

PEDIATRICS®

OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS

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

Rina Agustina, Frans J. Kok, Ondine van de Rest, Umi Fahmida, Agus Firmansyah, Widjaja Lukito, Edith J. M. Feskens, Ellen G. H. M. van den Heuvel, Ruud Albers and Ingeborg M. J. Bovee-Oudenhoven

Pediatrics 2012;129:e1155; originally published online April 9, 2012;

DOI: 10.1542/peds.2011-1379

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://pediatrics.aappublications.org/content/129/5/e1155.full.html>

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2012 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™



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

AUTHORS: Rina Agustina, MD,^{a,b} Frans J. Kok, PhD,^b Ondine van de Rest, PhD,^b Umi Fahmida, PhD,^a Agus Firmansyah, MD, PhD,^c Widjaja Lukito, MD, PhD,^a Edith J. M. Feskens, PhD,^b Ellen G. H. M. van den Heuvel, PhD,^d Ruud Albers, PhD,^e and Ingeborg M. J. Bovee-Oudenhoven, PhD^{f,g}

^aSEAMEO RECFON (Southeast Asian Ministers of Education Organization Regional Center for Food and Nutrition), and ^cFaculty of Medicine, Division of Pediatric Gastroenterology, Department of Child Health, University of Indonesia, Jakarta, Indonesia; ^bDivision of Human Nutrition, Wageningen University, Wageningen, Netherlands; ^dFrieslandCampina Research, Deventer, Netherlands; ^eUnilever Research and Development, Vlaardingen, Netherlands; ^fTop Institute Food and Nutrition, Wageningen, Netherlands; and ^gNIZO food research, Ede, Netherlands

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).

Dr Bovee-Oudenhoven's current affiliation is FrieslandCampina Research, Deventer, Netherlands.

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

doi:10.1542/peds.2011-1379

Accepted for publication Jan 5, 2012

Address correspondence to Ingeborg M.J. Bovee-Oudenhoven, PhD, FrieslandCampina Research, PO Box 87, 7400 AB Deventer, Netherlands. E-mail: ingeborg.bovee@frieslandcampina.com

(Continued on last page)



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 ultrahigh 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				
<i>L casei</i> CRL431	—	—	5 × 10 ⁸	—
<i>L reuteri</i> 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 debinding 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 calcium⁸ and rotavirus (diarrheal samples).³³

Before and at intervention end, non-fasting 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 socio-demographic 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

