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20<sup>th</sup> June, 2011

Dear Mr. Mathioudakis,

**Re: 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. (Claim serial No: 000288\_FI)**

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I am writing with regards to the above mentioned published Scientific Opinion by the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) which was published in EFSA Journal 2011;9(6):2167.

I write in my capacity as a clinician and a paediatrician who is most concerned about the negative opinion of the Panel and about the way the scientific and clinical evidence on Lactobacillus rhamnosus GG (L.GG) was evaluated by the Panel in reaching such an opinion.

My decision to write to you is motivated by my earnest desire to inform you that the Panel's decision to look away from the scientific and clinical evidence which supports this particular probiotic strain will gravely sabotage not only scientific advancement in research in L.GG but also set back further pursuit of non-pharmacological therapeutic alternatives in medicine.

The need for non-pharmacological therapeutic options is well-recognised by the medical community. Advancement in research in bacteria as therapeutic adjunct is welcomed by the medical profession and highlighted in a 2001 British Medical Journal editorial entitled, "Bacteriotherapy: the time has come. Bacterial interference is an increasingly attractive approach to prevention and therapy."<sup>1</sup>

Lactobacillus rhamnosus GG (L.GG) is the most researched and documented probiotic strain. It is a reference strain for other probiotic strains and its research has been replicated by other probiotic strains. The role of L.GG in the defence against gastrointestinal pathogens is its most fundamental health benefit and has been established by scientific research repeatedly.

The Panel's opinion that L.GG's numerous studies could not establish a cause and effect relationship between its consumption and the "maintenance of defence against pathogenic gastrointestinal microorganisms" implies that the research efforts of the scientific community has been misdirected, and the medical community has been misinformed these past quarter of a century.



While I appreciate that the Panel carries a heavy burden in ensuring that their evaluation is thorough for the sake of the safety of the general public, they should also ensure that their evaluation is fair to the food constituent under study, ie, L.GG, and is fair to the scientists and researchers who provided the scientific evidence.

Most of all, and I believe you will agree, this whole process of evaluation and the giving of a scientific opinion should be governed by impartiality and common sense.

In the light of far-reaching consequences resulting from the Panel's scientific opinion, it would be irresponsible of me, as a clinician, not to highlight the following observations to you and the EFSA.

## **EFSA PANEL'S OPINION**

### **A. Studies on the mechanism by which L.GG could maintain defence against pathogenic GI microorganisms.**

None of the published studies submitted were accepted by the Panel as evidence for an effect of L.GG consumption in maintaining defence against pathogenic GI microorganisms.

The Panel held the opinion that there was no evidence for an effect on L.GG consumption on the development of GI infections in the general population. Based on this opinion the Panel stated that these studies could not be used as a source of data for the scientific substantiation of the claim as their results could not predict the occurrence of an effect of L.GG on the development of GI infections in vivo in humans.

### **B. Human Intervention Studies**

The Panel evaluated 7 human intervention studies addressing the effect of L.GG consumption on the incidence/severity/duration of acute diarrhoea in subjects without diarrhoea at recruitment. Of the seven studies, 2 were considered as unsuitable to draw conclusions from (Hilton et al. 1997, Oberhelman et al. 1999,), 4 were evaluated as showing that L.GG had no effect (Oksanen et al. 1990, Hojsak et al. 2009, Mastretta et al. 2002, Szajewska et al. 2001) and 1 was evaluated and found to have an effect (Hojsak et al. 2010).

### **C. Immune responses after oral (viral) vaccination**

The Panel evaluated two studies on immune responses after oral (viral) vaccination. (De Vrese et al. 2005, Isolauri et al. 1995)

The Panel's conclusion was that both studies did not show an effect on L.GG consumption on immune responses after oral (viral) vaccination.

The final conclusion was that since only 1 out of the 5 human intervention studies accepted by the Panel was positive and that both the immune response studies were negative, the Panel was of the opinion that the evidence provided did not establish that consumption of L.GG had an effect on the defence against pathogenic GI microorganisms.

## COMMENTS ON THE PROCESS OF EVALUATION

### The Panel decided which study to include and which to discard

The Panel appeared to conduct its evaluation by first taking the clinical trials on the prevention of disease states (such as viral gastroenteritis and traveller's diarrhea) and deciding which ones were good enough to be evaluated and which should not be considered. Having chosen the clinical trials which it felt were of the appropriate quality to be evaluated, it then came up with a calculation of how many of the studies they had chosen were in their opinion, "positive" and how many were, in their opinion, "negative". Based wholly on their viewpoint and after simple arithmetic they came up with the opinion that 1 out of 5 plus 0 out of 2 equal "no effect". Based on this opinion, the Panel declared that all studies documenting the mechanisms by which L.GG could exert its claimed effect should not be evaluated.

