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Journal:	J Pediatr Gastroenterol Nutr.
Citations:	Jun 30.(SUPPL: [Epub ahead of print]): 2010
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Title:	Effects of Bifidobacterium lactis HN019 and Prebiotic Oligosaccharide Added
ISSN:	02772116

Effects of *Bifidobacterium lactis* HN019 and Prebiotic Oligosaccharide Added to Milk on Iron Status, Anemia, and Growth Among Children 1 to 4 Years Old

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ABSTRACT

Objective: To evaluate the effect of *Bifidobacterium lactis* HN019 and prebiotic-fortified milk on iron status, anemia, and growth among 1- to 4-year-old children.

Patients and Methods: In a community-based double-masked, controlled trial in a periurban population, 624 children were enrolled and randomly allocated to receive either milk fortified with additional probiotic and prebiotic (n = 312) or control milk (n = 312) for 1 year. Probiotic and prebiotic milk contained an additional 1.9×10^7 colony-forming units per day of probiotic *B lactis* HN019 and 2.4 g/day of prebiotic oligosaccharides milk. Hematological parameters were estimated at baseline and at the end of the study. Height and weight measurements were recorded at baseline, mid study, and the end of the study. Difference of means and multivariate regression models was used to examine the effect of intervention.

Results: Both study groups were similar at baseline. Compliance was high (>85%) and did not vary by intervention groups. As compared with non-fortified milk, consumption of probiotic- and prebiotic-fortified milk for a period of 1 year reduced the risk of being anemic and iron deficient by 45% (95% CI 11%, 66%; $P = 0.01$) and increased weight gain by 0.13 kg/year (95% CI 0.03, 0.23; $P = 0.02$).

Conclusions: Preschoolers are usually fed milk, which has good acceptance and can be easily fortified for delivery of probiotics. Consumption of *B lactis* HN019 and prebiotic-fortified milk resulted in a smaller number of iron-deficient preschoolers and increased weight gain.

Key Words: anemia, compliance, fortification, growth, iron status, probiotics

(*JPGN* 2010;00: 00–00)

Undernutrition is the major underlying cause of 35% of child deaths and 11% of the global burden of disease among children younger than 5 years of age and a major attributable risk factor for growth faltering and anemia (1). Developing countries in the southeast Asia region have a high burden of undernutrition, with approximately 51% of Indian preschool children experiencing stunting and 75% experiencing iron-deficiency anemia (1–3). Concurrent micronutrient deficiencies, especially of iron and zinc, coexist and often make children prone to growth failure, anemia, delayed development, increased morbidity due to infectious diseases, and risk of death (4–6). It is thus a public health priority to develop feasible and sustainable interventions to improve growth and reduce iron deficiency among children.

Research on alleviating iron deficiency and growth faltering among young children has focused mostly on improving nutrition by fortifying (7,8) or supplementing selected micronutrients (9,10), increasing intake of high-quality food products by health education (11), and improving absorption and bioavailability of iron from ingested foods (12). Limited data are available to evaluate the effect of probiotic supplementation on growth and anemia in the community setting.

Probiotics are a group of microorganisms that benefit the host by adhering to the gut epithelium, stimulating the host immune response, inhibiting epithelial and mucosal adherence of pathogens, and producing antimicrobial substances (13). The role of certain species of probiotics in shielding the gut epithelium, thereby preventing diarrheal conditions, has been well documented (14,15). Often probiotics are combined with prebiotics, nondigestible substrates that favor their proliferation and increase their effects (16).

Recent community-based randomized controlled trials have provided evidence that consumption of probiotic-supplemented formula or lactobacilli-acidophilus–fortified yogurt resulted in significantly accelerated growth of undernourished as well as adequately nourished preschool children (17,18). Studies on consumption of probiotic *Bifidobacterium lactis* HN019 for shorter duration among adults of all ages have shown an effect on immune functions (19). We have already reported the effect of prebiotics and probiotics on the morbidity outcomes among children (20). The present report is the first to provide evidence on the benefits of the long-term consumption of milk fortified with a combination of probiotic *B lactis* HN019 and prebiotics on growth and prevention of anemia among children 1 to 4 years of age.

