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Immune effects of probiotics in humans

Evaluation of efficacy and safety

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Abstract

Effects of probiotics on the immune system
Evaluation of efficacy and safety

There is currently insufficient scientific evidence demonstrating that probiotics can have beneficial effects on the immune system. The safety of these products also has not been demonstrated scientifically.

The number of commercially available products that contain probiotics, such as food supplements, dairy products and infant formulas, is steadily increasing. Although probiotics are claimed to have positive effects on gut microflora and immune resistance and to prevent allergies, most of these effects are not based on scientific evidence.

The National Institute for Public Health and the Environment (RIVM) conducted a literature study aimed at assessing the efficacy and safety of probiotics. The effects of probiotics, for example, on constipation or diarrhea, are currently subjects of extensive investigation. This literature study focuses on the effects of probiotics on the immune system, as determined from human clinical trials. No or insufficient evidence was found for supporting the claims that probiotics are effective in treating hay fever, rheumatoid arthritis or Crohn's disease. In contrast, data are available on the beneficial effects of certain probiotics on immune resistance, atopic eczema and the chronic inflammatory bowel disease, ulcerative colitis. However, more research is needed to substantiate these effects scientifically. The best substantiated evidence for positive effects of probiotics is currently available for two intestinal disorders, diarrhea caused by antibiotics and 'pouchitis' (a complication that can occur after surgery of colitis patients).

Little information is available on the possible adverse effects that can occur when people consume these products. A recent publication reported that probiotics induced an unwanted stimulation of the immune system in infants.

It is recommended that both the safety and efficacy of these products should be closely investigated.

Key words:
Probiotics, immune effects, safety, efficacy, humans

Rapport in het kort

Effecten van probiotica op het immuunsysteem

Evaluatie van werkzaamheid en veiligheid

Er is momenteel onvoldoende wetenschappelijk bewijs dat probiotica een positief effect hebben op het immuunsysteem. Evenmin is wetenschappelijk bewezen dat gebruik van dit type product veilig is.

Er komen steeds meer producten op de markt die probiotica bevatten, zoals voedingssupplementen, zuiveldrankjes en zuigelingenvoeding. Van probiotica wordt beweerd dat ze een positief effect kunnen hebben op de darmflora ,op de weerstand en dat ze allergieën kunnen voorkomen. De meeste van deze geclaimde effecten zijn echter niet wetenschappelijk onderbouwd.

Om de werkzaamheid en veiligheid van probiotica in kaart te brengen heeft het RIVM een literatuurstudie uitgevoerd. Momenteel vindt veel onderzoek plaats naar de effecten van probiotica, bijvoorbeeld bij obstipatie of diarree. In deze studie ligt de nadruk op effecten op het immuunsysteem, ontleend aan klinische studies bij mensen.

Uit het onderzoek is gebleken dat er geen of onvoldoende bewijs is dat probiotica helpen bij hooikoorts, reuma of de ziekte van Crohn. Er zijn gegevens bekend over positieve effecten van sommige probiotica op de weerstand, atopisch eczeem en de chronische darmontsteking ulceratieve colitis. Meer onderzoek is nodig om deze effecten met wetenschappelijke bewijzen te staven. Het beste onderbouwd is momenteel de positieve uitwerking van probiotica bij twee darmaandoeningen, te weten diarree veroorzaakt door antibiotica en ‘pouchitis’ (een complicatie die optreedt nadat patiënten met colitis zijn geopereerd).

Er is nog weinig bekend over mogelijke negatieve effecten als mensen dit soort producten consumeren. Een recente publicatie heeft aangetoond dat gebruik van probiotica bij zuigelingen tot een ongewenste stimulatie van het immuunsysteem kan leiden.

Het is aan te bevelen om zowel de veiligheid of werkzaamheid van dit soort producten nader te onderzoeken.

Trefwoorden:
Probiotica, immuuneffecten, veiligheid, werkzaamheid, humaan

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Summary

Probiotics are considered to convey health benefits to the host when ingested in sufficient numbers. The ability to induce health benefits is dependent on the probiotic strain used and most of the beneficial effects are related to the intestinal tract or the immune system. Although the number of scientific papers on this subject is increasing, evidence for efficacy is often not convincing. Furthermore, some properties of probiotics may induce unwanted effects in certain susceptible populations. However, information on safety of probiotics is lacking, because probiotics are considered as friendly and harmless bacteria. A literature study was conducted in order to obtain insight in both efficacy and safety of probiotics. The focus was on immune effects of probiotics.

The Pubmed database was used to search for placebo-controlled clinical trials on this subject. To obtain more information on efficacy, also meta-analyses that have studied effectiveness of probiotics were included. For additional data on safety, a search for case-reports on probiotics was conducted.

This literature search demonstrates that the best evidence for efficacy of probiotics has been obtained for two intestinal disorders: pouchitis and antibiotics-associated diarrhea. In addition, data published on effects of probiotics on immune resistance, vaccination responses, atopic eczema, and ulcerative colitis suggest that some probiotics may be effective. However, more research is needed to substantiate this. Data published on prevention of rhinitis and asthma, enhancement of the immune system, rheumatoid arthritis and Crohn's disease are usually not convincing and mostly inconclusive.

There are not many publications that have investigated safety of (long-term) ingestion of probiotics. A few trials have demonstrated that probiotics are well-tolerated by infants and induced no adverse short-term effects. However, unwanted immunostimulation was observed in infants from allergic parents who received the probiotic *Lactobacillus acidophilus* in the first six months of life. This intervention was intended to prevent development of atopic diseases, but induced increased sensitization to several allergens. It is unknown if this will eventually lead to an increase in the incidence of allergic diseases, this should be monitored by follow-up of these infants for a longer period. In addition, several case reports describe the occurrence of bacteremia or sepsis by lactobacilli. These effects are only observed in isolated cases, concerning patients with severe underlying diseases.

In conclusion, the public enthusiasm on efficacy of probiotics is for most of the immune effects not supported by scientific data. Although some promising results are obtained, there is a need for more controlled trials with sufficient numbers of patients/volunteers. In addition, there is a lack of data on safety, and the recent paper on increased sensitization in infants who received *L. acidophilus* suggests that short- and long-term efficacy and safety of probiotics should be evaluated before health claims can be substantiated.

1 Introduction

Probiotics are defined as living microorganisms which on ingestion of certain numbers exert health benefits. In general, lactic acid bacteria are often used as probiotics, for instance lactobacilli and bifidobacteria. In addition, certain yeasts are used as probiotics, e.g. *Saccaromyces boulardii*.

The idea that probiotics can exert health benefits has increased the interest in these microorganisms, both scientifically and commercially. The number of scientific papers on probiotics is rising, and in addition the amount of products that contain probiotics is increasing. In the Netherlands several probiotic products can be purchased, e.g. dairy products, food supplements, and infant formulas. It is thought that probiotics can beneficially influence the gut (59) and the immune system (31). Many of these positive effects have been found in animal studies, but evidence from clinical studies is limited.

Despite the lack of evidence from clinical trials the number of available probiotic products is increasing and the health claims that are made on these products suggest that consumption can have several beneficial effects. In general it is thought that probiotics are friendly bacteria that are harmless to the host. However, due to some properties of probiotics, consumption might not be safe for everybody. Many probiotics are claimed to have an effect on the immune system, which is often demonstrated in animal studies. Although some of these immune effects could beneficially influence the host, for instance by enhancing resistance or alleviation of allergic diseases, modulation of the immune system might induce unwanted effects.

This idea has initiated a RIVM project that is performed by order of the Food and Consumer's Safety Authority. The goal of this project was to develop a model to evaluate the efficacy and safety of probiotics. One important issue was the safety of specific products, such as infant formulas. Early in life the immune system is still developing and is more susceptible to immunotoxic compounds. It is unknown if early administration of probiotics, for instance present in infant formulas, can induce adverse effects. Furthermore, some probiotics have been shown to specifically stimulate Th1 immunity, which can be beneficial in allergic individuals, but might aggravate Th1 mediated diseases, such as autoimmune diseases. By using experimental animal models we have studied effects of *Lactobacillus casei* Shirota (LcS) and *Bifidobacterium* (*B.*) *animalis* on allergy and autoimmunity. In general, the probiotic LcS aggravated both allergic and autoimmune responses (6, 28, 30, 32). These immune effects were not different when probiotics were administered during lactation, except for the aggravation of lung inflammation in the allergy model which was only demonstrated when probiotics were given during lactation (30, 32). In contrast, *B. animalis* suppressed both allergic and autoimmune responses (29). These data show that immune effects of probiotics are strain-dependent and that immunomodulation not always results in beneficial effects.

Data from experimental animal studies can provide information on the immune effects that a probiotic can elicit, it is however difficult to extrapolate these data to the situation in humans. In order to confirm efficacy and safety of a probiotic strain data from clinical trials are needed. In the EU, the new Regulation on Nutrition and Health Claims has come into force since July 1st 2007. Health claims that are on reduction of diseases or are specific for infants have to be evaluated by a national authority and subsequently by the European Food Safety Authority (EFSA). The principles of assessment of efficacy are formed in the EU programme PASSCLAIM. According to PASSCLAIM, health claims cannot be substantiated by animal studies or small uncontrolled human studies. Small controlled human studies supported by laboratory data make the claim 'possible', but to substantiate a health claim large-scale controlled human studies are needed (3). This new regulation will provide valuable information on efficacy and will prevent misleading of consumers. According to this legislation, food companies do

not have to demonstrate safety of their products. One may wonder if applications that are somewhat different from initial applications and also extended use that is different from initial use, could possibly lead to safety issues. Therefore, in 2005, we have adapted a decision scheme from guidelines that were proposed previously(34, 92), to evaluate not only efficacy but also safety of probiotics. One important part of this decision scheme is the evaluation by expert judgment that should not only consider the plausibility of the health claim, but in the safety assessment should also take into account for which application the probiotics are meant (31).

To gain insight in the evidence for efficacy and safety of probiotics a literature study was conducted. This literature study has focussed on clinical trials that have investigated immune effects of probiotics. Furthermore, to obtain information on safety, all publication on this subject were included to. These data will be used to evaluate both efficacy and safety of probiotics.

2 Methods

The Pubmed database was used to search for human clinical trials. This was done by searching for ‘probiotics’ with the following limits: English, Dutch, Clinical Trial, Randomized Controlled Trial, Humans. The Pubmed search was restricted to papers that were published between January 1980 and January 2007.

The results from the Pubmed search were then screened via their abstracts. Papers that were not on probiotics were excluded. Also, trials that investigated combinations of probiotics with prebiotics, vitamins, or other compounds were excluded.

An additional search in the Pubmed database was done to find meta-analyses that have studied effectiveness of probiotics. Also, the Pubmed database has searched for case-reports on probiotics, in order to find more information on safety.

3 Results

3.1 Pubmed search

The search strategy resulted in 319 papers that were published between 1997 and January 2007. A total of 52 papers were excluded, for reason outlined in the methods section.

The majority of the studies has focused on effects of probiotics on the gut (137 studies) and immune system (71 studies). The effects of probiotics on the gut, include studies that have investigated survival and colonization of probiotics in the gastrointestinal tract, effects on gut microflora composition and metabolism, on diarrhea (acute, chronic, antibiotic-associated), inflammatory bowel disease (IBD: ulcerative colitis, Crohn's disease, pouchitis), irritable bowel syndrome, constipation, and enterocolitis.

This report will focus on immune effects of probiotics, both on efficacy and safety. The majority of studies have investigated effects on the immune system of healthy volunteers and on the prevention or alleviation of atopic eczema in infants. Furthermore, effects on rhinitis, asthma, resistance, common cold, respiratory infections, vaccination responses and rheumatoid arthritis have been studied. These studies, together with trials that have investigated effects on IBD, will be discussed in this report.

Until now, there are not many studies that can provide safety information (9 studies). Therefore, information from these studies will be compared with data from case-reports. Most of these case reports describe infections due to probiotics. Furthermore, 1 paper on the safety of probiotics in infants with cow's milk allergy was found. These papers are included in the evaluation of efficacy and safety of probiotics.

In addition to the papers that describe clinical trials and case-reports on probiotics, we have also searched for meta-analyses that were focussed on effectiveness of probiotics. A total of 10 meta-analyses have been published. These meta-analyses have studied effectiveness of probiotics on antibiotic-associated diarrhea (16, 20, 46, 63, 101, 102) acute diarrhea (43, 93, 110), and plasma cholesterol (2).

3.2 Atopic eczema and cow's milk allergy

Effects of probiotics on atopic eczema have been studied in 19 randomized clinical trials (summarized in Appendix 1). The study populations were either infants with diagnosed atopic dermatitis suspected of cow's milk allergy (14 papers) or pregnant mothers from atopic families (5 papers). The majority of papers have investigated effects of LGG (9, 45, 47, 48, 53, 60, 77, 84, 112, 113). In other studies effects of *B. lactis* Bb12 (45), *L. fermentum* (119), *L. rhamnosus* (9) *L. lactis* (1) and *L. acidophilus* (106) were investigated. A few studies have looked at efficacy of a combination of two or more probiotics: *L. rhamnosus* combined with *B. lactis* (97), *L. rhamnosus* combined with *L. lactis* (87, 88) and a mixture containing LGG, *L. rhamnosus* LC705, *B. breve* Bbi99, and *Propionibacterium freudenreichii ssp. Shermanii* JS (112-114).

One of the endpoints that is often used to assess effects of intervention is the SCORAD score (Severity Scoring of Atopic Dermatitis), an objective clinical scoring system of symptoms. In six studies other endpoints were measured, e.g. cytokines, proinflammatory markers and faecal IgA (77, 78, 86, 88, 112,

113). These parameters provide information on mechanisms involved, but do not give information on the improvement of eczema. Only the objective SCORAD score can provide information on efficacy.

Reduction of symptoms was observed in four studies, of which three used LGG as a probiotic (45, 52, 60) and one *L. fermentum* (119). It is important to note that only one study demonstrated a significant effect when the probiotic group was compared with the placebo group (52) while in the other studies improvement of SCORAD was observed within the probiotic group comparing symptoms before and after intervention, and not within the placebo group (45, 60, 119). Significant effects within the intervention group can reflect a normal reduction of symptoms, which often is observed in these patients and could therefore be unrelated to the intervention. Therefore, significant improvement of symptoms compared to the placebo group is a more reliable factor for the success of the intervention.

No improvement of eczema was found in five studies (1, 9, 87, 97, 114). However, in four of these studies a subgroup analyses revealed that probiotics improved eczema in children in which eczema was accompanied with increased serum IgE levels against several allergens (1, 87, 97, 114). Hence probiotics seem to be more beneficial in sensitized infants. The probiotics used in these studies were LGG (114), *L. rhamnosus* combined with *L. lactis* (87), *L. lactis* (1) or a combination of *L. rhamnosus* and *B. lactis* (97).