The evaluation process in which the Panel picked and chose which study to include and which to discard was in itself a process which could not be free of bias and preconceived opinion.

Of the 7 human intervention studies analysed by the Panel, 1 positive study was accepted as positive (Hojsak et al. 2010), 1 negative study was accepted as negative (Hojsak et al. 2009), 2 positive studies were thrown out on the basis of weakness in methodology (Hilton et al. 1997, Oberhelman et al. 1999), 1 positive study was deemed to be negative (Szajewska et al. 2001), 1 study with negative and positive results were considered as negative although the authors admitted randomisation failure in the negative group (Oksanen et al. 1990), 1 negative study with possible weakness in study design was accepted as negative (Mastretta et al. 2002).

Of the 2 human intervention studies on the stimulation of protective immune responses to oral viral vaccination, both were found to be negative although the studies clearly demonstrated enhanced immune responses in the L.GG group. (de Vrese et al. 2005, Isolauri et al. 1995)

The process adopted by the Panel comes across as if the judges looked at the evidence first, then picked and chose which evidence to admit based on how reliable they deemed the evidence to be and finally pronounced their judgment based on the evidence they had chosen.

### Evaluation process in reverse

Contrary to established evaluation algorithms where evidence from *in vitro* and animal studies would first be accepted before clinical trials were conducted, the Panel's evaluation process appeared to work in reverse. It first decided whether enough number of clinical trials they had chosen to evaluate were "positive" before considering whether or not published scientific data on how the physiological effect could be exerted would either be evaluated or jettisoned.

The health relationship under evaluation was not that of prevention of disease states but the physiological effect of "maintenance of defence against pathogenic gastrointestinal microorganisms". Therefore scientific studies which documented how this beneficial physiological effect could be exerted at the molecular, cellular and genomic levels should be examined and evaluated, without preconceived determination about which human intervention studies would subsequently be chosen to be evaluated.

Even if just one human clinical study showed that there was evidence that L.GG could indeed help in maintaining defence against intestinal pathogens, this would constitute sufficient grounds for the Panel to evaluate the studies which demonstrated the mechanisms by which this claimed effect was exerted.

As long as there existed data supporting the claimed statement, the data should be included in the body of evidence supporting the claim.

Even if the claim was for the “prevention of a disease”, which was not the case, the Panel’s process of evaluation appeared to be contrary to how evaluation of treatment options were conducted by the medical community ie, evaluation of all *in vitro*, animal, human interventional studies and meta-analyses on the treatment option. Recommendation would then be scaled on the strength of all research documents, each judged on its own merits.

The recommended algorithm for the evaluation of probiotic products, emulating the systematic evaluation of pharmaceutical substances, has been published in the FAO/WHO Guidelines for the Evaluation of Probiotics in Food, 2002.<sup>2</sup> (Fig. 1)

