

Enrichment of Formula in Probiotics or Prebiotics and Risk of Infection and Allergic Diseases up to Age 5.5 Years in the Nationwide Etude Longitudinale Française depuis l'Enfance (ELFE) Cohort

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ABSTRACT

Background: An increasing number of infant and follow-on formulas are enriched with probiotics and/or prebiotics; however, evidence for health effects of such enrichment in early childhood remains inconclusive.

Objectives: The present study aimed to assess whether the consumption of formula enriched with probiotics or prebiotics was associated with the risk of infection and allergic diseases in early childhood.

Methods: Analyses involved data for 8389 formula-fed children from the Etude Longitudinale Française depuis l'Enfance (ELFE) cohort. Enrichment of the formula with probiotics or prebiotics that was consumed from the age of 2–10 mo was identified by the formula ingredient list. Lower respiratory tract infection (LRTI), upper respiratory tract infection (URTI), gastrointestinal infection, wheezing, asthma, food allergy, and itchy rash were prospectively reported by parents up to the age of 5.5 y. Adjusted logistic regression models were used to assess associations between the consumption of enriched formula and risk of infection and allergic diseases.

Results: Aged 2 mo, more than half of formula-fed infants consumed the probiotic-enriched formula and only 1 in 10 consumed the prebiotic-enriched formula. Consumption of the *Bifidobacterium lactis*-enriched formula at 2 mo was associated with a lower risk of LRTI [OR (95% CI) = 0.84 (0.73–0.96)]. Consumption of the *Bifidobacterium breve*-enriched formula up to 6 mo was associated with a higher risk of LRTI [OR (95% CI) = 1.75 (1.29–2.38)] and asthma [OR (95% CI) = 1.95 (1.28–2.97)], whereas its consumption from 6 to 10 mo was associated with a lower risk of LRTI [OR (95% CI) = 0.64 (0.48–0.86)] and asthma [OR (95% CI) = 0.59 (0.40–0.88)]. Moreover, the consumption of *Streptococcus thermophilus* from 6 to 10 mo was associated with a higher risk of asthma [OR (95% CI) = 1.84 (1.29–2.63)]. No significant association was found for gastrointestinal infection, food allergy, and itchy rash. Overall, the consumption of prebiotic-enriched formula was not significantly associated with infection and allergy risk.

Conclusions: Associations between the consumption of probiotic-enriched formula and risk of respiratory symptoms differ according to the strain considered and consumption period. Further well-designed studies are needed to confirm these results. *J Nutr* 2022;152:1138–1148.

Keywords: infancy, enrichment, infant formula, prebiotic, probiotic, infection, allergy, birth cohort

Introduction

There is increasing evidence that the microbiome could influence the infant's immune system development and that an imbalanced microbial composition and reduced diversity (dysbiosis) could promote susceptibility to metabolic and immune-related diseases (1, 2). Colonization of the digestive tract and formation of the newborn's microbiota begins mainly at birth, and both its composition and function are variable for the first 3 y of life, after which it stabilizes (2, 3). The composition of the infant gut microbiome is affected by several factors such as the maternal microbiota composition, mode of delivery, feeding practices, environmental exposures, and antibiotic use (2, 4–7).

Breastfeeding is the preferred nutrition option for newborns because it provides all of their nutritional needs (8). Human breast milk is a complex biofluid that contains proteins, lipids, carbohydrates, and various minerals and vitamins (7, 9). It also contains a wide range of nonnutritional bioactive components and a variety of both prebiotics and probiotics that ensure neonate gut colonization by microbes beneficial for metabolism, immune development, and lifelong health (1, 5, 10). According to the FAO and the WHO, probiotics can be considered “living microorganisms, which when administered in adequate amounts confer health benefits on the host,” while prebiotics defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already established in the colon, and thus in effect improve host health” (11).

Although exclusive breastfeeding for the first 6 mo of life or ≥ 4 mo is the nutritional recommendation for healthy term infants (8), several studies of infant feeding practices have shown a high level of noncompliance with these recommendations (12–14). When exclusive breastfeeding is not attainable, infant formula is the preferred option. To reproduce some of the

beneficial aspects of breast milk, a large number of formulas are enriched with probiotics, prebiotics, or both (i.e. symbiotics) (15, 16).

Several randomized controlled trials (RCTs) have assessed the effect of probiotic-, prebiotic-, or symbiotic-enriched formula on infant health. Some showed a beneficial effect of probiotics and prebiotics on respiratory or gastrointestinal infections, antibiotic use, atopic dermatitis, or other types of allergies (17–22), but others did not report any association with incidence of diarrhea episodes, respiratory illnesses, and allergic manifestations (18–24). Moreover, systematic reviews concluded that evidence was not conclusive for recommending the routine use of probiotic-, prebiotic-, or symbiotic-enriched formula in healthy term infants (25–28). The Committee on Nutrition from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition also concluded lack of data to recommend their routine use (29).

In this study, we aimed to describe the consumption of probiotic- or prebiotic-enriched formula and its association with risk of infection and allergic diseases in early childhood.

Methods

Study design

This analysis was based on data from the Etude Longitudinale Française depuis l'Enfance (ELFE) study, a multidisciplinary nationwide birth cohort including 18,329 children born in 2011 in 320 participating maternity units among a random sample of 349 maternity units in mainland France (30). Inclusion began in April 2011 and took place during 25 selected recruitment days over 4 waves of 4–8 d each that covered all 4 seasons. Inclusion criteria were singleton or twins born after 33 weeks of gestation, from mothers aged 18 y or older and not planning to move outside of metropolitan France in the next 3 y. Foreign families also participated in the study if mothers were able to read French, Arabic, Turkish, or English.

Participating mothers had to provide written consent for their own and their child's participation. Fathers signed the consent form for the child's participation when present at inclusion or were informed about their rights to oppose it. The ELFE study was approved by the Advisory Committee for Treatment of Health Research Information (Comité Consultatif sur le Traitement des Informations pour la Recherche en Santé), the National Data Protection Authority (Commission Nationale Informatique et Libertés), and the National Statistics Council.

Data collection

Families were followed by phone interview at age 2 mo, 1 y, 2 y, 3.5 y, and 5.5 y. They also completed a monthly internet/paper-based questionnaire on infant feeding from the age of 3–10 mo.

Infant milk feeding.

At the 2-mo interview, if relevant, the age at first introduction of infant formula was collected.

From the age of 2–10 mo, the name and brand of the formula used was collected monthly for formula-fed infants. Classification of all reported infant formulas was based on their nutritional characteristics (ingredient list and nutritional composition). Enrichment of the formula with probiotics [*Bifidobacterium breve* (BC50), *Bifidobacterium lactis* (BB12), *Bifidobacterium infantis*, other *Bifidobacterium*, *Lactobacillus reuteri* (DSM 17938), *Lactobacillus fermentum* (CECT5716), *Lactobacillus rhamnosus*, other nonspecified *Lactobacillus*, *Streptococcus thermophilus*] or with prebiotics [galacto-oligosaccharides (GOSs) and fructo-oligosaccharides (FOSs)] was identified from the ingredient list.

Infants receiving both breast milk and formula were classified according to the type of formula they received.

