed in Fig. 1. A total of 1089 individuals were selected for inclusion in the study, with 1072 volunteers randomised to the fermented product (537) or the control product (535). Out of these volunteers, 125 were selected for blood sample assessment (sixty-three randomised to fermented product and sixty-two randomised to control). Sixty-three volunteers were selected for assessment of *L. casei* in stools (thirty-two randomised to fermented product and thirty-one randomised to control).

There were 208 (19·4 %) volunteers with a major protocol deviation in the study. This was well balanced between groups. Most frequently, the major deviations were relative to the time between planned visits (7·9 %), the concomitant treatments that could interfere with the study results (4·5 %) and the completeness of the protocol at the final visit (4·3 %).

The baseline characteristics for the ITT population were well balanced across the fermented product and control product groups (Table 2) regarding age (median age = 76·0 years, range 69·0–95·0), sex ratio, BMI, mini-mental state score, current diseases, concomitant treatments and history of CID. Current diseases were mainly vascular diseases, muscular–bone diseases and cardiovascular diseases. The results of the initial clinical examination were normal for all volunteers.

Compliance was 100 % in median for all volunteers (assessed by the number of consumed bottles of products), with no difference between the study groups (Table 2). The consumption duration was 84·0 (83; 85) (median (Q1; Q3)) days (expected value for the study: 84 ± 6 d), and the mean number of bottles consumed was 168·0 (164; 169) (median (Q1; Q3)) bottles per subject (expected value for the study: 168 ± 12 bottles per subject). Forty-six volunteers (4 %) withdrew from the study prematurely and each withdrawal was unrelated to the product consumption.

Primary outcome measure: cumulative number of episodes of common infectious diseases

The primary outcome measure was analysed in the ITT population using a generalised linear model assuming a Poisson distribution, with factors of age, sex, AGGIR score, history of CID in previous month, product group, centre (random effect) and taking into account the study duration. During the 3 months of product consumption, 217 volunteers (20·4 % of the whole population) experienced a total of 255 episodes of CID (Fig. 2).

In the fermented product group, 105 volunteers reported 120 CID during the product consumption phase, compared with 112 volunteers who reported 135 CID in the control group. Considering the whole population, the mean rate for this period was 0·2 (SD 0·5) in the fermented product group and 0·3 (SD 0·5) in the control group (rate reduction = 10·6 %), with an adjusted relative risk of 0·89 (95 % CI 0·70, 1·14, P=0·373). The difference between groups regarding the cumulative number of all CID was not statistically significant. In addition, there was no statistically significant difference between groups when the whole-study phase was considered (3-month consumption phase + 1-month follow-up phase).

There were no statistically significant interactions between fermented product and the included factors, and therefore

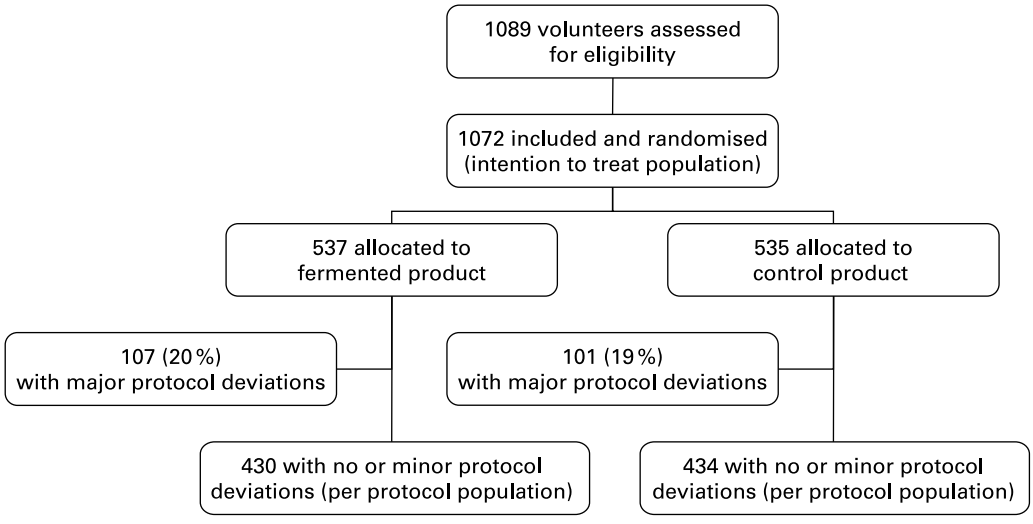

Fig. 1. Volunteer flow in the study.