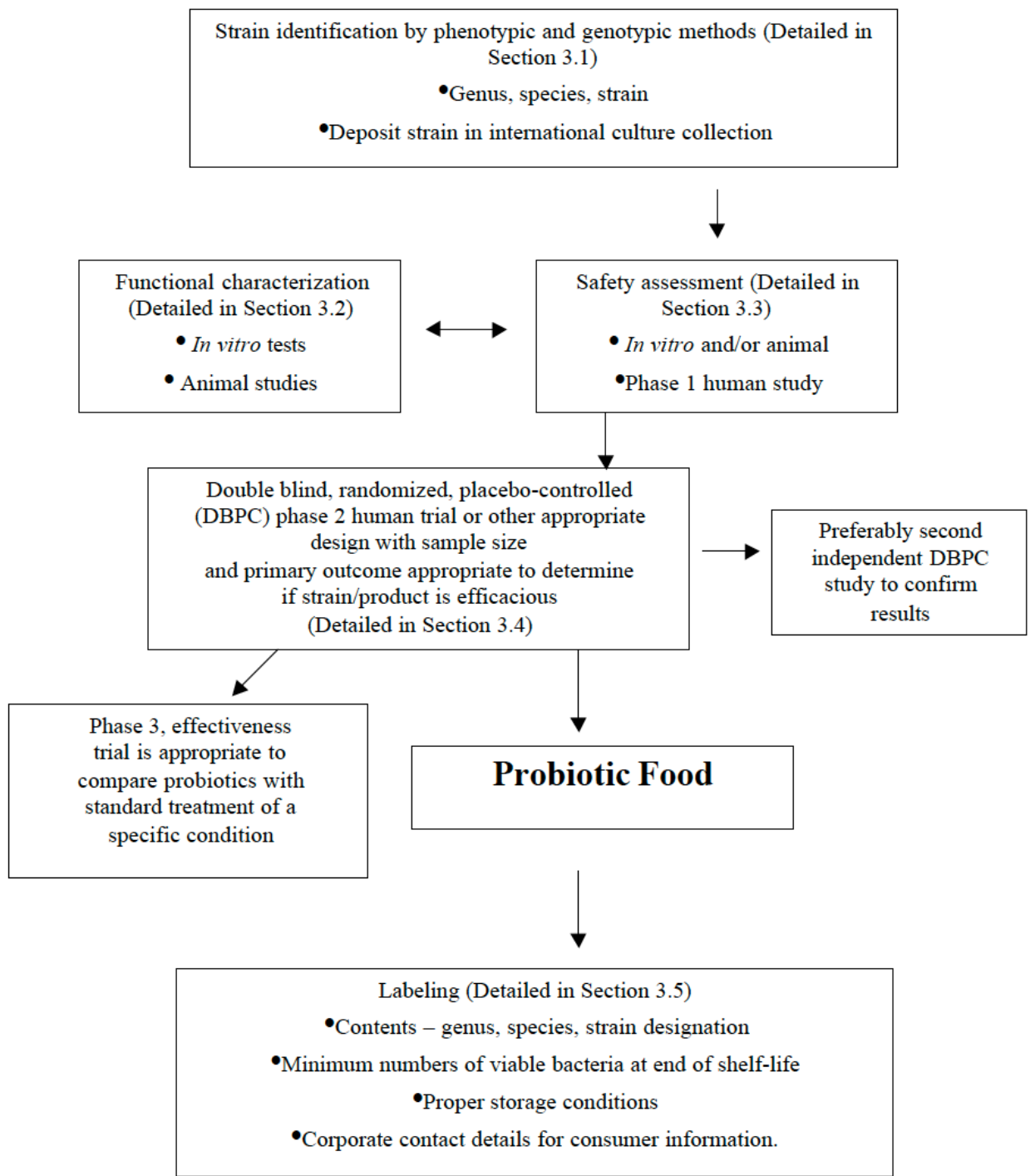


Fig. 1 FAO/WHO Guidelines for the Evaluation of Probiotics in Food, 2002

## COMMENTS ON THE SCIENTIFIC STUDIES

### A. Studies on the mechanism by which L.GG could maintain defence against pathogenic GI microorganisms

These studies were not human intervention studies but the data generated from these studies formed the basis for conducting the human intervention studies.

Therefore data from these scientific publications, which preceded human intervention studies, were important and valid scientific evidence in themselves and should not be disregarded, regardless of the results of subsequent human intervention studies.

Moreover, the claim in question was a beneficial physiological effect so scientific data which supported how this physiological effect came about should be assessed and acknowledged.

These studies documented L.GG's effect on four levels of defence against gut pathogens namely:-

1. its effect on gut mucosal integrity without, and in the presence of, gut pathogens,
  - maintaining intestinal mucosal barrier which could reduce pathogenic invasion<sup>3 4 5 6 7 8</sup>
  - enhancing mucosal regeneration which acts as a repair mechanism to ensure gut mucosal barrier integrity is maintained<sup>3 4</sup>
  - ameliorating pro-inflammatory damage to the intestinal barrier caused by pathogens<sup>9 10 11 12 13</sup>
2. its effect on preventing adherence and translocation of intestinal pathogens through the mucosal surface<sup>14 15 16 17 18 19 20 21 22</sup>
3. the production of antimicrobial substances against pathogenic microorganisms in the gut<sup>23 24 25 26 27 28 29 30 31 32</sup>
4. its effect on antibody response to gut pathogens or their attenuated surrogates, enteroviral vaccines.<sup>33 34 35</sup>

### B. Human Intervention Studies

Hilton et al. 1997

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The Panel's criticism of possible limitations in the study involved:

- High drop-out rate
- Statistics were performed in the "completers" only
- No data on symptoms accompanying diarrhoeal episodes were reported.

- High drop-out rate.

This was an intervention study. Only travellers who had completed the course of L.GG were considered to have undergone the intervention. Anyone who did not complete the intervention was rightly excluded from the statistical analysis. The “non completers” did not fulfill the inclusion criteria which was completion of the intervention. As long as the number of 245 travellers who fulfilled the criteria for inclusion into the statistical analysis had enough power to show a statistical difference, this study should be considered scientifically sound.

- The Panel raised its concern about what kind of diarrhoea the travellers suffered from and made a point about the possibility that antibiotics consumed by travellers could cause diarrhoea.

About 80% of travellers diarrhoea is due to bacterial enteropathogens with the rest due to viral or parasitic enteric pathogens.