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The ELFE cohort is a joint project between INED (Institut National d'Etudes Démographiques), INSERM (Institut National de la Santé et de la Recherche Médicale), EFS (Etablissement Français du Sang), InVS (Institut de Veille Sanitaire), INSEE (Institut National de la Statistique et des Etudes Economiques), the Ministry of Health (DGS, Direction Générale de la Santé), the Ministry of Environment (DGPR, Direction Générale de la Prévention des Risques), the Ministry of Health and Employment (DREES, Direction de la Recherche, des Etudes, de l'Evaluation et des Statistiques), and the CNAF (Caisse Nationale des Allocations Familiales), with the support of the Ministry of Research and CCDSHS (Comité de Concertation pour les Données en Sciences Humaines et Sociales) and the Ministry of Culture (DEPS, Département des études, de la prospective et des statistiques). As part of the RECONAI platform, the ELFE study received state funding from the ANR within the framework of the “Future Investments” program (reference: ANR-11-EQPX-0038, ANR-19-COHO-0001). Author disclosures: AD-C has been invited to allergology and pediatric congresses (inscription and accommodation) by Mead Johnson, Sodilac, and Nutricia. KA-P has been invited by Stallergènes to speak at a conference, outside of the submitted work. All other authors report no conflicts of interest.

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Supplemental Figure 1 and Supplemental Tables 1–12 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/jn/>.

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Abbreviations used: ELFE, Etude Longitudinale Française depuis l'Enfance; FOS, fructo-oligosaccharide; GBTM, group-based trajectory modeling; GOS, galacto-oligosaccharide; LRTI, lower respiratory tract infection; PANDiet, Probability of Adequate Nutrient intake-based Diet quality index; RCT, randomized controlled trial; URTI, upper respiratory tract infection.

Infections and allergies.

In this study, severe/frequent infections, as well as allergies from the age of 2 mo to 5.5 y were assessed as follows:

- Gastrointestinal infection: parental report of ≥ 1 hospitalization for gastroenteritis/dehydration or emergency consultation for diarrhea/vomiting/dehydration.
- Upper respiratory tract infection (URTI): parental report of ≥ 1 hospitalization for URTI or emergency consultation for otalgia, ≥ 3 otitis events from birth, ≥ 3 laryngitis events from birth, or ≥ 3 angina events from birth.
- Lower respiratory tract infection (LRTI): parental report of ≥ 1 bronchiolitis event or 1 hospitalization for bronchitis/bronchiolitis/pneumopathy.
- Wheezing, asthma, itchy rash, or food allergy: ≥ 1 parental report of wheezing in the chest, medical diagnosis of asthma, itchy rash, or medical advice to avoid certain foods due to a food allergy.

Other variables.

Parental sociodemographic characteristics considered in this study were: maternal age at delivery (18–24, 25–29, 30–34, ≥ 35 y), number of older children in the household (no sibling, 1 sibling, ≥ 2 siblings), maternal migration status [“majority population” which included women born to 2 French parents (inside or outside of France), “descendants of migrants” including women born in France with ≥ 1 non-French parent, “migrants” including women not born in France and without French citizenship at birth], maternal education level (up to lower secondary, upper secondary, intermediate, 3-y university degree, ≥ 5 -y university degree), employment status during pregnancy [employed, unemployed, not in the labor force (e.g. housewife, student, disabled, retired)], and monthly household income per consumption unit ($<€750$, $€751$ – 1111 , $€1112$ – 1500 , $€1501$ – 1944 , $€1945$ – 2500 , $>€2500$). From the postal code of residence, we determined the region of residence (Paris region, North, East, East Paris Basin, West Paris Basin, West, South-West, South-East, Mediterranean) and the city size (rural area, urban area; >2000 inhabitants for rural area).

Maternal health characteristics included self-reported height and prepregnancy weight used to calculate prepregnancy BMI (<18.5 , 18.5 – 24.9 , 25.0 – 29.9 , ≥ 30.0 kg/m²), smoking status (never smoker, smoker only before pregnancy, smoker only in early pregnancy, smoker throughout pregnancy), and diet quality during pregnancy [measured by using the Probability of Adequate Nutrient intake-based Diet quality index (PANDiet) score, which reflects nutrient-based reference guidelines adapted for pregnancy; total scores range from 0 to 100] (31).

Newborn characteristics were collected from the medical record: child sex, gestational age, birth weight, and delivery mode [Caesarian-section (C-section), vaginal]. Birth weight categories (small, adequate, or large for gestational age) were defined according to Audipog reference curves (32). The 2-mo questionnaire collected the type of the physician consulted for the first visit after delivery (pediatrician, other child doctor, general practitioner, other including emergency).

Parent and sibling history of allergy (hay fever, asthma, eczema) was collected at the 2-mo interview. Children were considered to have a family history of allergy if ≥ 1 parent or sibling had a history of allergy.

Study sample

Children whose parents withdrew their consent within the first year and requested deletion of their data ($n = 57$) were excluded from this study. We also randomly excluded 1 twin out of 2 ($n = 287$) to avoid family clustering.

For the present analysis, children not followed at the 2-mo interview ($n = 1696$), those exclusively breastfed at the age of 2 mo ($n = 5045$), those without sufficient data on the infant formula consumed at age 2 mo ($n = 667$), those with a medical diagnosis of cow milk protein allergy at age 2 mo ($n = 193$), or those without any data on infection and allergy from the age of 2 mo to 5.5 y ($n = 1995$) were excluded. The main analyses then involved 8389 children (Figure 1).

Statistical analyses

Descriptive statistics.

Differences between excluded children ($n = 9596$) and children included in the main analyses ($n = 8389$) were assessed with Student *t*-test and chi-square test for continuous and categorical variables, respectively.

To take into account the inclusion procedure and biases related to nonconsent and attrition, the data were weighted (33) in sensitivity analyses. Weighting also included calibration on margins from the state register's statistical data and the 2010 French National Perinatal study (34) for the following variables: age, region, marital status, migration status, education level, and primiparity.

Main analyses.

Multiple imputations were performed to deal with missing data (covariates and longitudinal trajectories of the type of formula consumed from the age of 2–10 mo) (35, 36). We assumed that data were missing at random, i.e. that missing values in the data set may depend on the value of other observed variables but cannot depend on the values of unobserved variables (37). We generated 5 independent data sets by using the fully conditional specification method (MI procedure) and the calculated estimates of pooled effects (MIANALYSE procedure; SAS software). Missing data for categorical variables were imputed by using a multinomial model, for ordinal or binary variables by using logistic regression, and for the continuous variables by using linear regression (36).

Adjusted associations between the consumption of enriched formula and risk of infection or allergic diseases were examined by logistic regression models adjusted for confounding factors. Potential confounding factors were identified first from the literature, then selected by using the directed acyclic graphs method (38). The main model was adjusted for maternal and household characteristics [maternal age, education level, migration status, employment during pregnancy, smoking status, city size, region of residence, maternal diet quality during pregnancy (PANDiet score), number of older children, household income, and family history of allergy], child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery, age at infant formula introduction), and variables related to study design (maternity size and recruitment wave). A second model was performed to additionally account for the use of antibiotics up to the age of 1 y.

Formula enriched with probiotics or prebiotics was first examined at the 2-mo follow-up (enriched compared with not enriched). Then, to account for the temporal evolution in the consumption of prebiotic- or probiotic-enriched formula between children aged 2–10 mo, we identified groups of children with similar longitudinal patterns of consumption by using the Nagin's method for group-based trajectory modeling (GBTM) (39) with the TRAJ procedure from SAS software. These trajectories were modeled for children who consumed formula for ≥ 2 follow-ups from the age of 2–10 mo.

