Different effects of probiotic species/strains on infections in preschool children: A double-blind, randomized, controlled study

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ABSTRACT

Treatment and prevention of pediatric infectious diseases of three commercial probiotic products were evaluated by a double-blind, randomized, controlled trial. Test subjects under age 5, 1062 in total, were distributed randomly into four groups. This investigation showed that *L. casei rhamnosus* can control bacterial, viral and respiratory infections; a multi-species probiotic reduced gastrointestinal disease significantly. Long-term consumption of *L. rhamnosus* T cell-1 decreased the incidence of bacterial infection.

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1. Introduction

Infectious diseases are the most significant illnesses for children under age 5, particularly for those attending preschool [1]. Poor hygiene facilities present risks for respiratory and gastrointestinal tract infectious pathogens [2,3]. Viral pathogens, such as respiratory syncytial virus [4], human metapneumovirus [5], influenza A virus [6], parainfluenza viruses, and rhinoviruses are considered the major viruses that can cause respiratory tract diseases in children [6]; furthermore, rotaviruses [7], adenoviruses [8], and astroviruses [9] are viral pathogens that cause gastrointestinal diseases in children. Important infectious bacteria that have been implicated in day care-associated respiratory and gastrointestinal disorders are *Streptococcus pneumoniae* [10], *Shigella*, *Salmonella*, *Escherichia coli* [11], and *Aeromonas* [11,12].

Effective remedies have been intensively investigated to reduce pediatric infectious diseases which cause illness, debility, and in extreme cases, loss of life. Managerial methods [13,14] and probiotic supplementation are currently employed to reduce the incidences of infectious disease [15,16].

It has been proven that children with mild diarrhea who consumed the combination of *L. rhamnosus* and *L. reuteri* experienced a reduction in the duration of the diarrhea [15]. A study of 6- and 36-month old children afflicted with rotavirus gastroenteritis showed that *L. reuteri* significantly shortened the duration of diarrhea [17]. *L. rhamnosus* and *L. acidophilus* significantly attenuated the neutrophil infiltration and lipid peroxidation during *Shigella* dysenteriae 1-induced diarrhea in rats [18]. In addition, it was shown with the use of newborn rabbits as an experimental infection model that preventive administration of *L. casei* may, due to acceleration of a specific humoral immune response, lead to enhanced resistance to acute *E. coli* infection [19]. These studies illustrated the various effects of different *Lactobacillus* strains on gastrointestinal infections. It has been observed that several strains of probiotics have positive influences on non-specific stimulation of the host's immunity, although the molecular mechanism has not been elucidated [20–22]. Many probiotics are capable of preventing respiratory infections and reducing their severity [23,24]. Also, it was reported that mitigation or prevention of pediatric infectious disease occurred when children in day care centers ingested *Lactobacillus* [16].

The efficacy of commercial probiotics has been brought under scrutiny, with doubt remaining that all of them possess sufficient potency necessary for adequate gastrointestinal colonization. Further evidence is required to demonstrate that strain-specific probiotics can prevent various diseases. The proper selection among mono-strain, multi-strain, or multi-species probiotics is critical for efficacy in clinical trials [25]. Previous investigations

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involving the function and efficacy of probiotics on the prevention of pediatric infectious diseases were focused only on evaluating a single probiotic. So, the aim of this double-blind, randomized, controlled study was to compare the efficacy of three different commercial probiotics–*Lactobacillus casei rhamnosus*, *Lactobacillus rhamnosus* T cell-1 and a multiple probiotic – during short- and long-term intervention. The parameters examined in this investigation were the effect of different probiotics on the incidences of bacterial and viral infectious diseases, and more specifically, gastrointestinal and respiratory infections in preschooler. We report here that various commercial probiotics have dissimilar effects on different infectious diseases. The *L. casei rhamnosus* probiotic reduced respiratory infections, but multispecies probiotic supplementation significantly reduced gastrointestinal disease.

2. Methods

2.1. Study design and ethics

This was a double-blind, randomized, controlled study, with four parallel arms, and consent letters were signed by well-informed parents. This study was approved by the Committees of the Protection of Human Subjects Institutional Review Board of Tzu-Chi University and Hospital, Hualien, Taiwan.

