JOURNAL CLUB

Effect of Micronutrient Sprinkles on Reducing Anemia

A Cluster-Randomized Effectiveness Trial

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Objective: To evaluate the effectiveness of Sprinkles alongside infant and young child feeding (IYCF) education compared with IYCF education alone on anemia, deficiencies in iron, vitamin A, and zinc, and growth in Cambodian infants.

Design: Cluster-randomized effectiveness study.

Setting: Cambodian rural health district.

Participants: Among 3112 infants aged 6 months, a random subsample (n = 1350) was surveyed at baseline and 6-month intervals to age 24 months.

Intervention: Daily micronutrient Sprinkles alongside IYCF education vs IYCF education alone for 6 months from ages 6 to 11 months.

Main Outcome Measures: Prevalence of anemia; iron, vitamin A, and zinc deficiencies; and growth via biomarkers and anthropometry.

Results: Anemia prevalence (hemoglobin level <11.0 g/dL [to convert to grams per liter, multiply by 10.0]) was reduced in the intervention arm compared with the control arm by 20.6% at 12 months (95% CI, 9.4-30.2; *P* = .001), and the prevalence of moderate anemia (hemoglobin level <10.0 g/dL) was reduced by 27.1% (95% CI, 21.0-31.8; *P* < .001). At 12 and 18 months, iron deficiency prevalence was reduced by 23.5% (95% CI, 15.6-29.1; *P* < .001) and 11.6% (95% CI, 2.6-17.9; *P* = .02), respectively. The mean serum zinc concentration was increased at 12 months (2.88 µg/dL [to convert to micromoles per liter, multiply by 0.153]; 95% CI, 0.26-5.42; *P* = .03). There was no statistically significant difference in the prevalence of zinc and vitamin A deficiencies or in growth at any time.

Conclusions: Sprinkles reduced anemia and iron deficiency and increased the mean serum zinc concentration in Cambodian infants. Anemia and zinc effects did not persist beyond the intervention period.

Trial Registration: anzctr.org.au Identifier: ACTRN12608000069358

Arch Pediatr Adolesc Med. 2012;166(9):842-850.
Published online July 16, 2012.
doi:10.1001/archpediatrics.2012.1003

INDICATORS OF CHILD SURVIVAL ARE improving globally and in Cambodia.[1] Nevertheless, 55% of Cambodian children younger than 5 years are anemic and 40% have stunted growth.[2] Those aged 6 to 23 months are at highest risk for anemia[2,3] and micronutrient deficiencies, which together may lead to impairments in growth and immune function, cognitive and learning difficulties, and increased mortality.[4,5] The etiology of anemia is multifactorial, including iron deficiency, other micronutrient deficiencies, infections, and genetic hemoglobin (Hb) disorders. The latter are found in 30% to 70% of Cambodian individuals.[6,7]

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In efficacy trials in Cambodia[8] and elsewhere,[9] Sprinkles mixed with complementary foods significantly reduced anemia in young children. However, the effectiveness of Sprinkles when delivered

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through existing government health services is uncertain. Also, the appropriate duration of Sprinkles interventions is unclear. Therefore, we conducted a cluster-randomized controlled trial in Cambodia to evaluate the effectiveness of daily use of Sprinkles mixed with home-based complementary foods in infants from ages 6 to 11 months alongside infant and young child feeding (IYCF) education. We hypothesized that a 6-month duration of the intervention would be adequate to reduce anemia prevalence by age 12 months and that this effect would persist until at least ages 18 to 24 months. We evaluated the effect on anemia, deficiencies in iron, vitamin A, and zinc, and wasting, underweight, and stunting of growth. Children were followed up to age 24 months to establish whether any observed effects were sustained.

Table 1. Nutrient Composition of Sprinkles

Nutrient	Study Composition
Iron, microencapsulated ferrous fumarate	12.5 mg
Zinc gluconate	10 mg
Vitamin A, retinol acetate	300 µg
Iodine	90 µg
Vitamin B_1	0.5 mg
Vitamin B_2	0.5 mg
Vitamin B_6	0.5 mg
Vitamin B_{12}	0.9 µg
Niacin	6 mg
Folate, folic acid	160 µg
Vitamin C, ascorbic acid	30 mg
Copper	0.3 mg
Vitamin D	5 µg
Vitamin E	6 IU

METHODS

STUDY SETTING AND DESIGN

All children residing in Svay Rieng Operational Health District, Cambodia, and turning 6 months of age between March and August 2008 who were identified through listings of infants at health center (HC) and village levels were eligible to participate. This district is representative of rural Cambodia with a reasonably well-functioning government health system and a low malaria incidence rate (<1 case/1000 population).[10] It has 20 HCs serving a population of 292 000. Rolling enrollment occurred in monthly cohorts. The study was a cluster-randomized trial with HC catchment area as the unit of randomization.