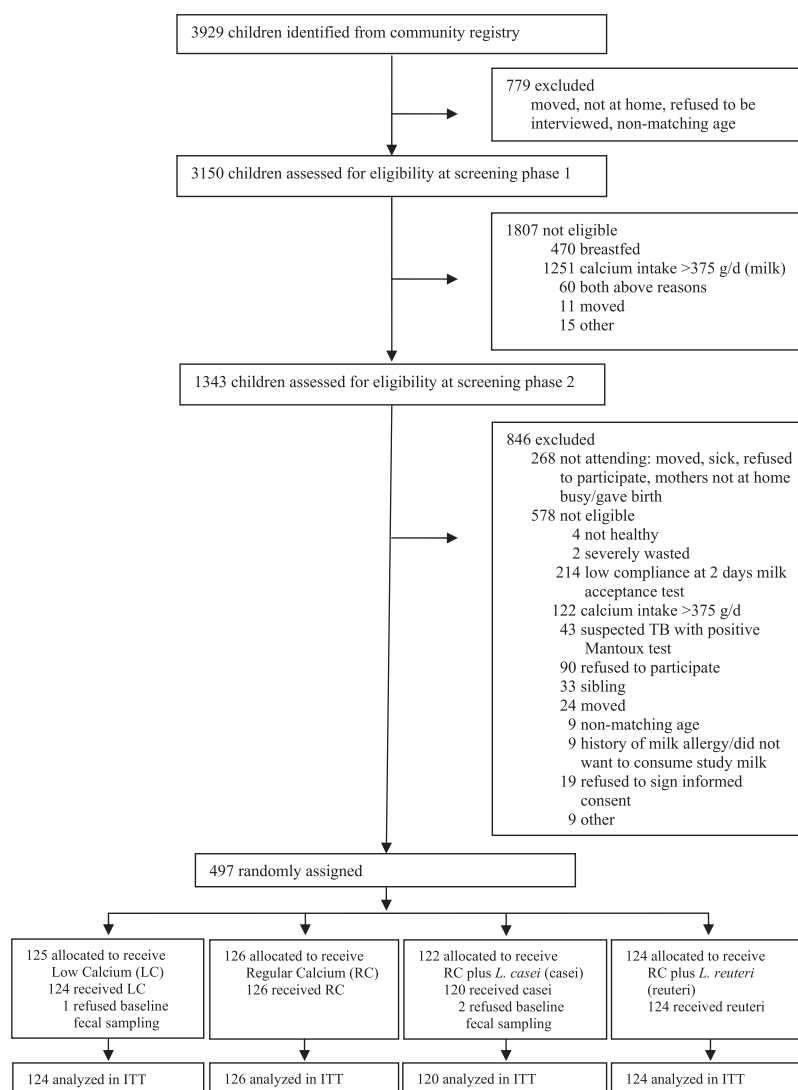


FIGURE 1
Flow diagram of study subjects. ITT, intention-to-treat; TB, tuberculosis.

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}

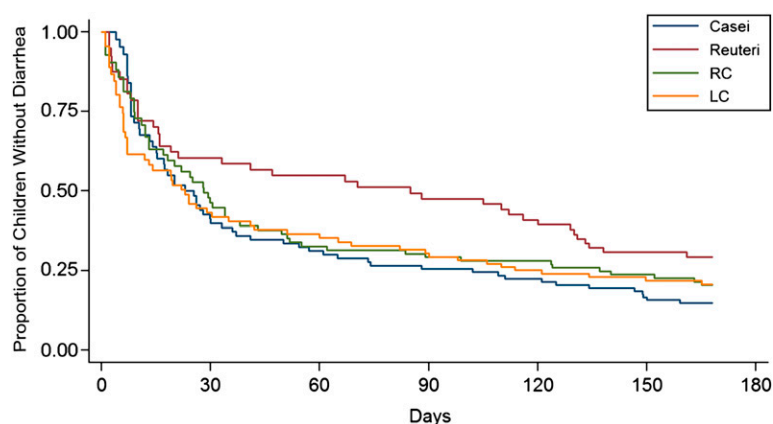
We did not only rely on mothers' perception but implemented an active surveillance program to verify mothers' daily records with twice-a-week visits of trained field workers and twice-a-month visits of field supervisors. The physician and monitoring expert accompanied the field workers on several of their home visits. All end points were assessed by using structured and pre-tested 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 pro-

biotics 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 newborns⁵⁸ 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 \pm SD	0.40 \pm 0.81	0.38 \pm 0.78	0.47 \pm 0.87	0.30 \pm 0.56
Adjusted RR (95% CI) ^a	1.00 (ref)	0.99 (0.62–1.58)	—	—
Duration of episodes, mean \pm SD, d	3.06 \pm 4.43	2.94 \pm 3.25	1.21 (0.76–1.92)	0.76 (0.46–1.25)
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 \pm SD	0.73 \pm 1.14	0.77 \pm 1.38	0.87 \pm 1.32	0.56 \pm 0.77
Adjusted RR (95% CI) ^a	1.00 (ref)	1.10 (0.77–1.59)	—	—
Duration of episodes, mean \pm SD, d	2.57 \pm 4.09	2.03 \pm 2.84	1.06 (0.74–1.53)	0.68 (0.46–0.99)
ARTIs episodes				
Mean incidence/child/y	7.22	7.52	7.07	7.45
No. of episodes, mean \pm SD	2.41 \pm 1.59	2.43 \pm 1.61	2.36 \pm 1.62	2.48 \pm 1.56
Adjusted RR (95% CI) ^b	1.00 (ref)	1.00 (0.86–1.18)	—	—
Duration of episodes, mean \pm SD, d	4.87 \pm 4.05	4.90 \pm 3.70	0.97 (0.82–1.14)	0.99 (0.84–1.16)

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.

