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Pediatrics 2012;129;e1155; originally published online April 9, 2012;
DOI: 10.1542/peds.2011-1379

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KEY WORDS

acute diarrhea, calcium, children, developing country, probiotics, Indonesia, *Lactobacillus casei* CRL431, *Lactobacillus reuteri* DSM17938

ABBREVIATIONS

ARTI—acute respiratory tract infection
CFU—colony-forming unit
CI—confidence interval
DSMB—data safety monitoring board
IQR—interquartile range
LC—low calcium
RC—regular calcium
RR—relative risk
WHO—World Health Organization

Dr Agustina was the principal investigator and responsible for study concept and design, data collection, laboratory analysis, accuracy and completeness of data analysis, and writing the manuscript; Drs Agustina, Kok, van de Rest, Fahmida, Firmansyah, Lukito, and Bovee-Oudenhoven had a major role in study design, interpretation of results, and writing of the report; Dr Feskens was involved in statistical data analyses, interpretation of results, and writing of the report; Drs van den Heuvel and Albers were involved in the study design and trial monitoring; and Drs Kok and Bovee-Oudenhoven coordinated and had final responsibility for the decision to submit for publication.

This trial has been registered at www.clinicaltrials.gov (identifier NCT00512824).

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www.pediatrics.org/cgi/doi/10.1542/peds.2011-1379

doi:10.1542/peds.2011-1379

Accepted for publication Jan 5, 2012

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WHAT'S KNOWN ON THIS SUBJECT: Some but not all randomized trials have shown effects of probiotics on incidence and duration of diarrhea and respiratory tract infections among children in developing countries. Calcium improves resistance to intestinal infections in adults, but efficacy in children is unknown.

WHAT THIS STUDY ADDS: *Lactobacillus reuteri* DSM17938 may prevent diarrhea, especially in children with lower nutritional status. Regular calcium milk, alone or with *Lactobacillus casei* CRL431, did not reduce diarrhea. None of the interventions affected respiratory tract infections in these Indonesian children.

abstract

OBJECTIVE: To investigate the effects of calcium and probiotics on the incidence and duration of acute diarrhea and acute respiratory tract infections (ARTIs) in low-socioeconomic communities of Jakarta, Indonesia.

METHODS: We conducted a 6-month, double-blind, placebo-controlled study in 494 healthy children aged 1 to 6 years who received low-lactose milk with low calcium content (LC; ~50 mg/day; $n = 124$), regular calcium content (RC; ~440 mg/day; $n = 126$), RC with 5.10^8 colony-forming units per day of *Lactobacillus casei* CRL431 (casei; $n = 120$), or RC with 5.10^8 colony-forming units per day of *Lactobacillus reuteri* DSM17938 (reuteri; $n = 124$). Number and duration of diarrhea and ARTIs episodes were primary and secondary outcomes, respectively.

RESULTS: Incidence of World Health Organization–defined diarrhea (≥3 loose/liquid stools in 24 hours) was not significantly different between RC and LC (relative risk [RR]: 0.99 [95% confidence interval (CI): 0.62–1.58]), between casei and RC (RR: 1.21 [95% CI: 0.76–1.92]), or between reuteri and RC (RR: 0.76 [95% CI: 0.46–1.25]) groups. Incidence of all reported diarrhea (≥2 loose/liquid stools in 24 hours) was significantly lower in the reuteri versus RC group (RR: 0.68 [95% CI: 0.46–0.99]). Irrespective of the definition used, reuteri significantly reduced diarrhea incidence in children with lower nutritional status (below-median height-and-weight-for-age z score). None of the interventions affected ARTIs.

CONCLUSIONS: RC milk, alone or with *L casei,* did not reduce diarrhea or ARTIs in Indonesian children. *L reuteri* may prevent diarrhea, especially in children with lower nutritional status. *Pediatrics* 2012;129:e1155–e1164

Acute diarrhea and acute respiratory tract infections (ARTIs) continue to lead the infectious cause of morbidity and mortality among children <5 years of age in developing countries.[1–3] In Indonesia, diarrhea and ARTIs (pneumonia) contribute to 25% and 16% of the mortality rate among young children, respectively.[4] Moreover, the prevalence of these diseases and malnutrition among children aged <5 years in low socioeconomic urban communities in Indonesia remained high.[5,6] Infection and malnutrition are interrelated,[7] and strategies to increase resistance to infections in this population are needed.

Preventive strategies (including provision of safe water and sanitation, exclusive breastfeeding, hand-washing, vitamin A and zinc supplementation, and vaccinations) are available in developing countries. However, these interventions are not always effective in reducing the burden of these diseases.[3]

Efforts to prevent diarrheal disease by dietary modulation of intestinal host defenses as an alternative strategy are promising.[8] A strictly controlled human study reported that supplementation of healthy adults with regular milk, naturally high in calcium, reduced foodborne enterotoxigenic *Escherichia coli*–induced diarrhea.[8] In addition to other micronutrient deficiencies, many Indonesian children aged <5 years unfortunately have a low dietary calcium intake not meeting their age-specific recommended daily allowance.[9,10] Whether calcium is equally beneficial in children with low dietary calcium intake and frequent episodes of intestinal and respiratory tract infections is currently unknown.