Table 2. Baseline characteristics and product compliance for volunteers in the intention to treat population
(Median and quartile values and number and percentage of subjects)

	Fermented product (*n* 537)			Control product (*n* 535)			
	Median	Q1; Q3	Number (% of subjects)	Median	Q1; Q3	Number (% of subjects)	*P*
Age (years)	76·0	72·0; 80·0		76·0	73·0; 81·0		0·262*
Sex (male)			198 (36·9)			202 (37·8)	0·764†
BMI (kg/m^2)	24·1	23·0; 25·0		24·9	23·0; 25·0		0·399*
MMS score	29·0	27·0; 30·0		28·0	27·0; 30·0		0·359*
Current diseases			1711 (98·4)			1697 (98·1)	0·505†
Concomitant products			1922 (96·4)			1954 (97·1)	0·259†
CID in last year (% yes)			(34·3)			(38·7)†	0·132†
CID in last month (% yes)			(2·6)			(4·3)†	0·129†
Product compliance (%)	100·0	99·0; 100·0		100·0	99·0; 100·0		0·935†

Q, quartile; MMS, mini-mental state.
* Mann–Whitney.
† χ^2 test.

subgroup analyses were not undertaken (sex: $P=0{\cdot}577$, age: $P=0{\cdot}555$, AGGIR score: $P=0{\cdot}974$, CID history: $P=0{\cdot}199$).

Analyses were conducted based on when the CID were reported. Among the 217 volunteers who have displayed CID, 82·9 % immediately reported CID and 17·1 % reported with delay. Whatever the time of reporting, there was no significant difference between groups for the cumulative number of CID.

Secondary outcome measures

Episodes of common infectious diseases by category and type (upper respiratory tract infections, lower respiratory tract infections and gastrointestinal tract infections). In ITT population, during the whole-study phase, URTI accounted for the majority of reported CID (54·5 %) followed by LRTI (29·1 %) and GITI (16·4 %), and this pattern was similar across groups. Rhinopharyngitis was the most frequently reported type of CID (50·3 %), followed by bronchitis (23·6 %), gastro-enteritis (16·4 %), flu and flu-like syndrome (5·5 %), sinusitis (3·1 %) and sore throat (1·0 %). Neither otitis nor pneumonia was reported during the study.

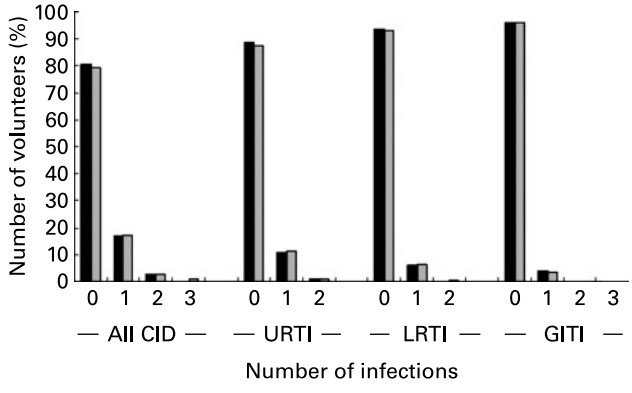

Fig. 2. Percentage of volunteers by the number of common infectious diseases (CID) experienced (intention to treat population) for all CID (any type), upper respiratory tract infections (URTI), lower respiratory tract infections (LRTI) or gastrointestinal tract infections (GITI) during the product consumption phase. The *y*-axis represents the percentage of volunteers. The *x*-axis describes the specific infection and number of infection experienced. Volunteers receiving fermented product (*n* 537) are represented by ■; volunteers receiving the control product (*n* 535) are represented by ▨. No volunteer experienced three URTI or LRTI whatever the group, 0·2 % of the volunteers in the control group and 0 % in the fermented product group experienced two or three GITI.

During the product consumption phase, the number of URTI, LRTI, GITI (Fig. 2) or of each type of CID (data not shown) was not statistically different between groups.

Common infectious diseases duration. The average duration of disease for each episode and the cumulative duration of CID were analysed in ITT population experiencing CID using a Mann–Whitney non-parametric test. When considering all the CID during the study product consumption phase, both the duration of CID episodes and the cumulative duration of CID were significantly lower in the fermented product group compared with the control group ($P=0{\cdot}008$ and 0·009, respectively; Table 3). For both episode and cumulative duration, significant differences between groups were also found when the whole-study phase (consumption phase plus follow-up phase) was considered ($P=0{\cdot}019$ and 0·018, respectively).

These differences were also significant in favour of fermented product for all URTI and for rhinopharyngitis during the product consumption phase (Table 3) and during the whole-study phase (all URTI: episode duration and cumulative duration, $P=0{\cdot}004$ for both; rhinopharyngitis: episode duration, $P=0{\cdot}01$; cumulative duration, $P=0{\cdot}009$).

For all CID, all URTI and rhinopharyngitis, the median episode duration was 1–1·5 d shorter in the fermented product compared with the control group (Table 3).

Regarding the LRTI and GITI, there was no significant difference in the episode duration or the cumulative duration.

All significant results were confirmed in the per protocol population presenting CID.