2.2. Participants

One thousand and sixty-two children were recruited and seventy-six children who did not remain in the study during the follow-up period were excluded from the investigation. Among the 986 children who completed the study, 193 were in the control group, 285 were in the *L. casei rhamnosus* group, 222 were in the *L. rhamnosus* T cell-1 group, and 286 were in the multiple probiotic group (Fig. 1). The characteristics of each study group are given in Table 1. No significant differences were observed in age, male/female ratio, duration of breast-feeding, smoking in the household, family income, house area, and history of allergy. This study excluded children who previously had complicated intestinal operations or immunosuppressive therapy, or those who suffered ill effects due to complex congenital heart disease, or low immune function syndromes.

2.3. Test preparations, blinding, and randomization

The intervention lasted 7 months, from October 20, 2003 to May 31, 2004. We regarded each class as a unit, and implemented a double-blind assignment of *L. casei rhamnosus* sachets, *L. rhamnosus* T cell-1 capsules, and multiple probiotic capsules to the children. The subjects were randomly assigned to one of four groups:

1. The *L. casei rhamnosus* group: Instructions for consumption: 2 sachets (3 g) of *L. casei rhamnosus* per day, 5 days a week;
2. The *L. rhamnosus* T cell-1 group: Instructions for consumption: 3 capsules (1.14 g) of *L. rhamnosus* T cell-1 per day, 5 days a week;
3. The multiple probiotic group: Instructions for consumption: 5 capsules (5 g) of a mix of 12 beneficial bacterial strains per day, 5 days a week;
4. The control group: no probiotic supplementation; no dietary inclusion criteria.

One *L. casei rhamnosus* sachet contained 1×10^8 cfu *L. casei rhamnosus*/g (Antibiophilus® Laboratoires Lyocentre Ltd, Aurillac, France), one *L. rhamnosus* T cell-1 capsule contained 1×10^{10} cfu *L. rhamnosus* T cell-1/g (T Cell-1 Probiotics, Chang Gung Biotechnology Corp, Taipei, Taiwan), and a multiple probiotic capsule contained 12 types of beneficial bacterial strains for the large and small intestines, including 7 different species of *Lactobacilli* (Neoangelac® 12A *Lactobacilli*, Multipower Enterprise Corp, Taipei, Taiwan). One capsule of the Neoangelac 12A *Lactobacilli* series contained 3 types of *Bifidobacteria* (2.4×10^9 cfu *B. bifidum*, 2.4×10^9 cfu *B. infantis*, 2.4×10^9 cfu *B. longum*); 7 types of *Lactobacilli* (2×10^9 cfu *L. casei*, 1.2×10^9 cfu *L. salivarius*, 1.6×10^9 cfu *L. brevis*, 2×10^9 cfu *L. plantarum*, 1.2×10^9 cfu *L. acidophilus*, 8×10^8 cfu *L. helveticus*, 2×10^9 cfu *L. rhamnosus*); 1 type of *Streptococcus* (1×10^9 cfu *S. thermophilus*) and 1 type of *Enterococcus* (1×10^9 cfu *E. faecium*). Dietary restrictions were not applied during the intervention periods.

2.4. Intervention

Nine hundred eighty-six participants were observed between January 1, 2001 and May 31, 2004. The baseline period was when these children attended day care centers from January 1, 2001 to December 31, 2002. Then, the intervention period lasted 7 months from October 20, 2003 to May 31, 2004. The short-term intervention period and the long-term intervention period were defined as interventions that lasted 3 months and 7 months, respectively, each beginning from October 20, 2003. The volunteers took the probiotic products, following the instructions on the package label. The investigated parameters were incidences of all pediatric diseases, bacterial infections, viral infections, gastrointestinal infections, and respiratory infections. Incidence frequency and episodes per person per month were described as the number of infection episodes relative to the corresponding population experience, and excluded routine immunization and other scheduled visits that were not related to infections. Infectious episode information during January 1, 2001 to May 31, 2004 was collected from the Bureau of National Health Insurance, Taiwan.

