EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of a health claim related to Lactobacillus rhamnosus GG and maintenance of defence against pathogenic gastrointestinal microorganisms pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to *Lactobacillus rhamnosus* GG and maintenance of defence against pathogenic gastrointestinal microorganisms pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Valio Ltd., submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG (LGG) and maintenance of defence against pathogenic gastrointestinal (GI) microorganisms. LGG is sufficiently characterised. The Panel considers that the health claim refers to the defence against GI pathogens in the general population without GI infections and does not include the treatment of GI infections. Maintenance of defence against pathogenic GI microorganisms is a beneficial physiological effect. Only one out of five human intervention studies showed an effect of LGG consumption on the development of GI infections, two human intervention studies did not show an effect of LGG consumption on the stimulation of protective immune responses after oral (viral) vaccination and, in the absence of evidence for an effect of LGG consumption on the development of GI infections in the general population, studies on the treatment of GI infections, on diarrhoea during antibiotic use, or mechanistic studies, cannot be used as a source of data for the scientific substantiation of the health claim. The Panel concludes that a cause and effect relationship has not been established between the consumption of LGG and maintenance of defence against pathogenic gastrointestinal microorganisms.

KEY WORDS

Lactobacillus rhamnosus GG, gastrointestinal infections, pathogens, acute diarrhea, health claims

[1] On request from the Competent Authority of Finland following an application by Valio Ltd., Question No EFSA-Q-2010-01028, adopted on 13 May 2011.

[2] Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. One member of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: nda@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Løvik, Ambroise Martin, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Inge Tetens, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

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SUMMARY

Following an application from Valio Ltd., submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG, ATCC 53103, and maintenance of defence against pathogenic gastrointestinal microorganisms.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.

The Panel considers that the food constituent, *Lactobacillus rhamnosus* GG (LGG), which is the subject of the health claim, is sufficiently characterised.

The claimed effect is "helps to maintain defence against intestinal pathogens". The target population proposed by the applicant is the general population (adults and children). The Panel considers that the health claim refers to the defence against intestinal pathogens in the general population without GI infections and does not include the treatment of gastrointestinal (GI) infections. The presence of pathogenic microorganisms in the GI tract may lead to the development of GI infections. Maintenance of the defence against pathogenic GI microorganisms may protect against the development of GI infections. The Panel considers that maintenance of defence against pathogenic GI microorganisms is a beneficial physiological effect.

The applicant submitted a total of 45 human studies and 41 non-human studies for the scientific substantiation of the health claim.

Out of the five human intervention studies which investigated the effect of LGG consumption on the development of GI infections from which conclusions could be drawn for the scientific substantiation of the health claim, one did not show an effect of LGG consumption on the incidence of Traveller's diarrhoea, one did not show an effect on the incidence or duration of GI infections in free-living children, and two out of three did not show an effect on the incidence or duration of GI infections in hospitalised children. The Panel notes that the evidence provided does not establish that consumption of LGG has an effect on the development of GI infections.

Two human intervention studies did not show an effect of LGG consumption on the stimulation of protective immune responses after oral (viral) vaccination.

A number of human intervention studies on the effect of LGG consumption on the duration and/or severity of diarrhoea in children with acute diarrhoea due to GI infections, as well as two meta-analyses and one consensus opinion including studies on the treatment of acute diarrhoea were provided. The Panel considers that the evidence provided by the applicant does not establish that results from studies using LGG as coadjutant in the treatment of GI infections provide information about the effect of LGG on the development of GI infections, that results obtained in young children provide information about the adult population, or that results obtained on the treatment of GI infections caused by viruses provide information about other types of GI infections.

Six studies in healthy adults and children under antibiotic treatment and two meta-analyses which included studies on the prevention of antibiotic-induced diarrhoea were presented. The Panel notes that these studies did not provide adequate information about the aetiology of diarrhoeal episodes, and that antibiotic treatment may induce diarrhoea by mechanisms unrelated to GI infections.

One study on the effects of LGG on vancomycin resistant enterococci (VRE) carrier rate in VRE-positive patients under multiple antibiotic treatments in a renal ward from which no conclusions could be drawn for the scientific substantiation of the claims and several human and non-human studies

which investigated the mechanisms by which LGG could exert the claimed effect were also provided. The Panel considers that, in the absence of evidence for an effect of LGG consumption on the development of GI infections in the general population that is the target of the health claim, these studies cannot be used as a source of data for the scientific substantiation of the claim because their results cannot predict the occurrence of an effect of LGG on the development of GI infections *in vivo* in humans.