Several meta-analyses and reviews have concluded that probiotics may prevent or reduce duration of diarrhea in children. However, the beneficial effects depend on the probiotic strain and dose, and evidence was obtained mainly in developed countries.[11–14] Moreover, several studies have explored benefits of probiotics in the prevention of ARTIs in children.[15–18] So far, recommendations to supplement with calcium or probiotics in community settings in developing countries are not justified.[13] Therefore, we investigated the efficacy of dietary calcium with or without 2 probiotic strains on the incidence and duration of acute diarrhea and ARTIs in children. The probiotic strains used are related to strains previously suggested to have antidiarrheal benefits in young children.[19–22]

METHODS

Study Design

A randomized, double-blind, placebo-controlled trial was conducted between August 2007 and September 2008 in low socioeconomic urban communities representing nonflooding and flooding areas of East Jakarta, Indonesia. The protocol was approved by the medical ethics committees of the Faculty of Medicine, University of Indonesia, and of Wageningen University. All parents provided written informed consent before inclusion.

Subjects

Children aged 1 to 6 years were selected from a community registry for the first screening phase to assess eligibility on the basis of the following inclusion criteria: apparently healthy, not being breastfed, and if consuming milk, calcium intake was <75% of the age-specific recommended daily allowances. In the second phase, registered physicians interviewed mothers and examined the children to check the exclusion criteria: symptoms of chronic/congenital diseases and disabilities, pulmonary tuberculosis, history of allergy, diarrhea on admission, antibiotic use within 2 weeks before study start, severe wasting (less than −3 SD of weight-for-height *z* score), calcium intake >375 mg/day according to a validated semiquantitative food-frequency questionnaire, not capable or willing to drink milk with a straw in a 2-day acceptance test, showing allergy or intolerance to the products, and/or sibling of included child (twins excepted).

Interventions

Children were randomly assigned to receive low-lactose milk as follows: with a low-calcium content (LC; ~50 mg/day), regular-calcium content (RC; ~440 mg/day), RC plus *Lactobacillus casei* CRL431 (5×10^8 colony-forming units [CFU]/day [casei]), or RC plus *Lactobacillus reuteri* DSM17938 (5×10^8 CFU/day [reuteri]). Milk was sweetened, chocolate-flavored, ambient stable (sterilized by using ultra-high temperatures), and packed in tetra paks (Frisian Flag, Indonesia, Jakarta, Indonesia). Milk was consumed with straws coated inside with the oil drop as placebo (BioGaia AB, Stockholm, Sweden) or with either *L casei* CRL431 (Chr Hanssen, Hørsholm, Denmark) or *L reuteri* DSM17938 (BioGaia AB) in vegetable oil. Probiotic dosage was based on supplier's information of efficacy, application in children, safety concerns when dosed for longer periods of time, and technical reasons (ie, straw coating). The different milk drinks and straws were indistinguishable to the investigators and participants. The composition of the milks and straws is described in Table 1.

Milks and straws were stored cooled (<10°C) at all times until delivery. Viability of the probiotics was checked each month by using selective plating. Field workers distributed milk and straws twice a week to the parents, who were instructed to store products refrigerated and prevent sun exposure. Parents without refrigerators obtained the products from the field workers' house on a daily basis and/or children consumed the products directly at the field workers' house.

TABLE 1 Composition of LC and RC Milk and Probiotic Straws

Composition	LC	RC	Casei	Reuteri
UHT milk (per 100 mL)				
Energy, kcal	93.8	98.0	98.0	98.0
Fat, g[a]	3.5	3.9	3.9	3.9
Protein, g[a]	3.9	3.8	3.8	3.8
Total carbohydrate, g[a]	11.7	12.0	12.0	12.0
Lactose, g	0.07	0.09	0.09	0.09
Vitamin A, μg	32	30	30	30
Calcium, mg[a]	15	129	129	129
Phosphor, mg	32	77	77	77
Magnesium, mg	6	6	6	6
Iron, mg	0.30	0.30	0.30	0.30
Zinc, mg	0.14	0.14	0.14	0.14
Straw probiotic, CFU/day				
L casei CRL431	—	—	5×10^8	
L reuteri DSM17938	—	—		5×10^8

UHT, ultra-high temperature.

[a] Based on chemical analyses.

Mothers were instructed to provide the children with 180 mL of milk twice daily (not with a meal) by using the straws provided. Mothers were requested to maintain the child's habitual diet but to exclude probiotic, prebiotic, or high-calcium foods/drinks other than the supplied ones. The amount of milk consumed was measured by using a calibrated stick put into the tetra paks to score the remaining volume by using a pretested 5-point scale. The field workers observed the children drinking milk at least once a week, and empty packages had to be shown during visits. During diarrheal episodes, children continued or restarted drinking milk as soon as possible but after being rehydrated with oral rehydration solution according to World Health Organization (WHO) guidelines.[23] We followed the local standard for outpatient and hospital care for diarrhea and ARTI, which were per WHO guidelines.[23–25] Liability insurance was provided for the children during the study. Activities with creative and educational contents were implemented to maintain compliance of both mothers and children.