2.5. Assessment of infectious disease

Average incidence densities of 167 types of diseases were estimated for each probiotic group. The pediatric diseases were defined as gastrointestinal disease, respiratory disease, atopic disease, and dermatologic disease. Bacterial infections were lymphadenitis, acute otitis media, pneumonia, sinusitis, urinary tract infection, meningitis, and bacterial gastroenteritis, etc. Viral infections were influenza, acute pharyngotonsillitis, acute laryngitis, croup, enterovirus infection, acute bronchitis, acute bronchiolitis, encephalitis, and viral gastroenteritis, etc. Respiratory infection included 28 categories such as the common cold, acute upper respiratory infections, acute bronchitis, acute bronchiolitis, acute sinusitis, acute pharyngitis, acute tonsillitis, acute laryngitis, acute epiglottitis and influenza, et al.; gastrointestinal infections included viruses and bacteria associated with diarrhea and vomiting; abdominal pain included 23 categories and non-infectious gastrointestinal disease; constipation included 22 categories, for a total of 50 classes.

2.6. Statistical analysis

Descriptive statistics, including the mean and standard error of the mean (S.E.M.), were determined for each of the four groups. Analysis of variance (ANOVA) was applied to all pediatric diseases, bacterial infections, viral infections, gastrointestinal infections, and respiratory infections that existed among the probiotic-treated groups and the control group during the same period and among the different periods in the same group. If a significant difference was present, the least significant difference (LSD) multiple comparison tests were used to identify specific significant groups. All statistical analyses were performed by using The Statistical Software Package for the Social Sciences, version 12.0.1 for Windows
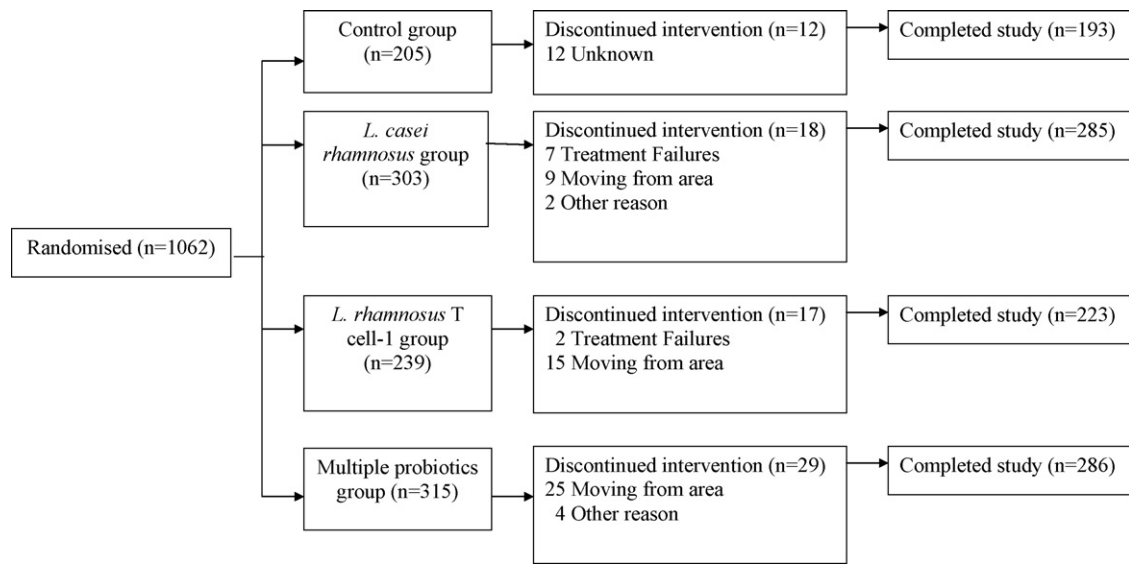
Fig. 1. Flow chart showing progress of the participants through the trial.

(SPSS Inc., Chicago, IL). A P-value of <0.05 was considered statistically significant for all analyses.