Lactobacillus casei *in stools.* Analysis of the amount of *L. casei* species in stools was performed at each planned visit in the ITT population. Comparison between groups was analysed by considering both the actual values and the change from baseline for each visit in both groups by using a Mann–Whitney test. At baseline, the results show no statistical difference between groups in the amount of *L. casei* species in stools ($P=0{\cdot}525$; Fig. 3). After 1, 2 or 3 months of product consumption, a statistically significant difference in the amount of *L. casei* was observed between the two groups (according to evolution from baseline and actual

Table 3. Cumulative and episode duration of common infectious diseases (CID) during product consumption phase in intention to treat population with CID

(Mean values and standard deviations; median values and quartile ranges)

	Fermented product					Control product					
	n	Mean	SD	Median	Quartiles (Q1–Q3)	*n*	Mean	SD	Median	Quartiles (Q1–Q3)	*P**
All CID											
Average duration per episode (d)	104	7·4	5·6	6·5	4–9	111	9·8	7·5	8·0	5–12	0·008
Cumulative duration (d)	104	9·1	10·4	7·0	4–11	111	12·1	11·4	8·0	6–16	0·009
All URTI											
Average duration per episode (d)	61	7·7	7·2	7·0	4–8	66	11·0	7·7	8·0	7–13	0·0002
Cumulative duration (d)	61	8·5	8·4	7·0	4–8	66	11·6	7·9	8·5	7–16	0·0003
Rhinopharyngitis											
Average duration per episode (d)	61	7·7	7·2	7·0	4–8	58	11·0	8·1	8·0	7–13	0·0007
Cumulative duration (d)	61	8·2	8·0	7·0	4–8	58	11·5	8·1	8·0	7–15	0·0006

Q, quartile; URTI, upper respiratory tract infections.
*Mann–Whitney test, median and quartiles must be considered as a summary statistic.

values at each point – $P<0·001$ for all comparison). The number of *L. casei* remained unchanged in control group throughout the whole study at about $4·9 \times 10^5$ eq. CFU/g of stools (baseline value; Fig. 3). In the fermented product group, the median value of *L. casei* amount increased from $2·9 \times 10^5$ (baseline) to $3·3 \times 10^7$ eq. CFU/g after 1-month consumption and was roughly maintained at the same level throughout the product consumption ($2–3·8 \times 10^7$ eq. CFU/g of stools, $P<0·001$). At the end of the follow-up phase, no statistical difference was found between groups and in each group compared with baseline value. This indicated a return to baseline level of *L. casei* amount in the fermented product group 1 month after the consumption has ceased. All results were confirmed in the per protocol population.

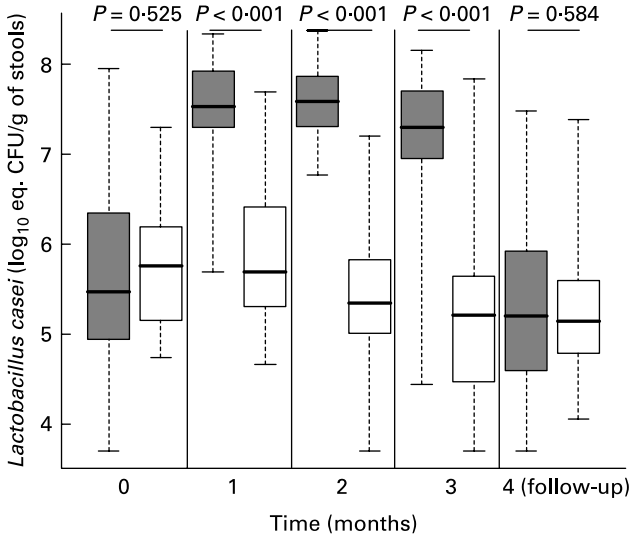

Fig. 3. Amount of *Lactobacillus casei* species in stools (in $\log_{10}$ equivalents of CFU/g of stools) during product consumption and after 1-month follow-up in intention to treat population (■, fermented product (*n* 32); □, control product (*n* 31)). In each box, upper limit, central line and lower limit indicate the quartile 3 (Q3), median and Q1 values, respectively. The upper and lower limits of the dotted lines indicate the minimum and maximum values, respectively. *P* value at 0 month (for comparison between groups of the data at baseline) and *P* values at 1–4 months (for the comparison between groups of the change from baseline) were calculated using a Mann–Whitney test.

Other secondary outcome measures in intention to treat population. There was no difference between the two groups of participants regarding the occurrence of CID (defined as the number of subjects having at least one CID), the time to first event, the severity, the intensity or duration of fever or CID-associated medication (prescribed and/or auto-administered). Only 3·6 % of the participants (*n* 4 in each group) experienced severe CID during the product consumption phase. In the same population and period, the proportion of volunteers who had fever associated with CID was 28·2 *v.* 28·3 %, and the proportion of volunteers receiving at least one prescription of medication was 67·5 *v.* 64·2 % in the fermented and the control product group, respectively.