In weighing the evidence, the Panel took into account that only one out of five human intervention studies showed an effect of LGG consumption on the development of GI infections, and that two human intervention studies did not show an effect of LGG consumption on the stimulation of protective immune responses after oral (viral) vaccination.

On the basis of the data presented, the Panel concludes that a cause and effect relationship has not been established between the consumption of LGG and maintenance of defence against pathogenic gastrointestinal microorganisms.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health), which are based on newly developed scientific evidence or include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Art 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

- The application was received on 05/08/2010.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence.
- The scientific evaluation procedure started on 10/09/2010.
- On 27/12/2010 and on 06/04/2011, the NDA Panel agreed on a list of questions which requested the applicant to provide additional particulars to accompany the application by 11/01/2011 and by 26/04/2011, respectively.
- The applicant submitted the responses to the NDA Panel's list of questions on 18/01/2011 and on 26/04/2011.
- During the meeting on 13/05/2011, the NDA Panel, after having evaluated the overall data submitted, adopted an opinion on the scientific substantiation of a health claim related to *Lactobacillus rhamnosus* GG (ATCC 53103) and maintenance of defence against pathogenic gastrointestinal microorganisms.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: *Lactobacillus rhamnosus* GG (ATCC 53103) and maintenance of defence against pathogenic gastrointestinal microorganisms.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of *Lactobacillus rhamnosus* GG (ATCC 53103), a positive assessment of its safety, nor a decision on whether *Lactobacillus rhamnosus* GG (ATCC 53103) is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

[4] European Parliament and Council (2006). Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Official Journal of the European Union OJ L 404, 30.12.2006. Corrigendum OJ L 12, 18.1.2007, p. 3–18.

It should also be highlighted that the scope, the proposed wording of the claim and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address: Valio Ltd., P.O.Box 30, 00039 VALIO, Finland.

The application does not include a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

Lactobacillus GG (*Lactobacillus rhamnosus* GG, ATCC53103)

Health relationship as claimed by the applicant

According to the applicant, consumption of probiotic *Lactobacillus* GG helps to maintain defence against intestinal pathogens, as demonstrated in reduced occurrence of intestinal infections following consumption of *Lactobacillus* GG in children and in adults. The applicant also states that mechanisms of action are multifunctional, e.g. immunological (enhanced specific and unspecific antibody formation and improved immune response), and improved mucosal barrier function, as demonstrated by eradication of pathogenic bacteria in a challenge model and with non-human data.

Wording of the health claim as proposed by the applicant

"Lactobacillus GG helps to maintain defence against intestinal pathogens".

Specific conditions of use as proposed by the applicant

For the general population, no restrictions of use. An effective daily dose of *Lactobacillus* GG of $>10^9$ CFU to be consumed in e.g., a glass of milk/fermented milk, juice, kefir, fruit- or whey-based drink or berry soup, or a cup of yoghurt or quark cream, 1-2 capsules or 5 drops of oil suspension.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is *Lactobacillus rhamnosus* GG (LGG).

The strain *L. rhamnosus* GG has been identified and characterised at species and strain level identity using both phenotypic and genotypic methods (Tynkkynen et al., 1999). The Panel notes that culture collection numbers from the American Type Culture Collection (ATCC53103) and from Belgian Co-ordinated Collections of Microorganisms (LMG 18243) are given. The genome sequence of LGG has been published (Kankainen et al., 2009).

The Panel considers that the food constituent, *L. rhamnosus* GG, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "helps to maintain defence against intestinal pathogens". The target population proposed by the applicant is the general population (adults and children).

The Panel considers that the health claim refers to the defence against gastrointestinal (GI) pathogens in the general population without GI infections and does not include the treatment of GI infections.

The presence of pathogenic micro-organisms in the GI tract (e.g., viruses, bacteria) may lead to the development of GI infections. Maintenance of defence against pathogenic GI microorganisms may protect against the development of GI infections.

The Panel considers that maintenance of defence against pathogenic gastrointestinal microorganisms is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search on February 22, 2010 which was updated on April 21, 2010 through PubMed to identify human intervention studies on *Lactobacillus* GG concerning intestinal infections with adequately diagnosed diarrhoea and antibiotic-associated diarrhoea in generally healthy subjects (including free-living asymptomatic *Helicobacter pylori* carriers and children with mainly respiratory infections), or investigating antibody formation and eradication of vancomycin-resistant enterococci. A total of 81 references were identified, 68 of which were considered pertinent to the application by the applicant. In a response to EFSA's request for additional information, the applicant submitted on 17/01/2011 a revised summary of all pertinent studies identified. The summary included 45 human studies and 41 non-human studies.