It is not clinically indicated to prescribe antibiotics to travellers as prophylaxis against travellers' diarrhoea. Furthermore, the majority of travellers' diarrhoea should not be treated by antibiotics, except when the condition is severe. Likewise, raising the possibility that the use of antibiotics among travellers could confound the incidence of diarrhoea among the travellers is unfounded and should not be a reason for ignoring the positive findings of this study.

### **Oksanen et al. (1990)**

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This study showed marked difference in the reported efficacy of L.GG in preventing traveller's diarrhoea in 2 separate locations.

- One of the reasons acknowledged by the researchers for this discrepancy was that there was uneven distribution of ages of the travellers to Marmaris, with more older travellers randomised to the placebo group. They also stated that older travellers were less likely to succumb to traveller's diarrhoea. This mismatch of the ages in the Marmaris group could skew the results and mask the efficacy of L.GG in the Marmaris group.

In contrast, the Alanya group's randomisation was successfully achieved as it had even age distribution in the L.GG and placebo groups and the difference in incidence of diarrhoea in the first week of travel was found to be significant.

Since the Panel appeared to be deeply concerned about all aspects of methodology, it follows that information from the Marmaris group which was not properly randomised should be disregarded and the results of the Alanya group which was successfully randomised, should be accepted as significant. Unfortunately the Panel's opinion was the reverse.

- The difference in L.GG's effect reported in this study calls for information on the possibility that diarrhoeal episodes were due to other causes and factors other than L.GG's efficacy. In this study no data was provided on the possibility that the diarrhoeal episodes were due to causes other than GI infections (antibiotic use was not reported, like in Hilton et al's study) or that the differences between L.GG and the placebo groups were due to factors other than the study products.

Yet the Panel accepted this study for evaluation and ranked it as a “negative” study.

On the basis of the Panel's previous concern regarding the absence of such information in Hilton et. al's study, it can be argued that this study should either also be fully excluded from its consideration or the statistical significance of L.GG's effect in reducing the incidence of traveller's diarrhoea in the Alanya group be acknowledged.

### **Oberhelman et al. (1999)**

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The Panel's criticism of possible limitations in the study involved:

- The number of drop-outs and how these were replaced to reach a total of 204 children from the 160 initially recruited and randomised was unclear.

The incidence of diarrhoea in the L.GG group was significantly reduced in the non-breastfed toddler age group.

The fact that the authors did not indicate why more children were entered into the study than the number initially randomised may weaken the strength of the conclusions but should not be the basis for dismissing the whole clinical trial.

### **Hojsak et al. (2009)**

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This study did not show any significant difference in the incidence of gastrointestinal infections in the community in the L.GG group.

This study was conducted between 19 November 2007 and 20 February 2008 but the peak occurrence of acute gastroenteritis and rotavirus gastroenteritis in Europe was between January and March.<sup>36</sup>

A study in Northern Italy (which shares the same latitude and climatic conditions of Zagreb), also found that the highest number of admissions for acute rotavirus gastroenteritis (a reflection of the incidence of rotavirus infection in the community) occurred in March and April.<sup>37</sup>

This study was conducted during the winter months when the incidence of rotavirus gastroenteritis was low while that of respiratory tract infections was high.

Hence it can be seen from the study that the incidence of gastroenteritis was indeed low in both the L.GG group (14.4%) and the placebo group (22.5%) and the difference could not reach statistical significance. In contrast, the incidence of respiratory tract infections which usually peaked during winter was high (43.2% in L.GG and 67.6% in placebo group).

The authors did point out this limitation of their study but it appeared that it was not taken into account by the Panel.

### **Mastretta et al. (2002)**

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This study was accepted by the Panel and considered as showing that L.GG was ineffective in preventing nosocomial rotavirus gastroenteritis.

This study's design had limitations which were not acknowledged by the Panel:

- a) The study included anyone who was hospitalised for >48 hours. But the length of stay in hospital was short (mean length of stay = 5.2 days). This meant that patients received L.GG

on average, for only 5 days. The authors mentioned that it took at least 2 days to colonise the gut so on average, the children experienced only 3 days of L.GG's protective effect.

As pointed out by Hojsak et al. (2010) the risk of nosocomial gastrointestinal infection rose as the length of hospital stay increased. In Hojsak's Cox proportional-hazards regression model the probability of survival without GI infection when given L.GG only started to become apparent after the third day of admission. Therefore, it is not surprising that Mastretta et al. could not find significant difference between the L.GG and placebo groups when their turnover of patients was so fast.

- b) The researchers defined nosocomial diarrhoea as the occurrence of diarrhoea at least 24 hours after admission. The usual definition of a nosocomial infection is one which occurs after 48 hours of admission.<sup>38</sup>

The timing is important because as the researchers pointed out the period of study (December 1, 1999 to May 31, 2000) was during the seasonal peak of rotavirus infections and the incubation period of rotavirus gastroenteritis was 24 - 72 hours. Since the researchers included diarrhoea occurring just 24 hours after admission, some of these children could have already contracted rotavirus in the community before admission and before L.GG was administered.