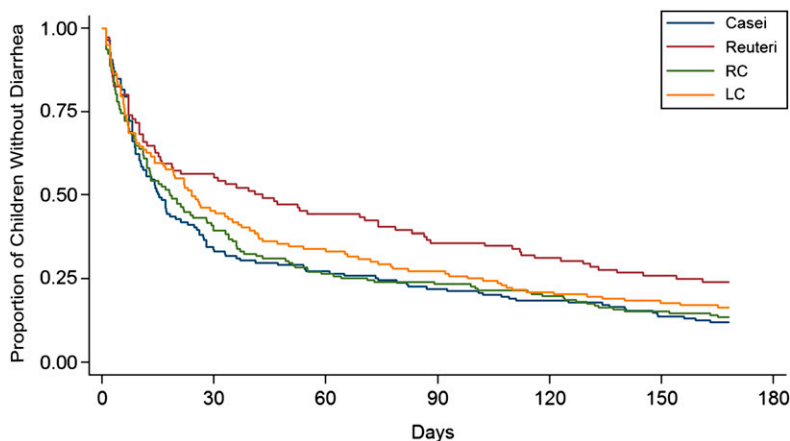


FIGURE 3

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 ($P = .036$).

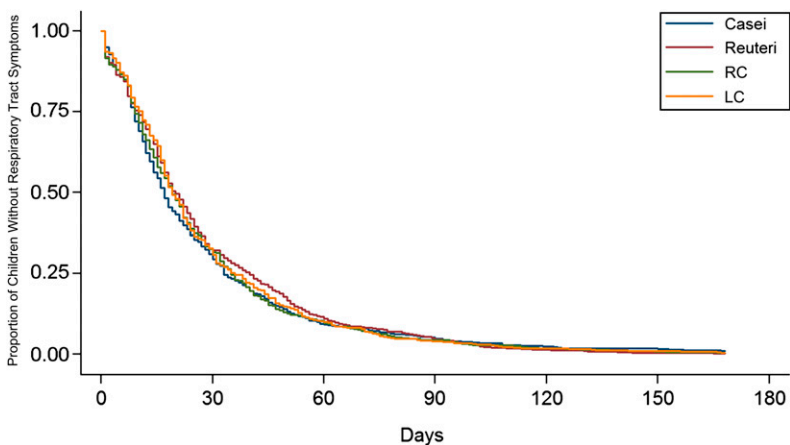


FIGURE 4

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 Purwastyastuti, 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.