Mechanisms that could explain the beneficial effects have been investigated in some studies. Rosenfeldt et al. (88) demonstrated that *L. rhamnosus* combined with *L. lactis* decreased gastrointestinal symptoms and intestinal permeability in infants with atopic eczema. In allergic patients the intestinal permeability is often increased and probably worsens the allergy, because food-allergy inducing proteins from the diet can more easily pass the gut barrier. Beneficial effects of LGG have also been associated with reduction of inflammation in the gut. The observed improvement of atopic eczema was accompanied by a decrease in faecal TNF-α in these infants (60). Viljanen et al. (113) demonstrated that in infants with eczema, LGG increased several inflammatory parameters in serum (C-reactive protein, E-selectin, interleukin-6 (IL-6). This was only observed in patients with IgE-associated eczema. In another study the improvement of eczema by LGG was associated with an increase of serum TGF-β, a cytokine involved in immunoregulation (45). LGG moreover increased IL-10, an immunoregulatory cytokine, in a small clinical trial, in which no placebo group was included (77).
Remarkably, a mixture of probiotics (LGG, *L. rhamnosus* LC705, *B. breve* Bbi99, and *Propionibacterium freudenreichii* ssp. *Shermanii* JS) was less effective than LGG alone (114). This demonstrates that consuming several strains of probiotics may not necessarily be better than consuming just one strain. The effects on gut inflammation were different when comparing the mixture with LGG, because LGG and not the mixture decreased faecal anti-trypsin (a marker for inflammation) and increased faecal IgA (112). Furthermore, in infants with IgE-associated eczema or cow's milk allergy LGG increased the production of IFN-γ (Th1) from stimulated peripheral blood mononuclear cells (PBMCs), which was not observed in infants that received the mixture (78). Hence, LGG appears to improve eczema by affecting the gut and by skewing the immune response towards Th1.

Although LGG seems to be the most studied and the most successful probiotic in the treatment of atopic eczema, there is one study that did not find any effects of LGG on atopic eczema (9). This discrepancy might be explained by differences between the clinical trials, such as severity of eczema at the start of the intervention (mild versus severe), duration of intervention, dose and age. Furthermore, in this study it was not possible to do a subgroup analyses, as has been done in other studies, because the number of infants with IgE-associated eczema was too small.

In all the above mentioned trials, no side-effects were reported, except for one study in which heat-inactivated LGG caused gastrointestinal problems and diarrhea and recruitment was stopped after the pilot phase of four weeks (52).

In addition, preventive effects of probiotics on the development of atopic eczema have also been investigated. This was done by selecting atopic pregnant mothers and starting intervention a few weeks before birth and continuing until the age of 6 months (either by given probiotics to breastfeeding mothers or to the infant). It was shown that LGG significantly reduced the prevalence of atopic dermatitis at age 2 and 4. Notably, at the age of 4 rhinitis was diagnosed twice as often in the LGG group, this was however not significant (47, 48). Rautava et al. (84) have shown that LGG intervention starting before birth and continuing until the age of 6 months had beneficial effects in infants that were exclusively breastfed for 3 months or more. In these infants the number of blood cells secreting IgM, IgA and IgG were significantly higher then in the placebo group. Prevalence of atopic disease was not established in this study, so it is unknown what the effects of this immune-enhancement are (84). In contrast, the probiotic *L. acidophilus* that was administered to infants born from allergic parents in the first six months of their lives, did not prevent atopic dermatitis at 6 and 12 months, but significantly increased sensitization against several allergens (as measured with a skin prick test) at 12 months (106).

3.3 Rhinitis and asthma

In Appendix 2 an overview is given of studies that have investigated the effects of probiotics on rhinitis or asthma. Only one of these twelve studies included patients with asthma and rhinitis (120), the other studies included only patients with rhinitis (4, 12, 13, 42, 67, 76, 105, 115, 123-125).

Three studies were performed with *B. longum* BB536 (123-125), two studies have used the probiotic *Bacillus clausii* (12, 13) and two other studies *L. paracasei* (76, 115). Other strains that have been used are *L. acidophilus* (120), *L. rhamnosus* (42), LcS (105), *L. gasseri* (67) and a combination of *L. acidophilus* with *Bifidobacterium* (unknown which substrain) (4).

Improvement of symptoms was reported in six studies. The combination of *L. acidophilus* and *Bifidobacterium* consumed by 4 patients with rhinitis for 4 months improved symptom score within the probiotic group. In the placebo group (n=7) no improvement was observed. PBMCs from the probiotic group showed a skewing towards Th1, but serum IgE levels were not changed in this group (4). These results are inconclusive, because they are based on very small groups and should be confirmed in a trial in which more patients are included. Effects of *L. paracasei*-33 were studied in larger groups and treatment for 30 days improved quality of life compared to placebo. Within the probiotic group nose symptoms were reduced during intervention (115). In a second study, it was shown that viable and heat-killed *L. paracasei*-33 were both effective in reducing symptom score and improving quality of life (76). These studies indicate that some probiotics can suppress subjective symptoms but give no information on the (immunological) mechanisms involved. In a pilot study with 15 patients with high IgE levels it was shown that *L. gasseri* reduced total and pollen-specific IgE in two subjects (67). Although there are not many studies that have shown an effect of probiotics on IgE, no conclusions can be drawn from such a small study which is not randomized and placebo controlled. Fluctuations in IgE might explain the observed effects.

Several studies have investigated the efficacy of *B. longum* BB536 on rhinitis triggered by Japanese cedar pollen. This is one of the most common allergic diseases in Japan with a prevalence of approximately 16%. In the first trial, *B. longum* reduced subjective eye symptoms, but not IgE. During the pollen season the number of blood eosinophils increased and the amount of blood IFN-γ decreased in the placebo group. However, in subjects that consumed *B. longum* the increase in eosinophils was suppressed. Furthermore, probiotics suppressed the decrease in IFN-γ at the early stage of the pollen season (125). In the first trial the placebo group consumed yoghurt, which also contains lactic acid bacteria. Therefore, a second study was conducted in which the placebo group received powder. This

second trial was done in a heavy pollen season and subjects dropped-out because they had to take medication. Significantly more subjects dropped out in the placebo group than in probiotic group. In this second trial *B. longum* reduced several subjective medical symptoms and also a tendency to reduced pollen-specific IgE was observed in the probiotic group (123), Data from both studies suggest that improvement is associated with a skewing of the immune response towards Th1. The efficacy of *B. longum* BB536 was further investigated in a double-blind two-way crossover study that was done outside the pollen season. Subjects were exposed to Japanese cedar pollen in an environmental exposure unit after the intervention period. Again, *B. longum* reduced several subjective symptoms, such as disruption of daily routine and total medication (124).

Consumption of probiotics had no effects on rhinitis or asthma in five studies (12, 13, 42, 105, 120). In some of these studies, probiotics do affect the immune system. *L. acidophilus* decreased eosinophils in patients with rhinitis and/or asthma and skewed the immune response towards Th1 (both almost reached significance) (120). *Bacillus claussi* significantly reduced nasal eosinophils (12) and skewed the immune response towards Th1 and immunoregulatory cytokines (13). Although these probiotics modulate the immune system, apparently this does not lead to reduction of symptoms. These effects were obtained in small trials, probably lacking sufficient statistical power.

3.4 Effects on the immune system of healthy volunteers

In 19 studies the effects of probiotics on several immune parameters in healthy volunteers have been investigated (summarized in Appendix 3). Different probiotics were used in these studies: LcS (99, 103, 104), LGG (75), *L.* casei DN11400 (64, 73, 74), *B. lactis* HN019 DR10 (5, 10, 36), *Escherichia* (*E.*) *coli* Nissle 1917 (18), *Bacillus polyfermenticus* (51), *L. johnsonii* La1 (89) and *L. rhamnosus* HN001 (95), or combination of probiotics: LGG and *B. lactis* Bb12 (83), *L. paracasei* CRL431 and *B. animalis lactis* Bb12 (11), *B. lactis* HN019DR10 and *L. rhamnosus* HN001 DR20 (35), *L. gasseri* CECT 5714 and *L. coryniformis* CECT 5711 (69, 71).

In two studies immune effects of probiotics were investigated in infants. In the first one premature infants were included that received the probiotic *E. coli* Nissle 1917. This intervention enhanced serum IgM levels. Clinical implications of this enhancement are unknown (18). In infants that received LGG and *B. lactis* Bb12 in the first year of their life an increase of serum sCD14 (a co-receptor for Toll-like receptor) and cow's milk-specific IgA-secreting cells was observed. In the probiotic group none of the infants developed atopic eczema, whereas 8% of the infants in the placebo group did. This difference was not significant and this study was not designed to study effects of probiotics on allergic disease. It is difficult to extrapolate these immune parameters to a health benefit (83).

In cross-over study the effects of LGG on healthy and milk-sensitive subjects was studied. After milk challenge several receptors on neutrophils and monocytes were upregulated in milk-hypersensitive subjects. When during challenge also LGG was given this increase was prevented. In contrast, milk challenge had no effects in healthy subjects, but LGG increased receptor expression on neutrophils (75). An increase in receptor expression cannot directly be translated in a health benefit or risk.

Several studies show that probiotics stimulate natural killer (NK) activity and/or phagocytosis (5, 10, 26, 35, 36, 51, 74, 95, 103, 104), reflecting an enhancement of natural immunity. NK cells play an important role in the defence against viruses and tumour cells. It is important to note that an increase in natural immunity cannot directly be translated in a specific health benefit, such as increased host resistance and reduction of infections. In contrast, two studies did not show an effect on NK activity and phagocytosis. In one trial LcS was consumed for 4 weeks (99) and in the other *B. animalis lactis* BB-12 and *L. paracasei* CRL-431 were consumed for 3 weeks (11).

It is hypothesized that in elderly, probiotics could beneficially influence the decline in immunity, which is a normal physiological process in ageing. In elderly, *B. lactis* increased natural immunity measured by increased NK activity, phagocytosis and cytokine production (5, 36). *B. lactis* was more effective in subjects with poor immune responses measured before intervention. This could imply that *B. lactis* could benefit elderly, and especially those with a poorly functioning immune system (36). In addition the probiotic *L. rhamnosus* also enhanced NK activity and tumoricidal activity in elderly. The increase in tumoricidal activity was more pronounced in individuals older then 70 years for both *L. rhamnosus* and *B. lactis* (35). These results are promising, but in these intervention studies no placebo group was included. The effects measured are before and after intervention and it is possible that they reflect fluctuations that could normally be present in individuals. The immunostimulation of *B. lactis* was further enhanced when this probiotic was given in combination with the prebiotic galacto-oligosaccharide (GOS) (10). In contrast, the increased NK activity and phagocytosis observed after *L. rhamnosus* intervention could not be further augmented with GOS (95). These data suggest that the stimulating effects of GOS on the immune effects of probiotics are strain-dependent.
The probiotic LcS enhanced NK activity in healthy elderly subjects (104). In contrast, in another trial LcS did not enhance natural immunity in healthy middle-aged volunteers (99). This difference might be explained by the difference in age in both interventions. LcS appears to be only effective in elderly individuals (>60 years) (104) and not in individuals younger than 65 yr (99). However, in another trial LcS effectively enhanced the NK activity in middle-aged individuals within intervention group, but not compared to placebo. In elderly (55-75 years) no effects of LcS were detected (103). All studies investigated effects of LcS in very small groups and it is possible that these trials did not have enough statistical power.

In an intervention study in young females the effects of conventional yoghurt was compared with yoghurt supplemented with *L.* casei. It was demonstrated that both yoghurt as well as yoghurt with *L.* casei increased NK activity during intervention. This suggest that both interventions stimulated the immune system, or that in the trial only time-dependent fluctuations in the immune system were measured (64). In another trial yoghurt was also used as a placebo. Again, yoghurt also modulated the immune system, by increasing monocytes and neutrophils. However, the increase in NK activity, serum IL-4, IL-10 and IgA were only measured within the probiotic group. The significance of this immunostimulation is not completely clear, especially since effects were more pronounced after 2 weeks of intervention than after 4 weeks (72).

Many trials included a low number of participants and it should be noted that these trials do not have enough statistical power. Furthermore, these general immune parameters cannot be translated in a health benefit, therefore clinical parameters should be assessed in such studies, e.g. decreased susceptibility to infections.

3.5 Resistance against infections

One of the health claims that is made for certain probiotic products, is that consumption will enhance resistance against infections. This health claim has been investigated in four trials (Appendix 4).

Two intervention studies have studied effects in infants attending day care centres. These infants are more prone to develop gastrointestinal and respiratory tract infections than children cared for at home. Consumption of LGG for seven months by infants from day care centres resulted in a lower use of antibiotics prescribed for respiratory tract infections and a lower absence due to illness compared to the placebo group. However, doctor's diagnosed respiratory tract illnesses did not differ significantly between the groups. The absence due to illness was 0.9 days lower in the LGG group, but when

adjusted for age it was only 0.6 days (41). Hence, the significant effects were modest and the clinical relevance is unclear (15). Another study in infants attending day care centres has studied effects of two different probiotics, *B. lactis* or *L. lactis*, on prevention of infections. It was demonstrated that both probiotics reduced days and episodes of fever and diarrhea. In addition, *L. lactis* also reduced the number of clinic visits, absence of day care and antibiotics prescriptions (118). In this study, the effect size of the significant changes is quite small. The authors have calculated number of days in the 12 week period per child. For example, days with diarrhea for this period were 0.59 in the placebo, 0.37 in the *B. lactis* and 0.15 in the *L. lactis* group. The clinical relevance of these effects are unclear (109).

A study in employees of Tetrapak in Sweden has shown that intake of *L. lactis* lowered the number of persons on sick leave from 23% in the placebo to 10% in the probiotic group. In shift-workers these effects were more pronounced, 33% of the placebo group was on sick leave during the intervention period and none of the probiotic group (108).

The effects of a mixture of *L. gasseri* PA16/8, *B. longum* SP07/3, and *B. bifidum* MF20/5 on common cold episodes has been studied in healthy volunteers. This study demonstrated that probiotics could not prevent common cold, as the incidence was not affected, but that the symptoms were less severe. In the probiotic group the symptom score, duration of symptoms and days with fever were lower than in the placebo group. In a subcohort additional parameters were assessed and it was shown that CD8+ T cells, which are involved in cellular immunity, were enhanced in the probiotic group. This could implicate that probiotics stimulated cellular immunity, which resulted in a faster recovery from common cold with milder symptoms (24, 25).

With the current knowledge it is too early to put a health claim on probiotic products, that consumption enhances resistance. The few studies that have been done, suggest that probiotics can enhance resistance, however, effect sizes are small and difficult to extrapolate into benefits for the general population.

3.6 Vaccination responses

Immunostimulation induced by probiotics can be assessed by measuring the effects on a vaccination responses. This has been done in four studies, summarized in Appendix 5.

LGG or *L. lactis* did not enhance the vaccination response induced after oral vaccination with Salmonella typhi on days 1, 3 and 5 (33). This study was performed in relative small groups and administration of the probiotics started one day before the first vaccination.

The probiotic LGG significantly enhanced neutralizing antibody titres against polio type 1 and significantly increased polio-specific IgA, but nog IgG. In contrast, *L. acidophilus* CRL431 did increase antibody titres, but these effects were not significant (23).

The probiotic *L. fermentum* increased the vaccination response against influenza, especially the influenza-specific IgA titres. In these volunteers several immune parameters were assessed and it was shown that in the probiotic group the number of NK cells was increased due to the probiotic treatment, which was not observed in the placebo group. Furthermore, TNF-α levels in serum were significantly higher in the probiotic group compared to the placebo. The incidence of influenza-like illnesses was assessed during 5 months after vaccination and was lower in the volunteers that consumed probiotics (70).

A study performed in infants that received *L. acidophilus* in the first six months after birth has investigated the effects of intake of probiotics on both allergen-specific and vaccine-specific responses.

This was done to assess if the anti-allergic properties that have been ascribed to probiotics could also decrease vaccination responses. The authors measured cytokine production after stimulation of PBMCs with vaccine components and food and respiratory allergens. Probiotics did not influence allergen-specific responses, except that PBMCs from this group less frequently produced TNF-α and IL-10 in response to house dust mite. Probiotics did reduce the IL-10 response to the vaccine antigen tetanus (107). The authors concluded that probiotics did not influence allergen-specific responses but did affect vaccine responses. However, results on alterations in cytokine production cannot be directly extrapolated to effects on vaccine responses. For this, it is necessary to measure antibody titres.