Randomization and Blinding

Eligible children were admitted to the study on enrollment basis and stratified according to area of living (flooding and nonflooding), age (<57 and ≥57 months), and gender. A randomization table with treatment codes and a block size of 8 was generated by using SAS version 9.1 (SAS Institute, Inc, Cary, NC) by an independent individual at Wageningen University. Twin siblings of subjects ($n = 3$) were allocated to the same treatment group. Researchers, mothers, children, and laboratory personnel were unaware of the treatment until all biochemical and data analyses were finished and until after the blind review meeting. The data safety monitoring board (DSMB) and an independent person at SEAMEO RECFON kept 3 sets of sealed envelopes allowing deblinding per subject without disclosing other children's treatments.

Outcomes

The primary outcomes were the number and duration of diarrheal episodes. The main secondary outcomes were the number and duration of ARTI episodes.

Diarrhea was identified according to the WHO definition (≥3 loose/liquid stools in 24 hours).[23] In addition, all reported diarrhea (broader definition: ≥2 loose/liquid stools in 24 hours) was evaluated. Stool frequency was counted when there was at least a 1-hour interval since the previous defecation.[26] An episode was considered to have ended on the last day of diarrhea followed by 2 diarrhea-free days.[27] Duration of diarrhea was defined as number of days from first until last excretion of the loose or liquid stool that was not followed by another abnormal stool in each episode.[26,28]

The presence of an ARTI was defined as when a child had ≥1 respiratory tract symptom(s) (runny nose, cough, or sore throat) and/or ≥1 additional respiratory tract symptom(s) or 1 constitutional symptom (fever, headache, restless, aphony, shortness of breath, acute ear pain, or discharge).[29,30] These symptoms were confirmed with a physician's diagnosis of acute-upper (rhinitis, pharyngitis, sinusitis, otitis, and common cold) and lower (pneumonia, bronchitis, and bronchiolitis) respiratory tract infection.[31] ARTI duration was the number of consecutive days with ≥2 defined signs and symptoms, with a 7-day symptom-free interval before a new episode could occur.[32]

Data Collection

Field workers collected fecal samples before and at the end of the intervention, as well as during diarrheal episodes. Diarrheal samples were collected from onset of diarrhea until maximally 3 days later. Stools contaminated with urine or that had fallen into the toilet or the child's underwear were discarded. Collected stools were kept cool (–20°C) at the field workers' house until storage in a freezer (–70°C) at the laboratory. Stools were freeze-dried and analyzed for calcium[8] and rotavirus (diarrheal samples).[33]

Before and at intervention end, nonfasting venous blood was drawn in the morning by trained phlebotomists. A study physician examined the health status of the children, and field workers performed anthropometric measurements. Lightly clothed children were weighed without shoes by using an

electronic scale (SECA model 890; SECA, Hamburg, Germany) with a precision of 0.1 kg. Body stature was measured by using a microtoise with a precision of 0.1 cm. Routine hematology testing was performed by using an automatic analyzer (Advia 120; Bayer Diagnostics, Tarrytown, NY).[34] A high-sensitivity chemiluminescent assay (Immulite; Dade Behring, Los Angeles, CA) was used to measure serum high-sensitivity C-reactive protein concentrations.[35] Serum α_1-acid glycoprotein was measured by using an enzyme-linked immunosorbent assay.[36]

Follow-up Observation for Diarrhea, ARTIs, and Adverse Events

During the trial, mothers recorded daily defecation patterns (time, frequency, and stool's visual appearance),[37] and feces were graded as 1 (normal), 2 (loose), 3 (semiliquid), and 4 (liquid) on a structured form.[38] Field workers verified records twice a week, and mothers or caregivers were instructed to report newly observed symptoms of intestinal infection immediately. In addition, the occurrences of ARTIs were determined and recorded by field workers on a structured, pretested form. Final diarrhea and ARTI diagnosis and recording in the trial database were verified by the physicians.

Adverse events were recorded by using International Classification of Diseases, 10th Revision codes.[39] Severity and likelihood of relation to the intervention were scored by the physician and continuously monitored by the DSMB. An independent expert monitored trial conduct and accordance to protocol.

Statistical Analysis

Sample size was calculated on the basis of mean episodes and duration of diarrhea, with a preset level of significance of 5% and a power of 80% allowing 2-sided testing and taking 20% dropouts and noncompliant cases into account. A minimum sample size of 480 patients for 4 treatment groups was required to detect a 21% reduction of mean number of diarrheal episodes and 0.7-day reduction of mean diarrhea duration over a 6-month intervention period. These effect sizes were based on meta-analyses of probiotics.[13,40]

Intention-to-treat analysis was performed for all outcomes and for all eligible children who were randomly allocated to treatment and had consumed the intervention products at least once. Analyses were conducted according to a predefined data analysis protocol.

The χ^2 test was used for comparison of categorical variables between groups, and Fisher's exact test was used when the expected count was <5. Student's t test was used to identify differences in normally distributed variables between predefined groups (between LC and RC; RC and casei; and RC and reuteri). The Mann–Whitney U test was applied when data were not normally distributed. PASW Statistic 17.0.3 for Windows (SPSS Inc, Chicago, IL) was used for analyses.