- c) Another possible error was that many of these children could have acquired rotavirus gastroenteritis after their discharge from hospital when L.GG was already terminated. It is a fact that being ill enough to be admitted to hospital and the use of antibiotics during admission predispose children to acquiring gastroenteritis especially when rotavirus infection is at its seasonal peak. Since the researchers extended their definition of nosocomial rotavirus gastroenteritis to 72 hours after discharge and the incubation period of rotavirus was 24 - 72 hours, these susceptible children could easily have acquired their rotavirus infection from the community after discharge when L.GG was already terminated, and not from their hospital stay. The result was an unusually high number of children (57.4% of gastroenteritis cases) who were diagnosed as having "nosocomial" gastroenteritis after discharge from hospital.

As a comparison, Szajewska et al's (2001) study which was conducted over 16 months did not report any case of diarrhoea up to 3 days after discharge from hospital.

The possibility of community acquired rotavirus gastroenteritis, contracted either before L.GG was administered or after L.GG was terminated, would confound the true incidence of hospital-acquired infections during which L.GG was consumed. This was reflected in the unusually high incidence of supposed nosocomial rotavirus gastroenteritis (25.4% in the L.GG group and 30.2% in the placebo group) reported by the authors when the incidence of nosocomial gastroenteritis was usually quoted as between 4.5% and 22.6%)<sup>39 40</sup>

- d) The Panel had, in its opinion on Szajewska et al. (2001), pointed out that there should be information on what treatment was given to the admitted patients so that diarrhoea due antibiotic-associated diarrhoea could be differentiated from infectious diarrhoea. This study did not give information on the number of patients on antibiotics and the length of administration of antibiotics but only mentioned that "owing to randomisation, antibiotic treated patients were homogeneously distributed into the treated group and placebo group". Without such information, as was brought up in the opinion on Szajewska et al. (2001), no conclusions should be drawn from this study.

Taking into consideration these weaknesses in the methodology, definite conclusions should not be drawn from this 'negative' study and the Panel should not accept this study as evidence against L.GG.

## Szajewska et al. (2001)

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The Panel's criticism of possible limitations in the study involved:

- Asymptomatic rotavirus carriers were not excluded from the analysis.
  - Power calculations were not reported.
  - Reasons for an unequal number of subjects being randomised to the L.GG group compared to the placebo group were unclear.
  - Antibiotic use was not reported or taken into account in the data analysis so that it was not possible to distinguish between diarrhoeal episodes of infectious versus non-infectious origin.
  - Incidence of diarrhoeal episodes cannot be used as a surrogate marker for GI infections.
- a) The L.GG dose of  $6 \times 10^9$  CFU was consumed twice, and not once a day, as stated by the Panel.
- b) Just as in the study by Mastretta et al. (2002), antibiotic use was not detailed or taken into account in the data analysis. In the study by Mastretta et al. the Panel did not find fault with their argument that "owing to randomisation, antibiotic treated patients were homogeneously distributed into the treated group and placebo group". This study was also a randomized study and the same argument as that put forward by Mastretta et al (2002) should also apply.
- c) This study differentiated between children with detectable rotavirus antigen in their stools and those who had rotavirus antigen in their stools and symptoms. The Panel considered that the detection of rotavirus antigen in the stools indicated that the child was "infected" and because the incidence of "infection" was not statistically different between the L.GG group and the placebo group, drew the conclusion that L.GG consumption had no effect on the incidence of GI (rotavirus) infection of hospitalised children.

The researchers had stated that the group termed as "Rotavirus infection" included both asymptomatic and symptomatic patients with antigen shedding in their stool. Put another way, this study found similar number of patients who had rotavirus in their GI tract which was to be expected, and was a consequence of, successful randomization since both groups were exposed to the same conditions in the hospital. What differentiated the L.GG children from the placebo group was symptomatic rotavirus infection ie, diarrhoea with shedding of rotavirus antigen in the stools, termed as "rotavirus gastroenteritis".

The claim being evaluated was that L.GG could "maintain defence against gastrointestinal pathogens". In this study L.GG's ability to "maintain defence against gastrointestinal pathogens" was clearly and statistically demonstrated because out of all the children who acquired rotavirus in their GI tract only 2.2% in the L.GG group as compared to 16.7% in the placebo group developed the diarrhoeal condition ie, gastroenteritis. (RR:0.13; 95% CI:0.02-0.79) These children ingested the gastrointestinal pathogen but the presence of L.GG in their intestinal tract provided defence against the pathogen and prevented them from developing the illness of gastroenteritis.