Sensitivity analyses.

Sensitivity analyses were conducted for children whose parents did not report any eczema, wheezing, gastrointestinal infection, or respiratory tract infection at the age of 2 mo ($n = 6502$), to address potential reverse causation bias.

Additional sensitivity analyses involved exclusively formula-fed infants at the age of 2 mo ($n = 6251$). Moreover, to deal with the issue of change in infant formula from birth to the 2-mo interview, a sensitivity analysis involved children without any change in infant formula up to the age of 2 mo ($n = 4399$). Finally, given our previous findings on the association between partially hydrolyzed formula and allergy (40), a sensitivity analysis was conducted among infants consuming nonhydrolyzed formula ($n = 7805$).

To deal with selection and attrition bias, a sensitivity analysis was conducted with weighted data according to the weighting described previously. Finally, as the main analyses used multiple imputation to deal with missing data, the main analyses were replicated on the complete-case sample.

All analyses were carried out with SAS 9.4 (SAS Institute).

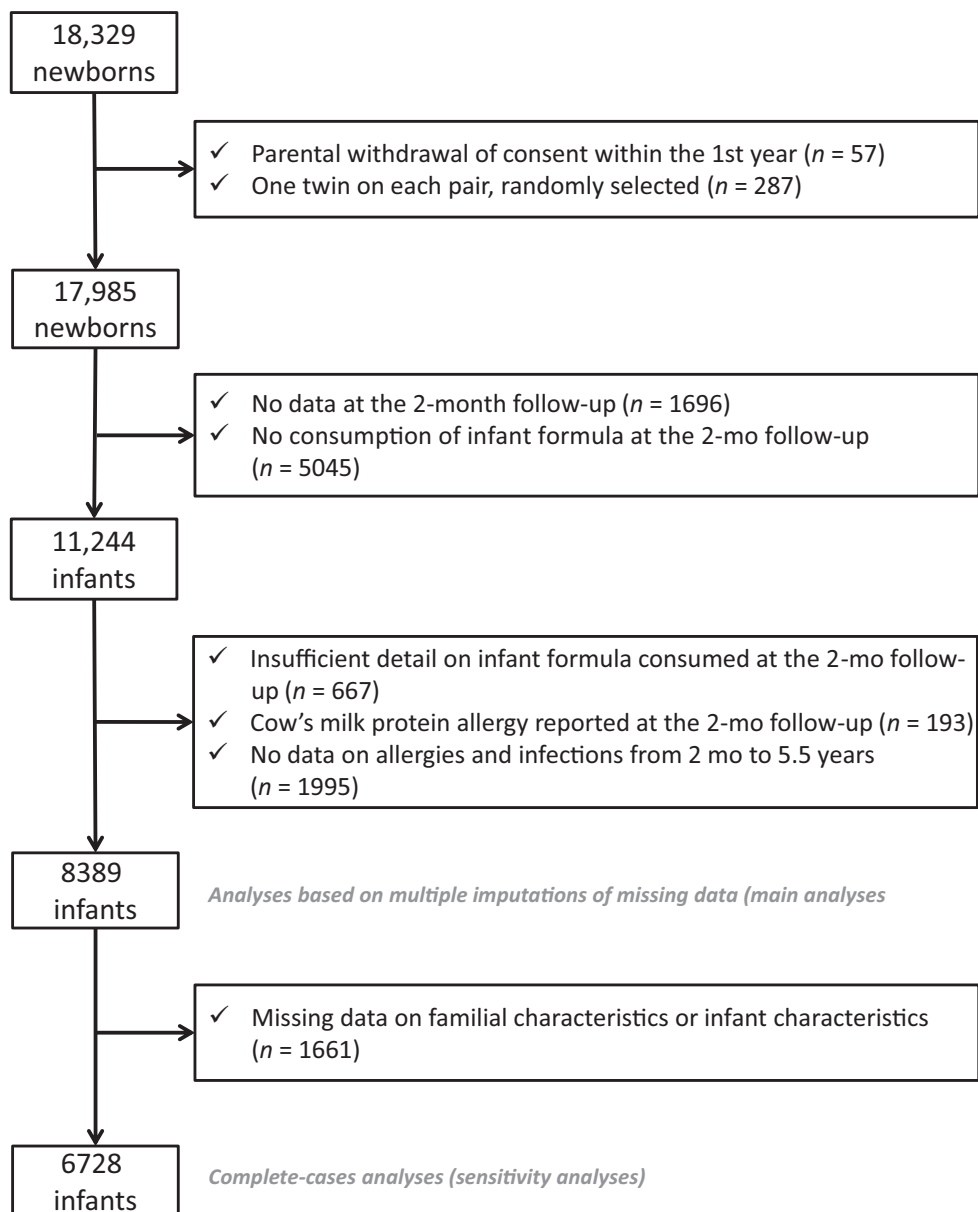


FIGURE 1 Flow chart of the study sample selection.

Results

Compared with excluded children, included children were frequently the first born (46.6% compared with 42.3%, $P < 0.0001$), were born to older mothers (mean age 31.0 ± 4.9 compared with 30.6 ± 5.2 y, $P = 0.00003$), mothers who were employed during pregnancy (75.2% compared with 67.3%, $P < 0.0001$), and had a higher education level (40.8% compared with 35.9% with ≥ 3 -y university degree, $P < 0.0001$). The characteristics of included children are described in [Table 1](#).

Enrichment of formulas with prebiotics and probiotics

At the 2-mo follow-up, more than half of formula-fed infants consumed probiotic-enriched formula. The enrichment with prebiotics usually using a mixture of GOS and FOS, concerned 9.1% (95% CI = 8.4–9.8%) of infants.

The weighted prevalence of formula-fed infants consuming probiotic-enriched formula at the 2-mo follow-up is presented

in [Figure 2](#). The main probiotics added to infant formula were *Streptococcus thermophilus*, *Lactobacillus reuteri* (DSM 17938), and *Bifidobacterium breve* (BC50), followed by *Lactobacillus fermentum* (CECT5716) and *Bifidobacterium lactis* (BB12). Enrichment with *S. thermophilus* was always associated with another enrichment (*B. breve*, *B. lactis*, or *L. reuteri*). The combined enrichment with *Bifidobacterium infantis* and *Lactobacillus rhamnosus* was consumed by <3% of infants and was not further examined.