The analysis of pathogens in case of CID could not be compared between groups due to an insufficient number of positive samples for the presence of the specific micro-organisms studied. Biological and immunological parameters were comparable between the two groups. They could not be analysed in the case of CID due to the insufficient number of infections. Quality of life was also assessed by change from baseline in SF-36® scores. No significant difference was reported between the two groups regarding the eight dimensions and the physical and mental component scores of the questionnaire.

Safety

The safety profile of the fermented product was comparable with the control product. During the study, 276 volunteers reported 416 adverse events. A summary of reported adverse events is given in Table 4. The most frequent categories of reported adverse events were the muscular–bone system (19 %), the gastrointestinal system (18·5 %) and infections other than CID (16·3 %), and there was no significant difference between groups in number of adverse event for any of these categories. Although a total of twenty-five serious adverse events were reported, none were related to fermented product consumption.

Regarding the vital signs during the study, there was no significant difference between the groups in the evolution from baseline of weight ($P=0·471$), of systolic ($P=0·902$)

Table 4. Summary table of adverse events (total number and percentage of volunteers with at least one event) during whole-study phase in intention to treat population

	Fermented product (*n* 537)	Control product (*n* 535)	All (*n* 1072)
AE	137 (25·5)	139 (25·9)	276 (25·7)
Emergent AE	128 (23·8)	129 (24·1)	257 (23·9)
Serious AE	11 (2·0)	11 (2·0)	22 (2·0)
Serious emergent AE	11 (2·0)	11 (2·0)	22 (2·0)
Severe emergent AE	14 (2·6)	9 (1·6)	23 (2·1)
Probably related emergent AE	6 (1·1)	5 (0·9)	11 (1·0)
Probably related serious emergent AE	0	0	0
Emergent AE leading to permanent withdrawal	8 (1·4)	10 (1·8)	18 (1·6)

AE, adverse event; emergent AE, AE that began or worsened during the product consumption phase.

or diastolic blood pressure ($P=0·554$) or of heart rate ($P=0·586$), and no relevant clinical evolution of these parameters was found in each group.

Discussion

The aim of this double-blind, randomised, controlled study was to assess the effect of a fermented dairy product containing the probiotic strain *L. casei* DN-114 001 on the resistance to common respiratory and gastrointestinal infections in an elderly free-living population. The baseline and demographic characteristics of the volunteers, the duration of study and the compliance to the product consumption were similar for the fermented product and the control product groups. There was also no difference between groups and no relevant modification in each group regarding the safety parameters including adverse events and vital signs. Furthermore, withdrawals from the study were rare and unrelated to the product consumption.

The study was performed from October 2006 to May 2007 and thus included the winter season when the rate of CID was expected to be high. However, instead of the estimated 1·5 CID we observed only 0·3 (SD 0·5) CID per subject in the control group. This was likely due to a particularly low incidence of infections during the winter 2006/2007 (as documented by the Groupes Régionaux d'Observation de la Grippe[40]). We can thus speculate that the fact that no difference was found between groups for the primary outcome measure, the cumulative number of all CID during the product consumption phase, may be attributed to a lower than expected number of events. Additionally, the low incidence of severe CID (4·2 %) could have been associated with an underreporting of total infections, which is consistent with the 17 % of delayed reported CID observed.

It is particularly interesting that the fermented product was found to significantly reduce both the average duration of CID per episode and the cumulative duration of CID. These reductions were statistically significant in the product consumption phase and also for the duration of the study as a whole (product consumption phase plus follow-up phase) when all the CID were considered. Significant reductions were also reported for all URTI and specifically for rhinopharyngitis. Fermented product consumption was found to reduce by 1–1·5 d (median) the duration of CID observed in the control group, which was comprised between 8 and 8·5 d. The present results confirm the preliminary observations on a similar ageing population showing that administration of the same product reduced the duration of respiratory and gastrointestinal infections[30]. Other studies also described the influence of probiotics on respiratory tract infections in children[41] or adults[29,42]. In an unblinded trial, elderly immunised by influenza and pneumococcal vaccination displayed a lower incidence of infections, especially respiratory, after consumption of a nutritional supplement containing *L. paracasei* and other nutrients such as vitamins[28]. In hospitalised enterally fed elderly, consumption of a fermented milk containing a probiotic strain of *Lactobacillus johnsonii* was shown to reduce the duration of infections requiring antibiotics (respiratory, intestinal and urinary infections taken together) and to improve respiratory symptoms[20]. The present study is the first randomised, double-blind, controlled trial indicating that a dairy probiotic product alone can be active against URTI in free-living elderly. The reduction of the duration of CID episodes by 1·5 d is in the range of the efficacy of neuraminidase inhibitors (1·0 d reduction) in influenza treatment[43]. The observed reduction of duration of respiratory infections could be associated with another benefit that is a lower risk of medical complication leading to severe symptoms since human rhinoviruses, the most common etiologic agents of URTI, were found to be associated with high frequency of prolonged illness and lower respiratory tract disease in elderly[44,45]. Such an effect could be investigated in a larger cohort of subjects.