Disease incidence was the number of episodes divided by child-years of observation.[41] For count outcomes, Poisson regression was used, or the negative binomial model in case of excess zeros and overdispersion, to estimate the relative risk (RR) and 95% confidence intervals (CIs) between groups.[42] For this purpose, Stata for Windows release 11 (Stata Corp, College Station, TX) was used. The dependent variable was number of episodes, and treatment group was the independent variable. The variables area, age, gender, diarrhea, and ARTI prevalence within the 2 weeks before study start, household monthly expenditure, and weight-for-height z score at baseline were included in the model as covariates. Potential effect modification by age, habitual calcium intake, and baseline nutritional status were assessed by adding interaction terms to the regression model. The adjusted Cox proportional hazards regression model for recurrent events was performed to compare the proportion of children without diarrhea and ARTIs in all groups.

RESULTS

A total of 3150 children were screened in phase 1 and 1343 in phase 2. Of 497 eligible children, 3 refused to have baseline measurements taken. In total, 494 children were randomly allocated to 4 treatment groups (Fig 1) and included in the intention-to-treat analysis.

At admission, all study groups were comparable with respect to sociodemographic characteristics, health and nutritional status, and habitual dietary intake (Table 2). About 21% of children were anemic, 23% underweight, 31% stunted, and 3% wasted. The compliance to study products was high (94%) and similar among groups. Both probiotic strains remained $>90\%$ viable during the intervention period.

The incidence of WHO-defined diarrhea was not significantly different among groups (Fig 2, Table 3). Duration of episodes also did not differ across groups.

For the outcome all reported diarrhea, children receiving RC and *L reuteri* (reuteri group) experienced a significant 32% reduction in diarrheal episodes compared with the RC group (RR: 0.68 [95% CI: 0.46–0.99]) (Table 3). In addition, the adjusted Cox probability curves showing the proportion of diarrhea-free children was better ($P = .036$) (Fig 3). For the other treatment groups, results from WHO-defined and all reported diarrhea were comparable.

Importantly, significant interactions with nutritional status were observed ($P < .05$) for both diarrhea outcomes. Stratified analysis showed a strong and significant effect of *L reuteri* in

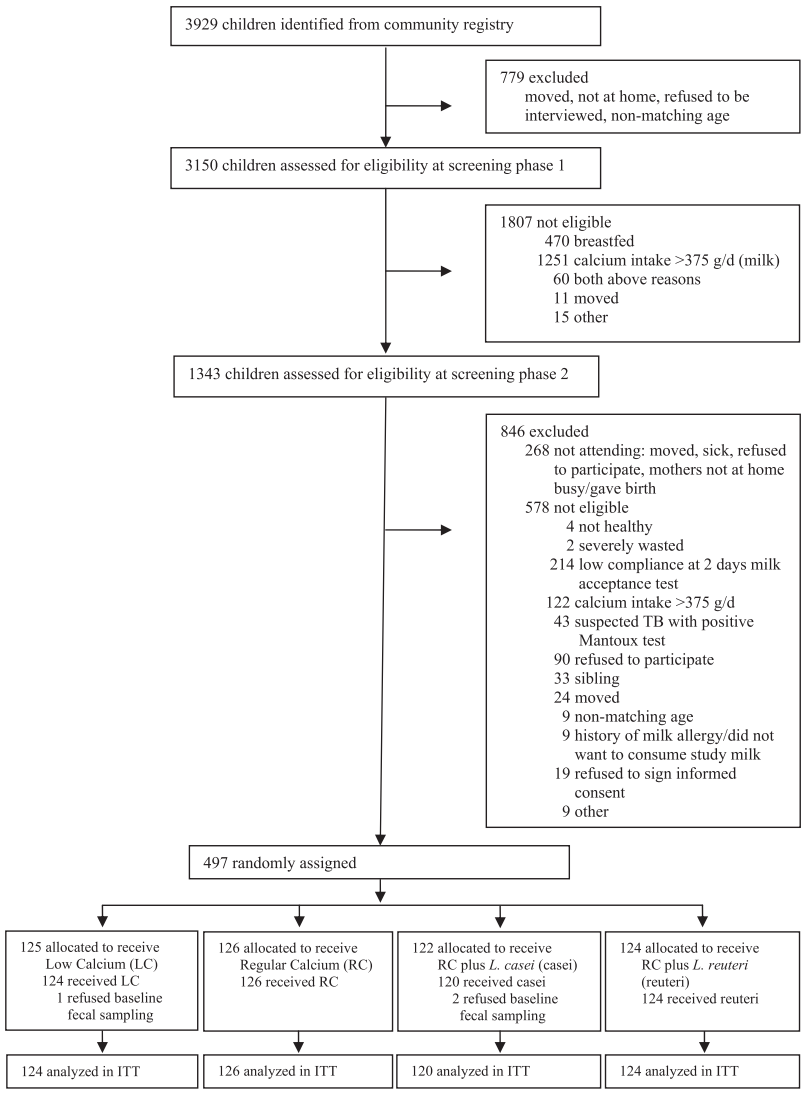

FIGURE 1
Flow diagram of study subjects. ITT, intention-to-treat; TB, tuberculosis.

children with below-median weight-for-age *z* score (RR for WHO-defined diarrhea compared with RC group: 0.44 [95% CI: 0.21–0.92]; RR for all reported diarrhea: 0.54 [95% CI: 0.31–0.94]) and in children with below-median height-for-age z score (RR for WHO-defined diarrhea: 0.44 [95% CI: 0.21–0.90]; RR for all reported diarrhea: 0.53 [95% CI: 0.30–0.92]). In children above the median *z* scores, the results for the reuteri group were not significantly different from the RC group. The prevalence of underweight and stunting was not significantly changed by the interventions (data not shown).