The study protocol was approved by the National Ethics Committee for Health Research, Ministry of Health, Cambodia, and the Human Ethics Committee, University of Otago, Dunedin, New Zealand. Verbal consent was obtained from all caregivers after full explanation of the study. Full informed consent by thumbprint signature was obtained from the subsample selected for follow-up at enrollment.

RANDOMIZATION

The HC catchment areas (population 10 000-20 000) (clusters) were randomized to an intervention arm (IYCF education plus Sprinkles; 10 clusters) or a control arm (IYCF education alone; 10 clusters), stratified by HC catchment village implementation of mother support groups (Baby Friendly Community Initiative), by a statistician not involved in the study implementation.

TRIAL SIZE

We estimated that 3600 eligible children were in the study area. The subsample size of 1350 was calculated based on a cluster-randomized study design allowing for a 20% dropout and an intraclass correlation coefficient of 0.15. Sample size was calculated to determine a difference of 25% for prevalence of anemia, taking into consideration the outcomes of deficiencies of vitamin A, serum zinc, and serum ferritin and *Z* scores of stunting and wasting, based on the outcome requiring the largest sample size with a power of 90% and a 5% level of significance.

INTERVENTION

Infants in the intervention arm received daily Sprinkles in single-dose sachets (**Table 1**), delivered monthly to their homes by government village health workers. Sprinkles were mixed with the infant's meal immediately before serving. Adherence was assessed monthly by a count of unused sachets from each household. The IYCF education was provided to caregivers of all children in both the intervention and control groups in verbal, written, and pictorial form together with cooking demonstrations, focusing on frequency, amount, consistency, and an increased consumption of animal-source foods. Immunizations, biannual vitamin A capsules, and mebendazole tablets (for deworming) were provided to all children according to Cambodia Ministry of Health guidelines.

SUBSAMPLE ENROLLMENT PROCEDURES

A subsample of 675 children in each arm was randomly selected within each monthly cohort at age 6 months. Data on sociodemographic status, antenatal practices, and postnatal practices were recorded via questionnaires, and anthropometric measurements and blood samples were collected. Children in the subsamples were followed up at 6-month intervals at ages 6, 12, 18, and 24 months at their local HC. On each occasion, a blood sample, information on feeding practices, and anthropometric measurements were collected. All children in the subsamples were invited to attend each round regardless of prior attendance (**Figure 1**).

BIOCHEMICAL ASSAYS

Nonfasting venous samples were taken using International Zinc Nutrition Consultative Group procedures.[11] Blood samples were drawn into an EDTA-containing tube for complete blood cell count and into a trace element–free tube (Becton, Dickinson, and Co) at least 30 minutes after applying topical anesthetic (Ametop gel [tetracaine hydrochloride, 4%]; Smith and Nephew). All blood samples were refrigerated immediately after collection. Complete blood cell counts were performed using an automated hematology analyzer (Sysmex Corp) at the National Institute of Public Health Laboratory, Phnom Penh, Cambodia. Serum aliquots were frozen in trace element–free polyethylene vials at –20°C and later at –70°C prior to shipment to the University of Otago for zinc analysis and to DBS-Tech, Willstaett, Germany, for serum ferritin, retinol binding protein (RBP), soluble transferrin receptor, C-reactive protein (CRP), and α1-acid glycoprotein (AGP) analyses.