In conclusion, there are only a few studies that have looked at the effects of probiotics on vaccination responses. Although the studies with *L. fermentum* (70)and LGG (23) suggest that some probiotics can enhance the vaccination responses, efficacy and clinical relevance should be demonstrated in clinical trials that use larger groups.

3.7 Rheumatoid arthritis

Effects of probiotics on autoimmunity have been studied in only one trial. Patients with mild and stable rheumatoid arthritis received either LGG ($1x10^{10}$ CFU/day; n=8) or placebo (n=13) for 12 months. LGG did not induce any statistical differences in clinical parameters and biochemical variables. In the LGG group a decrease of number of tender and swollen joints from 8.3 to 4.6 and in the placebo group from 5.5 to 4.8 (p=0.41) was observed after 12 months. Global assessment of activity of disease by a physician, demonstrated a reduction of 71% in the LGG and 30% in the placebo group (p=0.15). Serum IL-1β increased slightly in the LGG group (p=0.07), but other cytokines (IL-6, TNF-α, IL-10 or IL-12) were not affected (40). This study suggests that LGG can alleviate some of the subjective symptoms of rheumatoid arthritis. These effects were not significant and should be confirmed in a larger trial.

3.8 Inflammatory bowel disease

Appendix 6 summarizes results of clinical trials that have investigated effects of probiotics on IBD. Five trials have investigated effects on Crohn's disease (8, 39, 62, 79, 94). In three of these LGG was used as a probiotic, but in none of the studies LGG effectively maintained remission, since the relapse rate was similar compared to the placebo group (8, 79, 94). The yeast *S. boulardii* effectively maintained remission in patients treated with mesalazine. These results are promising, but were obtained in a small trial without a placebo group (39). In a larger placebo-controlled trial *L. johnsonii* LA1 did not affect recurrence of disease after surgical resection (62).

Effects on ulcerative colitis were studied in eight clinical trials (7, 17, 44, 50, 54, 85, 111, 121). In a small trial, a probiotic supplement Bifico, which contained undefined bifidobacterium strains, relapse rates improved significantly from 93% in the placebo to 20% in the Bifico group (17). A small trial in which *L. acidophilus* and *B. animalis lactis* were administered to patients with collagenous colitis demonstrated beneficial effects on bowel and stool frequency but not on histopathology or inflammation (121). Two small trials studied effects of a combination of *B. breve*, *B. bifidum* and

L. acidophilus on colitis. Consumption for one year lowered exacerbation of symptoms compared to the placebo group, but did not affect colonoscopic findings (44). Intake of this combination of probiotics for 12 weeks did not have effects on remission induced by standard medical treatment was found, but an improvement of histology score and endoscopic activity index was observed (50). In two

large trials the effects of *E. coli* Nissle 1917 were compared with the standard treatment mesalazine. Both trials demonstrated that *E. coli* Nissle 1917 was as effective in maintaining remission as mesalazine (54, 85). Two open label studies have demonstrated that VSL#3, a supplement that contains *L. casei, L. plantarum*, *L. acidophilus*, *L.* delbruecckii *bulgaricus*, *B. longum*, *B. breve*, *B. infantis* and *Streptococcus salivarius thermophilus*, maintained remission in 75 and 77% of the patients (7, 111). However without a placebo group no conclusions can be drawn from these results.

The supplement VSL#3 has also been used in patients with pouchitis, which is a complication that often occurs in IBD patients who have had an ileal-pouch-anal-anastomosis. After this surgery, the reservoir often becomes inflamed. In two RDBPCTs it was demonstrated that VSL#3 was more effective in maintaining remission compared to the placebo group. In both studies remission was maintained in 85% of the patients, whereas in the placebo group only 6% (65) or 0% (38) were still in remission after one year. In addition, VSL3# was also used in a trial that has investigated the ability of probiotics to prevent development of pouchitis after ileal-pouch-anal-anastomosis. In the placebo group 40% of the patients developed pouchitis whereas VSL#3 significantly lowered this, since only 10% developed pouchitis within one year (37).

In conclusion, there is no evidence for efficacy of probiotics in Crohn's disease. Fur ulcerative colitis results obtained with *E. coli* Nissle 1917, showed that it was as effective as conventional treatment. These results should be confirmed in a placebo-controlled trial. The supplement VSL#3 appears to effective in maintaining remission and preventing onset of pouchitis.

3.9 Meta-analyses on probiotics

3.9.1 Antibiotic-associated diarrhea

Antibiotic-associated diarrhea occurs in 5-30% of the patients that use antibiotics and severe cases are associated with *Clostridium difficile* infection. The rationale behind the use of probiotics is based on the assumption that antibiotics disturb the intestinal microflora and in that way can cause diarrhea. A total of six meta-analyses have evaluate the efficacy of probiotics on antibiotic-associated diarrhea (16, 20, 46, 63, 101). The results are summarized in Appendix 7

It is important to note that although all meta-analysis demonstrate that probiotics may prevent diarrhea, this does not mean that all studies included in these meta-analyses were protective. In the meta-analysis performed bij D'Souza et al. (20) only in six trials these beneficial effects were demonstrated, in three other trials no effects were observed. In the meta-analysis of Cremonini et al. (16) three studies demonstrated a significant reduction of diarrhea by probiotics, whereas two studies showed a reduction which was not significant, and in one study no effects were shown. In another meta-analysis only RDBPCTs with the yeast *S. boulardii* were included (101). In three of these five trials beneficial effects of *S. boulardii* were detected, whereas in two trials no effects were observed. Pooling all data showed that treatment with *S. boulardii* compared with placebo reduced the risk of diarrhea from 17.2% to 6.2%. This means that for every ten patients that receive antibiotics together with *S. boulardii* one fewer would develop diarrhea.

In the abovementioned meta-analyses most studies that were included involve adults. Szajewska et al. (102) have conducted a meta-analysis with six RDBPCTs that only have included children. Reduction of diarrhea was demonstrated significantly in four trials. Consumption of *L. acidophilus* + *B. infantis*, or *L. acidophilus* + *L. bulgaricus* did not result in a reduction of diarrhea. Overall, for every seven patients that develop antibiotic-associated diarrhea, one fewer will develop this when combining antibiotics with probiotics. A second meta-analysis that focused on pediatric patients was done with

data from six RDBPCTs (46). In this meta-analysis five of the trials included were the same as in the meta-analysis of Szajewska et al. (102). Significant reduction of risk to develop diarrhea was demonstrated in three trials. The combination of all trials showed that when probiotics are used one case of diarrhea would be prevented per six patients (46).

A drawback of all these meta-analyses is that they have used data from a limited number of trials. In a recent meta-analysis data from 31 RDBPCTs were included. Of these, 25 studied the effect on antibiotic-associated diarrhea and six studied the treatment of *Clostridium difficile* disease (63). In trials that investigated effects on antibiotic-associated diarrhea the most frequently used strains were *S. boulardii* (six trials) and LGG (six trials). A significant reduction of antibiotic-associated diarrhea was demonstrated in 13 of the 25 trials. The combined efficacy of these 25 trials showed that probiotics have a significant protective effect. When a subanalysis was done for the different probiotics used, it was shown that *S. boulardii*, LGG and mixtures of two different probiotics were significantly protective, while the use of single probiotic strains was not. In two of the six trials a reduction of recurrences of *Clostridium difficile* disease was demonstrated and in these trials *S. boulardii* was used. LGG and *L. plantarum* 299v were not effective. The combined efficacy of these six trials showed that probiotics have a significant protective effect. In 26 trials adverse effects of probiotics were monitored and only in two trials they were reported. *S. boulardii* was associated with thirst and constipation and LGG with bloating and gas.
In conclusion, these meta-analyses show that the probiotics LGG, *S. boulardii* and mixtures of probiotics may be effective in preventing antibiotic-associated diarrhea and that *S. boulardii* appears to be beneficial in *Clostridium difficile* disease.

3.9.2 Acute diarrhea

Appendix 8 summarizes the results of three meta-analyses that have investigated the effects of probiotics on acute diarrhea (43, 93, 110). Huang et al. (43) used 18 publications in their meta-analysis. This meta-analysis only included trials with children. In some publications more than one probiotic strain, different dosing groups, or viable or freeze-dried probiotics were used in one trial and this resulted in a total of 26 separate studies. All trials were randomized, but only ten were double-blind and placebo-controlled. This meta-analysis demonstrated that in children that received probiotics duration of diarrhea was shortened by 1 day. This was shown in 22 of the 26 studies. Subanalysis showed that LGG, used in 10 studies, reduced diarrhea by 1.2 days.

Efficacy of probiotics in preventing acute diarrhea was studied in another meta-analysis that included both adults and infants (93). In this meta-analysis the efficacy was evaluated per strain, age group, and cause of acute diarrhea. From the 34 included studies, 28 showed a significant protective effect and ten of these were statistically significant. This resulted in an overall reduction of diarrhea with 35%. When the cause of diarrhea was included in the analysis, it was shown that reduction was most convincing for antibiotic-associated diarrhea and inconclusive for traveller's diarrhea. Efficacy for diarrhea of diverse causes was significant. Furthermore, effects appeared to be more pronounced in children than in adults. *S. boulardii* seemed to be the most effective strain, followed by LGG. The combination of *L. acidophilus* and *L. bulgaricus* resulted in a non significant reduction. Furthermore, combination of strains appears to be more capable of reduction than single strains.
Van Niel et al. (110) only included trials that were randomized, double-blind and placebo controlled. Of the nine trials, seven could be used in the meta-analysis and six showed a reduced duration (0.7 day). In three studies the number of stools per day was reported and 1.6 less stools were calculated from this. Furthermore, a dose-response relationship between probiotic dose and reduction of diarrhea was found.

In conclusion, for acute diarrhea the results are not conclusive, since two meta-analysis included also trials that were not placebo-controlled. The meta-analysis of Van Niel et al. (110) suggests that probiotics can reduce the duration of acute diarrhea and this effect appears to be strain and dose dependent.

3.9.3 Effects on plasma cholesterol

One meta-analysis investigated the effects of a probiotic milk Gaio, containing *Enterococcus faecium* and two strains of *Streptococcus thermophilus*, on plasma cholesterol. In this meta-analysis five trials were enrolled, four were double blind and placebo controlled and one was a double blind cross-over study. Combining data from all trials showed that Gaio reduced total cholesterol with 0.23 mmol/liter and of LDL-cholesterol with 0.25 mmol/liter. This reduction was statistically significant and the reduction of LDL-cholesterol is clinically important.

3.9.4 Concluding remarks

It is important to bear in mind that the drawback of meta-analyses is the heterogeneity of the trials that are included. These differences include: age of the participants, number of patients, duration of treatment, probiotic strain used, single or multiple strains and dose of probiotics. It is well-documented that effects of probiotics are strain-dependent. Therefore, it would be preferable if a meta-analysis is performed with trials that use the same strain(s). However, this will reduce the number of studies that can be used and thereby the strength of the meta-analysis.

3.10 Safety of probiotics

In the papers discussed so far, hardly any adverse effects of probiotics are reported. The exception is the study performed by Taylor et al. (106). Administration of *L. acidophilus* to infants born from allergic parents in the first six months of their lives, significantly increased sensitization against several allergens at the age of 12 months. These results demonstrate that probiotics can stimulate the immune system. However, it is unknown if this increase in sensitization will also increase the incidence of allergic diseases in these infants. Follow-up of these infants for a longer period will provide insight in this.
Our Pubmed search has found some clinical trials that have investigated safety and tolerance of probiotics (summarized in Appendix 9).

Four studies have investigated safety and tolerance of infant formulas supplemented with probiotics (14, 82, 90, 117). All infant formulas were well-tolerated and no adverse effects were reported. Parameters that were assessed in these studies included growth, stool consistency and frequency, antibiotics use, colic, crying, respiratory infections and translocation to the blood. Intake of infant formula supplemented with *B. lactis* and *S. thermophilus* for an average of 7 months significantly reduced colic and also significantly lowered the use of antibiotics (90). Infant formula supplemented with *B. longum* and prebiotics significantly reduced constipation and these infants experienced less respiratory infections (not significant) (82).

Safety of the probiotic *L. lactis* was studied in HIV positive patients to investigate if probiotics are safe in these immunocompromised individuals. In this study no adverse effects were observed (122). Also, LGG was studied in HIV positive patients and no side effects were reported either (91). Colonization by and induction of bacteremia by LcS has been studied in critically ill children in the intensive care ward. Short-term exposure to LcS did not induce bacteremia in these patients (100). In healthy

volunteers, a combination of *B. longum* 2C and *B. longum* 46 consumed for 3 weeks did not induce any side effects. Safety of the yeast *S. boulardii* was studied in patients with peptic ulcers who were treated with antibiotics. No adverse effects were observed, but probiotic treatment significantly reduced the prevalence of diarrhea (27).

Adverse effects that can occur after ingestion of lactobacilli are bacteremia and sepsis. Several case-reports have described this. However, serious infections due to lactobacilli are rare and only isolated cases have been reported. These case-reports describe bacteremia, sepsis, and endocarditis in both infants and adults who received lactobacilli. For example, LGG has caused bacteremia in two infants (a 6 week old and a 6 year old) who were hospitalized for serious underlying diseases and received LGG to prevent antibiotics-associated diarrhea (57). In a 11 month old infant, with short gut syndrome, LGG induced bacteremia (21). Another probiotic, *L. acidophilus*, also caused bacteremia in an immunocompromised patient with AIDS and Hodgkin's disease (58). This probiotic also caused endocarditis in a 75-year old female (98). In an adult male with diabetes who consumed large quantities (up to 1.6 liters) of yoghurt supplemented with *L. rhamnosus* Lb12 endocarditis and septic arthritis was induced (81). Not only in immunocompromised individuals infections due to lactobacilli have been reported. The probiotic *L. paracasei* caused a pancreatic necrosis infection in an immunocompetent adult (126). The probiotic yeast *S. cerevisiae* can become pathogenic in immunocompromised individuals too (22, 68).

Hence, in patients with impaired immunity, gut anatomy or bowel integrity the use of probiotics can cause serious infectious and these patients should be either not treated with probiotics or be under strict surveillance.

One case report has described another adverse effect that can occur in infants that are allergic to cow's milk. Intake of two different probiotic brands caused allergic symptoms in four milk-allergic infants. These probiotic products contained residual milk proteins, which elicited the allergic reactions (66).

4 Discussion and conclusion

In this report an overview is given of the clinical trials that have been published on immune effects of probiotics. From this overview it can be concluded that evidence for most immune effects is inconclusive. In addition to immune effects, information from meta-analyses was included and these demonstrate that probiotics may be effective in the prevention of antibiotics-associated diarrhea.

Data published on prevention of rhinitis and asthma, enhancement of the immune system, rheumatoid arthritis and Crohn's disease are either not convincing or inconclusive. In addition, data published on effects of probiotics on immune resistance, vaccination responses, atopic eczema, and ulcerative colitis suggest that some probiotics may be effective, but more research is needed. The best evidence for efficacy has been obtained for pouchitis and antibiotics-associated diarrhea. Hence, based on the current literature evidence for direct effects of probiotics on the gut and intestinal disorders is more convincing than effects on the immune system.

It is important to note that many papers published in this field are based on small trials and that endpoints that are assessed are not always clinically relevant. For instance, probiotics appear to have beneficial effects on rhinitis, but only subjective symptoms are improved. The exception is *B. longum* BB536, which appears to be effective in reducing subjective symptoms and pollen-specific IgE in patients suffering from rhinitis triggered by Japanese cedar pollen (123-125).