REFERENCES

1. UNICEF/WHO. Diarrhoea: why children are still dying and what can be done. New York, NY: UNICEF, 2009. Available at: www.unicef.org/media/files/Final_Diarrhoea_Report_October_2009_final.pdf. Accessed September 28, 2010
2. World Health Organization. Acute respiratory infections. Updated September 2009. Available at: www.who.int/vaccine_research/diseases/ari/en/. Accessed September 28, 2010
3. World Health Organization. Coordinated approach to prevention and control of acute diarrhoea and respiratory infections. WHO SEARO. Available at: www.searo.who.int/LinkFiles/RC_63_a-11-SEA-RC63-10.pdf. Accessed September 28, 2010
4. Ministry of Health Republic of Indonesia. Report on Result of National Basic Health Research (RISKESDAS 2007). Available at: http://203.90.70.117/searo/Indonesia/LinkFiles/Health_Information_and_evidence_for_policy_Riskesdas_2007.pdf. Accessed April 1, 2011
5. Wasito E, Pritasari, Susilowati D, Iswarawanti DN, Schultink W, Gross R. Temporary stability of urban food and nutrition security: the East Jakarta study. *Asia Pac J Clin Nutr*. 2001;10(suppl):S29–S33
6. Sari M, de Pee S, Bloem MW, et al. Higher household expenditure on animal-source and nongrain foods lowers the risk of stunting among children 0-59 months old in Indonesia: implications of rising food prices. *J Nutr*. 2010;140(1):195S–200S
7. Scrimshaw NS, Taylor CE, Gordon JE. Interactions of nutrition and infection. *Manogr Ser World Health Organ*. 1968;57:3–329
8. Bovee-Oudenhoven IM, Lettink-Wissink ML, Van Doesburg W, Witteman BJ, Van Der Meer R. Diarrhea caused by enterotoxigenic *Escherichia coli* infection of humans is inhibited by dietary calcium. *Gastroenterology*. 2003;125(2):469–476
9. Santika O, Fahmida U, Ferguson EL. Development of food-based complementary feeding recommendations for 9- to 11-month-old peri-urban Indonesian infants using linear programming. *J Nutr*. 2009;139(1):135–141
10. Utomo B, Fitria L, Sulacha E, et al. Feeding patterns, nutrient intake and nutritional status among children 0-23 months of age in Indramayu, West Java, 1997. *Mal J Nutr*. 2000;6(2):147–170
11. Szajewska H, Mrukowicz JZ. Probiotics in the treatment and prevention of acute infectious diarrhea in infants and children: a systematic review of published randomized, double-blind, placebo-controlled trials. *J Pediatr Gastroenterol Nutr*. 2001;33(suppl 2):S17–S25
12. McFarland LV, Elmer GW, McFarland M. Meta-analysis of probiotics for the prevention and treatment of acute pediatric diarrhea. *Internl J Probiotics Prebiotics*. 2006;1:63–76
13. Sazawal S, Hiremath G, Dhingra U, Malik P, Deb S, Black RE. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infect Dis*. 2006;6(6):374–382
14. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Syst Rev*. 2010;(11):CD003048
15. Vouloumanou EK, Makris GC, Karageorgopoulos DE, Falagas ME. Probiotics for the prevention of respiratory tract infections: a systematic review. *Int J Antimicrob Agents*. 2009;34(3):197.e1–10
16. Hojsak I, Snovak N, Abdović S, Szajewska H, Misak Z, Kolacek S. Lactobacillus GG in the prevention of gastrointestinal and respiratory tract infections in children who attend day care centers: a randomized, double-blind, placebo-controlled trial. *Clin Nutr*. 2010;29(3):312–316
17. Sazawal S, Dhingra U, Hiremath G, et al. Prebiotic and probiotic fortified milk in prevention of morbidities among children: community-based, randomized, double-blind, controlled trial. *PLoS ONE*. 2010;5(8):e12164
18. Cáceres P, Montes S, Vega N, et al. Effects of Lactobacillus rhamnosus HN001 on acute respiratory infections and intestinal secretory IgA in children. *J Pediatr Infectious Dis*. 2010;5(4):353–362
19. Shornikova AV, Casas IA, Isolauri E, Mykkänen H, Vesikari T. Lactobacillus reuteri as a therapeutic agent in acute diarrhea in young children. *J Pediatr Gastroenterol Nutr*. 1997;24(4):399–404
20. Shornikova AV, Casas IA, Mykkänen H, Salo E, Vesikari T. Bacteriotherapy with Lactobacillus reuteri in rotavirus gastroenteritis. *Pediatr Infect Dis J*. 1997;16(12):1103–1107
21. 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
22. Gaón D, García H, Winter L, et al. Effect of Lactobacillus strains and Saccharomyces boulardii on persistent diarrhea in children. *Medicina (B Aires)*. 2003;63(4):293–298
23. World Health Organization. The treatment of diarrhoea: a manual for physicians and other senior health workers, WHO/CDR/95.3. Geneva, World Health Organization, 4th revision, 2005. Available at: <http://whqlibdoc.who.int/publications/2005/9241593180.pdf>. Accessed September 28, 2010
24. World Health Organization. *The Management of Acute Respiratory Infection in Children. Practical Guidelines for Outpatient Care*. Geneva, Switzerland: World Health Organization; 1995
25. World Health Organization. Pocket book of hospital care for children. Guidelines for the management of common illnesses with limited resources. Geneva, World Health Organization. Available at: www.who.int/child_adolescent_health/documents/9241546700/en/index.html. Accessed November 10, 2011
26. Agustina R, Lukito W, Firmansyah A, Suhardjo HN, Murniati D, Bindels J. The effect of early nutritional supplementation with a mixture of probiotic, prebiotic, fiber and micronutrients in infants with acute diarrhea in Indonesia. *Asia Pac J Clin Nutr*. 2007;16(3):435–442
27. Bhutta ZA, Bird SM, Black RE, et al. Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials. *Am J Clin Nutr*. 2000;72(6):1516–1522
28. Isolauri E, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. *Pediatrics*. 1991;88(1):90–97
29. Cabello C, Manjarrez ME, Olvera R, Villalba J, Valle L, Paramo I. Frequency of viruses associated with acute respiratory infections in children younger than five years of age at a locality of Mexico City. *Mem Inst Oswaldo Cruz*. 2006;101(1):21–24
30. McElhaney JE, Gravenstein S, Cole SK, et al. A placebo-controlled trial of a proprietary extract of North American ginseng (CVT-E002) to prevent acute respiratory illness in institutionalized older adults. *J Am Geriatr Soc*. 2004;52(1):13–19
31. Hojsak I, Abdović S, Szajewska H, Milosević M, Krznarić Z, Kolacek S. Lactobacillus GG in the prevention of nosocomial gastrointestinal and respiratory tract infections. *Pediatrics*. 2010;125(5). Available at: www.pediatrics.org/cgi/content/full/125/5/e1171
32. Koch A, Mølbak K, Homøe P, et al. Risk factors for acute respiratory tract infections in young Greenlandic children. *Am J Epidemiol*. 2003;158(4):374–384
33. Iturriza-Gómara M, Elliot AJ, Dockery C, Fleming DM, Gray JJ. Structured surveillance