The serum zinc concentration was analyzed using flame atomic absorption spectrophotometry (AAnalyst 800; Perkin Elmer Corp). The interassay coefficient of variation for zinc (as a percentage) was 5.8% (n = 278) and the mean (SD) value for the certified reference material was 86.21 (4.97) µg/dL (to con-

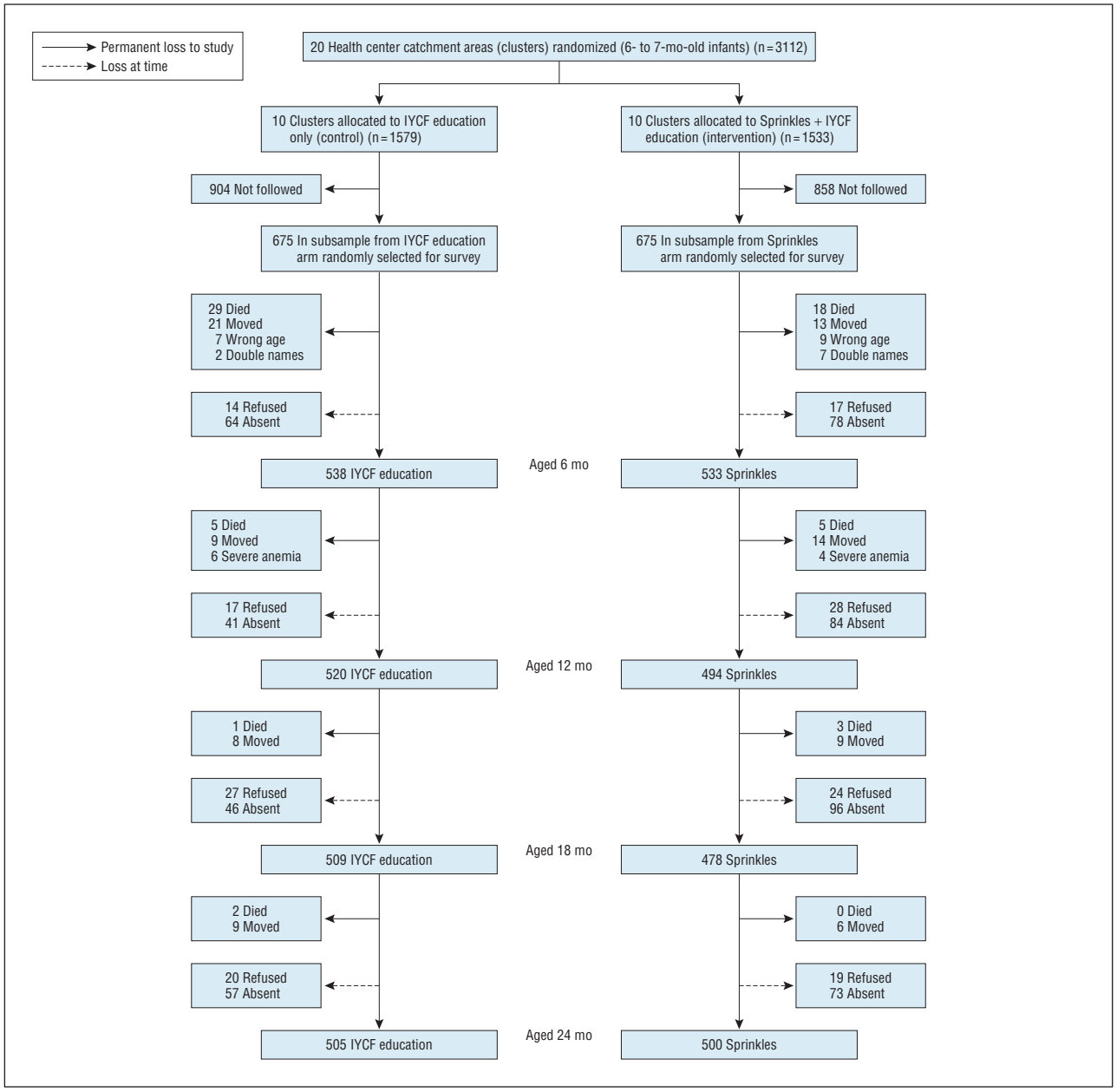

Figure 1. Study profile. All subsample children were invited to attend each round regardless of prior attendance. IYCF indicates infant and young child feeding.

vert to micromoles per liter, multiply by 0.153) (coefficient of variation, 5.8%; n = 31) compared with the certified value of 87.97 µg/dL (95% CI, 2.48-90.46). Serum ferritin, RBP, soluble transferrin receptor, CRP, and AGP concentrations were analyzed using a sandwich enzyme-linked immunosorbent assay technique.[12] Acute and chronic inflammation were assessed by a serum CRP concentration greater than 5 mg/L (to convert to nanomoles per liter, multiply by 9.524) and an AGP concentration greater than 1 g/L, respectively.[13]

Screening for genetic Hb disorders was performed at age 18 months using the Sebia MINICAP analyzer and HEMOGLOBIN(E) program, designed for separating normal Hb (A, A_2, and F) and detecting major Hb variants, including HbE, HbH, and Hb Constant Spring. In addition, α THAL IC Strip Test was performed for the determination of α-thalassemia.