3. Results

3.1. Effect of probiotic treatment on incidence of all pediatric disease

Preschoolers' incidences of all pediatric diseases increased after entering the day care center in every group compared with the baseline period ($P > 0.05$, Fig. 2). Preschoolers who received probiotic treatment experienced a decline in physician visits, although this was statistically insignificant between the groups during the intervention period ($P > 0.05$, Fig. 2).

3.2. Effect of probiotics on bacterial infectious disease

The incidence of bacterial infectious disease in preschoolers of every group increased significantly after entering the day care center compared to the baseline period ($P < 0.05$, Fig. 3). Preschoolers who received a single strain probiotic during the long-term intervention experienced a significant improvement in recovery from any bacterial infectious disease. The incidence of bacterial infection diagnosed by doctors in the *L. casei rhamnosus* group was fewer (mean difference, -0.15 times/month; CI_{95}, -0.27 to -0.03; $P = 0.021$, Fig. 3) as compared to the control group. The analysis of the *L. rhamnosus* T cell-1 group showed a significant decrease in those with a doctor-diagnosed bacterial infection as compared to the control group (-0.16; CI_{95}, -0.30 to -0.03; $P = 0.020$, Fig. 3). No

significant differences in the incidence of doctor-diagnosed bacterial infection were observed in the multiple probiotic and control groups ($P > 0.05$, Fig. 3).

### 3.3. Effect of probiotics treatment on viral infectious disease

Incidences of viral infectious diseases in preschoolers increased after entering the day care center in every group as compared to the baseline period ($P < 0.05$, Fig. 4). The preschoolers receiving the *L. casei rhamnosus* treatment had 0.30 times lower odds of doctor-diagnosed viral infection than the control group during short-term intervention (-0.16; CI_{95}, -0.54 to -0.06; $P = 0.015$, Fig. 4). No significant difference was observed in the *L. rhamnosus* T cell-1 and the multiple probiotic groups ($P > 0.05$, Fig. 4).

3.4. Effect of probiotic treatment on gastrointestinal disease

There was no difference in the incidence of gastrointestinal infectious disease after preschoolers entered the day care center in the control group compared with the baseline period ($P < 0.05$, Fig. 5). However, preschoolers in the multiple probiotic group experienced a significant reduction in gastrointestinal infection both during the short-term (-0.045; CI_{95}, -0.040 to -0.040; $P = 0.007$) and the long-term (-0.049; CI_{95}, -0.037 to -0.061; $P = 0.004$) intervention. Single strain probiotic (*L. casei rhamnosus* and *L. rhamnosus* T cell-1, respectively) administration showed an insignificant ability to prevent disease when compared to the control group ($P > 0.05$, Fig. 5). However, the mean incidence of gastrointestinal disease significantly decreased in the group that participated in the

Table 1
Baseline characteristics of the study groups.