The longitudinal trajectories of the consumption of formulas enriched with probiotics or prebiotics from the age of 2–10 mo are presented in [Supplemental Figure 1](#). For *B. breve*- and *S. thermophilus*-enriched formula, a 4-group solution was identified with infants consuming: 1) never-enriched formula, 2) enriched formula up to the age of 5–6 mo, 3) enriched formula from the age of 6–7 mo, and 4) enriched formula throughout infancy. For *L. fermentum*- and *L. reuteri*-enriched formula, a 3-group solution was identified with infants consuming:

TABLE 1 Characteristics of children and families included in the main analyses ($n = 8389$)

	Missing values (n)	% (n) or Mean $\pm$ SD
Family characteristics		
Maternal age at delivery, y	0	31 $\pm$ 4.9
Maternal education level	294	
Up to lower secondary		3.4% (279)
Upper secondary		36% (2911)
Intermediate		24.7% (1999)
3-y university degree		17.4% (1411)
≥ 5 -y university degree		18.5% (1495)
Maternal employment during pregnancy	2	
Employed		75.2% (6306)
Unemployed		11.3% (949)
Out of the labor force		13.5% (1132)
Household income (€/month/consumption unit)	247	1661.2 $\pm$ 974.8
Residence in rural area	2	23.3% (1951)
Smoking during pregnancy	100	
Never smoker		54.3% (4503)
Smoker only before pregnancy		24.7% (2047)
Smoker only in early pregnancy		4.1% (336)
Smoker throughout pregnancy		16.9% (1403)
Maternal prepregnancy BMI (kg/m^2)	127	23.7 $\pm$ 5
Children in household	0	
No sibling		46.6% (3907)
One sibling		36.2% (3040)
≥ 2 siblings		17.2% (1442)
Maternal diet quality during pregnancy (PANDiet 0–100 score)	978	54.9 $\pm$ 9
Child characteristics		
Boys	0	51% (4282)
Gestational age (weeks)	132	39.2 $\pm$ 1.5
Child's birth weight	241	
Small weight for gestational age		10% (811)
Adequate weight for gestational age		80.1% (6528)
Large weight for gestational age		9.9% (809)
Age at infant formula introduction, mo	3	0.4 $\pm$ 0.5
Ever infection from age 2 mo to 5.5 y		
Gastrointestinal infection	0	13.4% (1122)
Upper respiratory tract infection	0	32.4% (2716)
Lower respiratory tract infection	0	37.8% (3172)
Ever allergic diseases from age 2 mo to 5.5 y		
Wheezing	0	42.4% (3556)
Medical diagnosis of asthma	0	14.1% (1179)
Itchy rash	0	45.9% (3849)
Food allergy	0	6.3% (525)

PANDiet, Probability of Adequate Nutrient intake-based Diet quality index.

1) never-enriched formula, 2) occasionally enriched formula, and 3) enriched formula throughout infancy. For *B. lactis*-enriched formula, a 2-group solution was identified with infants consuming: 1) never-enriched formula and 2) enriched formula throughout infancy. For GOS-enriched formulas, a 3-group solution was identified, with infants consuming: 1) never-enriched formula, 2) early use of enriched formula, and 3) delayed use of enriched formula.

Probiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 y

The findings of unadjusted analyses are presented in Supplemental Tables 1 and 2.

Bifidobacterium enrichment.

In the main model, compared with infants consuming non-*Bifidobacterium*-enriched formula, those consuming *B. lactis*-enriched formula at the age of 2 mo were at lower risk of LRTI and wheezing from the age of 2 mo to 5.5 y (Table 2). We found no significant association between *Bifidobacterium* enrichment and URTI, asthma (Table 2), gastrointestinal infection, food allergy, or itchy rash up to the age of 5.5 y (Table 3). Further adjustment for antibiotic use up to the age of 1 y did not substantially modify the risk estimates, but the observed associations between the consumption of *B. lactis*-enriched formula and wheezing were no longer significant (data not shown).

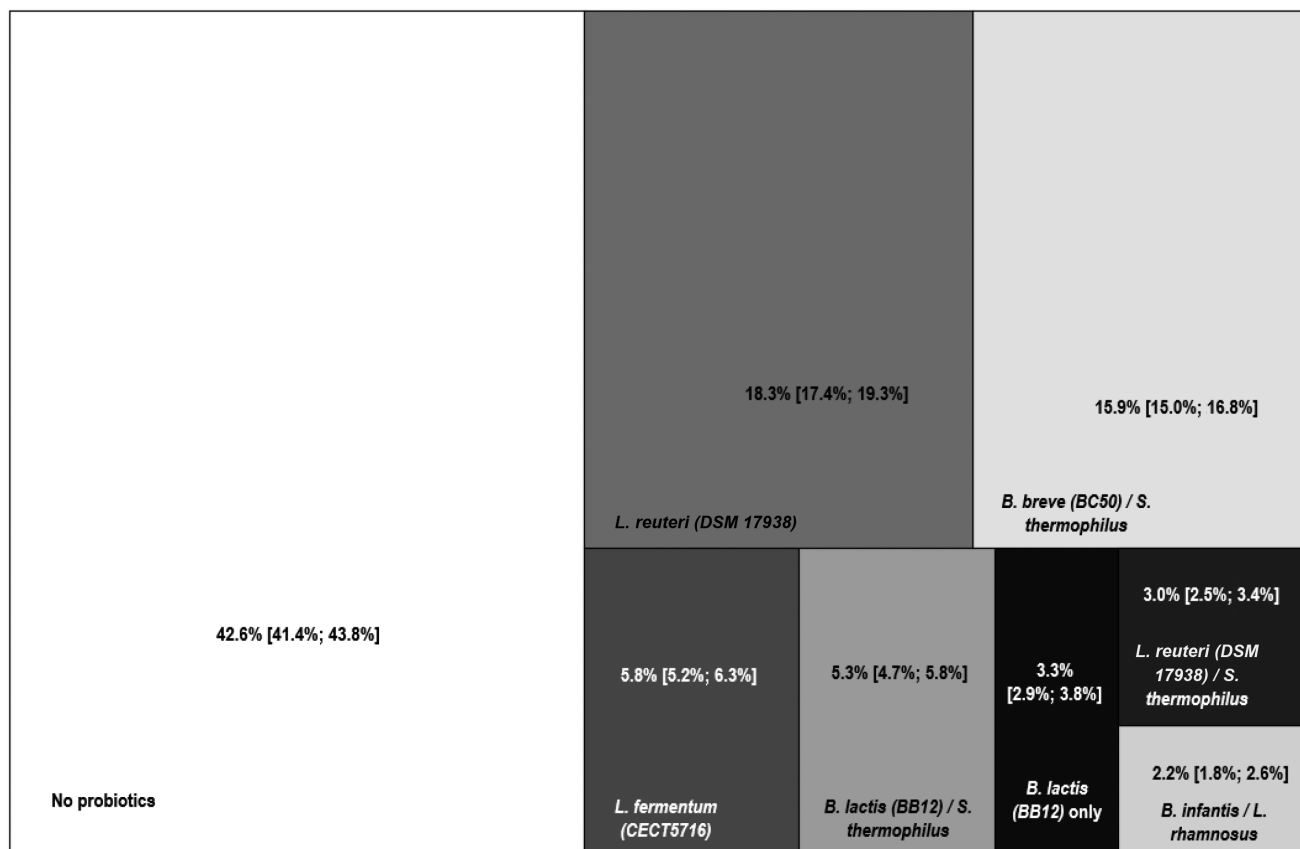


FIGURE 2 Weighted prevalence and CIs of formula-fed infants consuming probiotic-enriched formula at the 2-mo follow-up. *B.*, *Bifidobacterium*; *L.*, *Lactobacillus*; *S.*, *Streptococcus*.

The observed associations for the consumption of *Bifidobacterium*-enriched formula and the risk of respiratory symptoms, gastrointestinal infection, food allergy, and itchy rash were consistent in analyses among exclusively formula-fed infants (Supplemental Tables 3 and 4), in weighted analyses, and analyses in the complete-case sample (Supplemental Tables 5–8). Similar findings were also observed in the other sensitivity analyses [analyses conducted in subsamples of infants without any symptoms up to the age of 2 mo, infants without any change in formula up to the age of 2 mo (Supplemental Tables 9–12)], and infants consuming nonhydrolyzed formula (data not shown).