Anthropometry was conducted by trained interviewers using standardized procedures and calibrated equipment.[14] Recumbent length was measured using portable length-measuring boards (Shorr Board; Shorr Productions), weight was measured using electronic scales (UNIscale; UNICEF), and head and mid–upper arm circumferences were measured using nonstretch retractable circumference tapes (Chasmors CTM08 Circumference Measure; Chasmors Ltd). Measurements were taken in duplicate; a third measurement was taken if the difference between the first 2 measurements was outside the allowable difference for that measure[15] (0.5 cm for length, mid–upper arm circumference, and head circumference; 0.1 kg for weight). Computerized monthly data quality checks were conducted to ensure within- and between-interviewer consistencies of data.

STATISTICAL ANALYSIS

Data entry was blinded. Interviews and anthropometric data were double entered into an SPSS version 11.5 statistical software database (SPSS Inc). Complete blood cell count and bio-

Table 2. Baseline Household and Participant Characteristics

Characteristic	Control (n = 582)	Intervention (n = 578)
Female, No. (%)	275 (51.2)	269 (50.4)
Age, mean (SD), mo	6.16 (0.37)	6.13 (0.34)
Household members, mean (SD), No.	5.35 (1.80)	5.45 (1.88)
Children aged <5 y in household, mean (SD), No.	1.30 (0.50)	1.33 (0.53)
Electricity mains, No./total No. (%)	81/539 (15.0)	93/534 (17.4)
Television, No./total No. (%)	347/539 (64.4)	326/534 (61.0)
Motorcycle or scooter, No./total No. (%)	302/538 (56.1)	268/534 (50.2)
Ownership of agricultural land, No./total No. (%)	481/538 (89.4)	477/534 (89.3)
Literacy of mother, No./total No. (%)	232/537 (43.2)	219/530 (41.3)
Duration of pregnancy at first antenatal care visit, mean (SD), mo	5.3 (12.4)	5.7 (13.1)
Antenatal care visits, mean (SD), No.	5.8 (13.1)	5.5 (11.8)
Iron/folate tablets consumed during pregnancy, mean (SD), No.	72.4 (34.4)	70.6 (39.1)
Took mebendazole during pregnancy, No. (%)	323 (62.2)	282 (53.7)
Birth weight, mean (SD), kg	3.03 (0.52)	3.09 (0.60)
Still breastfeeding at baseline at age 6-7 mo, No. (%)	512 (95.5)	510 (96.0)
Times breastfed during previous 24 h, mean (SD), No.	10.3 (5.7)	10.4 (5.5)
Biomarker concentration at baseline at age 6-7 mo, mean (SD)		
α1-Acid glycoprotein, g/L	0.77 (0.22)	0.77 (0.21)
C-reactive protein, mg/L	2.96 (3.90)	3.00 (4.08)
Soluble transferrin receptor, mg/L	9.3 (3.1)	9.6 (3.7)
Retinol binding protein, μmol/L	1.07 (0.23)	1.08 (0.25)
Ferritin, ng/mL	39.5 (27.2)	41.1 (28.9)
Zinc, μg/dL	63.4 (13.1)	63.4 (13.7)
Genetic hemoglobin disorder, No./total No. (%)	247/480 (51.5)	217/439 (49.4)
Anthropometric measurements at baseline at age 6 mo, mean (SD)		
Weight, kg	7.2 (2.0)	7.15 (0.9)
Length, cm	65.5 (3.5)	65.6 (3.0)
Head circumference, cm	41.7 (1.8)	41.7 (1.8)
Mid–upper arm circumference, cm	13.8 (1.2)	13.8 (1.1)

SI conversion factors: To convert C-reactive protein to nanomoles per liter, multiply by 9.524; to convert ferritin to picomoles per liter, multiply by 2.247; and to convert zinc to micromoles per liter, multiply by 0.153.

chemical data were entered at the respective laboratories. Statistical analyses were performed using Stata version 11 statistical software (StataCorp LP). All means are reported with standard deviation. Differences between groups are reported with 95% CIs. Analysis was by randomized group, but no imputation was used for missing data. Linear mixed models were used for continuous variables and generalized linear mixed models were used for categorical variables, with HC as a random effect. Generalized linear mixed models via Poisson regression were used to estimate rate ratios (RRs). The RRs were calculated for anemia recovery rates. Separate analyses were performed at 12, 18, and 24 months. All models were adjusted for values at baseline (age 6 months).