In trials that have investigated effects on the immune system of volunteers effects on NK activity or phagocytosis were used as a read-out. Many of these trials were very small or did not include a placebo group. Therefore, no conclusions can be drawn, since they can reflect fluctuations that are normally present in individuals. In addition, an increase of NK activity or phagocytosis cannot be translated in a health benefit directly. This should be done by demonstrating reduced susceptibility to infections or effects on tumour development. Only a few trials have investigated the effects of probiotics on infections. In infants beneficial effects of probiotics were reported, such as reduced antibiotics use, reduced episodes of fever and diarrhea, and day care absence due to illness (41, 118). However, the effect sizes in these studies were very small and the clinical relevance for the general population is unknown. In adults, *L. reuteri* decreased sick leave, especially in shift workers (108) and a combination of *L. gasseri, B. longum* and *B. bifidum* reduced the duration of common cold with almost two days (24, 25). These results suggest that certain probiotics can beneficially influence the immune system and reduce illness due to infections. However, more studies should be done to confirm these results.

To assess effects of an intervention on the immune system, a read-out system that is often recommended (19), is measurement of vaccination responses. For probiotics, only a few studies have done this. Some probiotics did not affect vaccination responses, but LGG enhanced polio-specific antibody titres (23) and *L. fermentum* increased influenza-specific antibody titres and reduced the incidence of influenza-like illnesses (70). These results indicate that probiotics can affect immune responses, and that it appears to beneficially improve the effectiveness of influenza vaccination. Again, more clinical trials are needed to confirm these results and to demonstrate the clinical relevance.

Efficacy of probiotics in alleviation or prevention of atopic eczema has been studied in several trials. A number of of these studies suggest beneficial effects on alleviation of symptoms, especially in infants with IgE-associated eczema (1, 52, 87, 97, 114), whereas one study did not demonstrate any beneficial effects at all (9). For some probiotics the results are promising, but it is too early to advise the use of probiotics for therapeutic purposes. Many trials were small and in addition the positive effects seem to be confined to IgE-associated eczema. Hence, larger controlled studies with well-defined probiotic

strains or mixtures of strains should be performed to obtain reliable information. A few studies have investigated effects in prevention of atopic manifestations, by including high-risk infants, e.g. from atopic parents, in their intervention studies. It was demonstrated that LGG prevented development of atopic eczema at age of 2, 4 and 7. However, allergic rhinitis and asthma tended to be more common in the probiotic group. The frequency of atopic sensitization was similar between the groups, suggesting that the preventive effect on eczema was not IgE-mediated (47-49). *L. acidophilus* failed to induce any reduction of atopic eczema and induced a concerning increase in sensitization (106). This is the first trial that has reported adverse outcomes, although the significance in terms of development of allergic diseases later in life is unknown and should be further investigated in these infants. However, these data demonstrate that at the moment probiotics should not be recommended for allergy prevention. More insight in the effects of supplementation early in life should be obtained, both short-term and long-term effects. Allergic manifestations, such as asthma and rhinitis, develop at a later age (3-5 years), hence infants should be monitored for a longer period to assess effects on these allergic diseases. Currently, several similar trials are ongoing, for instance in the Netherlands (120 infants), UK (600 infants), Australia and New Zealand (450 infants), Singapore (300 infants) (80), which will provide more information on the efficacy and safety of probiotics in allergy prevention.

In all these trials high-risk infants are included and therefore it is impossible to establish if the use of probiotics has favourable effects in infants in the general population. A few studies have investigated if infant formulas supplemented with probiotics were safe. In these studies no adverse short-term effects were demonstrated and probiotics were well-tolerated (14, 82, 90, 117). In these studies predominantly general parameters were assessed, for instance growth. The benefits for the infants that received the probiotics include reduced colic and lower use of antibiotics (90) and reduced constipation (82). No information on long-term effects were assessed in these studies. Therefore, although probiotics appear to induce no adverse short-term effect in infants, studies should also assess long-term effects.

There is no evidence for efficacy of probiotics in Crohn's disease, but probiotics may be effective in ulcerative colitis. The probiotic *E. coli* Nissle 1917 was as effective in maintaining remission as standard treatment (54, 85). However, in both studies no placebo group was included and therefore it is unknown what the relapse rate is without any treatment at all. Especially, since relapse rates were very different in those studies, around 70% and 35%, a placebo group is necessary in order to correctly interpret these data. Probiotics may be effective in the prevention and treatment of pouchitis, which is one of the complications that can occur in IBD patients after surgery. A supplement called VSL#3 (which contains eight strains of bacteria) was more effective in maintaining remission than the placebo (38, 65) and VSL#3 furthermore prevented the onset of pouchitis after surgery (37). It can therefore be concluded that the probiotic VSL#3 shows the potential in both prophylactic and maintenance therapy for pouchitis.

In addition to clinical trials, this report has also included meta-analyses on probiotics. In a meta-analysis data from different trials are pooled in order to determine efficacy of an intervention. One of the complicating factors by doing meta-analysis on probiotics, is the strain-dependency of effects elicited by different probiotics. Therefore, it is unknown what the strength is of such a meta-analysis. However, some of the published meta-analysis have included many trials, making it possible to perform a subanalysis for a specific strain. It was demonstrated that *S. boulardii* and LGG may be effective in preventing antibiotic-associated diarrhea (63). For acute diarrhea less meta-analysis have been performed. In general, all meta-analysis showed that probiotics can shorten the duration of diarrhea with approximately 1 day and that especially *S. boulardii* and LGG were effective (43, 93, 110). In conclusion, probiotics can have beneficial effects in acute and antibiotic-associated diarrhea, and these effects depend on the strain used.

The new EU regulations on health claims will force manufacturer's that want to make a health claim intended for infants or on reduction of disease, to provide a dossier with scientific data that support this

claim. This will eventually improve the insight into efficacy and prevent misleading claims on products. However, in this new EU regulation there is no need to demonstrate if a probiotic strain is safe. In addition, probiotics that were on the market before 1997 are considered as safe (Novel Foods Regulation 258/97/EC). Until now, no adverse effects are reported in the general population, except for some isolated cases of infections due to lactobacilli in individuals who were severely immunocompromised. However, it might be possible that due to publication bias not all data on safety and efficacy obtained for probiotics are published. Furthermore, development of new applications, such as infant formulas, will change the population that uses probiotics. The assumption that probiotics can be considered as safe, because of long term consumption by adults cannot be extrapolated to infants, because they are more susceptible to exogenous factors, especially immunomodulating factors. The immune system is still in development early in life and is therefore very vulnerable. Therefore, safety of probiotics intended for supplementation of infant formulas should be thoroughly assessed.

In conclusion, the public enthusiasm on efficacy of probiotics is for most of the immune effects not supported by scientific data. Although some promising results are achieved, especially on intestinal disorders, there is a need for more controlled trials with sufficient numbers of patients/volunteers. In addition, there is a lack of data on safety, and the recent paper on increased sensitization in infants who received *L. acidophilus* suggests that probiotics are not always harmless, friendly bacteria that only induce beneficial effects. Therefore, before supplementing infant formulas with probiotics and promoting them as beneficial, more research is needed to exclude induction of adverse (long-term) immune effects.

References

1. Abrahamsson TR, Jakobsson T, Bottcher MF, Fredrikson M, Jenmalm MC, Bjorksten B, Oldaeus G. Probiotics in prevention of IgE-associated eczema: A double-blind, randomized, placebo-controlled trial. Journal of Allergy and Clinical Immunology. 2007;119(5):1174-80.

2. Agerholm-Larsen L, Bell ML, Grunwald GK, Astrup A. The effect of a probiotic milk product on plasma cholesterol: a meta-analysis of short-term intervention studies. Eur J Clin Nutr. 2000;54(11):856-60.

3. Aggett PJ, Antoine JM, Asp NG, Bellisle F, Contor L, Cummings JH, Howlett J, Muller DJ, Persin C, Pijls LT, Rechkemmer G, Tuijtelaars S, Verhagen H. PASSCLAIM: consensus on criteria. Eur J Nutr. 2005;44 Suppl 1:i5-30.

4. Aldinucci C, Bellussi L, Monciatti G, Passali GC, Salerni L, Passali D, Bocci V. Effects of dietary yoghurt on immunological and clinical parameters of rhinopathic patients. Eur J Clin Nutr. 2002;56(12):1155-61.

5. Arunachalam K, Gill HS, Chandra RK. Enhancement of natural immune function by dietary consumption of Bifidobacterium lactis (HN019). Eur J Clin Nutr. 2000;54(3):263-7.

6. Baken KA, Ezendam J, Gremmer ER, de Klerk A, Pennings JL, Matthee B, Peijnenburg AA, van Loveren H. Evaluation of immunomodulation by *Lactobacillus casei* Shirota: Immune function, autoimmunity and gene expression. Int J Food Microbiol. 2006;112(1):8-18.

7. Bibiloni R, Fedorak RN, Tannock GW, Madsen KL, Gionchetti P, Campieri M, De Simone C, Sartor RB. VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol. 2005;100(7):1539-46.

8. Bousvaros A, Guandalini S, Baldassano RN, Botelho C, Evans J, Ferry GD, Goldin B, Hartigan L, Kugathasan S, Levy J, Murray KF, Oliva-Hemker M, Rosh JR, Tolia V, Zholudev A, Vanderhoof JA, Hibberd PL. A randomized, double-blind trial of Lactobacillus GG versus placebo in addition to standard maintenance therapy for children with Crohn's disease. Inflamm Bowel Dis. 2005;11(9):833-9.

9. Brouwer ML, Wolt-Plompen SA, Dubois AE, van der Heide S, Jansen DF, Hoijer MA, Kauffman HF, Duiverman EJ. No effects of probiotics on atopic dermatitis in infancy: a randomized placebo-controlled trial. Clin Exp Allergy. 2006;36(7):899-906.

10. Chiang BL, Sheih YH, Wang LH, Liao CK, Gill HS. Enhancing immunity by dietary consumption of a probiotic lactic acid bacterium (Bifidobacterium lactis HN019): optimization and definition of cellular immune responses. Eur J Clin Nutr. 2000;54(11):849-55.

11. Christensen HR, Larsen CN, Kaestel P, Rosholm LB, Sternberg C, Michaelsen KF, Frokiaer H. Immunomodulating potential of supplementation with probiotics: a dose-response study in healthy young adults. FEMS Immunol Med Microbiol. 2006;47(3):380-90.

12. Ciprandi G, Vizzaccaro A, Cirillo I, Tosca MA. Bacillus clausii effects in children with allergic rhinitis. Allergy. 2005;60(5):702-3.

13. Ciprandi G, Vizzaccaro A, Cirillo I, Tosca MA. Bacillus clausii exerts immuno-modulatory activity in allergic subjects: a pilot study. Allerg Immunol (Paris). 2005;37(4):129-34.

14. Connolly E, Abrahamsson T, Bjorksten B. Safety of D(-)-lactic acid producing bacteria in the human infant. J Pediatr Gastroenterol Nutr. 2005;41(4):489-92.

15. Coulthard MG, Mellis CM. Does probiotic milk prevent infections in children attending daycare centres? Med J Aust. 2004;181(10):556-7.

16. Cremonini F, Di Caro S, Nista EC, Bartolozzi F, Capelli G, Gasbarrini G, Gasbarrini A. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. Alimentary Pharmacology & Therapeutics. 2002;16(8):1461-7.

17. Cui HH, Chen CL, Wang JD, Yang YJ, Cun Y, Wu JB, Liu YH, Dan HL, Jian YT, Chen XQ. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. World J Gastroenterol. 2004;10(10):1521-5.

18. Cukrowska B, LodInova-ZadnIkova R, Enders C, Sonnenborn U, Schulze J, Tlaskalova-Hogenova H. Specific Proliferative and Antibody Responses of Premature Infants to Intestinal Colonization with Nonpathogenic Probiotic E. coli Strain Nissle 1917. Scandinavian Journal of Immunology. 2002;55(2):204-9.

19. Cummings JH, Antoine J-M, Azpiroz F, Bourdet-Sicard R, Brandtzaeg P, Calder PC, Gibson GR, Guarner F, Isolauri E, Pannemans D, Shortt C, Tuijterlaars S, Watzl B. PASSCLAIM - Gut health and immunity. European Journal of Nutrition. 2004;43(Suppl 2):ii/118 - II/73.

20. D'Souza AL, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. BMJ. 2002;324(7350):1361.

21. De Groote MA, Frank DN, Dowell E, Glode MP, Pace NR. Lactobacillus rhamnosus GG bacteremia associated with probiotic use in a child with short gut syndrome. Pediatr Infect Dis J. 2005;24(3):278-80.

22. de Llanos R, Querol A, Peman J, Gobernado M, Fernandez-Espinar MT. Food and probiotic strains from the Saccharomyces cerevisiae species as a possible origin of human systemic infections. International Journal of Food Microbiology. 2006;110(3):286-90.

23. De Vrese M, Rautenberg P, Laue C, Koopmans M, Herremans T, Schrezenmeir J. Probiotic bacteria stimulate virus-specific neutralizing antibodies following a booster polio vaccination. Eur J Nutr. 2005;44(7):406-13.

24. De Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J. Effect of Lactobacillus gasseri PA 16/8, Bifidobacterium longum SP 07/3, B. bifidum MF 20/5 on common cold episodes: a double blind, randomized, controlled trial. Clin Nutr. 2005;24(4):481-91.

25. De Vrese M, Winkler P, Rautenberg P, Harder T, Noah C, Laue C, Ott S, Hampe J, Schreiber S, Heller K, Schrezenmeir J. Probiotic bacteria reduced duration and severity but not the incidence of common cold episodes in a double blind, randomized, controlled trial. Vaccine. 2006;24(44-46):6670-4.

26. Donnet-Hughes A, Rochat F, Serrant P, Aeschlimann JM, Schiffrin EJ. Modulation of Nonspecific Mechanisms of Defense by Lactic Acid Bacteria: Effective Dose. J Dairy Sci. 1999;82(5):863-9.

27. Duman DG, Bor S, Ozutemiz O, Sahin T, Oguz D, Istan F, Vural T, Sandkci M, Isksal F, Simsek I, Soyturk M, Arslan S, Sivri B, Soykan I, Temizkan A, Bessk F, Kaymakoglu S, Kalayc C. Efficacy and safety of Saccharomyces boulardii in prevention of antibiotic-associated diarrhoea due to Helicobacterpylori eradication. Eur J Gastroenterol Hepatol. 2005;17(12):1357-61.

28. Ezendam J, Baken KA, Van Loveren H. Immune effects of Lactobacillus casei Shirota. RIVM report 340320004. 2006.

29. Ezendam J, Van Loveren H. Immune effects of the probiotic *Bifidobacterium breve*. RIVM report 340320006. 2006.

30. Ezendam J, Van Loveren H. Long-term immune effects of Lactobacillus casei Shirota during lactation. RIVM report 340320005. 2006.

31. Ezendam J, van Loveren H. Probiotics: immunomodulation and evaluation of safety and efficacy. Nutr Rev. 2006;64(1):1-14.

32. Ezendam J, Van Loveren H. *Lactobacillus casei* Shirota administered during lactation increases the duration of autoimmunity in rats and enhances lung inflammation in mice. Br J Nutr. 2007;in press.

33. Fang H, Elina T, Heikki A, Seppo S. Modulation of humoral immune response through probiotic intake. FEMS Immunol Med Microbiol. 2000;29(1):47-52.

34. FAO/WHO. 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. Available at ftp://ftp.fao.org/es/esn/food/wgreport2.pdf. London, Ontario: FAO/WHO Working group; 2002.