According to the GBTM method, infants always consuming *B. lactis*-enriched formula from the age of 2–10 mo were also at lower risk of URTI and asthma up to the age of 5.5 y (Table 2). Consumption from the age of 6 mo of *B. breve*-enriched formula was related to a lower risk of LRTI and asthma up to the age of 5.5 y, whereas consumption in the first months only was related to a higher risk of LRTI and asthma up to the age of 5.5 y (Table 2). Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (Supplemental Tables 3–12).

Lactobacillus enrichment.

In the main model, compared with the consumption of non-*Lactobacillus*-enriched formula, the consumption of *L. reuteri*-enriched or *L. fermentum*-enriched formula at the age of 2 mo was not associated with occurrence of infection or allergic diseases up to the age of 5.5 y (Tables 2 and 3). After further

adjustment for antibiotic use, the consumption of *L. reuteri*-enriched formula at the age of 2 mo was related to a lower risk of URTI up to the age of 5.5 y (OR = 0.83 and 95% CI = 0.72–0.97; data not shown).

According to the GBTM method, the occasional or regular consumption of *L. reuteri*-enriched formula from the age of 2–10 mo was not associated with occurrence of infection or allergic diseases (Tables 2 and 3). The occasional consumption of *L. fermentum*-enriched formula from the age of 2–10 mo was related to a higher risk of URTI up to the age of 5.5 y, but not regular consumption. The consumption of *L. fermentum*-enriched formula was not related to other infections or allergic diseases. Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (Supplemental Tables 3–12).

Streptococcus thermophilus enrichment.

In the main model, compared with the consumption of non-*S. thermophilus*-enriched formula at the age of 2 mo, the consumption of *S. thermophilus*-enriched formula was not related to infections or allergic diseases up to the age of 5.5 y (Tables 2 and 3). Similar findings were observed after further adjustment for antibiotic use (data not shown).

According to the GBTM method, the consumption of *S. thermophilus*-enriched formula from the age of 2–10 mo was related to a higher risk of URTI up to the age of 5.5 y. In addition, the consumption of *S. thermophilus*-enriched formula up to the age of 6 mo was related to a lower risk of asthma up to the age of 5.5 y, whereas consumption from 6 mo was related to a higher risk of asthma (Table 2). Consumption

TABLE 2 Adjusted associations between the consumption of enriched formula at 2 mo and from 2 to 10 mo and the risk of respiratory diseases up to the age of 5.5 y (*n* = 8389)

	n	Upper respiratory tract infection		Lower respiratory tract infection		P	Wheezing		P	Asthma		P
		OR [95% CI]	P	OR [95% CI]	P		OR [95% CI]	P				
Consumption at 2 mo												
Enrichment with Bifidobacterium												
No Bifidobacterium	5805	1.00 [Ref]	0.1	1.00 [Ref]	0.03		1.00 [Ref]	0.2		1.00 [Ref]	0.3	
<i>Bifidobacterium breve</i> (BC50)	1372	1.19 [1.00; 1.41]		1.09 [0.93; 1.29]			1.06 [0.90; 1.24]			0.97 [0.77; 1.21]		
<i>Bifidobacterium lactis</i> (BB12)	732	0.95 [0.82; 1.10]		0.84 [0.73; 0.96]			0.87 [0.75; 0.99]			0.83 [0.68; 1.01]		
Other Bifidobacterium or unspecified strain	480	0.87 [0.71; 1.08]		1.15 [0.95; 1.40]			1.10 [0.91; 1.34]			1.25 [0.96; 1.62]		
Enrichment with Lactobacillus			0.2		0.5			0.8			0.1	
No Lactobacillus	6043	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
<i>Lactobacillus reuteri</i> (DSM 17938)	1679	0.86 [0.75; 1.00]		1.09 [0.95; 1.25]			1.00 [0.87; 1.14]			0.85 [0.70; 1.02]		
<i>Lactobacillus fermentum</i> (CECT5716)	480	1.04 [0.87; 1.24]		0.96 [0.81; 1.15]			1.08 [0.91; 1.28]			1.19 [0.95; 1.50]		
Other Lactobacillus or unspecified strain	187	1.18 [0.87; 1.60]		0.89 [0.67; 1.20]			0.95 [0.71; 1.26]			1.06 [0.72; 1.55]		
Enrichment with Streptococcus			0.5		0.6			0.3			0.3	
No Streptococcus	6295	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
<i>Streptococcus thermophilus</i>	2094	0.96 [0.86; 1.07]		0.97 [0.87; 1.08]			1.06 [0.95; 1.17]			1.08 [0.94; 1.26]		
Enrichment with prebiotics			0.6		0.9			0.5			0.7	
No prebiotics	7456	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
FOS/GOS or GOS only	933	0.98 [0.91; 1.06]		1.00 [0.93; 1.08]			1.03 [0.96; 1.11]			0.98 [0.89; 1.09]		
Consumption between 2 and 10 mo												
Enrichment with <i>B. breve</i> (BC50)			0.4		0.002			0.4			0.003	
Never	6053	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Up to 6 mo	284	1.16 [0.85; 1.60]		1.75 [1.29; 2.38]			1.28 [0.96; 1.72]			1.95 [1.28; 2.97]		
From 6 mo	748	1.01 [0.74; 1.40]		0.64 [0.48; 0.86]			0.90 [0.67; 1.20]			0.59 [0.40; 0.88]		
Always	1304	0.79 [0.62; 1.02]		0.90 [0.70; 1.15]			0.90 [0.71; 1.15]			0.74 [0.52; 1.05]		
Enrichment with <i>B. lactis</i> (BB12)			0.01		0.2			0.1			0.04	
Never	7598	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Always	791	0.80 [0.68; 0.95]		0.89 [0.76; 1.05]			0.89 [0.76; 1.04]			0.78 [0.62; 0.99]		
Enrichment with <i>L. fermentum</i> (CECT5716)			0.05		0.3			0.03			0.3	
Never	7717	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Sometimes	329	1.21 [1.02; 1.44]		1.13 [0.96; 1.34]			1.13 [0.95; 1.34]			1.15 [0.92; 1.44]		
Always	343	0.92 [0.78; 1.10]		0.90 [0.76; 1.06]			1.03 [0.87; 1.22]			0.95 [0.75; 1.20]		
Enrichment with <i>L. reuteri</i> (DSM 17938)			0.2		0.5			0.9			0.6	
Never	6468	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Sometimes	590	1.00 [0.88; 1.14]		1.07 [0.94; 1.22]			1.02 [0.90; 1.15]			0.98 [0.82; 1.17]		
Always	1331	0.94 [0.84; 1.05]		0.94 [0.85; 1.05]			1.00 [0.90; 1.10]			0.97 [0.84; 1.12]		
Enrichment with <i>S. thermophilus</i>			0.08		0.4			0.8			0.003	
Never	5218	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Up to 6 mo	471	0.97 [0.76; 1.25]		0.81 [0.64; 1.03]			0.97 [0.77; 1.22]			0.66 [0.47; 0.94]		
From 6 mo	812	0.81 [0.61; 1.09]		1.13 [0.86; 1.50]			0.99 [0.75; 1.29]			1.84 [1.29; 2.63]		
Always	1888	1.34 [1.06; 1.69]		1.10 [0.88; 1.37]			1.10 [0.89; 1.36]			1.15 [0.85; 1.57]		
Enrichment with GOS			0.1		0.5			0.2			0.3	
Never	7205	1.00 [Ref]		1.00 [Ref]			1.00 [Ref]			1.00 [Ref]		
Early use	487	0.87 [0.76; 0.99]		0.96 [0.85; 1.10]			1.08 [0.95; 1.22]			1.06 [0.88; 1.28]		
Delayed use	697	1.13 [0.97; 1.32]		0.99 [0.84; 1.16]			0.87 [0.75; 1.01]			0.88 [0.69; 1.12]		

ELFE, Etude Longitudinale Française depuis l'Enfance; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide.