PATIENTS AND METHODS

The study was undertaken in Sangam Vihar, a resettlement colony in New Delhi between April 2002 and 2004. The study was a double-blind, randomized controlled trial with 4 arms wherein we evaluated the effect of 2 separate interventions in comparison to their respective controls. The details of the study population, design, methods, eligibility, recruitment, and randomization have been published previously (21).

Received June 16, 2009; accepted February 17, 2010.

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In brief, children 1 to 3 years old, likely to remain in the area, not experiencing severe malnutrition, and nonallergic to milk were invited to participate in the study. Parents of eligible children were contacted and the purpose of the study explained to them, and consent was sought for participation. After obtaining consent, children were enrolled and scheduled to visit clinic for baseline assessments. At baseline, detailed physician examination, blood sampling for assessment of body iron stores and anemia, and weight (ATCO Weighing Solutions Co Ltd, Mumbai, India, and SecaCorp Columbia, MD, with a sensitivity of 10 g) and length/height (Shorr Productions, Olney, MD, for length and stadiometers for height with a sensitivity of 0.1 cm) measurements were undertaken. Venous blood was drawn by a trained nurse using trace element–free syringes. Blood samples were analyzed for hemogram using a Coulter automated flow cytometer (Beckman Coulter, Fullerton, CA), zinc protoporphyrin (ZnPP) using a hematoflorometer (AVIV Biomedical, Lakewood, NJ) (22), and serum ferritin (sFr) and serum transferrin (sTfr) using commercial enzyme-linked immunosorbant assay kits (Spectro Ferritin kit, Spectro Transferrin kit; Ramco Labs, Houston, TX) (23,24).

Children were randomized to 1 of the 4 groups by a computer-generated allocation sequence (permuted block randomization with block length of 16). Children were stratified into 2 strata (severely anemic and nonseverely anemic) based on their baseline hemoglobin (Hb) levels and were assigned a letter code (A through D) on the basis of randomization sequence number within each strata. Milk sachets were coded with these letter codes at the manufacturing unit and were only known to the supervisor at Fonterra Brands (Singapore). The investigators and field team were blinded to the intervention code during the study period. At the study site, the letter code was stripped off and labeled with child identification information. The sachets were similar in appearance, size, taste, and packaging.

Fortified and control (Co) milk powders were provided by Fonterra Brands and were packaged as 32-g single-serving sachets. The procedure for reconstitution of the milk sachet was explained to the mothers. Intervention was delivered weekly by household milk assistants and data on compliance were collected. Intervention (fortified milk per 3 serving per day) was designed to deliver 1.9×10^7 colony-forming unit/day of probiotic *B lactis* HN019 and 2.4 g of prebiotic oligosaccharides (PP milk). Probiotic Co milk powder sachets were similar in composition to the intervention sachets except for the presence of probiotics and prebiotics in the intervention sachets. The fortified and Co milk powders were formulated to safely deliver fortified nutrients within nutritional guidelines (Table 1).

Anthropometric assessments were repeated at 6 months and at 1 year of intervention, and end-of-study blood sample was collected after completion of intervention.

Sample Size and Power

For the main study, the sample size was calculated based on the morbidity due to common childhood illnesses. On the basis of the given sample size, we had more than 90% power to detect a change in mean Hb level by 0.50 g/dL over the baseline Hb, and a change in anthropometric indices of 0.5 *z* score over the baseline *z* scores, at $\alpha = 0.05$.

Data Management and Statistical Methods

The data were collected in a predesigned form and collated and stored in the relational database management system developed in Oracle 8i. Double data entry (to check for data entry errors) and manual checking of frequencies were conducted before the code was broken to ensure data quality. Weight and length of the children were transformed into height for age, weight for age, and weight for height *z* scores with reference standards using World Health Organization growth standards (25). In our study, children with Hb $\leq$10 g/dL were considered anemic and classified as iron deficient if they satisfied any 2 of the following 4 conditions: sFr <12 μg/L, sTfr >8.3 μg/mL, hematocrit $\leq$30%, or ZnPP $\geq$80 μmol/mol of heme (26,27). Differences in means of hematological and anthropometric parameters between 2 groups after 1 year of intervention were compared using the Student *t* test and proportion of anemic and iron-deficient children by the χ^2 test.