Duration and severity of CID could be related clinically. Here, we observed an effect of the fermented product on CID duration, but not on the severity of CID. This could be attributed to the very low number of severe episodes in the whole population, which might have prevented the detection of any significant difference between groups. The absence of a product effect on fever is also at variance with a previous result showing a significant decrease of the maximum temperature recorded during winter pathologies in the elderly consuming the same product[30]. However, in the latter, the incidence of infectious disease was much higher (0·38 per subject for 3 weeks only) and thus, more severe pathologies may have been reported as exemplified by the occurrence of bacterial bronchopneumonia.

Increase of *Lactobacilli* level in faeces and survival of *L. casei* DN-114 001 in the gut were previously reported in young adults[46–48] and children[49] after consumption of a dairy product containing *L. casei* DN-114 001. In the present study, we provide the first evidence in elderly consuming the fermented product for an increase of *L. casei* species in the gut as quantified in the stool analysis. A 100-fold higher

British Journal of Nutrition

level was observed throughout the product consumption and decreased to baseline level when consumption was stopped, suggesting the possibility of a transient presence in the gut of the probiotic strain as previously described in adults using selective culture methods[46]. In any case, this result indicates a good compliance of the subjects to the products consumption. Further investigations are needed to understand the origin of those *Lactobacilli* since they could be the probiotic strain *L. casei* DN-114 001 and/or commensal gut bacteria of the same species specifically favoured by the product.

The mechanism by which a fermented product taken orally can have an effect against infection of the airways is a matter of debate. A clinical study showed that consumption of a mix of probiotics decreases the occurrence in nasal cavity of potentially pathogenic bacteria known to be responsible for respiratory infectious disease[50]. Another possible mechanism could be the effect of probiotics on the innate and adaptive immune system[51], as previously reported for dairy product containing *L. casei* DN-114 001[37–39,52,53]. In the present study, none of the immune parameters tested was modulated during fermented product consumption. However, the same product can improve immune responses to influenza vaccination of the elderly[54]. Therefore, we cannot rule out the possibility of an action of the fermented product on other immune parameters than the one assessed. In addition, the analysis of the data on the blood immune parameters during infection could not be performed due to the too low number of subjects presenting a CID in the subset of population with blood samples collection. Several other mechanisms enhancing defence were described, which may have contributed to the clinical effect observed[55,56]. *In vitro* experiments on intestinal epithelium human cells showed that *L. casei* DN-114 001 can inhibit the cell adhesion and invasion by adherent-invasive *Escherichia coli*[57] and the increase in paracellular permeability induced by enteropathogenic *E. coli* infection[58]. Furthermore, innate defence may be increased by probiotics via enhancing defensin expression. Until now, however, this has been shown *in vitro* using *E. coli* Nissle only[59]. To what extent an improvement of mineral absorption and vitamin production by lactic acid producing bacteria may play a role is uncertain. There is some evidence for an increase in absorption of minerals with potential impact on defence such as Zn and Se by prebiotics. For probiotics, however, the evidence is still scarce[60].

All the active components of the product used in the present study have not been fully identified. The health effects are likely carried by the whole product containing not only the strains (*L. casei* DN-114 001 and yoghurt ferments), but also all of the metabolites resulting from the fermentation process. This explains the design of our control product as a non-fermented, acidified, sweetened, flavoured dairy drink with no bacteria. The contribution of the probiotic strain *L. casei* DN-114 001 is supported by two studies in children, which compared the effect of the same fermented product to the one of a standard yoghurt containing *S. thermophilus* and *Lactobacillus bulgaricus*[61,62]. The results showed that compared with jellied milk, the consumption of the probiotic fermented product significantly reduced the duration of diarrhoea, whereas standard yoghurt had no significant effect[61]. In addition, when directly compared with a standard yoghurt, the probiotic fermented milk also significantly decreased the incidence of diarrhoea[62]. There is therefore evidence that *L. casei* DN-114 001 may play a role in the effect of the fermented product we used, providing superiority to the yoghurt symbiosis present in the product. However, this symbiosis could also provide beneficial effects as illustrated by several reports describing the health benefit of yoghurt consumption on immune system and gut, especially gastrointestinal infections[22,23]. A further clarification of the extent to which the components of the fermented product used in the present study contribute to its effect would require a much higher sample size and separate studies with adequate, prior statistical estimations.