35. Gill HS, Rutherfurd KJ, Cross ML. Dietary Probiotic Supplementation Enhances Natural Killer Cell Activity in the Elderly: An Investigation of Age-Related Immunological Changes. Journal of Clinical Immunology. 2001;V21(4):264-71.

36. Gill HS, Rutherfurd KJ, Cross ML, Gopal PK. Enhancement of immunity in the elderly by dietary supplementation with the probiotic Bifidobacterium lactis HN019. Am J Clin Nutr. 2001;74(6):833-9.

37. Gionchetti P, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology. 2003;124(5):1202-9.

38. Gionchetti P, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology. 2000;119(2):305-9.

39. Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in Maintenance Treatment of Crohn's Disease. Digestive Diseases and Sciences. 2000;V45(7):1462-4.

40. Hatakka K, Martio J, Korpela M, Herranen M, Poussa T, Laasanen T, Saxelin M, Vapaatalo H, Moilanen E, Korpela R. Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis--a pilot study. Scand J Rheumatol. 2003;32(4):211-5.

41. Hatakka K, Savilahti E, Ponka A, Meurman JH, Poussa T, Nase L, Saxelin M, Korpela R. Effect of long term consumption of probiotic milk on infections in children attending day care centres: double blind, randomised trial. BMJ. 2001;322(7298):1327.

42. Helin T, Haahtela S, Haahtela T. No effect of oral treatment with an intestinal bacterial strain, Lactobacillus rhamnosus (ATCC 53103), on birch-pollen allergy: a placebo-controlled double-blind study. Allergy. 2002;57(3):243-6.

43. Huang JS, Bousvaros A, Lee JW, Diaz A, Davidson EJ. Efficacy of Probiotic Use in Acute Diarrhea in Children: A Meta-Analysis. Digestive Diseases and Sciences. 2002;V47(11):2625-34.

44. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized Controlled Trial of the Effect of Bifidobacteria-Fermented Milk on Ulcerative Colitis. J Am Coll Nutr. 2003;22(1):56-63.

45. Isolauri E, Arvola T, Sutas Y, Moilanen E, Salminen S. Probiotics in the management of atopic eczema. Clin Exp Allergy. 2000;30(11):1604-10.

46. Johnston BC, Supina AL, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized placebo-controlled trials. CMAJ. 2006;175(4):377-83.

47. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet. 2001;357(9262):1076-9.

48. Kalliomaki M, Salminen S, Poussa T, Arvilommi H, Isolauri E. Probiotics and prevention of atopic disease: 4-year follow-up of a randomised placebo-controlled trial. Lancet. 2003;361(9372):1869-71.

49. Kalliomaki M, Salminen S, Poussa T, Isolauri E. Probiotics during the first 7 years of life: A cumulative risk reduction of eczema in a randomized, placebo-controlled trial. Journal of Allergy and Clinical Immunology. 2007;119(4):1019-21.

50. Kato K, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A, Otsuka M, Hasunuma O, Kurihara R, Iwasaki A, Arakawa Y. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. Aliment Pharmacol Ther. 2004;20(10):1133-41.

51. Kim H-S, Park H, Cho I-Y, Paik H-D, Park E. Dietary Supplementation of Probiotic Bacillus polyfermenticus, Bispan Strain, Modulates Natural Killer Cell and T Cell Subset Populations and Immunoglobulin G Levels in Human Subjects. Journal of Medicinal Food. 2006;9(3):321-7.

52. Kirjavainen PV, Arvola T, Salminen SJ, Isolauri E. Aberrant composition of gut microbiota of allergic infants: a target of bifidobacterial therapy at weaning? Gut. 2002;51(1):51-5.

53. Kirjavainen PV, Salminen SJ, Isolauri E. Probiotic bacteria in the management of atopic disease: underscoring the importance of viability. J Pediatr Gastroenterol Nutr. 2003;36(2):223-7.

54. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut. 2004;53(11):1617-23.

55. Kuhbacher T, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, Gionchetti P, Blaut M, Campieri M, Folsch UR, Kamm MA, Schreiber S. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis

10.1136/gut.2005.078303. Gut. 2006;55(6):833-41.

56. Kuisma J, Mentula S, Jarvinen H, Kahri A, Saxelin M, Farkkila M. Effect of Lactobacillus rhamnosus GG on ileal pouch inflammation and microbial flora. Aliment Pharmacol Ther. 2003;17(4):509-15.

57. Land MH, Rouster-Stevens K, Woods CR, Cannon ML, Cnota J, Shetty AK. Lactobacillus sepsis associated with probiotic therapy. Pediatrics. 2005;115(1):178-81.

58. Ledoux D, Labombardi VJ, Karter D. Lactobacillus acidophilus bacteraemia after use of a probiotic in a patient with AIDS and Hodgkin's disease. Int J STD AIDS. 2006;17(4):280-2.

59. Macfarlane S, Macfarlane GT, Cummings JH. Review article: prebiotics in the gastrointestinal tract. Aliment Pharmacol Ther. 2006;24(5):701-14.

60. Majamaa H, Isolauri E. Probiotics: a novel approach in the management of food allergy. J Allergy Clin Immunol. 1997;99(2):179-85.

61. Makelainen H, Tahvonen R, Salminen S, Ouwehand AC. In vivo safety assessment of two Bifidobacterium longum strains. Microbiol Immunol. 2003;47(12):911-4.

62. Marteau P, Lemann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soule JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. Gut. 2006;55(6):842-7.

63. McFarland LV. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of Clostridium difficile disease. Am J Gastroenterol. 2006;101(4):812-22.

64. Meyer AL, Micksche M, Herbacek I, Elmadfa I. Daily intake of probiotic as well as conventional yoghurt has a stimulating effect on cellular immunity in young healthy women. Ann Nutr Metab. 2006;50(3):282-9.

65. Mimura T, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. Gut. 2004;53(1):108-14.

66. Moneret-Vautrin DA, Morisset M, Cordebar V, Codreanu F, Kanny G. Probiotics may be unsafe in infants allergic to cow's milk. Allergy. 2006;61(4):507-8.

67. Morita H, He F, Kawase M, Kubota A, Hiramatsu M, Kurisaki J, Salminen S. Preliminary Human Study for Possible Alteration of Serum Immunoglobulin E Production in Perennial Allergic Rhinitis with Fermented Milk Prepared with Lactobacillus gasseri TMC0356. Microbiol Immunol. 2006;50(9):701-6.

68. Munoz P, Bouza E, Cuenca-Estrella M, Maria Eiros J, Jesus Perez M, Sanchez-Somolinos M, Rincon C, Hortal J, Pelaez T. Saccharomyces cerevisiae Fungemia: An Emerging Infectious Disease. Clin Infect Dis. 2005;40(11):1625-34.

69. Olivares M, Diaz-Ropero MP, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, Martin R, Rodriguez JM, Xaus J. The consumption of two new probiotic strains, Lactobacillus gasseri CECT 5714 and Lactobacillus coryniformis CECT 5711, boosts the immune system of healthy humans. Int Microbiol. 2006;9(1):47-52.

70. Olivares M, Diaz-Ropero MP, Sierra S, Lara-Villoslada F, Fonolla J, Navas M, Rodriguez JM, Xaus J. Oral intake of Lactobacillus fermentum CECT5716 enhances the effects of influenza vaccination. Nutrition. 2007;23(3):254-60.

71. Olivares M, Paz Diaz-Ropero M, Gomez N, Sierra S, Lara-Villoslada F, Martin R, Miguel Rodriguez J, Xaus J. Dietary deprivation of fermented foods causes a fall in innate immune response. Lactic acid bacteria can counteract the immunological effect of this deprivation. J Dairy Res. 2006;73(4):492-8.

72. Ouwehand AC, Salminen S, Isolauri E. Probiotics: an overview of beneficial effects. Antonie Van Leeuwenhoek. 2002;82(1-4):279-89.

73. Parra D, Martinez De Morentin B, Cobo J, Mateos A, Martinez A. Monocyte function in healthy middle-aged people receiving fermented milk containing Lactobacillus casei. J Nutr Health Aging. 2004;8(4):208-11.

74. Parra MD, Martinez de Morentin BE, Cobo JM, Mateos A, Martinez JA. Daily ingestion of fermented milk containing Lactobacillus casei DN114001 improves innate-defense capacity in healthy middle-aged people. J Physiol Biochem. 2004;60(2):85-91.

75. Pelto, Isolauri, Lilius, Nuutila, Salminen. Probiotic bacteria down-regulate the milk-induced inflammatory response in milk-hypersensitive subjects but have an immunostimulatory effect in healthy subjects. Clin Exp Allergy. 1998;28(12):1474-9.

76. Peng G-C, Hsu C-H. The efficacy and safety of heat-killed Lactobacillus paracasei for treatment of perennial allergic rhinitis induced by house-dust mite. Pediatric Allergy and Immunology. 2005;16(5):433-8.

77. Pessi T, Sutas Y, Hurme M, Isolauri E. Interleukin-10 generation in atopic children following oral Lactobacillus rhamnosus GG. Clin Exp Allergy. 2000;30(12):1804-8.

78. Pohjavuori E, Viljanen M, Korpela R, Kuitunen M, Tiittanen M, Vaarala O, Savilahti E. *Lactobacillus* GG effect in increasing IFN-gamma production in infants with cow's milk allergy. J Allergy Clin Immunol. 2004;114(1):131-6.

79. Prantera C, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. Gut. 2002;51(3):405-9.

80. Prescott SL, Bjorksten B. Probiotics for the prevention or treatment of allergic diseases. Journal of Allergy and Clinical Immunology. 2007;120(2):255-62.

81. Presterl E, Kneifel W, Mayer HK, Zehetgruber M, Makristathis A, Graninger W. Endocarditis by Lactobacillus rhamnosus due to yoghurt ingestion? Scand J Infect Dis. 2001;33(9):710-4.

82. Puccio G, Cajozzo C, Meli F, Rochat F, Grathwohl D, Steenhout P. Clinical evaluation of a new starter formula for infants containing live Bifidobacterium longum BL999 and prebiotics. Nutrition. 2007;23(1):1-8.

83. Rautava S, Arvilommi H, Isolauri E. Specific Probiotics in Enhancing Maturation of IgA Responses in Formula-Fed Infants. Pediatr Res. 2006.

84. Rautava S, Kalliomaki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. J Allergy Clin Immunol. 2002;109(1):119-21.

85. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon ATR. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. The Lancet. 1999;354(9179):635-9.

86. Rinne M, Kalliomaki M, Arvilommi H, Salminen S, Isolauri E. Effect of probiotics and breastfeeding on the bifidobacterium and lactobacillus/enterococcus microbiota and humoral immune responses. J Pediatr. 2005;147(2):186-91.

87. Rosenfeldt V, Benfeldt E, Nielsen SD, Michaelsen KF, Jeppesen DL, Valerius NH, Paerregaard A. Effect of probiotic *Lactobacillus* strains in children with atopic dermatitis. J Allergy Clin Immunol. 2003;111(2):389-95.

88. Rosenfeldt V, Benfeldt E, Valerius NH, Paerregaard A, Michaelsen KF. Effect of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. J Pediatr. 2004;145(5):612-6.

89. Saarela M, Lahteenmaki L, Crittenden R, Salminen S, Mattila-Sandholm T. Gut bacteria and health foods--the European perspective. Int J Food Microbiol. 2002;78(1-2):99-117.

90. Saavedra JM, Abi-Hanna A, Moore N, Yolken RH. Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr. 2004;79(2):261-7.

91. Salminen MK, Tynkkynen S, Rautelin H, Poussa T, Saxelin M, Ristola M, Valtonen V, Jarvinen A. The efficacy and safety of probiotic Lactobacillus rhamnosus GG on prolonged, noninfectious diarrhea in HIV Patients on antiretroviral therapy: a randomized, placebo-controlled, crossover study. HIV Clin Trials. 2004;5(4):183-91.

92. Salminen S, von Wright A, Morelli L, Marteau P, Brassart D, de Vos WM, Fonden R, Saxelin M, Collins K, Mogensen G, Birkeland SE, Mattila-Sandholm T. Demonstration of safety of probiotics -- a review. Int J Food Microbiol. 1998;44(1-2):93-106.

93. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. Lancet Infect Dis. 2006;6(6):374-82.

94. Schultz M, Timmer A, Herfarth HH, Sartor RB, Vanderhoof JA, Rath HC. Lactobacillus GG in inducing and maintaining remission of Crohn's disease. BMC Gastroenterol. 2004;4:5.

95. Sheih Y-H, Chiang B-L, Wang L-H, Liao C-K, Gill HS. Systemic Immunity-Enhancing Effects in Healthy Subjects Following Dietary Consumption of the Lactic Acid Bacterium Lactobacillus rhamnosus HN001. J Am Coll Nutr. 2001;20(2):149-56.

96. Shen B, Brzezinski A, Fazio VW, Remzi FH, Achkar JP, Bennett AE, Sherman K, Lashner BA. Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. Aliment Pharmacol Ther. 2005;22(8):721-8.

97. Sistek D, Kelly R, Wickens K, Stanley T, Fitzharris P, Crane J. Is the effect of probiotics on atopic dermatitis confined to food sensitized children? Clinical & Experimental Allergy. 2006;36(5):629-33.

98. Soleman N, Laferl H, Kneifel W, Tucek G, Budschedl E, Weber H, Pichler H, Mayer HK. How safe is safe?--a case of Lactobacillus paracasei ssp. paracasei endocarditis and discussion of the safety of lactic acid bacteria. Scand J Infect Dis. 2003;35(10):759-62.

99. Spanhaak S, Havenaar R, Schaafsma G. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans. Eur J Clin Nutr. 1998;52(12):899-907.

100. Srinivasan R, Meyer R, Padmanabhan R, Britto J. Clinical safety of Lactobacillus casei shirota as a probiotic in critically ill children. J Pediatr Gastroenterol Nutr. 2006;42(2):171-3.

101. Szajewska H, Mrukowicz J. Meta-analysis: non-pathogenic yeast Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacol Ther. 2005;22(5):365-72.

102. Szajewska H, Ruszczynski M, Radzikowski A. Probiotics in the prevention of antibiotic-associated diarrhea in children: A meta-analysis of randomized controlled trials. The Journal of Pediatrics. 2006;149(3):367.

103. Takeda K, Okumura K. Effects of a Fermented Milk Drink Containing Lactobacillus casei Strain Shirota on the Human NK-Cell Activity. J Nutr. 2007;137(3):791S-3.

104. Takeda K, Suzuki T, Shimada SI, Shida K, Nanno M, Okumura K. Interleukin-12 is involved in the enhancement of human natural killer cell activity by Lactobacillus casei Shirota. Clin Exp Immunol. 2006;146(1):109-15.

105. Tamura M, Shikina T, Morihana T, Hayama M, Kajimoto O, Sakamoto A, Kajimoto Y, Watanabe O, Nonaka C, Shida K, Nanno M. Effects of Probiotics on Allergic Rhinitis Induced by Japanese Cedar Pollen: Randomized Double-Blind, Placebo-Controlled Clinical Trial. Int Arch Allergy Immunol. 2006;143(1):75-82.

106. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: A randomized controlled trial. Journal of Allergy and Clinical Immunology. 2007;119(1):184-91.

107. Taylor AL, Hale J, Wiltschut J, Lehmann H, Dunstan JA, Prescott SL. Effects of probiotic supplementation for the first 6 months of life on allergen- and vaccine-specific immune responses. Clinical & Experimental Allergy. 2006;36(10):1227-35.