Multivariate regression models were designed to estimate the adjusted effect of intervention on height velocity or weight velocity. Socioeconomic status (SES), sex, age at baseline, and anemia status, which could influence the treatment effect, were added as covariates. All of the statistical analyses were carried out on Stata version 9.2 (StataCorp, College Station, TX), and SPSS version 12 (SPSS Inc, Chicago, IL). The statistical significance for all of the analyses was ascertained at $\alpha = 0.05$.

TABLE 1. Composition of milk preparation

Nutritive value, per day	PP group	Co group
Energy, kJ	1890	1890
Protein, g	20.1	20.1
Carbohydrate, g	50.1	50.1
Fat, g	19.2	19.2
Vitamin A, μg*	300	300
Vitamin D, μg	5.1	5.1
Vitamin E, mg†	6	6
Vitamin K, μg	13.5	13.5
Vitamin C, mg	48	48
Folate (DFE), μg	114	114
Vitamin B_{12}, μg	2.7	2.7
Calcium, mg	720	720
Phosphorus, mg	540	540
Iron, mg	5.4	5.4
Zinc, mg	3.3	3.3
Prebiotic oligosaccharides, g	2.4	0
B lactis HN019 DR10, CFU	1.9×10^7	0

*Microgram retinol activity equivalent.
†Milligram α-tocopherol equivalents.
CFU = colony-forming unit; Co = control group; DFEs = dietary folate equivalents; PP = probiotic group.

Ethics

The study protocol was approved by the human research and ethical review committees at Johns Hopkins University and Annamalai University.

RESULTS

In this article, we present the results of a 1-year intervention of probiotic-fortified milk on growth and iron deficiency. Of 651 eligible children identified for the study, 27 (parent or caretaker) refused to participate. In all, 624 children were enrolled in the trial. Children were randomly allocated to *B lactis* HN019 and prebiotic-fortified milk group (PP; n = 312) and to Co milk group (n = 312) (Fig. 1). At enrollment, children assigned to the 2 groups were

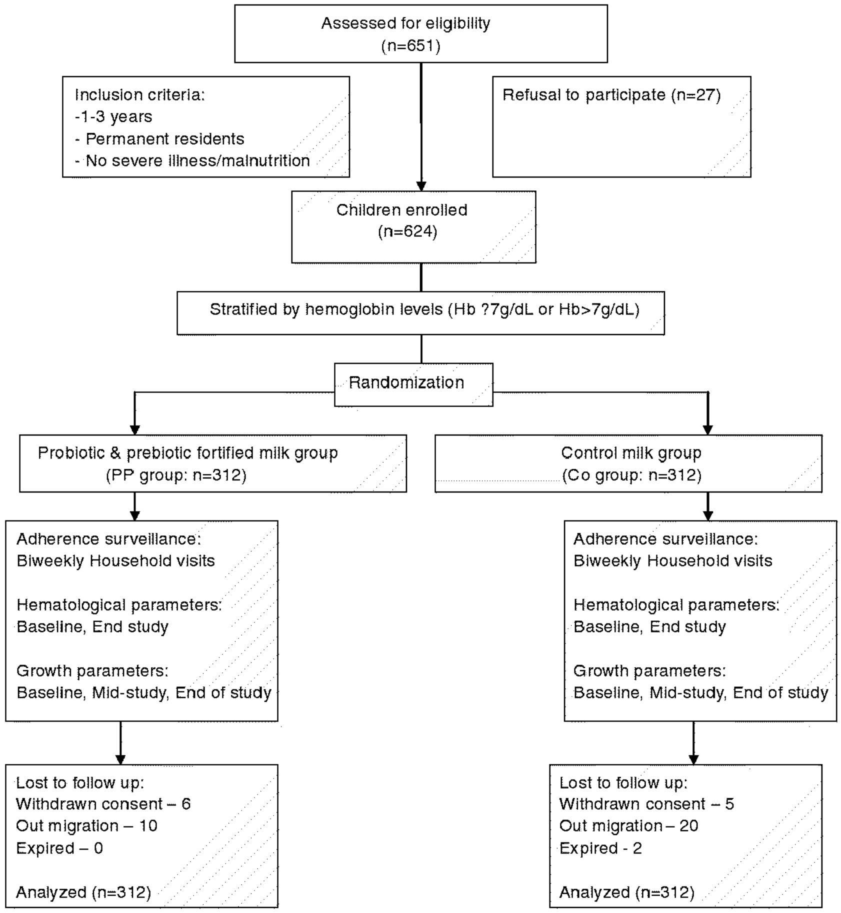

FIGURE 1. Schematic representation of double-blind randomized controlled trial.