The percentages of diarrheal samples positive for rotavirus according to study group were as follows: LC, 28%; RC, 25%; casei, 28%; and reuteri, 19%. Differences were not significant.

The incidence, number of episodes, and duration of ARTIs were not significantly different among treatments (Fig 4, Table 3).

Reported adverse events (International Classification of Diseases, 10th Revision codes) were comparable among groups, except for change in bowel habits (less regular defecation) and asthma. Nine children in the reuteri group experienced a change in bowel habits, compared with 2 in the RC group. Although based on a few cases, this difference was statistically significant. Three children had asthma in the reuteri group and none in the RC group ($P < .05$). The proportions of antibiotic use during the intervention according to study group were 9% in LC, 15% in RC, 15% in casei, and 9% in reuteri. The median duration of antibiotic use was higher in the RC group (median: 10 days; interquartile range [IQR]: 4–14) compared with the reuteri group (3 days; IQR: 2.5–4.5; $P = .025$), but did not differ from the other groups (LC, 4 days; IQR: 3–7.5; casei, 5 days; IQR: 3–11). One child died of bone tuberculosis 3.5 months after study end, which was unrelated to study participation according to the DSMB.

DISCUSSION

Neither calcium nor *L casei* CRL431 affected any of the diarrheal outcomes. In contrast, *L reuteri* DSM17938 supplementation significantly reduced the incidence of all reported diarrhea (–32% in ≥2 loose/liquid stools in 24 hours) and nonsignificantly reduced the incidence of WHO-defined diarrhea (24% in ≥3 loose/liquid stools in 24 hours). Notably, for both diarrhea outcomes, the protective effect of *L reuteri* DSM17938 was significant in children with lower nutritional status (below-median height-and-weight-for-age *z* score). None of the interventions affected incidence or duration of ARTIs. No serious adverse events related to the interventions were reported.

We applied the WHO definition of diarrhea to collect data on the primary outcome. Because the WHO considers fecal consistency more important than the number of stools[23,43] and their definition leaves room for registration of any increase in normal stool frequency, we also evaluated the outcome of all reported diarrhea (a broader definition of diarrhea). Although the WHO definition is the best validated, it

TABLE 2 Baseline Characteristics of the Indonesian Children According to Assigned Treatment

Characteristic	LC (n = 124)	RC (n = 126)	Casei (n = 120)	Reuteri (n = 124)
Living in flooding area, *n* (%)	81 (65)	82 (65)	78 (65)	82 (66)
Male, *n* (%)	67 (54)	68 (54)	66 (55)	68 (55)
Age, mean ± SD, mo	59.3 ± 14.3	58.9 ± 14.2	60.3 ± 13.7	58.9 ± 15.1
Family size, mean ± SD	5.1 ± 1.7	5.4 ± 1.7	5.2 ± 1.8	5.0 ± 1.8
Household expenditure, mean ± SD, US$/mo[a]	189 ± 97	194 ± 139	159 ± 69	203 ± 181
Mother's education <6 y, *n* (%)	43 (35)	43 (34)	52 (43)	50 (40)
Diarrhea 2 wk before study, *n* (%)	20 (16)	13 (10)	24 (20)	15 (12)
ARTI 2 wk before study, *n* (%)[b]	48 (39)	51 (40)	52 (43)	56 (45)
Serum HS-CRP, median (IQR), mg/L	0.79 (0.23–1.82)	0.75 (0.28–2.90)	0.75 (0.30–2.50)	0.66 (0.25–3.03)
Serum AGP, median (IQR), g/L	0.79 (0.69–0.93)	0.82 (0.70–0.95)	0.83 (0.70–0.97)	0.81 (0.71–0.94)
Anemia, *n* (%)	24 (19)	33 (26)	24 (20)	24 (19)
Nutritional status				
Weight-for-age z score, mean ± SD	−1.27 ± 1.1	−1.40 ± 0.9	−1.15 ± 1.1	−1.26 ± 1.2
Height-for-age z score, mean ± SD	−1.53 ± 1.0	−1.65 ± 0.9	−1.39 ± 1.0	−1.47 ± 1.1
Weight-for-height *z* score, mean ± SD	−0.51 ± 1.0	−0.59 ± 1.2	−0.58 ± 1.0	−0.65 ± 0.9
Fecal calcium, median (IQR), mg/g	7.6 (4.7–11.1)	7.5 (4.8–10.4)	6.6 (4.8–9.3)	7.8 (5.1–11.4)
Habitual dietary intake,[c] mean ± SD				
Energy, kcal/d	1033 ± 368	1066 ± 329	1024 ± 369	976 ± 310
Protein, g/d	34.3 ± 13.5	36.3 ± 12.9	33.5 ± 13.6	32.9 ± 11.0
Carbohydrate, g/d	155 ± 58	157 ± 48	156 ± 58	146 ± 49
Fat, g/d	32.2 ± 13.2	34.5 ± 13.9	31.8 ± 13.7	30.9 ± 11.4
Fiber, g/d	4.5 ± 3.1	5.1 ± 3.5	4.9 ± 3.7	4.6 ± 2.8
Calcium, mg/d	235 ± 95	241 ± 97	228 ± 105	228 ± 94
Iron, mg/d	6.1 ± 2.7	6.6 ± 2.6	6.2 ± 2.7	6.1 ± 2.4
Zinc, mg/d	4.4 ± 2.0	4.8 ± 1.9	4.4 ± 2.0	4.4 ± 1.6