3.1. Development of gastrointestinal infections

Seven human intervention studies addressing the effects of LGG consumption on the incidence/severity/duration/ of acute diarrhoea in subjects without diarrhoea at recruitment were provided. Two studies related to Traveller's diarrhoea and were conducted in healthy adult subjects (Hilton et al., 1997; Oksanen et al., 1990), two studies were conducted in free-living children ((Hojsak et al., 2009; Oberhelman et al., 1999) and three studies were on children hospitalised for reasons other than gastro-intestinal infections (Hojsak et al., 2010; Mastretta et al., 2002; Szajewska et al., 2001).

Studies on Traveller's diarrhoea (adults)

In a randomised, double-blind, placebo-controlled (DBPC) intervention study, Hilton et al. (1997) studied the occurrence of Traveller's diarrhoea in 400 (245 completers) US subjects aged 17-80 years (mean age 50 years) travelling to 14 geographical destinations in Africa, India, Central and South America for periods of one to three weeks. Participants received $2x10^9$ CFU LGG in capsules or identical placebo capsules (ethyl cellulose powder) once daily starting two days prior to departure and for the whole duration of the trip (i.e., for 9 to 23 days). Subjects were given a diary card to record diarrhoea episodes and were interviewed after return from the trip. If at least one diarrhoea episode was reported, then subjects were asked about the number of stools per day, duration of diarrhoea and associated symptoms, and medications taken. The Panel notes that medication use, including use of antibiotics and of "anti-diarrheal" mediations, was not reported. Diarrhoea was defined as three or more loose stools/day in subjects whose normal number of bowel movements/day was two or less. Compliance was defined as taking the study products as prescribed. Compliance with dietary measures (drink exclusively bottled or boiled water, avoid fresh fruits and vegetables) advised before the trip was not assessed. Days on which subjects failed to take the study products were excluded from analysis. A total of 245 subjects (n=126 in the LGG group) and 2743 travel days (defined as days in which subjects took the study products both that day and the previous day while not taking "anti-diarrheal" products that day) were included in the statistical analysis (completers only). Power calculations were not performed. The dependent variable for analysis was the proportion of days that a traveller had diarrhoea.

The Panel notes that this study has significant methodological limitations (e.g., high drop out rate; statistics performed in the population of completers only; no data on symptoms accompanying diarrhoeal episodes were reported). The Panel also notes that the information provided does not allow excluding the possibility that the diarrhoeal episodes were due to causes other than GI infections (antibiotic use not reported) or that differences between LGG and placebo groups were due to factors

other than the study products (e.g., different exposure to hazardous food and drink). The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

In a randomised, DBPC intervention study, Oksanen et al. (1990) assessed the occurrence of Traveller's diarrhoea in 820 eligible Finnish travellers (756 completers) aged 10 to 80 years (mean age 43.8 years) to two destinations in Turkey (Mamaris and Alaya) for periods of one or two weeks. Participants received $2x10^9$ CFU of LGG/day in sachets or identical placebo sachets (ethyl cellulose powder) once daily starting two days prior to departure and for the whole duration of the trip (i.e., for 9 to 16 days). Participants received no living, eating or drinking restrictions. A physician was available during the trip to register the cases of diarrhoea. During the return flight participants completed a questionnaire to record incidence of diarrhoea and related symptoms. Diarrhoea was defined as three or more unformed stools per day for periods lasting more than 24 hours or 1-2 unformed watery stools in less than 24 hours. A total of 756 subjects (n=127 in the LGG group) were included in the statistical analysis (completers only). A total of 497 subjects were on a one-week trip (147 to Alanya, n=71 in the LGG and n=76 in the placebo groups, 350 to Mamaris, n=175 on LGG and placebo groups), and of these, 259 continued the trip for an additional week (81 to Alanya, n=40 in the LGG and n=41 in the placebo group, 178 to Mamaris, n=87 on LGG and n=91 in the placebo group). Power calculations were not performed. Upon a request from EFSA, the applicant stated that power calculations leading to a sample size of 820 subjects needed were performed on the basis of several assumptions about protection rate, but no further details were provided. The total number of subjects with diarrhoea during the trip was 331 (out of 756) of whom 153 were in the LGG group and 178 were in the placebo group (p=0.065, RR=0.88, 95 % CI: 0.75-1.04). Upon a request from EFSA, the applicant stated that stratified analyses were conducted by travel destination and travel duration, as these were variables affecting the outcome measure. Of the total number of diarrhoeal cases, 189 (57.1 %) were reported during the first week and 142 (42.9 %) during the second week. The number of cases of diarrhoea in travellers going to Marmaris was 74 in the placebo group and 68 in the LGG (p=0.51) during the first week. In those staying two weeks, 47 cases were recorded in the placebo group and 49 in the LGG (p=0.53). The number of cases of diarrhoea in travellers going to Alanya was 30 in the placebo group and 17 in the LGG (p=0.04) during the first week. In those staying two weeks, 47 cases were recorded in the placebo group and 49 in the LGG (p=0.10).