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, pre-pregnancy BMI), diet quality during pregnancy, city size, region of residence, household income, family history of allergy, and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery, and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.

TABLE 3 Associations between the consumption of enriched formula at 2 mo and from 2 to 10 mo and the risk of gastrointestinal infections, food allergies, or itchy rash up to the age of 5.5 y ($n = 8389$)

	Gastrointestinal infection OR [95% CI]	<i>P</i>	Food allergy OR [95% CI]	<i>P</i>	Itchy rash OR [95% CI]	<i>P</i>
Consumption at 2 mo						
Enrichment with Bifidobacterium		0.3		0.4		0.7
No Bifidobacterium	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Bifidobacterium breve</i> (BC50)	0.93 [0.75; 1.17]		0.87 [0.64; 1.18]		1.07 [0.91; 1.25]	
<i>Bifidobacterium lactis</i> (BB12)	0.92 [0.76; 1.12]		0.91 [0.68; 1.21]		1.00 [0.88; 1.15]	
Other Bifidobacterium or unspecified strain	1.27 [0.98; 1.64]		1.01 [0.67; 1.52]		1.00 [0.82; 1.21]	
Enrichment with Lactobacillus		0.5		0.2		0.8
No Lactobacillus	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Lactobacillus reuteri</i> (DSM 17938)	1.15 [0.95; 1.39]		0.78 [0.59; 1.02]		0.95 [0.83; 1.08]	
<i>Lactobacillus fermentum</i> (CECT5716)	0.94 [0.72; 1.21]		0.79 [0.55; 1.12]		1.00 [0.84; 1.19]	
Other Lactobacillus or unspecified strain	0.87 [0.58; 1.29]		1.74 [1.01; 3.01]		1.11 [0.83; 1.47]	
Enrichment with Streptococcus		0.9		0.06		0.3
No Streptococcus	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
<i>Streptococcus thermophilus</i>	1.01 [0.88; 1.17]		1.21 [0.99; 1.49]		0.94 [0.85; 1.04]	
Enrichment with prebiotics		0.3		0.1		0.5
No prebiotics	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
FOS/GOS or GOS only	1.05 [0.95; 1.17]		0.89 [0.76; 1.04]		0.98 [0.91; 1.05]	
Consumption between 2 and 10 mo						
Enrichment with <i>B. breve</i> (BC50)		0.6		0.7		0.2
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 mo	1.21 [0.78; 1.88]		1.20 [0.69; 2.08]		1.29 [0.96; 1.73]	
From 6 mo	1.06 [0.70; 1.62]		1.02 [0.56; 1.87]		0.91 [0.69; 1.20]	
Always	0.87 [0.62; 1.24]		1.00 [0.62; 1.63]		0.84 [0.66; 1.06]	
Enrichment with <i>B. lactis</i> (BB12)		0.8		0.6		0.5
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Always	1.03 [0.82; 1.28]		1.08 [0.81; 1.44]		0.95 [0.81; 1.10]	
Enrichment with <i>L. fermentum</i> (CECT5716)		0.5		0.1		0.9
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	1.14 [0.89; 1.46]		0.79 [0.54; 1.16]		0.98 [0.83; 1.16]	
Always	0.86 [0.67; 1.11]		1.00 [0.69; 1.44]		1.03 [0.87; 1.21]	
Enrichment with <i>L. reuteri</i> (DSM 17938)		0.7		0.1		0.2
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Sometimes	1.08 [0.90; 1.30]		1.15 [0.90; 1.47]		1.02 [0.90; 1.16]	
Always	0.96 [0.83; 1.13]		0.82 [0.66; 1.01]		0.93 [0.84; 1.03]	
Enrichment with <i>S. thermophilus</i>		0.5		0.4		0.3
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Up to 6 mo	0.84 [0.59; 1.20]		1.27 [0.83; 1.96]		0.83 [0.66; 1.04]	
From 6 mo	0.89 [0.60; 1.33]		0.73 [0.41; 1.31]		1.08 [0.83; 1.41]	
Always	1.17 [0.86; 1.59]		0.82 [0.54; 1.26]		1.14 [0.92; 1.40]	
Enrichment with GOS		0.1		0.2		0.6
Never	1.00 [Ref]		1.00 [Ref]		1.00 [Ref]	
Early use	1.18 [0.99; 1.42]		0.83 [0.62; 1.10]		0.95 [0.83; 1.07]	
Delayed use	0.80 [0.63; 1.02]		1.03 [0.72; 1.49]		1.08 [0.93; 1.26]	

ELFE, Etude Longitudinale Française depuis l'Enfance; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide.

Logistic regressions adjusted for maternal and household characteristics (maternal age, education level, migration status, employment during pregnancy, smoking status, prepregnancy BMI, diet quality during pregnancy, city size, region of residence, household income, family history of allergy, and number of older children), child characteristics (sex, gestational age, birth weight category, type of physician consulted after discharge, C-section delivery, and age at infant formula introduction) and ELFE study design variables (maternity size and recruitment wave). For a given period, all enrichments were considered simultaneously.

of *S. thermophilus*-enriched formula was not related to other infections or allergic diseases (Tables 2 and 3). Similar findings were observed after further adjustment for antibiotic use (data not shown) and in sensitivity analyses (Supplemental Tables 3–12).

Prebiotic-enriched formula and risk of infection or allergic diseases up to age 5.5 y

Consumption of prebiotic-enriched formula at the age of 2 mo or between 2 and 10 mo was not related to infection and allergic diseases, except for the association between early use of GOS-enriched formula and a lower risk of URTI (Tables 2 and 3). However, this association was no longer significant after further adjustment for antibiotic use up to the age of 1 y (data

not shown). The observed findings for the consumption of prebiotic-enriched formula and the risk of infection and allergic diseases were similar in sensitivity analyses (Supplemental Tables 3–12).

Discussion

Among children born in 2011, more than half of formula-fed infants had consumed probiotic-enriched formula at the age of 2 mo. The infant formula consumed was often enriched with *L. reuteri* (DSM 17938) or *B. breve* (BC50) combined with *S. thermophilus*, followed by *L. fermentum* (CECT5716) and *B. lactis* (BB12) combined with *S. thermophilus*. Overall,

the consumption of probiotic-enriched formula was related to the risk of respiratory diseases (respiratory tract infection, wheezing, asthma) up to the age of 5.5 y, but not with gastrointestinal infection, food allergy, and itchy rash. However, the observed associations for respiratory diseases were not consistent depending on the strains used for enrichment, as well as the consumption period of the formula. No significant association was found for the consumption of prebiotic-enriched formula.