AGP, α_1-acid glycoprotein; HS-CRP, high-sensitivity C-reactive protein.

[a] Student's *t* test; significantly different, RC versus casei and reuteri versus casei ($P < .05$).

[b] χ^2 test; significantly different, RC versus reuteri ($P < .05$).

[c] Assessed by using a semiquantitative food-frequency questionnaire.

may not be generalizable to different settings such as our intervention, which included children of older age and in an urban community setting.[44] Moreover, mothers in the study area usually reported diarrhea when their child defecated ≥2 loose/liquid stools, and broader diarrhea definitions were applied by other clinical trials.[45–47]

We did not only rely on mothers' perception but implemented an active surveillance program to verify mothers' daily records with twice-a-week visits of trained field workers and twice-a-month visits of field supervisors. The physician and monitoring expert accompanied the field workers on several of their home visits. All end points were assessed by using structured and pretested forms as applied by others.[26,29,37,38] The forms were adapted to the local situation and were used by field workers who were rigorously trained and supervised on their application.

Previous evidence on the preventive effect of probiotics on diarrhea and ARTIs has been limited to small studies, mainly hospital or day care center based, with a short follow-up period and performed in developed countries.[11–13,40,48] Therefore, our study in a low socioeconomic community of a developing country, with a much higher number of subjects and longer follow-up, provides critical data to help establish the relevance of these interventions for the prevention of diarrhea in developing countries. To our knowledge, our study is the first large randomized controlled trial, focusing on the effect of calcium with or without 1 of 2 specific probiotics to reduce diarrhea and respiratory tract infections in these settings. Our results indicate that the effect of a probiotic, such as *L reuteri* DSM17938, on diarrhea is modified by nutritional status and is confined to children with lower nutritional status.

The rationale for using calcium in children is based on a proof-of-principle study with adults orally challenged with live but attenuated enterotoxigenic *Escherichia coli*. Dietary calcium strongly reduced infection-induced diarrhea in that study.[8] Animal studies show protective effects against *Salmonella* as well,[49,50] but human verification for that finding is still lacking. Rotavirus is responsible for 60% of hospitalized and 41% of outpatient clinic diarrheal cases in Indonesian children.[51] Important bacterial pathogens among children in developing countries are *E coli* (10%–20%), *Salmonella* (<5%), *Shigella* (5%–10%), *Campylobacter*, and *Vibrio cholerae* (exact %'s unknown).[52] The absence of a beneficial effect of calcium in our trial may indicate a difference in efficacy between children and adults and/or that protective effects are pathogen dependent.

The application of probiotics to prevent or treat acute diarrhea is based on the

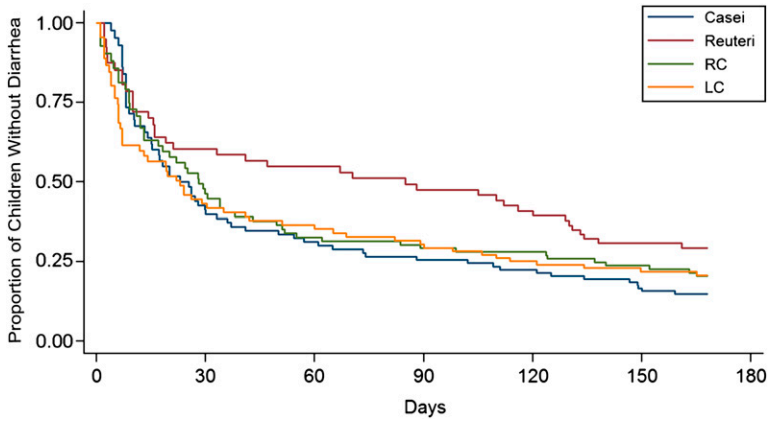

FIGURE 2

Adjusted Cox survival curve of the WHO-defined diarrhea (≥3 loose/liquid stools in 24 hours) episodes. Adjusted for area of living, gender, age, diarrhea and ARTIs 2 weeks before the study, household expenditure. and weight-for-height *z* score. No significant differences between interventions were observed. Probability of survival without diarrhea in relation to duration of diarrheal episodes (days) for 4 groups. No significant differences between interventions were observed.