The Panel notes that no effect of LGG on the prevention of Traveller's diarrhoea was observed when all subjects were considered together, that a significant effect of LGG on the incidence of diarrhoea compared to placebo was only observed in travellers going to the destination with the smallest sample size and only during the first week, and that although the authors speculate that distinct microbial environments in the two locations could have explained the differences observed, no evidence for this contention has been provided. The Panel considers that this study does not show an effect of LGG consumption on the incidence of Traveller's diarrhoea.

Studies in free-living children

In a randomised, DBPC intervention study, Oberhelman et al. (1999) investigated the effects of consuming LGG at a dose of $3.7x10^{10}$ CFU (n=99) in flavoured gelatine once daily 6 times per week for 15 months versus placebo (same gelatine without LGG, n=105) on the incidence of diarrhoea in infants and young children (age range 6 to 24 months) with first degree malnutrition from an indigent peri-urban Peruvian town. Only one child per household was recruited and participants who dropped out were replaced. It is unclear from the publication why the initial cohort consisted of 160 children but results are provided for a total of 204 children. Diarrhoea episodes were defined as at least one day with at least four liquid stools for children within the age group included in the protocol, with at least two days free of symptoms separating distinct episodes. Diarrhoea episodes were identified by the investigators during daily home visits based on standardised questionnaires. The primary outcome was incidence of diarrhoea, but power calculations were not reported. Outcome variables were

analysed for the whole sample and for subgroups stratified by age, breast milk intake, and nutrition status in children with at least one month of follow up. It is unclear from the publication whether these post-hoc comparisons were pre-planned and no justification is provided for them.

The Panel notes that the number of drop outs and how these were replaced to reach a total of 204 children from the 160 initially recruited and randomised is unclear from the publication and this omission limits the conclusions which can be drawn from the study with respect to randomisation, blinding and comparability of the LGG and placebo groups. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim due to its methodological limitations.

In a randomised, DBPC intervention study, Hojsak et al. (2009) investigated the effect of consuming 100 ml of a fermented milk product containing LGG at a dose of 10^9 CFU (n=139) once daily for three months versus placebo (same fermented milk product without LGG, n=142) in 281 Croatian children (mean age 52 months, age range 13-86 months) who attended day care centres. Primary outcomes were number of children with physician-diagnosed GI infections defined as diarrhoea with three or more loose or watery stools within 24 hours and/or vomiting and number of children with physician-diagnosed respiratory tract infections. It was calculated that 133 subjects per group were needed to observe a 20 % difference between intervention and placebo groups with a power of 80 % and an α=0.05. Intention to treat (ITT) analyses were performed. Compared with the placebo group, children in the LGG group had no significant reduction in the risk of GI infections (RR=0.63, 95 % CI: 0.38-1.06), vomiting episodes (RR=0.60, 95 % CI: 0.29-1.24) or diarrhoeal episodes (RR=0.63, 95 % CI: 0.35-1.11), nor in the number of days with GI symptoms (p=0.063).

The Panel notes that microbiological confirmation of the infectious nature of the vomiting and diarrhoea episodes was not obtained. However, the Panel considers that GI infections clinically diagnosed by the primary care physician following well defined criteria can be used as an appropriate outcome measure for the scientific substantiation of the claim, provided that adequate exclusion criteria for the most common non-infectious causes of diarrhoea have been applied, as was done in this study. The Panel considers that this study does not show an effect of LGG on the incidence or duration of GI infections in free-living children.

Studies in hospitalised children (nosocomial infections)

In a randomised, DBPC intervention study, Mastretta et al. (2002) investigated the effects of consuming LGG in one capsule daily (dissolved in water prior to administration) at a dose of 10^{10} CFU (n=134) during the entire hospital stay versus placebo (same capsule without LGG, n=135) in Italian children aged one to 18 months who were hospitalised for reasons other than infections for at least 48 hours in a study period of six months. Subjects who developed diarrhoea within 24 hours of admission, had a history of gastroenteritis in the previous two weeks or immunodeficiency, or were readmitted to hospital within 72 hours of discharge, were excluded. The aim of the study was to assess the effectiveness of LGG and breast-feeding in the prevention of nosocomial rotavirus infections. Primary and secondary outcomes were not identified in the study and power calculations were not reported. Nosocomial diarrhoea was defined as three or more loose stools per day at least 24 hours after admission. Rotavirus was tested for in faecal specimens from each child collected at admission, on every diarrhoea episode (during hospitalisation and up to three days after discharge), at discharge, and 72 hours afterward in every patient who did not develop nosocomial diarrhoea. A total of 220 subjects were included in data analysis (completers only, 114 in the LGG and 106 in the placebo group). Reasons for exclusion were asymptomatic rotavirus carrier (n=20), voluntary interruption of hospital stay (n=22) and no show after discharge (n=7). No significant differences between the LGG and placebo groups were reported with respect to the incidence of symptomatic or asymptomatic nosocomial rotavirus infections either during hospitalisation or after discharge in the total sample of children, in breastfed or in non-breast fed infants, or on the number of days of hospitalisation.