As in our study, most RCTs highlighted the null or beneficial effect of probiotic enrichment on respiratory diseases or respiratory infection-related outcomes (25, 29). However, when considering the strains, results are less consistent across studies. Indeed, only 1 RCT including 80 healthy children aged 6 mo in Spain showed a lower number of episodes of respiratory infections with the consumption of formula enriched with *L. salivarius* CECT5713, compared with nonenriched formula (17). Another RCT involving 81 healthy infants in Finland who consumed infant formula before the age of 2 mo also showed a protective effect of formula enriched with *B. lactis* BB12 combined with *L. rhamnosus* GG ATCC53103 on the incidence of recurrent respiratory infections during the first year of life (19). However, in this study, probiotics were not added to the formula during manufacturing, but rather as capsules (prebiotics/placebo) directly added to the formula. In addition, the consumption of probiotic-enriched formula was not related to the incidence of respiratory infections.

In the ELFE study, the consumption of probiotic-enriched formula was not associated with the risk of itchy rash and food allergy, which is consistent with the findings observed in other studies (29). The consumption of probiotic-enriched formula was also not associated with gastrointestinal infection in this study. Although some RCTs showed that the consumption of probiotic-enriched formula (e.g. enrichment with *B. lactis* BB12 alone or combined with *S. thermophilus*, *L. reuteri* ATCC 55730, or *L. salivarius* CECT5713) had a protective effect on gastrointestinal infection outcomes (17, 18, 25), others examining the effect of formula enriched with *B. lactis* BB12 combined with *L. rhamnosus* GG ATCC53103, *B. longum* BL999 combined with *L. rhamnosus* LPR, or *B. breve* C50 combined with *S. thermophilus* or with *L. johnsonii* La1 found nonsignificant associations (19, 24, 25, 29).

In the present study, the effect of probiotic enrichment was evaluated for each probiotic strain, although some formulas were enriched with a combination of strains. This methodological approach does not consider the synergistic effect of probiotics strains; however, the different strains studied were adjusted for each other in all models. Interactions between strains were also tested and were not significant in any model.

Overall, studies that evaluated the effect of probiotic-enriched formula on childhood health showed inconsistent findings. This observation could be explained by the considerable heterogeneity in the RCT designs. Indeed, the types of probiotics and strains as well as the combinations used differed across studies, with the different probiotic strains having specific properties. The measured outcomes and their definitions, duration and timing of the intervention also differ across studies. Further studies with a long follow-up, large sample size, and using similar methodology (same inclusion criteria, duration and timing of intervention, probiotic strains, combinations and dose used) are needed to establish the effects of probiotic-enriched formula on childhood health.

Regarding the effect of prebiotic-enriched formula consumption on infection and allergy in childhood, available studies are

limited and the results are inconclusive (20–23, 26, 27, 29). We found no significant association between prebiotic-enriched formula consumption and the risk of gastrointestinal infection, respiratory symptoms, and allergic diseases, consistent with the findings observed in some RCTs (20, 21, 23). In contrast, 1 RCT involving healthy term infants in Italy, from parents with a history of allergy (atopic eczema, allergic rhinitis, or asthma) found lower number of episodes of URTI during the 2-y follow-up among infants consuming hypoallergenic formula enriched with a mixture of short-chain GOS and long-chain FOS than those consuming the same formula without enrichment (21). In addition, 1 RCT found a significantly lower number of children with ≥ 1 episode of acute diarrhea and lower number of diarrheal episodes during the 12-mo follow-up in the group receiving GOS/FOS-enriched formula compared with the control group (20), and another found that the cumulative incidences of allergic manifestations (atopic dermatitis, recurrent wheezing, and allergic urticaria) were lower in the intervention group than in the control group (21).

To our knowledge, no other longitudinal cohort has assessed the association between probiotic or prebiotic formula enrichment and infection/allergic manifestations, which hinders international comparison of our results.

The present prospective study involved children included in a large French birth cohort with detailed data on infant diet, which allowed us to examine among formula-fed infants and under real conditions of use, the effect of formula enrichment with probiotics or prebiotics on infection and allergic diseases in childhood. In this cohort, the number of infants exposed to probiotic-enriched formula was higher than that observed in the trials (mostly small sample sizes). This high proportion could possibly be attributable to popular beliefs about the health benefits of probiotics. However, a previous study using the ELFE cohort data showed few social- and health-related factors associated with the use of prebiotic- or probiotic-enriched formula (41), which suggests that reverse causality bias is limited in the present study. Indeed, the authors found that only family income and breastfeeding duration were related to the use of prebiotic- or probiotic-enriched formula at the age of 2 mo and the type of infant formula used was fairly stable between 2 and 10 mo.

Although a wide range of sociodemographic and economic data was considered in the present study, other unmeasured factors such as dietary supplements containing probiotics or prebiotics consumed by mothers or infants and intake of prebiotics from complementary foods might have led to potential residual confounding. In this cohort, health data were reported by parents and not validated by medical records, which could lead to a potential measurement bias. However, the items used were derived from international ones (42) to limit this bias. In addition, repeated data on health-related outcomes in childhood limited memory bias. The various sensitivity analyses performed to examine the robustness of our results and address selection and reverse causation bias showed very similar findings, which suggests that if there were any potential biases, they should have a limited impact on our findings.

In conclusion, this observational study showed that probiotic-enriched formulas are often consumed, but few show a real health benefit. The consumption of prebiotic-enriched formula does not have convincing effects on health. Replication in other observational studies and larger well-designed RCTs are needed to confirm these results and possibly identify the most interesting strains and optimal “window of opportunity” for prebiotic- and probiotic-enriched formula consumption.

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The authors' contributions were as follows—CR, M-ND, M-AC, SL, and BL-G: were responsible for the development of the design and protocol of the study; MA, CD-P, and BL-G: performed the statistical analyses; MA: wrote the manuscript; BLG: provided methodological guidance; MA, CD-P, AD-C, KA-P, CR, M-ND, SL, M-AC, and BL-G: were involved in interpreting the results and editing the manuscript for important intellectual content; and all authors: read and approved the final manuscript.

Data Availability

The data underlying the findings cannot be made freely available for ethical and legal restrictions imposed because this study includes a substantial number of variables that together could be used to re-identify the participants based on a few key characteristics and then used to access other personal data. Therefore, the French ethics authority strictly forbids making these data freely available. However, they can be obtained upon request from the ELFE principal investigator. Readers may contact marie-aline.charles@inserm.fr to request the data. The code book and analytic code will be made available upon request pending application and approval.