| Characteristic	Control group ($n = 193$)	L. casei rhamnosus group ($n = 285$)	L. rhamnosus T cell-1 group ($n = 222$)	Multiple probiotic group ($n = 286$)
Age (years)	4.74 ± 1.07	4.54 ± 1.04	5.16 ± 1.05	4.64 ± 0.95
Male/female	1.31	1.23	1	1.07
Duration of breast feeding (months)	1.51 ± 3.35	1.73 ± 3.89	1.62 ± 3.84	2.39 ± 5.01
House area (m^2)	44.3 ± 20.2	40.4 ± 20.1	43.9 ± 21.0	50.3 ± 25.38
Smoking in household	57%	63%	65%	55%
Family income (10^4 NT/year)	66.5 ± 32.2	69.0 ± 34.2	65.9 ± 32.5	71.8 ± 40.8
History of allergy (diagnosed by doctor)				
Asthma	4%	6%	5%	4%
Allergic rhinitis	15%	20%	18%	13%
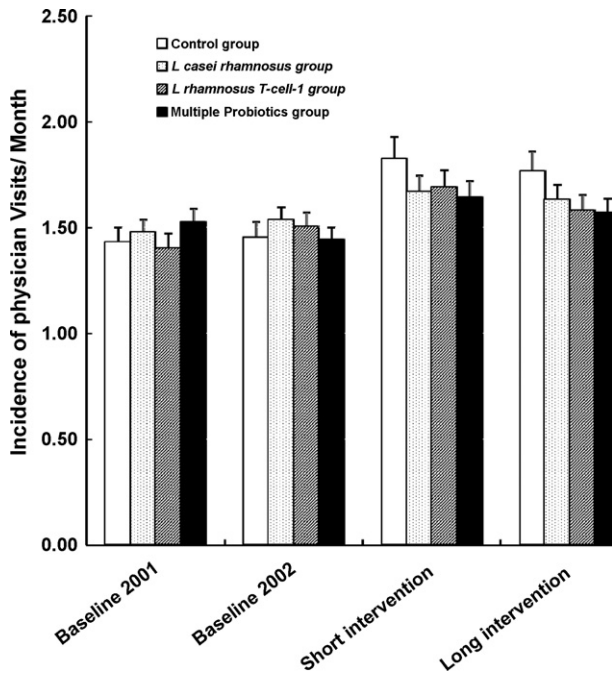
Fig. 2. Effects of oral administration of the three different commercial probiotics on physician visits in preschool children. Mean number of physician visits per month during the baseline period and intervention period in preschool children that received *L. casei rhamnosus* (▦), *L. rhamnosus* T cell-1 (▨) or the multiple probiotic (■), compared to the control group (□). Baseline 2001 and 2002: Period before entrance to preschool and treatment of children allocated to the probiotic and control groups. Short-term: Period that the children had been treated with different commercial probiotics in the first 3.3 months. Long-term: Period that the children had been treated with different commercial probiotics during the whole 7.3 months.

short-term consumption of *L. casei rhamnosus* as compared to the group that consumed *L. casei rhamnosus* before entering preschool (-0.034; CI$_{95}$, -0.041 to -0.026; $P = 0.031$ and -0.040; CI$_{95}$, -0.046 to -0.034; $P = 0.011$, Fig. 5).
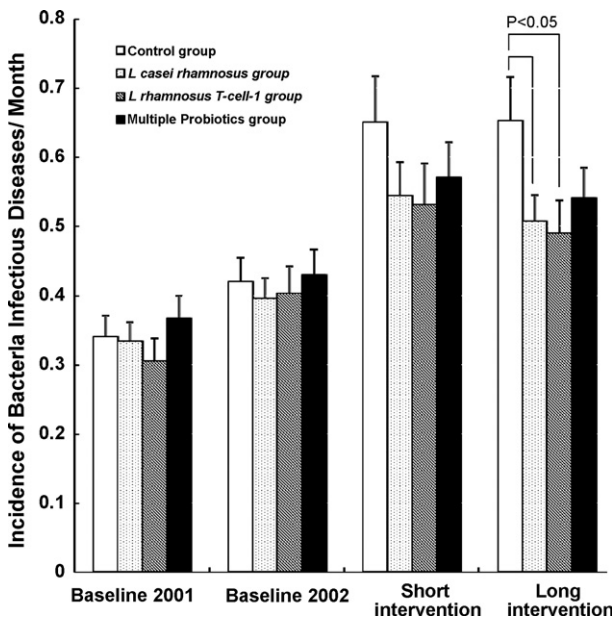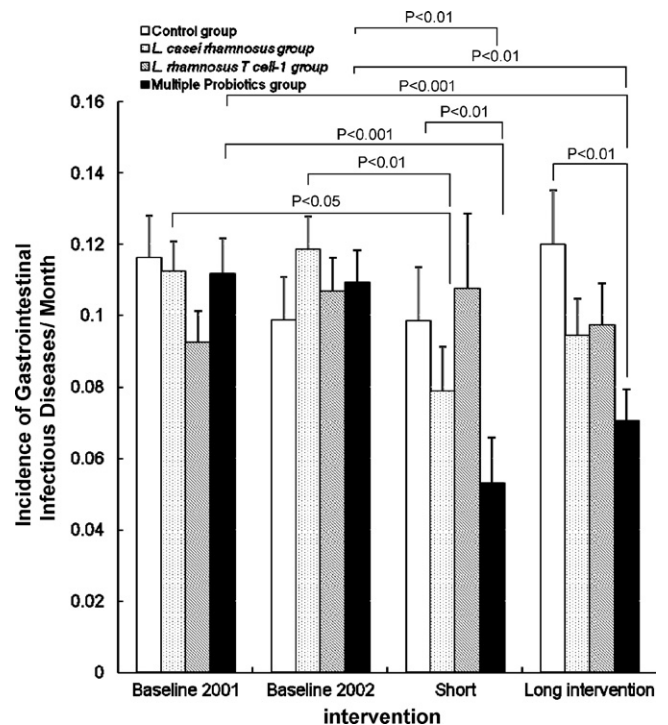