The Panel notes that this study does not show an effect of LGG consumption on the incidence of GI rotavirus infection in hospitalised children.

In a randomised, DBPC intervention study, Szajewska et al. (2001) investigated the effects of consuming LGG at a dose of $6x10^9$ CFU (n=45) in sachets (reconstituted in water) once daily during the entire hospital stay versus placebo (same sachets without LGG, n=36) in 81 Polish children of one to 36 months of age who were hospitalised for reasons other than diarrhoea. Primary outcome was incidence of diarrhoea defined as three or more loose or watery stools within 24 hours. Secondary outcomes were age of children with diarrhoea, onset time of diarrhoea after admission, duration of diarrhoea, number of watery stools per 24 hours in children with diarrhoea. Rotavirus infection was diagnosed when rotavirus antigen was detected in a stool specimen. Rotavirus gastroenteritis was diagnosed when the rotavirus antigen was detected in the stool specimen of a child who presented with acute gastroenteritis. The Panel notes that asymptomatic rotavirus carriers were not excluded from the analysis. Patients were evaluated for stool number and consistency in the hospital and parents were advised to contact the hospital physicians if loose or watery stools occurred within three days of hospital discharge. Stool samples were collected weekly and during each diarrhoea episode for bacteria and rotavirus antigen. The Panel notes that power calculations were not reported and that the reasons for an unequal number of subjects being randomised to the LGG group compared to the placebo group are unclear. The information provided by the applicant on these two points upon EFSA's request was insufficient. Main reasons for hospitalisation were ear, nose and throat disorders, respiratory, and urinary tract infections. The authors acknowledge that antibiotic use was not reported nor taken into account in data analysis, so that it was not possible to distinguish between diarrhoeal episodes of infectious *vs*. non-infectious origin. The Panel considers that the incidence of diarrhoeal episodes in this study cannot be used as a surrogate marker for GI infections (see also section 3.4) and considers that no conclusions can be drawn from this outcome measure for the scientific substantiation of the claim. The incidence of rotavirus infection was not significantly different between the LGG (n=9, 20 %) and the placebo (n=10, 27.8 %) groups. No significant differences between the LGG and placebo groups were noted for any of the secondary outcomes tested.

The Panel notes that this study does not show an effect of LGG consumption on the incidence of GI (rotavirus) infection of hospitalised children.

In a randomised, DBPC intervention study, Hojsak et al. (2010) investigated the effects of consuming 100 ml of a fermented milk product containing LGG at a dose of 10^9 CFU (n=376) once daily during the entire hospital stay versus placebo (same pasteurised fermented milk product without LGG, n=366) in Croatian children older than 12 months (mean age 10±5 years) who were hospitalised for reasons other than infections for at least three days. Primary outcomes were physician-diagnosed (nosocomial) GI infections, defined as diarrhoea with three or more loose or watery stools within 24 hours and/or vomiting, and number of children with physician-diagnosed respiratory tract (nosocomial) infections. Secondary outcomes were number of vomiting episodes, number of diarrheal episodes, number of GI infections which lasted for more than two days, number of respiratory tract infections which lasted for more than three days, and duration of hospitalisation. All infections were diagnosed by a paediatrician on the basis of clinical signs and symptoms. Stools of subjects having a GI infection were tested for bacteria, rotavirus, adenovirus and norovirus. Seven days after discharge subjects were contacted to check for new diarrhoeal episodes which could have been incubated in the hospital, but no episodes were recorded after discharge. Antibiotic associated diarrhoea without a positive stool test was excluded from analyses. It was calculated that 242 subjects per group were needed to observed a 15 % difference between intervention and placebo groups with a power of 90 % and an α=0.05. The Panel notes that recruitment exceeded by 50 % the sample sized calculated.

Upon request of EFSA, the applicant stated that the recruitment period was established at the beginning of the study (6 months), that all eligible subjects were recruited during that period, and that no statistical analysis was undertaken before the end of the study.