- of infectious intestinal disease in pre-school children in the community: 'The Nappy Study'. *Epidemiol Infect.* 2009;137(7):922–931
34. Kotisaari S, Romppanen J, Penttilä I, Punnonen K. The Advia 120 red blood cells and reticulocyte indices are useful in diagnosis of iron-deficiency anemia. *Eur J Haematol.* 2002;68(3):150–156
 35. Roberts WL, Sedrick R, Moulton L, Spencer A, Rifai N. Evaluation of four automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. *Clin Chem.* 2000;46(4):461–468
 36. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J Nutr.* 2004;134(11):3127–3132
 37. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol.* 1997;32(9):920–924
 38. Canani RB, Cirillo P, Terrin G, et al. Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *BMJ.* 2007;335(7615):340
 39. World Health Organization. International Statistical Classification of Diseases (ICD) and Related Health Problem, 10th Revision, Version for 2007. Available at: <http://apps.who.int/classifications/apps/icd/icd10online/>. Accessed September 28, 2010
 40. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics.* 2002;109(4):678–684
 41. Brooks WA, Santosham M, Naheed A, et al. Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomised controlled trial. *Lancet.* 2005;366(9490):999–1004
 42. Coxe S, West SG, Aiken LS. The analysis of count data: a gentle introduction to poisson regression and its alternatives. *J Pers Assess.* 2009;91(2):121–136
 43. World Health Organization. Diarrhoea. 2011. Available at: www.who.int/topics/diarrhoea/en/. Accessed November 9, 2011
 44. Baqui AH, Black RE, Yunus M, Hoque AR, Chowdhury HR, Sack RB. Methodological issues in diarrhoeal diseases epidemiology: definition of diarrhoeal episodes. *Int J Epidemiol.* 1991;20(4):1057–1063
 45. Johnston BC, Shamseer L, da Costa BR, Tsuyuki RT, Vohra S. Measurement issues in trials of pediatric acute diarrheal diseases: a systematic review. *Pediatrics.* 2010;126(1). Available at: www.pediatrics.org/cgi/content/full/126/1/e222
 46. Vanderhoof JA, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr.* 1999;135(5):564–568
 47. Veenemans J, Mank T, Ottenhof M, et al. Protection against diarrhea associated with Giardia intestinalis is lost with multi-nutrient supplementation: a study in Tanzanian children. *PLoS Negl Trop Dis.* 2011;5(6):e1158
 48. Guarino A, Lo Vecchio A, Canani RB. Probiotics as prevention and treatment for diarrhea. *Curr Opin Gastroenterol.* 2009;25(1):18–23
 49. Bovee-Oudenhoven IM, Termont DS, Weerkamp AH, Faassen-Peters MA, Van der Meer R. Dietary calcium inhibits the intestinal colonization and translocation of Salmonella in rats. *Gastroenterology.* 1997;113(2):550–557
 50. Bovee-Oudenhoven IM, Wissink ML, Wouters JT, Van der Meer R. Dietary calcium phosphate stimulates intestinal lactobacilli and decreases the severity of a salmonella infection in rats. *J Nutr.* 1999;129(3):607–612
 51. Soenarto Y, Aman AT, Bakri A, et al. Burden of severe rotavirus diarrhea in Indonesia. *J Infect Dis.* 2009;200(suppl 1):S188–S194
 52. Larson CP, Henning L, Luby S, Faruque ASG. Chapter 17. Infectious childhood diarrhea in developing countries. In: Krämer A, Kretzschmar M, Krickeberg K, eds. *Modern Infectious Disease Epidemiology Concepts, Methods, Mathematical Models, and Public Health.* New York, NY: Springer; 2010:291–308
 53. Hajela N, Nair GB, Ganguly NK. Are probiotics a feasible intervention for prevention of diarrhoea in the developing world? *Gut Pathog.* 2010;2(1):10
 54. Oberhelman RA, Gilman RH, Sheen P, et al. A placebo-controlled trial of Lactobacillus GG to prevent diarrhea in undernourished Peruvian children. *J Pediatr.* 1999;134(1):15–20
 55. Sur D, Manna B, Niyogi SK, et al. Role of probiotic in preventing acute diarrhoea in children: a community-based, randomized, double-blind placebo-controlled field trial in an urban slum. *Epidemiol Infect.* 2011;139(6):919–926
 56. Tubelius P, Stan V, Zachrisson A. Increasing work-place healthiness with the probiotic Lactobacillus reuteri: a randomised, double-blind placebo-controlled study. *Environ Health.* 2005;4:25
 57. Savino F, Pelle E, Palumeri E, Oggiero R, Miniero R. Lactobacillus reuteri (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. *Pediatrics.* 2007;119(1). Available at: www.pediatrics.org/cgi/content/full/119/1/e124
 58. Connolly E, Abrahamsson T, Björkstén B. Safety of D(-)-lactic acid producing bacteria in the human infant. *J Pediatr Gastroenterol Nutr.* 2005;41(4):489–492
 59. Rosander A, Connolly E, Roos S. Removal of antibiotic resistance gene-carrying plasmids from Lactobacillus reuteri ATCC 55730 and characterization of the resulting daughter strain, L. reuteri DSM 17938. *Appl Environ Microbiol.* 2008;74(19):6032–6040
 60. Gonzalez S, Albarracin G, Locascio de Ruiz Pesce M, et al. Prevention of infantile diarrhoea by fermented milk. *Microbiol Aliments Nutr.* 1990;8:349–354
 61. Gonzalez SN, Cardozo R, Apella MC, Oliver G. Biotherapeutic role of fermented milk. *Biotherapy.* 1994;8(2):129–134
 62. Reid G. *Regulatory and clinical aspects of dairy probiotics. Background paper for the Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria.* FAO, Rome (Italy). Geneva, Switzerland: World Health Organization; 2001
 63. Bauer TM, Lalvani A, Fehrenbach J, et al. Derivation and validation of guidelines for stool cultures for enteropathogenic bacteria other than Clostridium difficile in hospitalized adults. *JAMA.* 2001;285(3):313–319