comparable for SES, demographic descriptors, body iron stores, anemia, and growth parameters (Table 2). Of 312 children in both arms, we were able to estimate baseline iron deficiency for 292 children in the PP group and 295 children in the Co group (Table 2) because in a few children the minimum of 2 parameters needed for iron deficiency classification were not available. The adherence to intervention was similar in the groups. The consumption did not vary significantly during a period of 1 year, and no adverse effects were reported or observed.

Effect on Iron Stores and Anemia

Compared with the consumption of Co milk, consumption of PP milk resulted in a 45% (95% CI 11%, 66%; $P=0.01$) lower risk of being anemic and iron deficient. No significant effect of fortified milk was observed on iron status indicators such as Hb, ZnPP, RDW, sFr, and sTfr levels (Table 3).

Effect on Growth

The mean weight velocity among children consuming PP milk was higher than that in children consuming Co milk by 0.13 kg/year (95% CI 0.03–0.23; $P=0.02$). There was no significant difference in change in z scores (baseline to end of the study) between the 2 groups (Table 4). In multivariate models, after adjusting for age, SES, sex, and severe anemic status at baseline, we found that children in the PP group had significantly greater weight gain (B $=0.11$; 95% CI 0.006–0.21]; $P=0.04$) as compared with children in the Co group (Table 5).

DISCUSSION

In this study, we found a significant beneficial effect on weight velocity and a 45% lower risk of being iron deficient of the consumption of probiotic- and prebiotic-fortified milk for 1 year. No effect was observed on individual iron deficiency indicators or on other anthropometric z scores. High compliance with the probiotic- and prebiotic-fortified milk powder for a long duration suggests that milk can easily be used as a food vehicle and fortification of commonly consumed foodstuffs can be an effective strategy at the population level.

The health effects can vary by the genera of the probiotic used (13). *B lactis* HN019 was used in this study because this has shown extensive safety and immunostimulant activity in animal models (28–30) and among healthy adult volunteers (31–33). Further, studies have also shown that the fecal microbial flora of breast-fed infants largely consists of bifidobacteria and human milk stimulates the production of bifidobacteria. The presence of *Bifidobacterium* in the intestinal environment was associated with lower rates of morbidity and mortality in breast-fed infants (34,35). The presence of such bacterial strains in fortified milk would thus produce a sort of similar intestinal environment and would confer health benefits.

TABLE 2. Baseline sociodemographic, hematological, plasma zinc, and anthropometric parameters among probiotic- and prebiotic-fortified milk versus control milk group children

	PP group (n = 312)	Co group (n = 312)
Age, mo	22.2 ± 6.4	22.9 ± 6.8
Illiterate mother*	152 (48.7)	155 (49.7)
Occupation father, daily wage labor*	105 (33.7)	112 (35.9)
Occupation mother, housewives*	299 (95.8)	301 (96.5)
Socioeconomic status score	7.66 ± 2.57	7.10 ± 2.45
Hematological parameters		
Hemoglobin, g/dL	9.11 ± 1.56	9.10 ± 1.49
Hematocrit, %	31.00 ± 4.10	30.87 ± 4.03
Zinc protoporphyrin, μmol/mol heme	193.46 ± 125.65	199.12 ± 124.99
Red cell distribution width, %	19.36 ± 2.75	19.35 ± 2.68
Serum ferritin, μg/L	9.23 ± 7.96	9.87 ± 9.09
Serum transferrin, μg/mL	15.25 ± 8.85	15.21 ± 8.79
Anemic† and iron deficient*,‡ (n = 292,295)§	158 (54.1)	168 (56.9)
Anemic and noniron deficient*	55 (18.8)	56 (19.0)
Nonanemic and iron deficient*	27 (9.2)	19 (6.4)
Nonanemic and noniron deficient*	52 (17.8)	52 (17.6)
Plasma zinc, μg/dL	63.37 ± 25.21	62.27 ± 26.07
Anthropometric parameters		
Weight, kg	9.07 ± 1.43	9.13 ± 1.46
Height, cm	78.07 ± 5.87	78.47 ± 6.06
Normal*	107 (34.3)	95 (30.4)
Wasted*	15 (4.8)	14 (4.5)
Stunted*	137 (43.9)	157 (50.3)
Wasted and stunted*	53 (17.0)	46 (14.7)