108. Tubelius P, Stan V, Zachrisson A. Increasing work-place healthiness with the probiotic Lactobacillus reuteri: a randomised, double-blind placebo-controlled study. Environ Health. 2005;4:25.

109. Van Niel CW. Probiotics: not just for treatment anymore. Pediatrics. 2005;115(1):174-7.

110. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus Therapy for Acute Infectious Diarrhea in Children: A Meta-analysis. Pediatrics. 2002;109(4):678-84.

111. Venturi A, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther. 1999;13(8):1103-8.

112. Viljanen M, Kuitunen M, Haahtela T, Juntunen-Backman K, Korpela R, Savilahti E. Probiotic effects on faecal inflammatory markers and on faecal IgA in food allergic atopic eczema/dermatitis syndrome infants. Pediatr Allergy Immunol. 2005;16(1):65-71.

113. Viljanen M, Pohjavuori E, Haahtela T, Korpela R, Kuitunen M, Sarnesto A, Vaarala O, Savilahti E. Induction of inflammation as a possible mechanism of probiotic effect in atopic eczema-dermatitis syndrome. Journal of Allergy and Clinical Immunology. 2005;115(6):1254-9.

114. Viljanen M, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. Allergy. 2005;60(4):494-500.

115. Wang J. The Efficacy and Safety of Heat-Killed Lactobacillus paracasei for Treatment of Perennial Allergic Rhinitis Induced by House-Dust Mite. Pediatrics. 2006;118(Supplement 1):S23-.

116. Wang MF, Lin HC, Wang YY, Hsu CH. Treatment of perennial allergic rhinitis with lactic acid bacteria. Pediatr Allergy Immunol. 2004;15(2):152-8.

117. Weizman Z, Alsheikh A. Safety and tolerance of a probiotic formula in early infancy comparing two probiotic agents: a pilot study. J Am Coll Nutr. 2006;25(5):415-9.

118. Weizman Z, Asli G, Alsheikh A. Effect of a probiotic infant formula on infections in child care centers: comparison of two probiotic agents. Pediatrics. 2005;115(1):5-9.

119. Weston S, Halbert A, Richmond P, Prescott SL. Effects of probiotics on atopic dermatitis: a randomised controlled trial. Arch Dis Child. 2005;90(9):892-7.

120. Wheeler JG, Shema SJ, Bogle ML, Shirrell MA, Burks AW, Pittler A, Helm RM. Immune and clinical impact of Lactobacillus acidophilus on asthma. Ann Allergy Asthma Immunol. 1997;79(3):229-33.

121. Wildt S, Munck LK, Vinter-Jensen L, Hanse BF, Nordgaard-Lassen I, Christensen S, Avnstroem S, Rasmussen SN, Rumessen JJ. Probiotic treatment of collagenous colitis: a randomized, double-blind, placebo-controlled trial with Lactobacillus acidophilus and Bifidobacterium animalis subsp. Lactis. Inflamm Bowel Dis. 2006;12(5):395-401.

122. Wolf BW, Wheeler KB, Ataya DG, Garleb KA. Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus. Food and Chemical Toxicology. 1998;36(12):1085-94.

123. Xiao J-Z, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, Miyaji K, Iwatsuki K, Togashi H, Enomoto K, Enomoto T. Probiotics in the treatment of Japanese cedar pollinosis: a double-blind placebo-controlled trial. Clinical & Experimental Allergy. 2006;36(11):1425-35.

124. Xiao JZ, Kondo S, Yanagisawa N, Miyaji K, Enomoto K, Sakoda T, Iwatsuki K, Enomoto T. Clinical Efficacy of Probiotic Bifidobacterium longum for theTreatment of Symptoms of JapaneseCedar Pollen Allergy in SubjectsEvaluated in an EnvironmentalExposure Unit. Allergol Int. 2007;56(1):67-75.

125. Xiao JZ, Kondo S, Yanagisawa N, Takahashi N, Odamaki T, Iwabuchi N, Iwatsuki K, Kokubo S, Togashi H, Enomoto K, Enomoto T. Effect of probiotic Bifidobacterium longum BB536 in relieving clinical symptoms and modulating plasma cytokine levels of Japanese cedar pollinosis during the pollen season. A randomized double-blind, placebo-controlled trial. J Investig Allergol Clin Immunol. 2006;16(2):86-93.

126. Z'Graggen WJ, Fankhauser H, Lammer F, Bregenzer T, Conen D. Pancreatic necrosis infection due to Lactobacillus paracasei in an immunocompetent patient. Pancreatology. 2005;5(1):108-9.

Appendix 1: Effects of probiotics on atopic eczema and/or cow's milk allergy

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LGG, ATCC 53103)	1. 31 infants, 2.5-15.7 mo with atopic eczema	1. RDBPCT, a. extensively hydrolyzed whey, n=16 b. extensively hydrolyzed whey+LGG, n=15	1. $5x10^8$ CFU per day	1. 1 mo	1. Improvement of SCORAD in LGG group (p=0.008) not in placebo. Decrease of faecal TNF-α in LGG group (p=0.03), not in placebo.	60
	2. 11 breast-fed infants, 0.6-8.5 mo with atopic eczema	2. Open trial: LGG was given to mothers	2. 10^{10} CFU twice/day	2. 1 mo	2. Improvement of SCORAD from 26 to 11	
LGG, (ATCC 53103) or *B. lactis* Bb12	27 infants with early onset atopic eczema, exclusively breastfed, mean age 4.6 mo	RDBPCT 1. extensively hydrolyzed whey 2. same formula with LGG 3. same formula with *B. lactis* Bb12	$3x10^8$ CFU LGG/day $1x10^9$ CFU/*B. lactis*/day	6 mo	After 2 mo improvement of SCORAD in both probiotic groups compared to placebo (p=0.002) and decrease of serum sCD4 (p=0.005). Serum TGF-β decreased in *B. lactis* (p=0.04) and increased in LGG (p=0.07)	45
LGG (ATCC 53103)	9 infants 7-24 mo, with atopic eczema and CMA	RCT; no placebo group; LGG was given in capsules	10^{10} CFU twice/day	4 wk	Serum IL-10 increased between before and after treatment (p<0.001)	77
LGG (ATCC 53103)	62 pregnant women from atopic families; exclusively breastfed infants	RDBPCT: 1. Placebo n=32; 2. LGG n=30. Dosing 2-4 wk before expected delivery until infant was 3 mo	$2x10^{10}$ CFU/day	±4 mo	Higher concentration of TGF-β in breast milk in LGG group (p=0.018). Reduction of prevalence of atopic eczema in LGG group (relative risk 0.32; p=0.098)	84

Appendix 1 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LGG (ATCC 53103)	159 pregnant mothers from atopic families; 132 infants completed 4 yr follow-up	RDBPCT: 1. Placebo n=68; 2. LGG n=64. Dosing 2-4 wk before expected delivery until infant was 6 mo. In case of breastfeeding: mothers were given capsules, otherwise infants received capsule	10^{10} CFU twice/day.	±7 mo	Reduction of prevalence of atopic eczema at age of 2 in LGG group (relative risk 0.51; p=0.008) and at age of 4 (relative risk 0.57). At age of 4 rhinitis was diagnosed twice as often in LGG group (p=0.15)	47, 48
LGG (ATCC 53103)	35 infants, age 3.5-6.8 mo with suspected CMA	RDBPCT: 1. extensively hydrolyzed whey, n=8; 2. same formula with viable LGG, n=14; 3. same formula with heat-killed LGG, n=13	$3x10^{10}$ CFU/kg body weight/day	4 wk	Heat-inactivated LGG caused gastrointestinal symptoms, study was terminated earlier. Viable LGG decreased SCORAD (p=0.02) compared to placebo	52
LGG (ATCC 53103)	96 pregnant women from atopic families, breastfeeding was strongly encouraged	RDBPCT 1. Placebo, n=? 2. LGG, n=? Dosing 4 wk before expected delivery until infant was 6 mo of age	$1x10^{10}$ CFU/day	± 7 mo	No effect on number of IgM-, IgA- and IgG-secreting cells in blood. Combined effect of exclusive breastfeeding for at least 3 mo + probiotics: increase of number of IgM- (p=0.005), IgA- (p=0.03) and IgG-secreting cells (p=0.04)	86

Appendix 1 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LGG (ATCC 53103) MIX*	230 infants aged 1.4-11.9 mo with atopic dermatitis and symptoms suggestive of CMA	RDBPCT: 1. placebo, n=74, 2. LGG, n=80, 3. MIX, n=76	LGG: $5x10^9$ CFU/twice/day; MIX* twice/day	4 wk	SCORAD decreased in all groups, no significant differences. Subgroup analysis showed that LGG decreased SCORAD compared to placebo (p=0.036) in infants with IgE-associated eczema	114
LGG (ATCC 53103) MIX*	132 infants aged 1.4-11.5 mo with atopic dermatitis and symptoms suggestive of CMA	RDBPCT: 1. placebo, n=38, 2. LGG, n=52, 3. MIX, n=42	LGG: $5x10^9$ CFU/twice/day; MIX* twice/day	4 wk	Infants with IgE-associated eczema: LGG and MIX increased C-reactive protein and E-selectin compared to placebo (significant). MIX increased serum IL-10 (p=0.016).	113
LGG (ATCC 53103) or MIX*	129 infants aged 1.4-11.5 mo with atopic dermatitis and symptoms suggestive of CMA	RDBPCT: 1. placebo, n=36, 2. LGG, n=42, 3. MIX, n=41	LGG: $5x10^9$ CFU/twice/day; MIX* twice/day	4 wk	LGG: increased secretion of IFN-γ by PBMCs from infants with IgE-associated eczema (p=0.017) and with CMA (p=0.006). MIX: increase of IL-4 by PBMCs from infants with CMA	78
LGG (ATCC 53103) MIX*	230 infants aged 1.4-11.9 mo with atopic dermatitis and symptoms suggestive of CMA	RDBPCT: 1. placebo, n=74, 2. LGG, n=80, 3. MIX, n=76	LGG: $5x10^9$ CFU/twice/day; MIX* twice/day	4 wk	Faecal IgA higher in LGG (NS) and MIX (NS) compared to placebo. Faecal anti-trypsin decreased in LGG, not in other groups. Challenge with milk in IgE-associated CMA: LGG increased faecal IgA (p=0.014) and decreased TNF-α (NS) compared to placebo	112

Appendix 1 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. rhamnosus 19070-2 and *L. reuteri* DSM 122460	43 children from 1 to 13 yr with moderate to severe atopic dermatitis	Double blind crossover design A. Placebo – washout – probiotics B. Probiotics – washout – placebo 2 wk run-in, then 6 wk period for treatment, placebo or washout	10^{10} CFU of each strain twice/day	6 wk	Improvement of self-reported symptoms compared to placebo (p=0.001); SCORAD decreased during active treatment (p=0.06), not during placebo. Effect was more pronounced in patients with positive skin prick test and increased IgE levels	87
L. rhamnosus 19070-2 and *L. reuteri* DSM 122460	41 children from 1 to 13 yr with atopic dermatitis	Double blind crossover design A. Placebo – washout – probiotics B. Probiotics – washout – placebo 2 wk run-in, then 6 wk period for treatment, placebo or washout	10^{10} CFU of each strain twice/day	6 wk	Decrease in gastrointestinal symptoms compared to placebo (p=0.002); decrease in intestinal permeability compared to placebo (p=0.001).	88
L. rhamnosus and *B. lactis*	59 children aged 1.1-10.9 yr with atopic dermatitis	RDBPCT 1. Placebo, n=30 2. Probiotics, n=29	$2x10^{10}$ CFU/day	12 wk	Reduction of SCORAD score was greater in probiotic group compared to placebo (NS). In subpopulation of food sensitized children SCORAD improved more in probiotic group compared to placebo (p=0.01)	97

Appendix 1 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. acidophilus (LAVRI-A1)	178 pregnant atopic mothers	RDBPCT 1. Placebo n= 89 2. *L. acidophilus* n=89 Dosing started after birth	$3x10^9$ CFU/day	6 mo	Probiotics did not affect atopic dermatitis rate at 6 and 12 mo of age. Increased sensitization in probiotic group compared to placebo at 12 mo (p=0.045)	106
L. rhamnosus or LGG	50 infants less than 5 mo old with atopic dermatitis	RDBPCT 1. Placebo, n=17 2. *L.rhamnosus*, n=17 3. LGG, n=16	$5x10^9$ CFU/ml formula	3 mo	No effect on SCORAD, sensitization, inflammatory parameters, cytokine production	9
L. reuteri	188 pregnant atopic mothers	RDBPCT 1. Placebo n=93 2. *L. reuteri* n=95 Dosing 4 wk before expected delivery until infant was 12 mo.	$1x10^8$ CFU/day	±13 mo	No effect on incidence of atopic eczema until age of 2. IgE-associated eczema was lower in *L. reuteri* group (17%) than in placebo (28%). IgE-associated eczema and sensitization rates were significantly lower in infants with allergic mothers.	1
L. fermentum VRI003 PCC™	53 children aged 6-18 mo with moderate or severe atopic dermatitis	RDBPCT 1. Placebo, n=26 2. *L. fermentum,* n=27	$1x10^9$ CFU/twice/day	8 wk	Reduction of SCORAD in probiotic group at wk 8 (p=0.03) and 16 (p=0.06). SCORAD reduction in placebo group was not significant	119

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; RCT: randomized controlled trial; LGG: *Lactobacillus* GG; *B.: Bifidobacterium; L.: Lactobacillus*; CFU: colony forming units; mo: months, wk: weeks; CMA: cow's milk allergy; SCORAD: scoring atopic dermatitis; PBMCs : peripheral blood mononuclear cells; NS: not significant. * MIX: combination of probiotics LGG $5x10^9$ CFU, *L. rhamnosus* LC705 $5x10^9$ CFU, *B. Breve* Bbi99 $2x10^8$ CFU and *Propionibacterium freudenreichii* ssp. *Shermanii* JS $2x10^9$ CFU

Appendix 2: Effects of probiotics on rhinitis and asthma

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. acidophilus	15 patients with clinically confirmed asthma and/or rhinitis	Double-blind crossover 1. yoghurt 2. yoghurt with *L. acidophilus* 1 mo yoghurt, 4 wk washout, 1 mo *L. acidophilus* or vice versa	~ $9.3x10^{10}$ CFU/day	1 mo	Increase of IFN-γ production by lymphocytes (p=0.054) and decrease of eosinophilia (p=0.09) in *L. acidophilus* group. No improvement of quality of life and clinical parameters.	120
L. acidophilus and *Bifidobacterium*	11 patients with clinically confirmed allergic rhinitis	Study design unknown 1. Probiotics n=4 2. skimmed milk n=7 3. healthy volunteers, n=3	$1x10^6$ – $2x10^7$ CFU/g	4 mo	PBMCs released more IFN-γ and less IL-4 in probiotic group. Symptom score improved within the probiotic group (p=0.004) and not in milk group. No effect on serum IgE.	4
L. paracasei-33	80 patients with clinically confirmed allergic rhinitis	RDBPCT 1. fermented milk n=20 2. fermented milk+*L. paracasei* n=60	$2x10^9$ CFU/day	30 days	Improvement of quality of life in probiotic group compared to placebo (p=0.037). Improvement of nose symptoms within probiotic group (p=0.041)	116

L. paracasei-33	90 patients with clinically confirmed allergic rhinitis	RDBPCT 1. placebo, n=30 2. Living *L. paracasei* , n=30 3. Heat-killed *L. paracasei*, n=30	$5x10^9$ CFU/day	30 days	Symptom score reduced and quality of life improved within both probiotic groups (p=0.002 and p=0.011). Symptom score was reduced in both probiotic groups compared to placebo (p=0.000).	76