Fig. 3. Effects of the oral administration of the three different commercial probiotics on bacterial infectious disease in preschool children. Mean number of bacterial infectious diseases per month during the baseline period and intervention period in preschool children that received *L. casei rhamnosus* (▦), *L. rhamnosus* T cell-1 (▨) or the multiple probiotic (■), compared to the control group (□). *Significantly different from the control group ($P < 0.05$).
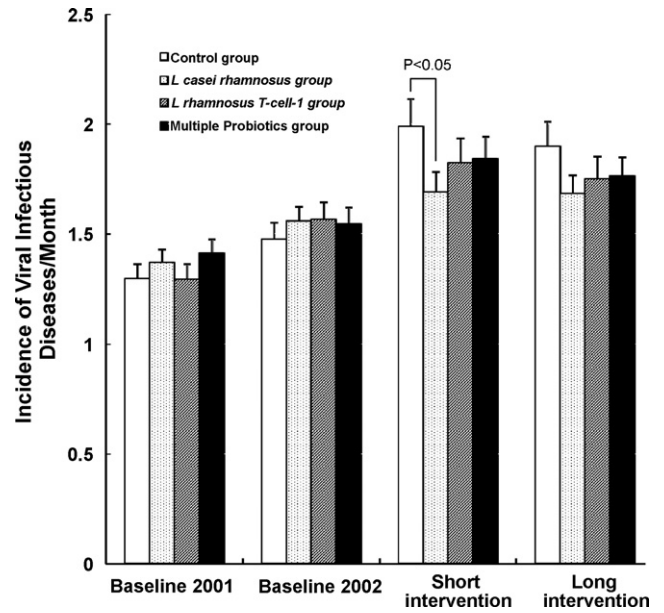
Fig. 4. Effects of the oral administration of the three different commercial probiotics on viral infectious disease in preschool children. Mean number of viral infectious diseases per month during the baseline period and intervention period in preschool children that received *L. casei rhamnosus* (▦), *L. rhamnosus* T cell-1 (▨) or the multiple probiotic (■), compared to the control group (□). *Significantly different from the control group ($P < 0.05$).
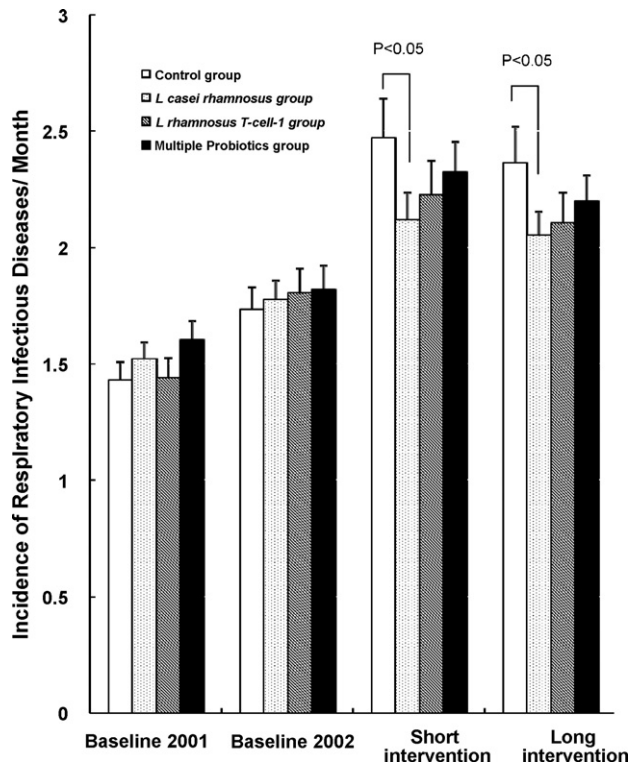
3.5. Effect of probiotics on respiratory disease