In conclusion, consumption of a fermented dairy product containing *L. casei* DN-114 001 is associated with a significant decrease in the duration of CID, especially URTI and particularly rhinopharyngitis providing the first evidence that a dairy fermented product containing a probiotic may have a beneficial effect against respiratory infections in the free-living elderly. It is possible that the low number of CID observed in the study as a whole contributed to the lack of effect of the product on the primary and some secondary outcomes. Based on the present results and on previous observations[30] and given the high morbidity and mortality associated with infectious diseases especially of the airways in the elderly, dietary intervention using a dairy product containing the probiotic strain *L. casei* DN-114 001 could be considered as a mean to improve health status of this population.

Acknowledgements

We thank particularly Leila Mhamdi (Keyrus, Levallois-Perret, France) for statistical analyses, Bénédicte Borgies, Caroline Caty and Isabelle Seksek (Danone Research) for the study coordination, Stéphane Doat and Tanveer Hayat (Danone Research) for the logistic organisation of products supply, Thomasz Gawlas (Danone-Poland, Bierun, Poland) for management of products manufacture and Pierre Burguière and Jérôme Combrisson for their support on *L. paracasei* analysis. We also thank all the general practitioner members of MGRecherches who participated to the study as investigators and the members of the contract organisation research Advanced Drug Development Services (Boulogne-Billancourt-France) involved in the management of the general study organisation (Séverine Guigui), the data management (Viriginie Gomis) and the statistical calculations (Vincent Houé). We thank Sophie Costello and Ebony Samuels for their help in manuscript preparation (Heron Evidence Development Ltd, Letchworth Garden City, UK). E. G. (research scientist at Danone Research) participated in the development of study concepts and designs, the writing of the protocol, the analyses and interpretation of the data, the writing of the study report and of the publication. F. T. (biostatistician at Danone Research) participated in the study design, in the writing of study protocol, in statistical management, in the statistical analyses and interpretation of the data, in the writing of the study report and of the publication. F. L. received funding from Danone Research as main investigator of the study. J. S. contributed to the design of the study, to the evaluation and interpretation of all data and to writing of the manuscript. He received fees for contributing to the Advisory Board of Danone, Germany. The Department of Physiology and Biochemistry of Nutrition,

MRI was funded by Danone Research for performing several studies. Its institute was also funded by Campina, Chr. Hansen, Danisco, Müller, Nestlé, Bauer and Merck for studies on probiotics, and he received fees as a speaker at a symposia sponsored by Yakult, Danone, Nestlé, Orthomol and Merck. The work was undertaken at General practitioner centres of Quintiles/MGRecherches, France. The study was sponsored and funded by Danone Research.