Intention to treat (ITT) analyses were performed. There was no significant difference regarding the duration of hospitalisation between the LGG and placebo groups. The risk for GI infections was significantly reduced in the LGG group compared with the placebo group (19 in LGG and 44 in placebo; RR=0.40, 95 % CI: 0.25–0.70, number needed to treat (NNT) =15, 95 % CI: 9-36). In relation to secondary end points related to GI infections, children in the LGG group had a reduced risk for vomiting episodes (17 in LGG and 33 in placebo, RR=0.5, 95 % CI: 0.3–0.9), for diarrheal episodes (7 in LGG and 33 in placebo, RR=0.24, 95 % CI: 0.10–0.50), and reduced risk for episodes of GI infections which lasted more than two days (19 in LGG and 45 in placebo, RR=0.40, 95 % CI: 0.25–0.70) compared with the placebo group. None of the subjects had a bacterial infection. In five subjects, rotavirus (two patients in the placebo group) or norovirus (three patients, two in the placebo group and one in the LGG group) was isolated. All subjects were treated symptomatically and none required antibiotic treatment.

The Panel notes that only in five of the 40 cases of diarrhoea an infectious (viral) aetiology was ascertained. However, the Panel considers that GI infections clinically diagnosed by the hospital physician following well defined criteria can be used as an appropriate outcome measure for the scientific substantiation of the claim, provided that adequate exclusion criteria for the most common non-infectious causes of diarrhoea have been applied, as was done in this study. The Panel considers that this study shows an effect of LGG on the incidence and duration of (viral) GI infections in hospitalised children.

Summary of the studies on the development of gastrointestinal infections

The Panel notes that, out of the five human intervention studies which investigated the effect of LGG consumption on the development of GI infections from which conclusions could be drawn for the scientific substantiation of the health claim, one did not show an effect of LGG consumption on the incidence of Traveller's diarrhoea, one did not show an effect on the incidence or duration of GI infections in free-living children, and only one out of three showed an effect on the incidence or duration of GI infections in hospitalised children.

The Panel notes that the evidence provided does not establish that consumption of LGG has an effect on the development of GI infections.

3.2. Immune responses after oral (viral) vaccination

In a randomised, DBPC parallel, three-arm intervention study, de Vrese et al. (2005) investigated the effects of consuming LGG (n=21) or *Lactobacillus paracasei* subspecies *paracasei* (strain CRL431, n=21) at a dose of 10^{10} CFU daily inoculated in 100 g of an acidified milk product with the appearance of yogurt (i.e., in order to exclude any effect from living or inactivated yogurt bacteria (*Streptococcus thermophilus* and *Lactobacillus delbrückii* subspecies *bulgaricus* on the immune system) versus placebo (same acidified milk product without inoculation of bacteria, n=22) on the immune response to oral polio vaccine types 1-3 in 64 male volunteers aged 20–30 years. Volunteers consumed the acidified milk products for 5 weeks and were vaccinated orally against polio on day 8 with commercially available live attenuated poliomyelitis viruses of type 1 strain LSc1, type 2 strain P2712, and type 3 strain Leon 12a1b at dosages guaranteed by the manufacturer to provide immune protection (NT>1:4) in 95 % of vaccinated subjects. Blood samples were taken for antibody determinations 4 weeks before, immediately before, and 2, 4 and 7 weeks after vaccination. Poliovirus serotype 1, 2 and 3 neutralising antibody titres (NT) and poliovirus-specific IgG, IgM and IgA were assessed. Poliovirus binding-inhibition test was performed in addition to the conventional poliovirus neutralisation test to detect and quantify IgG antibodies to poliovirus serotypes 1, 2 and 3, which are predominantly exerting the neutralising effect of serum. The primary outcome was subjects developing immune protection after vaccination. Power calculations were not reported and adjustment for multiple testing was not performed. Efficacy of vaccination was determined by a chi-square analysis of the number of subjects with protective neutralising antibody titres before and after

vaccination, whereby Log2 titres ≥ 3 on sampling days 1 and 2 (four weeks and immediately before vaccination) and on sampling day 5 (four weeks after vaccination) were considered protective. Vaccination significantly increased the percentage of subjects whose neutralising antibody titres indicated protection against polio, but the change was not significantly different between groups. NT significantly increased in the LGG group compared to placebo for poliovirus serotypes 1 (p=0.048) and 2 (p=0.014), but not for serotype 3. Specific IgA titres significantly increased in the LGG group compared to placebo for serotype 1, whereas no significant differences were observed between groups on IgA for serotypes 2 and 3 or on IgM and IgG for any serotype.