All values in means, unless specified. Co = control; PP = probiotic group; sFr = serum ferritin; sTfr = serum transferrin.
* Percentage in parentheses.
† Anemic if hemoglobin <10 g/dL.
‡ Iron deficient if 2 conditions out of 4 are satisfied: serum ferritin <12 μg/L, serum transferrin >8.3 μg/mL, hematocrit ≤30%, zinc protoporphyrin ≥80 μmol/mol heme.
§ Baseline iron deficiency levels were estimated for 292 children in the PP group and 295 children in the Co group.

The Co milk provided energy (1890 kJ/day) and protein (20.1 g/day), and the fortified milk (intervention milk) was designed to provide additional *B lactis* HN019 (1.9×10^7 colony-forming units per day) prebiotic oligosaccharide (2.4 g/day) over the Co milk. The results among children consuming fortified milk compared with children consuming Co milk would reflect effects of consuming a combination of *B lactis* HN019 and prebiotic oligosaccharide over and above the energy and protein supplement.

The evidence on the effect of probiotics on growth and anemia among normal children is scarce. The growth effects observed in our study are similar to the recently conducted randomized controlled trials in infants using formulas fortified with *Lactobacillus rhamnosus GG* (36) and *B lactis BB* with *Streptococcus thermophilus* (18), in which an improvement in weight/height/length *z* score was observed. Studies using fermented foods supplemented with probiotic *Lactobacillus* among undernourished or normal children also documented improvements in weight and height gain (17,37,38). Few studies on prebiotics documented that supplementation of synbiotics or prebiotics among acutely ill children receiving antibiotics resulted in greater weight gain and an increase in bifidobacteria levels, which may reduce the growth and virulence of pathogens (39,40). In contrast to the findings of our study, a long-term supplementation trial with only probiotics of different strains did not find any growth effects (41), thus suggesting a plausible synergistic effect of probiotics and prebiotics in delivering the health benefits. The beneficial effects observed in growth could be due to regeneration of gut epithelium, leading to better absorption of nutrients or mineral absorption (42). There is a possibility that the protective effects of probiotic and prebiotic supplementation against diarrhea may also lead to improved production of immunoglobulins and, consequently, may promote compensatory growth in children who often lose weight due to recurrent illnesses (20,43).

Long-term consumption of *B lactis* HN019 and prebiotic-fortified milk did not have any additional effect on the individual iron indicators, but it reduced the proportion of children with iron-deficiency anemia. One of the reasons for noneffect on iron status indicators could be that because both fortified and Co milk consumed 5.4 mg of iron for 1 year, the effect related to improved gut functions was limited to the tail of distribution of iron-deficiency anemia. Similar beneficial effects on prevention of anemia were observed with short-term consumption of probiotic *Lactobacillus acidophilus* (La1) yogurt among Egyptian school-age children (44). The biological plausibility of this effect could be due to the immunomodulatory effects of probiotics, which could reduce enteric infections, thus leading to better absorption of micronutrients (45,46). Another possible mechanism demonstrated in animals could be that consumption of probiotic *L acidophilus*

TABLE 3. Effect of probiotic *B lactis* HN019 and prebiotic oligosaccharide fortified milk on iron status and anemia among children 1 to 4 years of age