Appendix 2 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. rhamnosus ATCC53103	31 young adults and teenagers allergic to birch pollen and apple	RDBPCT 1. Placebo, n=16 2. *L. rhamnosus*, n=15	$5x10^9$ CFU/day	5.5 mo	No difference in nose, eye or lung symptoms	42
Bacillus clausii	20 children with allergic rhinitis due to pollen sensitization	Investigators were blinded 1. No treatment, n=10 2. *Bacillus clausii*, n=10	$2x10^9$ CFU/day	3 wk	No difference in symptom relief. Probiotics reduced nasal eosinophils (p=0.042) compared to placebo	12
Bacillus clausii	10 children with allergic rhinitis and 10 non-atopic healthy adults	Investigators were blinded, no placebo group	$2x10^9$ CFU/day	4 weeks	In allergic infants IFN-γ (p=0.038), TGF-β (p=0.039) and IL-10 (p=0.009) increased and IL-4 (p=0.004) decreased during treatment.	13
LcS	109 patients with allergic rhinitis triggered by Japanese cedar pollen	RDBPCT 1. Placebo, n=54 2. LcS, n=55	$4x10^{10}$ CFU/day	8 wk	No effect	105
L. gasseri TMC0356	15 subjects with high serum IgE levels and allergic rhinitis	No placebo group	$8.6x10^{10}$ CFU/day	28 days	Total IgE and pollen-specific IgE decreased in two subjects ($p<0.05$). Mean Th1/Th2 ratio increased during intervention ($p<0.01$)	67

Appendix 2 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
B. longum BB536	40 patients with allergic rhinitis triggered by Japanese cedar pollen	RDBPCT 1. Yoghurt, n=20 2. Yoghurt + *B. longum*, n=20	$3.5\pm2.4x10^{8}$ CFU/twice/day	14 wk	Probiotics reduced eye symptoms (p=0.044). No effect on IgE. Reduction blood eosinophils and increased IFN-γ production by PBMCs	125
B. longum BB536	44 patients with allergic rhinitis triggered by Japanese cedar pollen	RDBPCT 1.Placebo, n=22 2. *B. longum*, n=22	$5x10^{10}$ CFU/twice/day	13 wk	Probiotics reduced sneezing (p=0.023), rhinorrhea (p=0.006), nasal blockage (p=0.012), nasal itching (p=0.008), throat symptoms (0.028). Specific IgE tended to be reduced (p=0.067) and blood eosinophils were lower (NS). Decrease of Th2 chemokines in wk 4 and 8 (p=<0.05), no effect on IFN-γ	123
B. longum BB536	21 patients with allergic rhinitis triggered by Japanese cedar pollen	Double-blind crossover study in environmental exposure unit 1. Placebo – 2 wk washout – *B. longum, n*= 10 2. *B. longum* -2 wk washout – placebo, n=11	$5x10^{10}$ CFU/twice/day	4 wk	During pollen exposure: probiotics reduced ocular symptoms (p=0.033), After pollen exposure: probiotic group had less disruptions of daily routine (p=0.011), used less eye drops (p=0.047) and less total medication (p=0.041).	124

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; *L.: Lactobacillus*; LcS: *Lactobacillus casei* Shirota; *B.: Bifidobacterium;* CFU: colony forming units, mo: months, wk: weeks; PBMCs : peripheral blood mononuclear cells, NS: not significant

Appendix 3: Effects of probiotics on the immune system of healthy volunteers

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
E.coli Nissle 1917	61 premature infants	RDBPCT 1. placebo n=27 2. *E. coli* n=34	10^8 CFU for 5 days, then 10^8 CFU/3 times/wk	26 days	*E. coli* increased serum IgM at day 14 compared to placebo ($p<0.05$). No effect on serum IgA.	18
L. johnsonii La1	42 healthy subjects aged 21-57 yr	RDBPCT 1. Fermented milk 2. Fermented milk + *L. johnsonii* 3. Fermented milk + *L. johnsonii*	150 ml of fermented milk 2. $1.5x10^9$ CFU/day 3. $1.5x10^8$ CFU/day	3 wk	Increased phagocytic activity in the $1.5x10^9$ group ($p=0.019$) compared to placebo and not in the $1.5x10^8$ group. Six weeks after intervention, increased phagocytic activity decreased to pretreatment values	26
LGG (ATCC 53103) and *B. lactis* Bb12	72 infants that received artificial feeding before 2 mo of age	RDBPCT 1. infant formula n= 40 2. infant formula with LGG & *B.lactis* n=32	$1x10^{10}$ CFU/day of both probiotics	10-12 mo	Increase of cow's milk-specific IgA-secreting cells at 7 mo ($p=0.043$) compared to placebo. Increased sCD14 at 12 mo compared to placebo ($p=0.046$).	83
LGG (ATCC 53103)	9 healthy subjects 8 milk-hypersensitive subjects	Double blind placebo controlled cross-over study: 1 wk milk challenge – 1 wk washout - 1 wk milk challenge	$2x10^8$ CFU/twice/day	1 wk	In milk-hypersensitive subjects LGG prevented the increase of receptors on neutrophils and monocytes. In controls LGG increased receptors on neutrophils and monocytes	75

Appendix 3 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LcS	20 healthy males aged 40-65 yr	RDBPCT 1. unfermented milk, n= 10 2. LcS fermented milk, n=10	10^{11} CFU/three times/day	4 wk	No effects on immune parameters (NK cell activity, phagocytosis, cytokine production, humoral parameters)	99
LcS	10 healthy volunteers aged 69-97 years	Double blind placebo controlled cross-over study: 3 wk intervention – 7 wk washout – 3 wk intervention	$4x10^{10}$ CFU/day	3 wk	NK activity was higher during probiotic intervention compared to placebo ($p<0.05$),	104
LcS	9 healthy volunteers aged 30-45 yr and 10 volunteers aged 55-75	Double blind placebo controlled cross-over study 1. unfermented milk 2. LcS-fermented milk	$4x10^{10}$ CFU/day	3 wk	In probiotic group NK activity increased ($p<0.01$) in middle-aged group from wk 1 (NS) and wk 3 ($p=0.005$) and in elderly group NK activity was not affected by LcS but in controls NK activity decreased ($P<0.01$)	103
B. lactis HN019 DR10 *L. rhamnosus* HN001 DR20	27 healthy volunteers aged 60-84 yr	Double blind three-stage before-and-after intervention trial; Group 1: *L. rhamnosus*, n=13; Group 2: *B. lactis*, n=14; 3 wk run-in – 3 wk intervention – 3 wk washout	*L. rhamnosus* $5x10^{10}$CFU/ day; *B. lactis*: $5x10^{9}$ CFU/day	3 wk	Increase in NK cells in blood by *L. rhamnosus* ($p=0.0003$) and *B. lactis* ($p=0.0016$) and significant increase in NK activity by *L. rhamnosus* and *B. lactis*. Tumoricidal activity of PBMCs increased 101 and 62% for *B. lactis* and *L. rhamnosus*	35

Appendix 3 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
B. lactis HN019 DR10	50 healthy volunteers aged 41-81 yr	Double blind three-stage before-and-after intervention trial 1. 3 wk run-in – 2 wk *B. lactis* - 3 wk washout; n=27 2. 3 wk run-in -2 wk *B. lactis* + GOS – 3 wk washout; n=23	$2.5x10^{10}$ CFU/day	2 wk	*B. lactis* significantly increased phagocytosis at week 6 and 9 and NK activity at wk 9 ($p<0.05$). *B.lactis* + GOS significantly increased phagocytosis and NK activity at wk 6 and 9. Effects of *B.lactis* + GOS on NK activity were higher then *B. lactis* ($p=0.043$)	10
B. lactis HN019 DR10	25 healthy volunteers with minimum age of 60-83 yr	RDBPCT 1. milk 2. milk with *B. lactis*	$1.5x10^{11}$ CFU/twice/ day	6 wk	Mitogen-stimulated PBMCs produced more IFN-γ compared to placebo at wk 6 ($p=0.036$). Phagocytic capacity increased at wk 6 and 12 compared to placebo ($p<0.05$).	5
B. lactis HN019 DR10	30 healthy volunteers aged 63-85 yr	Double blind three-stage before-and-after intervention trial; 2 groups (n=15 per group) with different dosing: 3 wk run-in – 3 wk intervention – 3 wk washout	1: $5x10^{10}$CFU/ day 2. $5x10^{9}$CFU/ day	3 wk	Both groups at wk 6: increase of T lymphocytes ($p<0.05$) and NK cells ($p<0.01$) in blood and increased phagocytosis ($p<0.005$) and NK activity ($p<0.01$) compared to wk 0.	36
Bacillus polyfermenticus	25 healthy males aged 20-35 yr	RDBPCT 1. Placebo, n=12 2. *B. polyfermenticus*, n=13	$3.1x10^{8}$ CFU/day	8 wk	Increase of serum IgE ($p<0.05$), NK cells ($p<0.05$) and T cells ($p<0.05$) compared to placebo	51

Appendix 3 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. casei DN114001	45 healthy volunteers aged 51-58 yr	RDBPCT 1. placebo, n=22 2. *L. casei*, n=23	$9.5x10^{10}$ - 10^{12}/three times/day	8 wk	Increase in oxidative burst capacity (p=0.021), NK activity (p=0.023) within the probiotic group and not in placebo group.	74
L. casei DN114001	45 healthy volunteers aged 51-58 yr	RDBPCT 1. placebo, n=22 2. *L. casei*, n=23	$9.5x10^{10}$ - 10^{12}/three times/day	8 wk	Increase in oxidative burst capacity within the probiotic group (p=0.029) and not in placebo group	73
L. casei DN114001	33 healthy women aged 22-29 yr	RDBPCTs 1. conventional yoghurt, n=16 2. yoghurt with *L. casei*, n=17	$3.7x10^{10}$ CFU/day for 2 wk, followed by $3.7x10^{10}$/ twice/day	4 wk	In probiotic group increase of Tc cells after 2 wk (p=0.001) and 6 wk (p=0.002). Yoghurt and *L.* casei increased NK activity at wk 2 and 6 ($p<0.05$).	64
L. rhamnosus HN001	52 healthy volunteers aged 44-80 yr	Double blind before-and-after intervention trial 1. 3 wk run-in – 2 wk *L.rhamnosus* - 3 wk washout; n=25 2. 3 wk run-in -2 wk *L.rhamnosus* + GOS – 3 wk washout; n=27.	$2.5x10^{10}$ CFU/day	3 wk	*L. rhamnosus* and *L.rhamnosus* + GOS increased phagocytosis at week 6 and 9 ($p<0.01$) and NK activity at wk 6 and 9 ($p<0.01$). No differences between *L. rhamnosus* with or without GOS.	95

Appendix 3 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. gasseri CECT 5714 and *L. coryniformis* CECT 5711	30 healthy volunteers aged 23-43 yr	RDBPCT 1. conventional yoghurt, n=15 2. yoghurt with probiotics, n=15	$2x10^9$ CFU/day of each strain	2 wk	Probiotics and yoghurt increased phagocytic activity ($p<0.05$). NK cells increased by probiotics ($p<0.05$).	71
L. gasseri CECT 5714 and *L. coryniformis* CECT 5711	30 healthy volunteers aged 23-43 yr	RDBPCT 1. conventional yoghurt, n=15 2. yoghurt with probiotics, n=15	$2x10^9$ CFU/day of each strain	4 wk	Blood monocytes and neutrophils were increased at wk 2 in both groups. NK cells were increased in probiotic group at wk 2 and 4 compared to yoghurt group ($p<0.05$). In probiotic group serum IL-4 and IL-10 were increased at wk 2 ($p<0.05$) and serum IgA was increased at wk 4 and IgE was decreased at wk 2.	69
L. paracasei CRL431 and B. *animalis lactis* Bb-12	75 healthy volunteers aged 18-40 years	RDBPCT, n=15 1. Placebo 2. 10^8 Bb-12 and CRL431 3. 10^9 Bb-12 and CRL431 4. 10^{10} Bb-12 and CRL431 5. 10^{11} Bb-12 and CRL431	Dose-response	3 wk	No effects on phagocytic activity, fecal IgA, cytokine production by PBMCs.	11

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; *L.:Lactobacillus,* LGG: *Lactobacillus* GG; LcS: *L casei* Shirota; *E.: Escherichia; B.: Bifidobacterium;* GOS: galacto-oligosaccharides; Tc: cytotoxic T cells: CFU: colony forming units, yr: years; mo: months, wk: weeks; PBMCs : peripheral blood mononuclear cells; NK cells: natural killer cells

Appendix 4: Effects of probiotics on resistance against infections

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LGG (ATCC 53103)	Healthy infants attending day care centers aged 1-6 yr	RDBPCT 1. Milk, n=261 2. Milk with LGG, n=252	±1-2 10^8 CFU/day	7 mo (winter period)	LGG reduced antibiotics use for respiratory infections (p=0.08) and absence due to illness (p=0.03) .	41
B. lactis Bb12 or *L. reuteri* ATCC 55730	Infants attending day care centers aged 4 to 10 mo without atopic and chronic diseases	RDBPCT 1. Infant formula, n=60 2. Formula+*B.lactis*, n=73 3. Formula+*L. reuteri*, n=68	$1x10^7$ CFU/g formula	12 wk	*B. lactis* and *L. reuteri* (p<0.001) reduced days and episodes with fever and with diarrhea. *L. reuteri* reduced clinic visits (p=0.002), absences from child care (p=0.015), antibiotics prescriptions (p=0.037).	118
L. reuteri ATCC 55730	Healthy volunteers from employees of Tetrapak, day workers and shift workers	RDBPCT 1. Placebo, n=87 2. *L. reuteri*, n=94	$1x10^8$ CFU/day	80days	Reduction of persons reporting sick leave from 23% in placebo to 10% in *L. reuteri* group (p<0.01). Shift-workers reported 33% sick-leave in placebo and 0% in *L. reuteri* group (p<0.005)	108
L. gasseri PA16/8, *B. longum* SP07/3, *B. bifidum* MF20/5	Healthy volunteers aged 18-67 yr	RDBPCT 1. Placebo, n=241 2. Probiotics mixture, n=238	*L. gasseri* $5x10^7$, *B. longum* $5x10^6$, *B. bifidum* $5x10^6$ CFU/day	3 - 5.5 mo	Incidence of common cold was not affected. Probiotic group had lower total symptom score (p=0.056), duration of common cold (p=0.045), and days with fever (p=0.017); Increase of CD8+ T cells (p=0.035).	24, 25

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; *L.:Lactobacillus,* LGG: *Lactobacillus* GG; *B.: Bifidobacterium;* CFU: colony forming units, yr: years; mo: months, wk: weeks; PBMCs : peripheral blood mononuclear cells; NK cells: natural killer cells