RESULTS

A total of 3112 infants were listed and invited to participate in the study; 1350 children were recruited to the subsample (Figure 1). All 20 clusters remained in the study for its entirety. The groups were comparable for all household and participant characteristics at baseline (**Table 2**). Among the eligible children, 93.3% used Sprinkles; the median number of Sprinkles sachets consumed per month per child was 23.8 (range, 0-30).

ANEMIA AND Hb RESPONSE

Prevalence of any anemia (Hb level <11.0 g/dL [to convert to grams per liter, multiply by 10.0]) at baseline was 84.0% (**Table 3**). Anemia prevalence at 12 months was reduced by 20.6% in the intervention group compared with the control group. Prevalence of moderate anemia (Hb level <10.0 g/dL)[16] at 12 months was reduced by 27.1% (Table 3). At later follow-ups, there were no statistically significant differences in any anemia between the 2 groups. The overall mean (SD) baseline Hb level was 10.03 (0.91) g/dL and did not differ between the groups. Mean Hb levels increased significantly from baseline to 12 and 18 months in the intervention group compared with the control group by 0.61 and 0.22 g/dL, respectively, with no statistically significant difference between the groups at 24 months.

For children who were anemic at baseline (Hb level <11.0 g/dL), the rate of recovery from anemia at 12 months was 94 of 330 children (28.5%) in the intervention group compared with 27 of 350 children (7.7%) in the control group (RR = 0.84; 95% CI, 0.73-0.96; P = .01). For moderately anemic children at baseline (Hb level <10.0 g/dL), the rate of recovery from anemia at 12 months was 77 of 109 children (70.6%) in the intervention group vs 50 of 141 children (35.5%) in the control group (RR = 2.13; 95% CI, 1.57-2.88; P < .001). At later follow-ups, there were no statistically significant differences between the groups.

Sprinkles had a similar proportional effect in decreasing anemia whether the child had an Hb disorder or not at 12 months (20.9% for no genetic Hb disorder vs 16.8% with an Hb disorder; P = .46).

Table 3. Anemia and Mean Hemoglobin Level as Primary Outcomes

Study Point	Control	Intervention	Difference (95% CI)[a]	RR (95% CI)	*P* Value[b]	ICC
Anemia, No./total No. (%)						
Hemoglobin <11.0 g/dL						
Baseline, 6 mo	411/491 (83.7)	410/486 (84.4)				
End, 12 mo	410/484 (84.7)	294/441 (66.7)	20.6 (9.4 to 30.2)	0.76 (0.64 to 0.89)	.001	0.000
Follow-up, 18 mo	287/397 (72.3)	231/361 (64.0)	10.0 (–2.9 to 20.6)	0.86 (0.71 to 1.04)	.12	0.000
Follow-up, 24 mo	259/457 (56.7)	219/443 (49.4)	8.9 (–1.2 to 17.2)	0.84 (0.70 to 1.02)	.08	0.000
Hemoglobin <10.0 g/dL						
Baseline, 6 mo	238/491 (48.5)	218/486 (44.9)				
End, 12 mo	229/484 (47.3)	95/441 (21.5)	27.1 (21.0 to 31.8)	0.43 (0.33 to 0.56)	<.001	0.000
Follow-up, 18 mo	109/397 (27.5)	93/361 (25.8)	3.3 (–5.4 to 9.7)	0.88 (0.65 to 1.20)	.42	0.000
Follow-up, 24 mo	68/457 (14.9)	55/443 (12.4)	–0.9 (–11.3 to 9.4)	0.88 (0.60 to 1.30)	.52	0.000
Hemoglobin level, mean (SD), g/dL						
Baseline, 6 mo	9.98 (0.93)	10.08 (0.89)				
End, 12 mo	10.00 (1.00)	10.58 (0.88)	0.61 (0.47 to 0.74)		<.001	0.016
Follow-up, 18 mo	10.43 (0.99)	10.58 (0.90)	0.22 (0.08 to 0.37)		.003	0.014
Follow-up, 24 mo	10.79 (0.88)	10.90 (0.88)	0.10 (–0.01 to 0.22)		.08	0.002

Abbreviations: ICC, intraclass correlation coefficient; RR, rate ratio.
SI conversion factor: To convert hemoglobin to grams per liter, multiply by 10.0.
[a] Mean difference between groups (expressed as percentage for anemia and as grams per deciliter for hemoglobin level; with 95% CI), adjusted for cluster design and values at baseline (age 6 months).
[b] *P* value for comparison between groups, adjusted for values at baseline (age 6 months).