This report clearly demonstrated L.GG's defence capability in action.

Clearly, the conclusion of the Panel that this study did not support the claim that L.GG could help to "maintain defence against gastrointestinal pathogens" is erroneous.

## **C. Immune responses after oral (viral) vaccination**

### **De Vrese et al. (2005)**

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The Panel stated that this study did not show an effect of L.GG consumption on immune responses after oral (viral) vaccination because there was no significant difference in the proportion of protected persons after vaccination, measured by the percentage of subjects whose neutralising antibody titres indicated protection against polio.

This study's aim was to demonstrate whether L.GG affected the immune response to a standardised enterovirus challenge (ie, polio vaccine). It was not designed to show a difference in the "proportion of protected persons after vaccination" as stated by the Panel.

In fact, it would be impossible for this study to show any significant difference in the "proportion of protected persons after vaccination" because

- (i) as pointed out by the Panel, volunteers were administered the booster vaccine at dosages "guaranteed by the manufacturer to provide immune protection in 95% of vaccinated subjects" and
- (ii) this was a booster dose which by definition meant that every subject already had immunisation during their primary immunisation and his or her immune system was already primed to respond to a polio virus challenge (ie the booster vaccine). As expected, this study demonstrated the efficacy of the booster vaccine in inducing the anamnestic immune response in the volunteers, whether they were given L.GG or not.

It was the extent to which the volunteers' neutralising antibodies responded to the booster vaccine which was studied. The researchers clearly demonstrated significantly higher poliovirus-specific IgA in the subjects taking L.GG. Poliovirus serotype-specific neutralizing antibody titres for Serotype 1 and 2 were also significantly higher when L.GG was consumed. This study demonstrated that L.GG could increase mucosal antibody (ie, IgA) production when challenged with an enterovirus (in this case, polio vaccine), a form of defence against gut pathogens.

The Panel's conclusion that the study did not show effect of L.GG consumption on immune responses after oral viral vaccination is incorrect.

### **Isolauri et al. (1995)**

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The Panel noted that there was no significant differences in the seroconversion rates or specific IgA, IgM or IgG concentrations between the L.GG and placebo groups. Based on this observation, it considered that the study did not show an effect of L.GG on the immune response after oral (viral) vaccination.

This study was conducted to find out if L.GG had any effect on boosting the immunogenicity of the test vaccine (DxRRV reassortant rotavirus vaccine) ie, whether it could enhance the immune system's response to the vaccine. It was not designed to test if L.GG could increase seroconversion rate because seroconversion capability depended primarily and ultimately on the immunogenicity of the test vaccine itself, and not the adjunctive immunostimulant measures. Consequently L.GG's effect on the immune system's response should not be a difference in seroconversion rates which were effected by the vaccine but rather a difference in any aspect of antibody production when challenged by the viral vaccine. It is not crucial whether serum antibodies were IgA, IgG or neutralising antibodies because as long as their production was

induced to a level great enough to be extruded onto the intestinal mucosal surface, it should be deemed a marker of protection.<sup>41</sup>

The vaccine in this study was a test vaccine and was not a commercially available vaccine which was guaranteed to produce seroconversion. In 1995 when this study was conducted, the scientific community was just testing candidate vaccines to document their immunogenicity so seroconversion rates were still being measured experimentally.

Isolauri et al. found significantly higher IgA seroconversion and mean number of IgM sASC against rotavirus in the L.GG group.

For the Panel to conclude that this study did not show any effect of L.GG on immune response after rotavirus challenge in the form of a test vaccine is unacceptable.

#### **D. Study on immune response after Rotavirus gastroenteritis was not accepted as evidence**

**Kaila M et al. 1992<sup>33</sup>**

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A human intervention study documenting L.GG's enhancement of circulating antibody secreting cell response in the convalescence period after an episode of acute rotavirus gastroenteritis was not considered for evaluation.

The reasons given by the Panel were:

- this study used L.GG in the treatment of GI infections and could not provide information about its effect in the development of GI infections,
- the results obtained in young children could not provide information on adults
- the results obtained on the treatment of GI infections caused by viruses could not provide information about other types of GI infections
- according to their opinion, L.GG consumption did not have an effect on the development of GI infections in the general population.

This study showed that children who consumed L.GG during acute rotavirus gastroenteritis mounted a significantly enhanced non-specific humoral response during the acute phase of infection and a significantly higher specific IgA response to the virus at convalescence.

Significantly more number of children in the L.GG group (90% of L.GG group vs 46% of placebo group) developed IgA specific antibody-secreting cell response to rotavirus at convalescence.