Appendix 5: Effects of probiotics on vaccination

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
LGG or *L. lactis*	29 healthy adult volunteers aged 20-50 yr; oral vaccination with *Salmonella typhi* on days 1, 3 and 5	RDBPCT 1. Placebo, n=9 2. LGG, n=10 3. *L. lactis*, n=10	LGG: $4x10^{10}$ CFU/day; *L. lactis* $3.4x10^{10}$ CFU/day	7 days	No significant differences between the groups for IgA, IgG and IgM secreting cells	33
LGG or *L. acidophilus* CRL431	64 healthy male volunteers; orally vaccinated with polio vaccine type 1, 2 and 3 at day 8	RDBPCT 1. Milk, n=22 2. Milk with LGG, n=21 3. Milk with *L. acidophilus*, n=21	$1x10^{10}$ CFU/day	5 wk	Both probiotics increased neutralizing antibody titers against polio type 1 (2-fold; p=0.048 for LGG and p=0.91 for *L. acidophilus*) and type 2 (1.3-fold; p=0.014 for LGG and p=0.4 for *L. acidophilus*). Polio-specific IgG was increased 2.2-fold by LGG (p=0.083) and 1.7-fold by *L. acidophilus* (p=0.22). Polio-specific IgA was increased 3.9-fold by LGG (p=0.036) and 1.7-fold by. *L. acidophilus* (p=0.12)	23
L. acidophilus LAVRI-A1	231 pregnant women with a history of allergic disease	RDBPCT 1. Placebo, n=60 2. *L. acidophilus*, n=58 Dosing started directly after birth	$3x10^{9}$ CFU/day	6 mo	Probiotics attenuated IL-10 production by tetanus toxoid-stimulated PBMCS (p=0.03) at 6 mo of age and decreased frequency of detectable TNF-α (p=0.046) and IL-10 (p=0.014) responses against house dust mite	107

Appendix 5 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
L. fermentum CECT5716	50 healthy volunteers aged 22-56 yr; intramuscular vaccinated with influenza	RDBPCT 1. Placebo, n=25 2. *L. fermentum*, n=25	$1x10^{10}$ CFU/day	28 days	Increase influenza-specific IgA in the probiotic group compared to placebo group ($p<0.05$). Increase of NK cells within the probiotic group ($p<0.05$) not in the placebo group. TNF-α production was higher in the probiotic group compared to the placebo ($p<0.05$). Probiotics lowered influenza-like illness 5 mo after vaccination ($p<0.05$).	70

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; *L.*:*Lactobacillus,* LGG: *Lactobacillus* GG; CFU: colony forming units, yr: years; mo: months, wk: weeks; PBMCs : peripheral blood mononuclear cells; NK cells: natural killer cells

Appendix 6: Effects of probiotics on inflammatory bowel disease

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
S. boulardii	32 patients with Crohn's disease in remissionaged 23-49 yr	RCT 1. Mesalazine, n=16 2. Mesalazine+ *S. boulardii*, n=16	500 mg	6 mo	Clinical relapse in 6 of the 16 patients that received mesalazine and 1 of the 16 patients that received mesalazine with *S. boulardii* (p=0.04)	39
LGG	45 patients with Crohn's disease in remission scheduled ofr curative resection aged 22-71 yr	RDBPCT 1. Placebo, n=22 2. LGG, n=23	$6x10^9$ CFU/twice/day	1 yr	No significant difference in percentage of clinical recurrence	79
LGG	75 infants with Crohn's disease in remission aged 5-21 yr	RDBPCT 1. Placebo, n=36 2. LGG, n=39	$1x10^{10}$ CFU/twice/day	2 yr	Time to relapse and number of patients relapsing was similar in study groups	8
LGG	11 patients with moderate to active Crohn's disease	RDBPCT 1. Placebo, n=6 2. LGG, n=5	$2x10^9$ CFU/day	6 mo	Remission rate was not affected by LGG. Time to relapse was shorte in LGG group (NS)	94
L. johnsonii LA1	98 patients with Crohn's disease who underwent surgical resection	RDBPCT 1. Placebo, n=50 2. *L. johnsonii,* n=48	$2x10^9$ CFU/twice/day	6 mo	Recurrence was 64% in placebo and 49% in probiotic group (NS)	62

Appendix 6 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
Bifico	30 patients with active ulcerative colitis in remission	RDBPCT 1. Placebo, n=15 2. Bifico, n=15	1.25 gram/day	8 wk	Relapse was 20% in Bifico and 93% in placebo group ($p<0.01$). Bifico increased fecal lactobacilli and bifidobacteria and lowered NF-κB compared to placebo ($p<0.05$)	17
E. coli Nissle 1917	117 patients with ulcerative colitis in remission	RDBT` 1. Mesalazine, n=59 2. *E. coli*, n=57	$2.5x10^{10}$ CFU/twice/day	12 mo	Relapse rate was similar in mesalazine (73%) and *E. coli* group (67%)	85
E. coli Nissle 1917	222 patients with ulcerative colitis in remission	RDBT` 1. Mesalazine, n=112 2. *E. coli*, n=110	$2.5\text{-}25x10^{9}$ CFU/day	12 mo	Relapse rate was similar in mesalazine (36%) and *E. coli* group (34%)	54
L. acidophilus and *B. animalis lactis*	29 patients with collagenous colitis	RDBPCT 1. Placebo, n=8 2. Probiotics, n=21	$1x10^{10}$ CFU of each probiotic/twice/day	12 wk	Reduction in bowel frequency was 29% in probiotic and 13% in placebo group (NS). Within probiotic group a reduction of stool frequency was observed ($p<0.05$).	121
B. breve, B. bifidum, L. acidophilus	22 patients with ulcerative colitis	RDBPCT 1. Placebo, n=10 2. Probiotics, n=11	$1x10^{10}$ CFU of each probiotic/day	12 mo	Exacerbation of symptoms was 90% in placebo and 27% in probiotic group ($p=0.0075$). No difference in colonoscopic findings.	44

Appendix 6 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
B. breve, B. bifidum, L. acidophilus	19 patients with mild to moderate ulcerative colitis	RDBPCT 1. Placebo, n=9 2. Probiotics, n=10	$1x10^{10}$ CFU of each probiotic/day	12 wk	Clinical remission achieved by medication was 40% in probiotic and 33% in placebo group. Endoscopic activity index and histology score decreased in probiotic ($p<0.01$) and not in placebo group	50
VSL#3	34 patients with active ulcerative colitis not responsive to normal treatment	Open label study	$1.8x10^{12}$ CFU/twice/day	6 wk	Remission rate 77%	7
VSL#3	20 patients with ulcerative colitis not responsive to normal treatment	Open label study	$1.5x10^{12}$ CFU/twice/day	12 mo	Remission rate 75%	111
VSL#3	31 patients with pouchitis in remission	Open label study	6 gram/day	6 mo	Recurrence of pouchitis in 81%	96
VSL#3	36 patients with recurrent or chronic pouchitis in remission	RDBPCT 1. Placebo, n=16 2. VSL#3, n=20	$6x10^{11}$ CFU/day	12 mo	Remission was maintained in 85% of the VSL#3 and 6% of the placebo group ($p<0.0001$)	65

Appendix 6 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
VSL#3	40 patients with recurrent or chronic pouchitis in remission	RDBPCT 1. Placebo, n=20 2. VSL#3, n=20	$6x10^{11}$ CFU/day	12 mo	Remission was maintained in 85% of the VSL#3 and 0% of the placebo group ($p<0.001$). VSL#3 increased total number of intestinal bacteria and restored diversity	38, 55
VSL#3	40 patients with ulcerative colitis who underwent ileal-pouch-anal anastomosis	RDBPCT 1. Placebo, n=20 2. VSL#3, n=20	$9x10^{11}$ CFU/day	12 mo	Pouchitis developed in 10% of the VSL#3 and 40% of the placebo group ($p<0.05$). VSL#3 improved quality of life score ($p<0.05$), placebo did not	37
LGG	20 patients with recurrent and chronic pouchitis	RDBPCT 1. Placebo, n=10 2. LGG, n=10	$1\text{-}2x10^{10}$ CFU/twice/day	3 mo	LGG did not improve clinical, histological and endoscopic symptoms of pouchitis	56

Abbreviations: RCT: randomized clinical trial; RDBPCT: randomized double-blind placebo controlled trial; RDBT: randomized double-blind trial; *S.: Saccharomyces; L.:Lactobacillus,E.: Escherichia;* LGG: *Lactobacillus* GG; CFU: colony forming units, yr: years; mo: months, wk: weeks

Appendix 7: Meta-analyses: effects on antibiotic-associated diarrhea

Number of RDBPCTs	*Probiotics*	*Odds ratio/relative risk*	*Ref.*
9	*S. boulardii* (n=4) *L. acidophilus+L. bulgaricus* (n=2) LGG (n=1) *L. acidophilus+B. longum* (n=1) *Enterococcus faecium* (n=1)	Combined OR: 0.37 OR *S. boulardii*: 0.39 OR other: 0.34	20
7	*S. boulardii* (n=3) LGG (n=3) *Lactobacillus* (n=1)	Combined RR: 0.40	16
5	*S. boulardii*	RR: 0.43	101
6	LGG (n=2) *S. boulardii* (n=1) *B. lactis+Streptococcus thermophilus* (n=1) *L. acidophilus+B.infantis* (n=1) *L .acidophilus + L. bulgaricus* (n=1)	Combined RR: 0.44	102

Appendix 7 continued

Number of RDBPCTs	Probiotics	Odds ratio/relative risk	Ref.
6	LGG (n=2) *S. boulardii* (n=1) *L. sporogens* (n=1) *L. acidophilus+B.infantis* (n=1) *L .acidophilus + L. bulgaricus* (n=1)	Combined RR: 0.43	46
31	LGG (n=6) *S. boulardii* (n=8) Single strain (n=9) Mixtures of two probiotics (n=8)	AAD: RR=0.43 CDC: RR=0.59	63

Abbreviations: RDBCTs: randomized, double-blind placebo controlled trial; n: number of trials; *S.: Saccaromyces; L.: Lactobacillus; B.:Bifidobacterium*; LGG: *L. rhamnosus* GG; OR: odd ratio; RR: relative risk; AAD: antibiotic-associated diarrhea; CDC: *Clostridium difficile* disease

Appendix 8: Meta-analyses: effects on acute diarrhea

Number of RDBPCTs	Probiotics	Results	Ref.
18	*LGG* (n=10) *L. reuteri* (n=3) *L. acidophilus* (n=2)*;* *L. rhamnosus* (n=2) *Enterococcus* SF68 (n=1); *S. boulardii* (n=1) *B. subtilis* (n=1) *L. rhamnosus+L. reuteri* (n=2) *Streptococcus thermophilus+L. acidophilus +L. bulgaricus* (n=1) *Streptococcus thermophilus+L. acidophilus +L. bulgaricus, B. bifidum* (n=1) *L. acidophilus + B. infantis* (n=1) *L. rhamnosus+L. delbruckii+ L. bulgaricus* (n=1)	Duration of diarrhea was 1 day shorter	20
9	LGG (n=4) *L. reuteri* (n=2) *L. acidophilus* (n=1) *L. acidophilus + L. bulgaricus* (n=2)	Duration of diarrhea was 0.7 day shorter Number of stools was 1.6 fewer	110

Appendix 8 continued

Number of RDBPCTs	***Probiotics***	***Results***	***Ref.***
34	LGG (n=10)	Pooled efficacy: 35% reduction	93
	S. boulardii (n=5)	AAD: 52% reduction	
	B. lactis Bb12 (n=2)	Traveller's diarrhea: 8% reduction (NS)	
	L. reuteri (n=1);	Other causes: 34% reduction	
	L. acidophilus (n=1)	LGG: 28% reduction	
	L. casei (n=1);	*S. boulardii*: 52% reduction	
	L. fermentum (n=1)	*L. acidophilus +L. bulgaricus*: 30% reduction (NS)	
	B.longum (n=1);		
	Enterococcus SF68 (n=1)		
	L. acidophilus +L. bulgaricus (n=7)		
	L. acidophilus + B. bifidum (n=1)		
	L. acidophilus + B. infantis (n=1)		
	Streptococcus thermophilus + B.lactis (n=1)		
	Streptococcus thermophilus + B.bifidum (n=1)		
	B.longum + L. acidophilus (n=1)		
	Combination of bifidobacteria (n=1)		
	Streptococcus thermophilus+L. acidophilus+L. bulgaricus (n=1)		

Abbreviations: RDBCTs: randomized, double-blind placebo controlled trial; S.: Saccaromyces; L.: Lactobacillus; B.:Bifidobacterium; LGG: L. rhamnosus GG; AAD: antibiotic-associated diarrhea; NS: not significant

Appendix 9: Safety of probiotics

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
B. lactis Bb12 and *St. thermophilus*	118 healthy infants aged 3-24 mo	RDBPCT 1. Infant formula, n=40 2. Infant formula with 1x106 CFU/g, n=39 3. Infant formula with 1x107 CFU/g, n=39	$1x10^6$ or $1x10^7$ CFU/g for both probiotics	210 ±127 days	Supplemented formulas were well tolerated and reduced colic ($p<0.001$) and antibiotic use ($p<0.001$). No significant differences in growth, day care absenteeism, or other health variables.	90
B. lactis Bb12 *L. reuteri* ATCC55730	59 healthy infants aged 3-65 days	RDBPCT 1. Infant formula, n=19 2. Infant formula with *B. lactis* n=20 3. Infant formula with *L. reuteri*, n=20	$1x10^7$ CFU/g for both probiotics	4 wk	Supplemented formulas were well tolerated and induced no adverse effects. Both probiotics did not affect stool, flatulence, crying, and daily restlessness score	117
L. reuteri ATCC55730	39 healthy infants born to mothers with a history of allergy	RDBPCT 1. Placebo, n=19 2. *L. reuteri*, n=20 Starting from day of birth	$1x10^8$ CFU per day (in oil)	12 mo	No adverse effects were observed. *L. reuteri* was not detected in the blood (assessed by measuring D(-) lactic acid in blood)	14

Appendix 9 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
B. longum BL999	97 healthy infants who were not breastfed	RDBPCT 1. Infant formula, n=59 2. Infant formula + *B. longum*, n=42 Starting before age of 14 days	$2x10^7$ CFU/l	7 mo	*B. longum* was well-tolerated, did not affect weight gain and did not induce adverse effects. Infants receiving *B. longum* had less constipation (p=0.03) and less respiratory infections (p=0.14)	82
L. reuteri SD2112	35 HIV+ adults	RDBPCT 1. Placebo, n=20 2. *L. reuteri*, n=15	$1x10^{10}$ CFU per day	21 days	*L. reuteri* did not affect safety parameters that were assessed.	122
LcS	28 critically ill infants in intensive care wards, aged 6 mo – 16 yr	Prospective descriptive pilot study	$1x10^7$ CFU/day	Max 5 days	LcS did not colonize or cause bacteremia in all subjects	100
B. longum 2C and *B. longum* 46	39 healthy volunteers aged 19-60 yr	RDBPCT 1. Placebo, n=20 2. *B. longum* 2C and 46, n=19	$2x10^9$ CFU/day both probiotics	3 wk	Probiotics were well tolerated without any adverse effects. Probiotics did not affect phagocytic activity.	61
LGG	17 HIV+ adults	Double-blind crossover design 2 wk washout – 2 wk LGG (n=8) or placebo (n=9) – 2 wk washout – 2 wk LGG (n=9) or placebo (n=8) – 1 wk washout	1-5 $x10^{10}$ CFU/day	2 wk	No effects of LGG on stool frequency or diarrheal bowel movements. No side effects reported and LGG had no effect on CD4 cell counts	91

Appendix 9 continued

Probiotic	Study population	Study design	Dosing	Duration	Results	Ref
S. boulardii	376 patients with peptic ulcer disease treated with antibiotics	RDBPCT 1. Placebo, n=185 2. *S. boulardii*, n=204	1 gram	2 wk	Probiotics reduced the prevalence of diarrhea during treatment and follow-up ($p<0.05$). No adverse effects induced by probiotics	27

Abbreviations: RDBPCT: randomized double-blind placebo controlled trial; *St: Streptococcus; L.:Lactobacillus, B.: Bifidobacterium; S.: Saccharomyces*; LcS: *Lactobacillus casei* Shirota; LGG: *Lactobacillus rhamnosus* GG; CFU: colony forming units, yr: years; mo: months, wk: weeks