References

1. Yoshikawa TT (2000) Epidemiology and unique aspects of aging and infectious diseases. *Clin Infect Dis* **30**, 931–933.
2. Gavazzi G & Krause KH (2002) Ageing and infection. *Lancet Infect Dis* **2**, 659–666.
3. LaCroix AZ, Lipson S, Miles TP, *et al.* (1989) Prospective study of pneumonia hospitalizations and mortality of U.S. older people: the role of chronic conditions, health behaviors, and nutritional status. *Public Health Rep* **104**, 350–360.
4. Djuretic T, Ryan MJ, Fleming DM, *et al.* (1996) Infectious intestinal disease in elderly people. *Commun Dis Rep CDR Rev* **6**, R107–R112.
5. Mouton CP, Bazaldua OV, Pierce B, *et al.* (2001) Common infections in older adults. *Am Fam Physician* **63**, 257–268.
6. Goodwin K, Viboud C & Simonsen L (2006) Antibody response to influenza vaccination in the elderly: a quantitative review. *Vaccine* **24**, 1159–1169.
7. McElhaney JE (2005) The unmet need in the elderly: designing new influenza vaccines for older adults. *Vaccine* **23**, Suppl. 1, S10–S25.
8. Targonski PV, Jacobson RM & Poland GA (2007) Immunosenescence: role and measurement in influenza vaccine response among the elderly. *Vaccine* **25**, 3066–3069.
9. Nichol KL (2003) The efficacy, effectiveness and cost-effectiveness of inactivated influenza virus vaccines. *Vaccine* **21**, 1769–1775.
10. Nikolich-Zugich J (2008) Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nat Rev Immunol* **8**, 512–522.
11. Delpedro AD, Barjavel MJ, Mamdouh Z, *et al.* (1998) Signal transduction in LPS-activated aged and young monocytes. *J Interferon Cytokine Res* **18**, 429–437.
12. McLachlan JA, Serkin CD, Morrey-Clark KM, *et al.* (1995) Immunological functions of aged human monocytes. *Pathobiology* **63**, 148–159.
13. Wenisch C, Patruta S, Daxbock F, *et al.* (2000) Effect of age on human neutrophil function. *J Leukoc Biol* **67**, 40–45.
14. Mysliwska J, Mysliwski A, Romanowski P, *et al.* (1992) Monocytes are responsible for depressed natural killer (NK) activity in both young and elderly low NK responders. *Gerontology* **38**, 41–49.
15. Solana R & Mariani E (2000) NK and NK/T cells in human senescence. *Vaccine* **18**, 1613–1620.
16. Riezzo G, Chiloiro M & Russo F (2005) Functional foods: salient features and clinical applications. *Curr Drug Targets Immune Endocr Metabol Disord* **5**, 331–337.
17. Jones PJ & Varady KA (2008) Are functional foods redefining nutritional requirements? *Appl Physiol Nutr Metab* **33**, 118–123.
18. Gill HS & Guarner F (2004) Probiotics and human health: a clinical perspective. *Postgrad Med J* **80**, 516–526.
19. de Vrese M & Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol* **111**, 1–66.
20. Fukushima Y, Miyaguchi S, Yamano T, *et al.* (2007) Improvement of nutritional status and incidence of infection in hospitalised, enterally fed elderly by feeding of fermented milk containing probiotic *Lactobacillus johnsonii* La1 (NCC533). *Br J Nutr* **98**, 969–977.
21. Hickson M, D'Souza AL, Muthu N, *et al.* (2007) Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial. *BMJ* **335**, 80.
22. Adolfsson O, Meydani SN & Russell RM (2004) Yogurt and gut function. *Am J Clin Nutr* **80**, 245–256.
23. Meydani SN & Ha WK (2000) Immunologic effects of yogurt. *Am J Clin Nutr* **71**, 861–872.
24. Nova E, Warnberg J, Gomez-Martinez S, *et al.* (2007) Immunomodulatory effects of probiotics in different stages of life. *Br J Nutr* **98**, Suppl. 1, S90–S95.
25. Ouwehand AC, Bergsma N, Parhiala R, *et al.* (2008) *Bifidobacterium microbiota* and parameters of immune function in elderly subjects. *FEMS Immunol Med Microbiol* **53**, 18–25.
26. Klein A, Friedrich U, Vogelsang H, *et al.* (2008) *Lactobacillus acidophilus* 74-2 and *Bifidobacterium animalis* subsp. *lactis* DGCC 420 modulate unspecific cellular immune response in healthy adults. *Eur J Clin Nutr* **62**, 584–593.
27. Kaila M, Isolauri E, Soppi E, *et al.* (1992) Enhancement of the circulating antibody secreting cell response in human diarrhea by a human *Lactobacillus* strain. *Pediatr Res* **32**, 141–144.
28. Bunout D, Barrera G, Hirsch S, *et al.* (2004) Effects of a nutritional supplement on the immune response and cytokine production in free-living Chilean elderly. *J Parenter Enteral Nutr* **28**, 348–354.
29. de Vrese M, Winkler P, Rautenberg P, *et al.* (2005) 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* **24**, 481–491.
30. Turchet P, Laurenzano M, Auboiron S, *et al.* (2003) Effect of fermented milk containing the probiotic *Lactobacillus casei* DN-114001 on winter infections in free-living elderly subjects: a randomised, controlled pilot study. *J Nutr Health Aging* **7**, 75–77.
31. Aggett PJ, Antoine JM, Asp NG, *et al.* (2005) PASSCLAIM: consensus on criteria. *Eur J Nutr* **44**, Suppl. 1, i5–i30.
32. Farnworth ER (2008) The evidence to support health claims for probiotics. *J Nutr* **138**, 1250S–1254S.
33. Haarman M & Knol J (2006) Quantitative real-time PCR analysis of fecal *Lactobacillus* species in infants receiving a prebiotic infant formula. *Appl Environ Microbiol* **72**, 2359–2365.
34. Ware J, Kosinski M & Dewey J (2000) *How to Score Version 2 of the SF-36(R) Health Survey*. Lincoln, RI: QualityMetric Incorporated.
35. Chavance M, Herbeth B, Lemoine A, *et al.* (1993) Does multivitamin supplementation prevent infections in healthy elderly subjects? A controlled trial. *Int J Vitam Nutr Res* **63**, 11–16.
36. Graat JM, Schouten EG & Kok FJ (2002) Effect of daily vitamin E and multivitamin–mineral supplementation on acute respiratory tract infections in elderly persons: a randomized controlled trial. *JAMA* **288**, 715–721.
37. Parra D, De Morentin BM, Cobo JM, *et al.* (2004) Monocyte function in healthy middle-aged people receiving fermented milk containing *Lactobacillus casei*. *J Nutr Health Aging* **8**, 208–211.
38. Parra MD, Martínez de Morentin BE, Cobo JM, *et al.* (2004) Daily ingestion of fermented milk containing *Lactobacillus casei* DN114001 improves innate-defense capacity in healthy middle-aged people. *J Physiol Biochem* **60**, 85–91.
39. Marcos A, Warnberg J, Nova E, *et al.* (2004) The effect of milk fermented by yogurt cultures plus *Lactobacillus casei* DN-114 001 on the immune response of subjects under academic examination stress. *Eur J Nutr* **43**, 381–389.
40. GROG – Groupes Régionaux d'Observation de la Grippe (2007) Bulletin 2007/27 – Saison grippe 2006/2007: une épidémie