References

1. Mortensen MS, Rasmussen MA, Stokholm J, Brejnrod AD, Balle C, Thorsen J, Krogfelt KA, Bisgaard H, Sørensen SJ. Modeling transfer of vaginal microbiota from mother to infant in early life. *Elife* 2021;10. doi: 10.7554/eLife.57051.
2. van Best N, Hornef MW, Savelkoul PH, Penders J. On the origin of species: factors shaping the establishment of infant's gut microbiota. *Birth Defects Research Part C: Embryo Today: Reviews* 2015;105(4):240–51.
3. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, Ross MC, Lloyd RE, Doddapaneni H, Metcalf GA, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 2018;562(7728):583–8.
4. Cukrowska B, Bierla JB, Zakrzewska M, Klukowski M, Maciorkowska E. The relationship between the infant gut microbiota and allergy. The role of *Bifidobacterium breve* and prebiotic oligosaccharides in the activation of anti-allergic mechanisms in early life. *Nutrients* 2020;12(4):946.
5. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, Adisetiyo H, Zabih S, Lincez PJ, Bittinger K, et al. Association between breast milk bacterial communities and establishment and development of the infant gut microbiome. *JAMA Pediatrics* 2017;171(7):647–54.
6. Thompson AL, Monteagudo-Mera A, Cadenas MB, Lampl ML, Azcarate-Peril MA. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. *Frontiers in Cellular and Infection Microbiology* 2015;5:3.
7. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. *Open Biology* 2019;9(9):190128.
8. WHO. Feeding and nutrition of infants and young children, guidelines for the WHO European region, with emphasis on the former Soviet countries. Copenhagen (Denmark), 2003.
9. Richard C, Lewis ED, Field CJ. Evidence for the essentiality of arachidonic and docosahexaenoic acid in the postnatal maternal and infant diet for the development of the infant's immune system early in life. *Applied Physiology, Nutrition, and Metabolism* 2016;41(5):461–75.
10. Le Doare K, Holder B, Bassett A, Pannaraj PS. Mother's milk: a purposeful contribution to the development of the infant microbiota and immunity. *Front Immunol* 2018;9:361.
11. FAO/WHO. Probiotics in Food: Health and Nutrition Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria and Guidelines for the Evaluation. Rome (Italy): FAO food and nutrition paper; 2006.
12. Victora CG, Bahl R, Barros AJD, França GVA, Horton S, Krasevec J, Murch S, Sankar MJ, Walker N, Rollins NC. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet North Am Ed* 2016;387(10017):475–90.
13. Schiess S, Grote V, Scaglioni S, Luque V, Martin F, Stolarczyk A, Vecchi F, Koletzko B. Introduction of complementary feeding in 5 European countries. *Journal of Pediatric Gastroenterology & Nutrition* 2010;50(1):92–8.
14. Cai X, Wardlaw T, Brown DW. Global trends in exclusive breastfeeding. *International Breastfeeding Journal* 2012;7(1):12.
15. Vandenplas Y, De Greef E, Veereman G. Prebiotics in infant formula. *Gut Microbes* 2014;5(6):681–7.
16. Federik M. Use of probiotic, prebiotic and symbiotic in infant formulas. *J Nutr Hum Health* 2019;3(1):12–8. DOI:10.35841/nutrition-human-health.3.1.12-18.
17. Maldonado J, Lara-Villoslada F, Sierra S, Sempere L, Gómez M, Rodríguez JM, Boza J, Xaus J, Olivares M. Safety and tolerance of the human milk probiotic strain *Lactobacillus salivarius* CECT5713 in 6-month-old children. *Nutrition* 2010;26(11–12):1082–7.
18. 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.
19. Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infancy – a randomised, double-blind, placebo-controlled study. *Br J Nutr* 2009;101(11):1722–6.
20. Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Bisceglia M, Lionetti P, Cinquetti M, Iacono G, Amarri S, et al. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. *Clin Nutr* 2009;28(2):156–61.
21. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. *J Nutr* 2008;138(6):1091–5.
22. Cuello-Garcia C, Fiocchi A, Pawankar R, Yepes-Nuñez JJ, Morgano GP, Zhang Y, Agarwal A, Gandhi S, Terracciano L, Schünemann HJ, et al. Probiotics for the prevention of allergies: a systematic review and meta-analysis of randomized controlled trials. *Clinical & Experimental Allergy* 2017;47(11):1468–77.
23. Sierra C, Bernal MJ, Blasco J, Martínez R, Dalmau J, Ortuño I, Espín B, Vasallo MI, Gil D, Vidal ML, et al. Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial. *Eur J Nutr* 2015;54(1):89–99.
24. Thibault H, Aubert-Jacquín C, Goulet O. Effects of long-term consumption of a fermented infant formula (with *Bifidobacterium breve* c50 and *Streptococcus thermophilus* 065) on acute diarrhea in healthy infants. *J Pediatr Gastroenterol Nutr* 2004;39(2):147–52.
25. Skórka A, Pięścik-Lech M, Kołodziej M, Szajewska H. To add or not to add probiotics to infant formulae? An updated systematic review. *Beneficial Microbes* 2017;8(5):717–25.
26. Skórka A, Pięścik-Lech M, Kołodziej M, Szajewska H. Infant formulae supplemented with prebiotics: are they better than unsupplemented formulae? An updated systematic review. *Br J Nutr* 2018;119(7):810–25.
27. Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutrition Journal* 2012;11(1):81.
28. de Silva D, Halken S, Singh C, Muraro A, Angier E, Arasi S, Arshad H, Beyer K, Boyle R, du Toit G, et al. Preventing food allergy in infancy and childhood: systematic review of randomised controlled trials. *Pediatr Allergy Immunol* 2020;31(7):813–26.

29. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, Pięścik M, Puntis J, Shamir R, Szajewska H, et al. Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition. *Journal of Pediatric Gastroenterology & Nutrition* 2011;52(2): 238–50.
30. Charles MA, Thierry X, Lanoe JL, Bois C, Dufourg MN, Popa R, Cheminat M, Zaros C, Geay B. Cohort profile: the French national cohort of children (ELFE): birth to 5 years. *Int J Epidemiol* 2020;49(2):368–9jj.
31. Bianchi CM, Mariotti F, Verger EO, Huneau JF. Pregnancy requires major changes in the quality of the diet for nutritional adequacy: simulations in the French and the United States populations. *PLoS One* 2016;11(3):e0149858.
32. Mamelle N, Munoz F, Grandjean H. [Fetal growth from the AUDIPOG study. I. Establishment of reference curves]. *J Gynécologie Obstétrique Biol Reprod* 1996;25(1):61–70.
33. Juillard H. Weighting of ELFE survey data at time 0. pandora.vjf.inserm.fr/public/, 2015.
34. Blondel B, Lelong N, Kermarrec M, Goffinet F, National Coordination Group of the National Perinatal Surveys. Trends in perinatal health in France from 1995 to 2010. Results from the French national perinatal surveys. *Journal de Gynécologie Obstétrique et Biologie de la Reproduction* 2012;41(4):e1–e15.
35. Sterne JA, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, Wood AM, Carpenter JR. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009;338(jun29 1):b2393.
36. van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res* 2007;16(3):219–42.
37. Briggs A, Clark T, Wolstenholme J, Clarke P. Missing... presumed at random: cost-analysis of incomplete data. *Health Econ* 2003;12(5):377–92.
38. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. *BMC Med Res Method* 2008;8(1):70.
39. Nagin D. Group-based modeling of development. Cambridge (MA): Harvard University Press;2005.
40. Dависсе-Ратурет C, Раһерисон С, Адель-Ратент К, Диварет-Шаувейо А, Вое С, Дуфурж МН, Лиорет S, Charles MA, de Lauzon-Guillain B. Use of partially hydrolysed formula in infancy and incidence of eczema, respiratory symptoms or food allergies in toddlers from the ELFE cohort. *Pediatr Allergy Immunol* 2019;30(6):614–23.
41. de Lauzon-Guillain B, Dависсе-Ратурет C, Лиорет S, Ksiazek E, Вое С, Дуфурж М-Н, Бурнез М, Nicklaus S, Wagner S, Charles MA. Use of infant formula in the ELFE study: the association with social and health-related factors. *Matern Child Nutr* 2018;14(1): e12477.
42. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. *Lancet North Am Ed* 2006;368(9537):733–43.