assumption that they antagonize intestinal pathogens. Possible mechanisms include the synthesis of antimicrobial substances, competitive inhibition of pathogen adhesion, competition with pathogens for growth substrates, modification of toxin and nontoxin receptors involved in bacterial recognition, and stimulation of the immune responses to pathogens.[53] Thus far, only 3 randomized trials have focused on the role of probiotics in prevention of acute diarrhea in a community setting in developing countries.[17,54,55] These studies found inconsistent effects and differed in probiotic strain and dose, intervention duration, and study subject's age. In our study, the effect size of diarrhea reduction by *L reuteri* was higher compared with a 14% reduction by supplementing *L casei* shirota in a comparable study in India,[55] a 6% reduction by *Bifidobacterium lactis* HN019 combined with prebiotic oligosaccharides in India,[17] and a 6% reduction using *Lactobacillus rhamnosus* GG in Peru.[54]

Strains of *L reuteri* have been used safely as a probiotic in adults,[56] children,[19] infants,[21,57] and newborns[58] in developed countries. The original strain of *L reuteri* (American Type Culture Collection strain 55730), from which *L reuteri* DSM17938 has been derived by removal of antibiotic resistance gene-carrying plasmids,[59] has been shown to significantly reduce duration of watery diarrhea associated with rotavirus in children aged 6 to 36 months[19,20] and diarrheal episodes in infants in day care centers.[21] In our study, children supplemented with *L reuteri* experienced a few adverse events, mainly associated with a less regular defecation pattern. *L reuteri* did not lead to any serious event related to the intervention, and positive results included a lower proportion and shorter duration of antibiotic use.

Milk fermented with *L casei* CRL431 and *Lactobacillus acidophilus* has reduced

TABLE 3 Effects of Probiotics and Calcium on Incidence of Diarrhea and ARTIs Among Indonesian Children

Outcome Measures	LC (*n* = 124)	RC (*n* = 126)	Casei (*n* = 120)	Reuteri (*n* = 124)
WHO-defined diarrhea episodes (≥3 loose/liquid stools in 24 h)				
Mean incidence/child per year	0.91	0.86	1.05	0.67
No. of episodes, mean ± SD	0.40 ± 0.81	0.38 ± 0.78	0.47 ± 0.87	0.30 ± 0.56
Adjusted RR (95% CI)[a]	1.00 (ref)	0.99 (0.62–1.58)	—	—
	—	1.00 (ref)	1.21 (0.76–1.92)	0.76 (0.46–1.25)
Duration of episodes, mean ± SD, d	3.06 ± 4.43	2.94 ± 3.25	2.37 ± 2.68	2.68 ± 3.05
All diarrhea episodes (2 and ≥3 loose/liquid stools in 24 h)				
Mean incidence/child/y	1.73	1.86	2.04	1.28
Number of episodes, mean ± SD	0.73 ± 1.14	0.77 ± 1.38	0.87 ± 1.32	0.56 ± 0.77
Adjusted RR (95% CI)[a]	1.00 (ref)	1.10 (0.77–1.59)	—	—
	—	1.00 (ref)	1.06 (0.74–1.53)	0.68 (0.46–0.99)
Duration of episodes, mean ± SD, d	2.57 ± 4.09	2.03 ± 2.84	2.08 ± 2.40	1.91 ± 2.52
ARTIs episodes				
Mean incidence/child/y	7.22	7.52	7.07	7.45
No. of episodes, mean ± SD	2.41 ± 1.59	2.43 ± 1.61	2.36 ± 1.62	2.48 ± 1.56
Adjusted RR (95% CI)[b]	1.00 (ref)	1.00 (0.86–1.18)	—	—
	—	1.00 (ref)	0.97 (0.82–1.14)	0.99 (0.84–1.16)
Duration of episodes, mean ± SD, d	4.87 ± 4.05	4.90 ± 3.70	4.96 ± 3.71	4.58 ± 3.43

ref, reference group of comparison.

[a] Negative binomial model, adjusted for area of living, gender, age, diarrhea and ARTI 2 weeks before the study, household expenditure, and weight-for-height z score.

[b] Poisson model, adjusted for area of living, gender, age, diarrhea and ARTI 2 weeks before the study, household expenditure, and weight-for-height *z* score.

—, presents an irrelevant comparison, which was not included in the analysis.

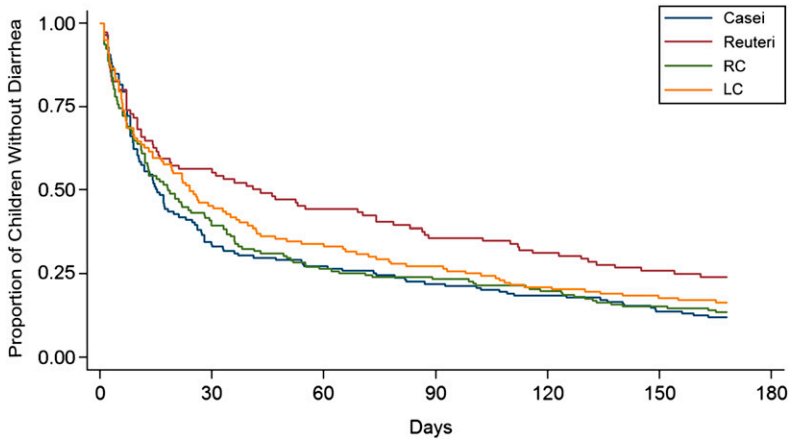

FIGURE 3

Adjusted Cox survival curve of all reported diarrhea (≥2 loose/liquid stools in 24 hours) episodes. Adjusted for area of living, gender, age, diarrhea and ARTIs 2 weeks before the study, household expenditure, and weight-for-height *z* score. Probability of survival without diarrhea in relation to duration of diarrheal episodes (days) for 4 groups. Significant differences occurred between the RC and reuteri groups ($P = .036$).