The incidence of respiratory infectious disease in preschoolers increased after entering the day care center in every group as com-

Fig. 5. Effects of oral administration of the three different commercial probiotics on gastrointestinal infectious disease in preschool children. Mean number of gastrointestinal infectious diseases per month during the baseline period and intervention period in preschool children that received *L. casei rhamnosus* (▦), *L. rhamnosus* T cell-1 (▨) or the multiple probiotic (■), compared to the control group (□). **Significantly different from the control group ($P < 0.01$).

Fig. 6. Effects of oral administration of the three different commercial probiotics on respiratory infectious disease in preschool children. Mean number of respiratory infectious diseases per month during the baseline period and intervention period in preschool children that received *L. casei rhamnosus* (▒), *L. rhamnosus* T cell-1 (▨) or the multiple probiotic (■), compared to the control group (□). *Significantly different from the control group ($P < 0.05$).

pared to the baseline period ($P < 0.05$, Fig. 6). There was a significant difference in doctor-diagnosed respiratory infection between the *L. casei rhamnosus* and the control group, and there was also a significant reduction in both the short-term(-0.352; CI$_{95}$, -0.243 to -0.460; $P < 0.000$) and the long-term(-0.309; CI$_{95}$, -0.200 to -0.418; $P < 0.000$) intervention groups (Fig. 6).

4. Discussion

Many studies have highlighted the benefits of probiotic bacteria for infectious disease prevention. However, the efficacy of many commercial probiotics is suspect, due to insufficient growth of various strains in the human intestinal tract, and hardly any probiotic manufacturers have solid evidence to match their claims. But *Lactobacillus spp.* have earned the most attention and have been investigated intensively, as this bacterial genus is among the few that confer many positive rewards to the test subjects. Three *Lactobacillus spp.*, *L. casei rhamnosus*, *L. rhamnosus* T cell-1, and the Neoangelac 12A *lactobacilli* multipower are major components of probiotic formulae and are very popular in the probiotic market of Taiwan. In the interest of the good health of preschoolers and the welfare of society, the pediatric benefits of these probiotics were further investigated in this study.

The incidence of all pediatric disease increased after entering day care centers in every group. These observations support the belief that attendance at day care centers increases the risk of infections [1,2]. An interesting result was that probiotic supplementation tended to diminish the number of physician visits, especially in reducing the number of children who had a high incidence of physician visits, and concomitantly increased the number of children

who had no physician visits during the intervention period (data not shown).

Gastrointestinal infections in preschool can be attributed to different factors, such as the transmission of enteropathogens by fomites, or ingestion of contaminated food or drink. Diligent hygiene was practiced by the preschoolers' families, and at all the day care centers. Furthermore, the gastrointestinal disease analysis suggested that preschool attendance did not lead to an increased risk of infections. Meanwhile, this study clearly showed the effectiveness of the multiple probiotics in preventing gastrointestinal disease in preschoolers. Reductions of 42% and 44% were found in gastrointestinal disease in the short- and long-term intervention periods, respectively, of the multiple probiotics group. In the *L. casei rhamnosus* group, there was a decreased frequency of gastrointestinal disease in preschoolers, although statistically insignificant when compared to the control group. The children who received the single strain *L. rhamnosus* T cell-1 supplementation did not exhibit any statistically significant difference as compared to the control group.