The Panel notes that no significant differences were observed in the primary outcome of the study (proportion of protected persons after vaccination), and that the large number of secondary comparisons performed was not adjusted for multiple testing, so that chance findings (e.g., borderline differences in NT) cannot be excluded. The Panel considers that this study does not show an effect of LGG consumption on immune responses after oral (viral) vaccination.

In a randomised, DBPC parallel, three-arm intervention study, Isolauri et al. (1995) investigated the effects of consuming LGG (n=29) at doses $5x10^{10}$ twice daily as freeze-dried powder mixed with water versus placebo (same microcrystalline cellulose without LGG, n=28) on the immune response to oral vaccination with Rhesus-human reassortant rotavirus vaccine strain D x RRV, corresponding to human rotavirus VP7 serotype 1, in full-term infants 2-5 months of age (mean 4.1 months). Before vaccination, the infants were fed with 30 ml of soy milk mixed with 5 ml of 7.5 % sodium bicarbonate solution to provide a buffer against gastric acidity. Thereafter, the first dose of LGG or placebo was given in 5 ml of water and twice daily in the five following days. The Panel notes that the dose of LGG used in this study is about 100 times higher than proposed in the CoU and that the administration of sodium bicarbonate before vaccination could have affected the number of live LGG during the GI passage. Blood was collected before and 30 days after vaccination. In 14 subjects per group, blood was also collected at day 8 after vaccination for specific rotavirus antibody-secreting cells (sASC) determination using ELISPOT. Primary and secondary outcomes were not stated. No significant differences in seroconversion rates or specific IgA, IgM or IgG concentrations were observed between groups. The mean number of IgM sASC against rotavirus increased significantly in the LGG group compared to placebo (F=5.78, p=0.02, ANOVA for repeated measurements), whereas no differences were observed on IgA sASC responses between groups.

The Panel notes that no significant differences in seroconversion rates or specific IgA, IgM or IgG concentrations were reported between the LGG and placebo groups, and that the large number of secondary comparisons performed in the study was not adjusted for multiple testing, so that chance findings (e.g., differences in IgM sASC against rotavirus) cannot be excluded. The Panel considers that this study does not show an effect of LGG consumption on immune responses after oral (viral) vaccination.

The Panel notes that the two human intervention studies provided did not show an effect of LGG consumption on the stimulation of protective immune responses after oral (viral) vaccination.

3.3. Treatment of gastrointestinal infections

A number of human intervention studies on the effect of LGG consumption on the duration and/or severity of diarrhoea in children with acute diarrhoea due to GI (mostly viral) infections were provided (Basu et al., 2007a, 2007b; Basu et al., 2009; Canani et al., 2007; Costa-Ribeiro et al., 2003; Guandalini et al., 2000; Guarino et al., 1997; Isolauri et al., 1994; Jasinski et al., 2002; Kaila et al., 1992; Kaila et al., 1995; Majamaa et al., 1995; Misra et al., 2009; Raza et al., 1995; Ritchie et al., 2010; Salazar-Lindo et al., 2004; Shornikova et al., 1997). Most of the studies were conducted in young children (1-36 months) and LGG was administered as coadjutant for the treatment of acute diarrhoea (e.g., in conjunction with oral or parenteral re-hydration) in a hospital setting. Also two meta-analyses of randomised controlled trials (Allen et al., 2010; Szajewska et al., 2007) and one

consensus opinion (Guarino et al., 2008) including studies on the effects of LGG on the treatment of acute diarrhoea were provided.

The Panel notes that the status of the GI tract in subjects with acute diarrhoea due to a GI infection may not be comparable to the status of the GI tract in subjects without a GI infection. The Panel also notes that the development and functions of the GI tract in young children is not comparable to those of the GI tract in adults. In addition, results obtained on the treatment of GI infections caused by viruses may not provide information about GI infections caused by e.g., bacteria, because the mechanisms of infections and the subject's response may differ.

The evidence provided by the applicant does not establish that results from studies using LGG as coadjutant in the treatment of GI infections provide information about the effect of LGG on the development of GI infections, that results obtained in young children provide information about the adult population, or that results obtained on the treatment of GI infections caused by viruses provide information about other type of GI infections.

The Panel considers that, in the absence of evidence for an effect of LGG consumption on the development of GI infections in the general population that is the target of the health claim (see section 3.1), studies on the treatment of GI infections (or meta-analyses/consensus opinions based on these studies) cannot be used as a source of data for the scientific substantiation of the claim.