Acute rotavirus infection is usually caused by any one of the 5 common serotypes of rotavirus. Anti-rotavirus IgA is the strongest marker of protection against subsequent rotavirus re-infection caused by other serotypes.<sup>42</sup>

Therefore, the finding of a significant increase in specific IgA response to a rotavirus infection in those who consumed L.GG supports the claim that L.GG helps maintain defence against GI pathogens.

The argument put forward by the Panel to ignore this study, that results obtained in young children could not provide information on adults, is invalid because certain pathogens cause illness most commonly in children. Rotavirus gastroenteritis is an infection of infants and young children so

human intervention studies to evaluate the effect of L.GG in rotavirus infections must recruit children and not adults. The results from these childhood studies were valid to the claim and should not be disregarded just because adults were not included in these studies.

The Panel's argument that results obtained on the treatment of GI infections caused by viruses could not provide information about other types of GI infections is true but does not invalidate the study's support of the claim since the claim is that of defence against gastrointestinal pathogens in general and not defence against any specified bacterium or virus.<sup>42</sup>

## CONCLUSION

The simple question that stands before the EFSA is this - "Is there scientific evidence to indicate that L.GG has an effect in helping to maintain defence against gastrointestinal pathogens?"

The Panel's choice of scientific evidence seemed to say "no" but the bulk of evidence published in the scientific and medical world seemed to say "yes".

In its search for health advancement, it is not often that mankind finds a new avenue in its therapeutic armamentarium for health as well as disease. A negative opinion from the Panel in the face of such an abundance of scientific evidence on the most basic health effect of this most-researched probiotic strain will derail further research on preventive bacteriotherapy.

Therefore I sincerely urge you and the European Commission to take steps to review and re-evaluate the scientific evidence supporting this reference strain for bacteriotherapy before this valuable and promising health-promoting option is sidelined and pushed into irrelevance by EFSA's opinion.

Yours faithfully,



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## Reference

1. Huovinen P. Bacteriotherapy: the time has come. Bacterial interference is an increasingly attractive approach to prevention and therapy. Br Med J 2001;323:353-354.
2. FAO/WHO Guidelines for the Evaluation of Probiotics in Food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food.2002.
3. Yan F et al. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. Gastroenterology 2007;132:562-75

4. Tao Y et al. Soluble factors from *Lactobacillus* GG activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. *Am J Physiol Cell Physiol* 2006;290:C1018-30
5. Sanchez B et al. Identification of novel proteins secreted by *Lactobacillus rhamnosus* GG grown in de Mann-Rogosa-Sharpe broth. *Lett Appl Microbiol* 2009;48(5):616-622
6. Banasaz M et al. Increased enterocyte production in gnotobiotic rats mono-associated with *Lactobacillus rhamnosus* GG. *Appl Environ Microbiol* 2002;68(6):3031-3034
7. Seth, A et al. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2008; 294:G1060–G1069.
8. Johnson-Henry KC et al. *Lactobacillus rhamnosus* strain GG prevents Enterohemorrhagic *Escherichia coli* O157:H7-induced changes in epithelial barrier function *Inf and Immunity* 2008;76(4):1340-1348
9. Yan F et al. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. *J Biol Chem* 2002;277(5):50959-65
10. Nandakumar NS et al. Effects of enteropathogenic bacteria & lactobacilli on chemokine secretion & Toll like receptor gene expression in human colonic epithelial cell lines. *Indian J Med Res* 2009;130(2):170-178
11. Ghadimi D et al. Effect of natural commensal-origin DNA on Toll-like receptor 9 (TLR9) signaling cascade, chemokine IL-8 expression, and barrier integrity of polarized intestinal epithelial cells. *Inflamm Bowel Dis* 2009;PMID:19714766
12. Roselli M et al. Probiotic bacteria *Bifidobacterium animalis* MB5 and *Lactobacillus rhamnosus* GG protect intestinal Caco-2 cells from the inflammation-associated response induced by enterotoxigenic *Escherichia coli* K88. *Br J Nutr* 2006;95:1171-84
13. Donato KA et al. *Lactobacillus rhamnosus* GG attenuates interferon- $\gamma$  and tumor necrosis factor- $\alpha$ -induced barrier dysfunction and pro-inflammatory signalling. *Microbiology* 2010;156:3288-3297
14. Mack DR et al. Probiotics inhibit enteropathogenic *E. coli* adherence in vitro by inducing intestinal mucin gene expression. *Am J Physiol* 1999;276:G941-950
15. Mattar AF et al. Effect of probiotics on enterocyte bacterial translocation in vitro. *Pediatr Surg Int* 2001;17:265-68
16. Hirano J et al. The effect of *Lactobacillus rhamnosus* on Enterohemorrhagic *Escherichia coli* infection of human intestinal cells in vitro. *Microbiol Immunol* 2003;47(6):405-409
17. Xu H et al. Assessment of cell surface properties and adhesion potential of selected probiotic strains. *Lett Appl Microbiol* 2009;49(4):434-442
18. Burkholder KM et al. *Salmonella enterica* serovar Typhimurium adhesion and cytotoxicity during epithelial cell stress is reduced by *Lactobacillus rhamnosus* GG. *Gut Pathog* 2009;1(1):14
19. Huang S-H et al. *Lactobacillus rhamnosus* GG suppresses meningitic *E. coli* K1 penetration across human intestinal epithelial cells In vitro and protects neonatal rats against experimental hematogenous meningitis. *Int J Microbiol* 2009;647862. doi:10.1155/2009/647862
20. Lee YK et al. Quantitative approach in the study of adhesion of lactic acid bacteria to intestinal cells and their competition with enterobacteria. *Appl Environ Microbiol* 2000;66(9):3692-3697
21. Lee YK et al. Displacement of bacterial pathogens from mucus and Caco-2 cell surface by lactobacilli. *J Med Microbiol* 2003;52:925-930
22. Lee YK et al. Competition for adhesion between probiotics and human gastrointestinal pathogens in the presence of carbohydrate. *Br J Nutr* 2002;88 (Suppl):S101-S108