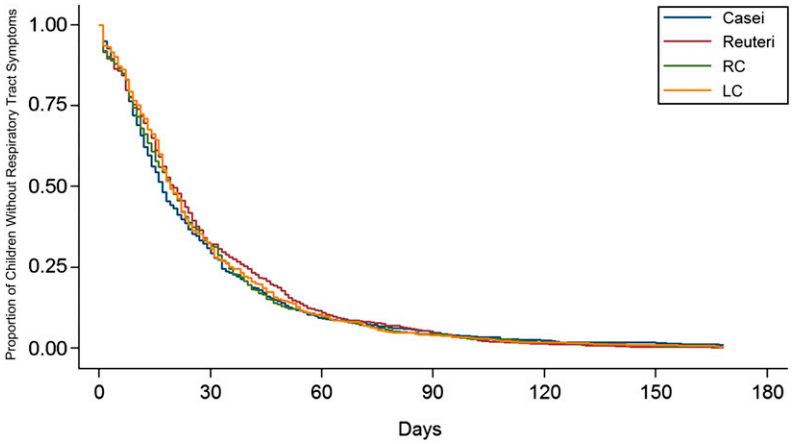

FIGURE 4

Adjusted Cox survival curve of ARTI episodes. Adjusted for area of living, gender, age, diarrhea and ARTIs 2 weeks before the study, household expenditure, and weight-for-height *z* score. Probability of survival without acute respiratory infections in relation to duration of episodes (days) for 4 groups. No significant differences between interventions were observed.

the incidence of diarrhea in children,[60] eliminated diarrhea due to post-gastroenteritis syndrome of malnourished hospitalized children,[61] and significantly reduced the number of daily stools, diarrheal duration, and vomiting of children with persistent diarrhea.[22] Our results underline that probiotic effects are strain specific, as we found protective effects of *L reuteri* DSM17938 against acute diarrhea in children, whereas supplementation of *L casei* CRL431 (without other strains) was without effect. The dosage of our probiotic strains (5×10^8 CFU/day) is within the effective dosage recommended by the Food and Agriculture Organization of the United Nations/WHO.[62]

The major strength of this study was its focus on prevention in contrast to most previous studies, which aimed at treatment of institutionalized children. Additional strengths were its double-blind design, the strict adherence to a rigorous protocol, the use of validated instruments in the assessment of diarrheal episodes, long duration of the intervention, and the excellent compliance rate. Per-protocol analysis, excluding the few noncompliant subjects (6%) and subjects having chronic antibiotic usage, did not change the outcome. A weakness of this study is the lack of microbiologic data identifying the diarrhea-inducing pathogens. This subject was not pursued because such stool analysis generally has poor diagnostic yield and incurs high costs.[63] As a consequence, specific effects of calcium or probiotics, if any, against specific diarrheal pathogens may have been missed.

CONCLUSIONS

Supplementation of *L reuteri*, at least on a diet including regular calcium milk, is 1 of the potential interventions to reduce the burden of acute infectious diarrhea in children. These results need to be confirmed by at least 1 other independent study in a comparable community.

ACKNOWLEDGMENTS

We thank Dr Christien van Beusekom, Mr Peter Spiekstra, Mr Jan van der Leij, and Ms Vicky Valentina (FrieslandCampina Research) for their contribution to study milk production, and Martin Jäkel, MD (Unilever Research and Development) for his advice on adverse-event analysis. We thank the highly dedicated and motivated children, parents, physicians, and research team members, especially Ms Ratna Wulanti, Ms Imas Maliha, Ms Siti Mulyani, Santi Sinarwati, MD, and Ms Devy Davelyna. We acknowledge the support of the head, elders, women leaders, and volunteers in Kampung Melayu and Rawabunga. We also thank Prof Purwantyastuti, Prof Arini Setiati, Sri Lestari, MD, Dr Moesijanti Soekarti, Ms Yulianti Wibowo, Mr Jeroen Sterken, Iwan Setiawan, MD, Dr Miren Iturriza-Gómara, and Mr Ahmad Sadariskar.

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

FINANCIAL DISCLOSURE: *Drs van den Heuvel and Albers are employed by FrieslandCampina and Unilever, respectively; the other authors have indicated they have no financial relationships relevant to this article to disclose. No funding was obtained from manufacturers providing the probiotic strains. Moreover, they had no influence on strain selection, study design, conduct, or conclusions.*

FUNDING: This trial was funded by the Top Institute Food and Nutrition, FrieslandCampina Research, and Unilever Research and Development. Doctoral scholarship (R. Agustina) was provided by the International Nutrition Foundation, USA.

Randomized Trial of Probiotics and Calcium on Diarrhea and Respiratory Tract Infections in Indonesian Children

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Pediatrics 2012;129;e1155; originally published online April 9, 2012;
DOI: 10.1542/peds.2011-1379