3.4. Diarrhoea during antibiotic use

Four studies in healthy adults under antibiotic treatment for the eradication of *H. pylori* (2001a; Armuzzi et al., 2001b; Cremonini et al., 2002) or under antibiotic treatment for other reasons (Siitonen et al., 1990), and two studies in children (Arvola et al., 1999; Vanderhoof et al., 1999) under antibiotic treatment for acute infections of the respiratory tract, urinary tract or soft tissues on the effects of LGG in preventing antibiotic-induced diarrhoea were presented. Also two meta-analyses of randomised controlled trials which included studies on the effects of LGG in preventing antibiotic-induced diarrhoea were presented (Sazawal et al., 2006; Szajewska et al., 2006). The Panel notes that these studies did not provide adequate information about the aetiology of diarrhoeal episodes, and that antibiotic treatment may induce diarrhoea by mechanisms unrelated to GI infections.

The Panel considers that studies on diarrhoea during antibiotic use (or meta-analyses including these studies) cannot be used as a source of data for the scientific substantiation of the claim.

3.5. Eradication of vancomycin resistant enterococci

One randomised, controlled intervention study on the effects of LGG on vancomycin resistant enterococci (VRE) carrier rate in VRE-positive patients under multiple antibiotic treatments in a renal ward was provided (Manley et al., 2007). The Panel notes that the dose of LGG used in the study was not reported, that the VRE strains found in faecal specimens were not reported and, therefore, it is not possible to ascertain whether these micro-organisms were pathogenic, that no statistical analyses were provided and that more subjects in the LGG group compared to the placebo group were under antibiotic therapy. The Panel considers that no conclusions can be drawn from this study for the scientific substantiation of the claim.

3.6. Studies on the mechanisms by which LGG could exert the claimed effect

Three intervention studies which investigated the effects of LGG consumption on markers of acute immune response to rotavirus infection in children were provided (Kaila et al., 1992; 1995; Majamaa et al., 1995). These studies have also been considered in section 3.3. The Panel notes that none of these studies assessed the effect of LGG on rotavirus re-infection and that no information was

provided on the kinetics of specific immune responses to rotavirus or on the levels of specific antibodies required for protection against rotavirus. Three human intervention studies in adults investigated the effects of LGG on markers of inflammation and gene expression (Di Caro et al., 2005; Kekkonen et al., 2008; Schultz et al., 2003), one intervention study in infants assessed the effects of LGG consumption on non-specific IgM, IgA and IgG secreting cells (Rinne et al., 2005), and 11 human intervention studies investigated the survival of LGG in the human GI tract consumed in different matrices. In addition, 41 animal and *in vitro* studies on the adhesion properties of LGG to the intestinal wall, on the inhibition of pathogen adhesion to the intestinal wall, on the antibacterial and antiviral activity of LGG, on the effects of LGG on intestinal mucosa integrity, proliferation, and repair, and on the effects of LGG on *inter alia* cytokine and antibody production, were provided.

The Panel considers that, in the absence of evidence for an effect of LGG consumption on the development of GI infections in the general population which is the target of the health claim (see section 3.1), these studies cannot be used as a source of data for the scientific substantiation of the claim as their results cannot predict the occurrence of an effect of LGG on the development of GI infections *in vivo* in humans.

In weighing the evidence, the Panel took into account that only one out of five human intervention studies showed an effect of LGG consumption on the development of GI infections, and that two human intervention studies did not show an effect of LGG consumption on the stimulation of protective immune responses after oral (viral) vaccination.

The Panel concludes that a cause and effect relationship has not been established between the consumption of LGG and maintenance of defence against pathogenic gastrointestinal microorganisms.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food constituent, *Lactobacillus rhamnosus* GG (LGG), which is the subject of the health claim, is sufficiently characterised.
- The claimed effect is "helps to maintain defence against intestinal pathogens". The health claim refers to the defence against intestinal pathogens in the general population without GI infections and does not include the treatment of GI infections. The target population proposed by the applicant is the general population (adults and children). Maintenance of defence against pathogenic gastrointestinal microorganisms is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of LGG and maintenance of defence against pathogenic gastrointestinal microorganisms.

DOCUMENTATION PROVIDED TO EFSA

Health claim application on *Lactobacillus rhamnosus GG* and maintenance of defence against pathogenic gastrointestinal microorganisms pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 000288_FI). September 2010. Submitted by Valio Ltd.

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GLOSSARY / ABBREVIATIONS

CFU	Colony forming units
DBPC	Double blind placebo controlled
GI	Gastrointestinal
ITT	Intention to treat
LGG	*Lactobacillus rhamnosus* GG
NNT	Number needed to treat
NT	Neutralising antibody titres
sASC	Specific antibody-secreting cells
VRE	Vancomycin resistant enterococci