23. Lu R et al. Isolation, identification, and characterization of small bioactive peptides from *Lactobacillus* GG conditional media that exert both anti-Gram-negative and Gram-positive bactericidal activity. *J Pediatr Gastroenterol Nutr* 2009;49(1):23-30
24. De Keersmaecker SCJ et al. Strong antimicrobial activity of *Lactobacillus rhamnosus* GG against *Salmonella typhimurium* is due to accumulation of lactic acid. *FEMS Microbiol Lett* 2006;259:89-96
25. Hudault S et al. Antagonistic activity exerted in vitro and in vivo by *Lactobacillus casei* (strain GG) against *Salmonella typhimurium* C5 infection. *App Environ Microbiol* 1997;63:513-18
26. Hutt P et al. Antagonistic activity of probiotic lactobacilli and bifidobacteria against entero- and uropathogens. *J Appl Microbiology* 2006;100:1324-1332
27. Carey CM et al. The effect of probiotics and organic acids on Shiga-toxin 2 gene expression in enterohemorrhagic *Escherichia coli* 0157:H7.
28. Fayol-Messaoudi D et al. pH-, lactic acid-, and non-lactic acid-dependent activities of probiotic lactobacilli against *Salmonella enterica* serovar typhimurium. *Appl Environ Microb* 2005;71(10):6008-6013
29. Isolauri E et al. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine* 1995;13:310-312
30. Marianelli C et al. Evaluation of antimicrobial activity of probiotic bacteria against *Salmonella enterica* subsp. *enterica* serovar typhimurium 1344 in a common medium, under different environmental conditions. *Res Microbiol* 2010; doi:10.1016/j.resmic.2010.06.07
31. Makras L et al. Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonella enterica* serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds. *Res Microbiol* 2006 157(3):241-247
32. Silva M et al. Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrob Agents Chemother* 1987;31(8):1231-1233
33. Kaila M et al. Enhancement of the circulating antibody secreting cell response in human diarrhea by a human lactobacillus strain. *Pediatr Res* 1992;32(2):141-144
34. de Vrese M et al. Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. *Eur J Nutr* 2005;44(7):406-413
35. Isolauri E et al. Improved immunogenicity of oral D x RRV reassortant rotavirus vaccine by *Lactobacillus casei* GG. *Vaccine* 1995;13:310-312
36. Van Damme P et al. Multicenter prospective study of the burden of rotavirus acute gastroenteritis in Europe, 2004-2005: The REVEAL Study. *J Infect Dis* 2007;195:S4-16
37. Zuccotti G et al. Epidemiological and clinical features of rotavirus among children younger than 5 years of age hospitalized with acute gastroenteritis in Northern Italy. *BMC Infect Dis* 2010;10:218-22]
38. World Health Organization; 2002. Publication WHO/CDS/CSR/EPH/2002.12
39. Ford-Jones et al. The incidence of viral-associated diarrhea after admission to a pediatric hospital. *Am J Epidemiol* 1990;131:711-718.
40. Ponce et al. Use of a prospectively measured incidence rate of nosocomial diarrhea in an infant/toddler ward as a meaningful quality assessment tool. *Clin Perform Qual Health Care* 1995;3:128-23
41. Jiang et al. The role of serum antibodies in the protection against rotavirus disease: An Overview. *Clin Infect Dis* 2002;34:1351-1361
42. Valezquez FR et al. Serum antibody as a marker of protection against natural rotavirus infection and disease. *J Infect Dis* 2000;182:1602-1609