Variables	PP group (n = 230)*	Co group (n = 213)	Difference of means (95% CI)	*P*
Hemoglobin, g/dL	10.84 ± 1.33	10.73 ± 1.34	0.11 (−0.14 to 0.36)	0.37
Hematocrit, %	35.85 ± 3.13	35.47 ± 3.67	0.38 (−0.26 to 1.02)	0.24
Zinc protoporphyrin, μmol/mol heme	90.43 ± 73.53	87.62 ± 63.95	2.81 (−10.1 to 15.7)	0.67
Red cell distribution width, %	16.47 ± 2.46	16.57 ± 2.41	−0.01 (−0.55 to 0.36)	0.68
Serum transferrin, μg/mL	8.09 ± 5.02	7.68 ± 3.74	0.41 (−0.44 to 1.27)	0.35
Serum ferritin, μg/L	13.53 ± 10.14	14.44 ± 11.16	−0.91 (−2.99 to 1.18)	0.39
Plasma zinc, μg/dL	63.55 ± 29.09	63.59 ± 32.70	−0.04 (−5.94 to 5.86)	0.99
Anemic† and iron deficient‡ (n, %)	33 (14.3)	50 (23.5)	0.55 (0.34–0.89)§	0.01
Anemic and noniron deficient (n, %)	16 (7.0)	8 (3.8)	1.92 (0.82–4.47)§	0.14
Nonanemic and iron deficient (n, %)	40 (17.4)	39 (18.3)	0.94 (0.58–1.52)§	0.80
Nonanemic and noniron deficient (n, %)	141 (61.3)	116 (54.5)	1.32 (0.91–1.93)§	0.14

Co = control; PP = probiotic group.
*End-of-study hematological data were available for 230 and 213 children in the PP group and in the Co group, respectively.
†Anemic if hemoglobin < 10 g/dL.
‡Iron deficient if 2 conditions out of 4 are satisfied: serum ferritin <12 μg/L, serum transferrin >8.3 μg/mL, hematocrit ≤30%, zinc protoporphyrin ≥80 μmol/mol heme.
§Odds ratio (95% CI).

TABLE 4. Effect of probiotic *B lactis* HN019 and prebiotic oligosaccharide fortified milk on anthropometric parameters among children 1 to 4 years of age

Variables	PP group (n = 257)*	Co group (n = 245)	Difference of means (95% CI)	*P*
Weight velocity†	2.13 ± 0.59‡	2.00 ± 0.59	0.13 (0.03–0.23)	0.02
Height velocity§	8.49 ± 1.41	8.28 ± 1.35	0.20 (−0.04 to 0.45)	0.09
Change in *z* scores between baseline and end of study				
Difference in weight for height score	0.44 ± 0.65	0.34 ± 0.63	0.09 (−0.01 to 0.21)	0.09
Difference in weight for age score	0.34 ± 0.54	0.26 ± 0.54	0.08 (−0.02 to 0.17)	0.12
Difference in height for age score	0.21 ± 0.42	0.18 ± 0.49	0.03 (−0.06 to 0.10)	0.55

Co = control; PP = probiotic group.
*End-of-study anthropological data were available for 257 and 245 children in the PP group and in the Co group, respectively.
†Rate of gain in body weight during a period of 1 year of intervention.
‡Mean ± SD.
§Rate of gain in height during a period of 1 year of intervention.

TABLE 5. Multiple linear regression* evaluating the effect of probiotic and prebiotic fortification on weight velocity and height velocity of children

	Weight velocity (n = 497)			Height velocity (n = 496)		
	B	95% CI	*P*	B	95% CI	*P*
PP group	0.11	0.006–0.21	0.04	0.15	−0.073 to 0.377	0.19
SES	−0.005	−0.025 to 0.015	0.61	0.003	−0.041 to 0.047	0.90
Sex	−0.04	−0.142 to 0.062	0.44	−0.22	−0.444 to 0.006	0.06
Age at baseline	−0.01	−0.018 to −0.002	0.01	−0.083	−0.100 to −0.066	0.000
Severe anemia	0.23	0.056–0.398	0.009	0.42	0.043–0.795	0.03
Constant	2.28		0.000	10.3		0.000
r^2	0.04			0.17		

PP = probiotic group; SES = socioeconomic status.
*Adjusted for SES, sex, age, and severe anemic status at baseline.

resulted in acidification of lumen and, thereby, could be effective in improving iron bioavailability, resulting in improved absorption of iron (47).

CONCLUSIONS

Our data thus suggest that in addition to other benefits, long-term consumption of probiotics and prebiotics may have an effect on growth and iron-deficiency anemia; however, the magnitude of this effect is not sufficient to recommend global probiotic and prebiotic use for the purpose of growth and anemia prevention as such. More studies are required for conclusive evidence.

Acknowledgments: We gratefully acknowledge the contributions of the parents of the children enrolled in the study, and the study team including field workers, supervisors, physicians, data management, and other support staff. We acknowledge the contributions of Anchor Institute, part of Fonterra Brands, Singapore in funding the study as well as providing the milk used in the trial.

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