The variety of commensal bacteria is essential to the development of gut mucosal immunity [13,26]. Previous research has shown that a combination of probiotic bacteria can stimulate the mucosal immune system, with similar conclusions being made from animal studies, and mixtures of gut microbial species can more efficiently stimulate the immune system than a single strain [27,28]. Other reports that are in agreement with our results are the findings that the combination of *L. rhamnosus* and *L. acidophilus* offered better protection compared to a single strain of *Lactobacilli* during a *Shigella* infection [17]; and probiotic products containing one strain of *Lactobacillus* had less positive effects on gastrointestinal diseases because of a decreased ability to successfully colonize the gastrointestinal tract [29].

This investigation clearly showed that single strain probiotic supplementation significantly reduced the incidence of bacterial infections by an average of 1.8 times for *L. casei rhamnosus*, and 1.92 times for *L. rhamnosus* T cell-1 during the experimental period. Some of the mechanisms that probiotics use to promote health include the synthesis of anti-microbial substances [30], reduction of the nutrients available for bacterial pathogens [31], inhibition of adhesion and invasion of pathogens [32], modification of toxin receptors [33], and stimulation of immune responses [20–22,34]. However, the multiple probiotic supplement had no significant effect on preventing bacterial infections. This might be attributed to antagonism among the different strains of probiotics in the multi-strain supplement [25].

Consumption of *L. casei rhamnosus* reduced viral infectious disease by 18% in the short-term intervention group. In the multiple probiotic and the *L. rhamnosus* T cell-1 groups, the effects of the probiotics were not strong enough to prevent viral infections. Much research has been done to study the effect of probiotics on bacterial or gastrointestinal infectious diseases, but only a few studies have examined the effect of probiotics on viral or systemic infectious diseases. de Vrese et al. [35] have envisaged probiotics positively influencing systemic organs by modulating immune function, stimulating virus-specific antibody production, and affecting intestinal mucosa absorption and secretion [36].

In preventing respiratory infections, children of the *L. casei rhamnosus* group had a reduction of 17% and 18% during the short- and long-term interventions, respectively, compared to the control group. Our current work was supported by a previous study showing that probiotics reduce respiratory infections and their severity among preschoolers [16]. There was a reduction in the occurrence of recurrent respiratory infections in the multiple probiotic and the *L. rhamnosus* T cell-1 group, but it was insignificant [16]. The diverse outcomes we observed among the three commercial probiotics may have resulted from probiotic strain-dependent effectiveness. Previ-

ous investigations showed that when research subjects were given a mixture of probiotics, there was an insignificant effect in preventing the incidence of respiratory infections [23,37].

Together with increasing reports of clinical effects against infectious diseases, there is a growing interest of the role of probiotics in bacterial and viral infectious disease prevention. This large population study has successfully demonstrated that probiotics could induce differential effects upon infectious disease in preschoolers among the three orally administered commercial probiotics. However, the benefits of probiotics were small in reducing the incidence of disease in some subgroups. Various probiotics can be efficient immune modulators whose effectiveness varies among strains and species, such as *Lactobacillus* and *Bifidobacterium*. Our findings exhibit similarities to studies showing that *Lactobacilli* species can affect antigen-specific IgG1/IgG2 Ab and cytokine responses [38–39]. Certain strains of *Lactobacilli* can activate myeloid dendritic cells to stimulate T cells and then induce Th1 cytokines, and could be useful for the delivery of bio-therapeutic agents [40]. This investigation strongly suggests that there is a need for rational probiotic selection and detailed evaluation prior to application in food or health care products. It also implies that the bacterial growth phase is a crucial parameter allowing for additional manipulation of immune responses by oral administration of *Lactobacilli*. A larger scale of investigation will be required to obtain more information about the effect of these parameters upon a study population.

In conclusion, this randomized, double-blind study shows that bio-therapeutic agents may be useful in preventing viral and bacterial infectious disease. However, different commercial probiotics have dissimilar effects on diverse infectious disease. The *L. casei rhamnosus* strain may reduce most infectious diseases, especially respiratory infections. Multiple probiotic supplementation may significantly reduce gastrointestinal disease, and long-term consumption of *L. rhamnosus* T cell-1 could decrease the incidence of bacterial infection.

Acknowledgements

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