(Continued from first page)

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

Copyright © 2012 by the American Academy of Pediatrics

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

Rina Agustina, Frans J. Kok, Ondine van de Rest, Umi Fahmida, Agus Firmansyah, Widjaja Lukito, Edith J. M. Feskens, Ellen G. H. M. van den Heuvel, Ruud Albers and Ingeborg M. J. Bovee-Oudenhoven

Pediatrics 2012;129:e1155; originally published online April 9, 2012;

DOI: 10.1542/peds.2011-1379

Updated Information & Services	including high resolution figures, can be found at: http://pediatrics.aappublications.org/content/129/5/e1155.full.html
References	This article cites 50 articles, 17 of which can be accessed free at: http://pediatrics.aappublications.org/content/129/5/e1155.full.html#ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Therapeutics & Toxicology http://pediatrics.aappublications.org/cgi/collection/therapeutics_and_toxicology
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://pediatrics.aappublications.org/site/misc/Permissions.xhtml
Reprints	Information about ordering reprints can be found online: http://pediatrics.aappublications.org/site/misc/reprints.xhtml

PEDIATRICS is the official journal of the American Academy of Pediatrics. A monthly publication, it has been published continuously since 1948. PEDIATRICS is owned, published, and trademarked by the American Academy of Pediatrics, 141 Northwest Point Boulevard, Elk Grove Village, Illinois, 60007. Copyright © 2012 by the American Academy of Pediatrics. All rights reserved. Print ISSN: 0031-4005. Online ISSN: 1098-4275.

American Academy of Pediatrics

DEDICATED TO THE HEALTH OF ALL CHILDREN™