NS British Journal of Nutrition

modeste. (Bulletin 2007/27 – 2006/2007 influenza season: a modest epidemic). http://www.grog.org/bullhebdo.php
41. Hatakka K, Savilahti E, Ponka A, *et al.* (2001) Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. *BMJ* **322**, 1327.
42. Cox AJ, Pyne DB, Saunders PU, *et al.* (2008) Oral administration of the probiotic *Lactobacillus fermentum* VRI-003 and mucosal immunity in endurance athletes. *Br J Sports Med* (Epublication ahead of print version 13 February 2009).
43. Cooper NJ, Sutton AJ, Abrams KR, *et al.* (2003) Effectiveness of neuraminidase inhibitors in treatment and prevention of influenza A and B: systematic review and meta-analyses of randomised controlled trials. *BMJ* **326**, 1235.
44. Hayden FG (2004) Rhinovirus and the lower respiratory tract. *Rev Med Virol* **14**, 17–31.
45. Wald TG, Shult P, Krause P, *et al.* (1995) A rhinovirus outbreak among residents of a long-term care facility. *Ann Intern Med* **123**, 588–593.
46. Oozeer R, Leplingard A, Mater DD, *et al.* (2006) Survival of *Lactobacillus casei* in the human digestive tract after consumption of fermented milk. *Appl Environ Microbiol* **72**, 5615–5617.
47. Rochet V, Rigottier-Gois L, Sutren M, *et al.* (2006) Effects of orally administered *Lactobacillus casei* DN-114 001 on the composition or activities of the dominant faecal microbiota in healthy humans. *Br J Nutr* **95**, 421–429.
48. Rochet V, Rigottier-Gois L, Levenez F, *et al.* (2008) 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* **54**, 660–667.
49. Guerin-Danan C, Chabanet C, Pedone C, *et al.* (1998) Milk fermented with yoghurt cultures and *Lactobacillus casei* compared with yoghurt and gelled milk influence on intestinal microflora in health infants. *Am J Clin Nutr* **67**, 111–117.
50. Glück U & Gebbers JO (2003) Ingested probiotics reduce nasal colonization with pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and beta-hemolytic streptococci). *Am J Clin Nutr* **77**, 517–520.
51. Corthesy B, Gaskins HR & Mercenier A (2007) Cross-talk between probiotic bacteria and the host immune system. *J Nutr* **137**, 781S–790S.
52. Pujol P, Huguet J, Drobnic F, *et al.* (2000) The effect of fermented milk containing *Lactobacillus casei* on the immune response to exercise. *Sports Med Training and Rehab* **9**, 209–223.
53. Tiollier E, Chennaoui M, Gomez-Merino D, *et al.* (2007) Effect of a probiotics supplementation on respiratory infections and immune and hormonal parameters during intense military training. *Mil Med* **172**, 1006–1011.
54. Boge T, Rémigy M, Vaudaine S, *et al.* (2009) A probiotic fermented dairy drink improves antibody response to influenza vaccination in the elderly in two randomised controlled trials. *Vaccine* (epublication ahead of print version 15 July 2009).
55. Sherman PM, Ossa JC & Johnson-Henry K (2009) Unraveling mechanisms of action of probiotics. *Nutr Clin Pract* **24**, 10–14.
56. Bourlioux P, Koletzko B, Guarner F, *et al.* (2003) The intestine and its microflora are partners for the protection of the host: report on the Danone Symposium 'The Intelligent Intestine,' held in Paris, June 14, 2002. *Am J Clin Nutr* **78**, 675–683.
57. Ingrassia I, Leplingard A & Darfeuille-Michaud A (2005) *Lactobacillus casei* DN-114 001 inhibits the ability of adherent-invasive *Escherichia coli* isolated from Crohn's disease patients to adhere to and to invade intestinal epithelial cells. *Appl Environ Microbiol* **71**, 2880–2887.
58. Parassol N, Freitas M, Thoreux K, *et al.* (2005) *Lactobacillus casei* DN-114 001 inhibits the increase in paracellular permeability of enteropathogenic *Escherichia coli*-infected T84 cells. *Res Microbiol* **156**, 256–262.
59. Wehkamp J, Harder J, Wehkamp K, *et al.* (2004) NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. *Infect Immun* **72**, 5750–5758.
60. Scholz-Ahrens KE, Ade P, Marten B, *et al.* (2007) Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J Nutr* **137**, 838S–846S.
61. Pedone CA, Bernabeu AO, Postaire ER, *et al.* (1999) 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* **53**, 179–184.
62. Pedone CA, Arnaud CC, Postaire ER, *et al.* (2000) Multicentric study of the effect of milk fermented by *Lactobacillus casei* on the incidence of diarrhoea. *Int J Clin Pract* **54**, 568–571.

British Journal of Nutrition