Table 4. Zinc, Retinol Binding Protein, and Ferritin Concentrations as Secondary Outcomes

	Mean (SD)			
Study Point	Control	Intervention	Difference (95% CI)[a]	*P* Value[b]
Zinc, µg/dL				
Baseline, 6 mo	63.4 (13.1)	63.4 (13.7)		
End, 12 mo	62.7 (11.1)	66.0 (15.7)	2.88 (0.26 to 5.42)	.03
Follow-up, 18 mo	63.4 (15.0)	64.7 (11.8)	0.59 (–1.44 to 2.68)	.56
Follow-up, 24 mo	63.4 (10.5)	62.7 (12.4)	–0.13 (–2.55 to 2.22)	.89
Retinol binding protein, µmol/L				
Baseline, 6 mo	1.1 (0.2)	1.1 (0.2)		
End, 12 mo	1.1 (0.2)	1.2 (0.3)	0.07 (0.03 to 0.11)	<.001
Follow-up, 18 mo	1.2 (0.2)	1.2 (0.3)	0.05 (0.01 to 0.09)	.008
Follow-up, 24 mo	1.2 (0.3)	1.2 (0.3)	0.01 (–0.10 to 0.07)	.72
Ferritin, ng/mL				
Baseline, 6 mo	39.5 (27.2)	41.1 (28.9)		
End, 12 mo	19.1 (17.3)	29.0 (21.2)	10.4 (6.3 to 14.5)	<.001
Follow-up, 18 mo	21.0 (14.8)	25.8 (16.7)	5.5 (1.4 to 9.5)	.008
Follow-up, 24 mo	33.1 (17.7)	33.7 (17.2)	0.3 (–3.3 to 4.0)	.85

SI conversion factors: To convert zinc to micromoles per liter, multiply by 0.153; to convert ferritin to picomoles per liter, multiply by 2.247.
[a] Mean difference between groups (with 95% CI), adjusted for infection by excluding cases with α1-acid glycoprotein levels greater than 1 g/L and C-reactive protein levels greater than 5 mg/L (to convert to nanomoles per liter, multiply by 9.524) and adjusted for values at baseline (age 6 months).
[b] *P* value for comparison between groups, adjusted for values at baseline (age 6 months).

MICRONUTRIENT RESPONSE

The overall mean (SD) baseline serum zinc concentration was 63.4 (13.7) µg/dL (**Table 4**). There was a significant difference in the mean (SD) serum zinc concentration in the intervention group compared with the control group at 12 months (*P* = .03) but no statistically significant difference at 18 or 24 months. After adjusting for baseline values and infection by excluding children with AGP levels greater than 1 g/L and CRP levels greater than 5 mg/L, there was no statistically significant difference in the prevalence of zinc deficiency (zinc concentration <64.7 µg/dL)[11] for the intervention group compared with the control group at 12 months or at later follow-ups (**Table 5**).

The overall mean (SD) baseline RBP concentration was 1.1 (0.2) µmol/L (Table 4). The prevalence of vitamin A deficiency (RBP concentration <0.7 µmol/L),[17] again after excluding children with elevated CRP and AGP concentrations, was low (<3.5% at any time). There was no statistically significant difference between the groups at any time (Table 5).

The overall mean (SD) ferritin concentration was 40.3 (28.0) ng/mL (to convert to picomoles per liter, multiply by 2.247) at baseline. Iron deficiency prevalence, as measured by a ferritin concentration lower than 12 ng/mL in the absence of infection,[18] was 10.1% at baseline (Table 5). There was a significant difference in iron deficiency for the intervention group vs the control group

Table 5. Iron, Zinc, and Vitamin A Deficiencies as Secondary Outcomes

Study Point	No./Total No. (%)		Difference, % (95% CI)[a]	RR (95% CI)	*P* Value[b]
	Control	Intervention			
Iron deficiency, ferritin <12 ng/mL[c]					
Baseline, 6 mo	47/384 (12.2)	35/380 (9.2)			
End, 12 mo	117/273 (42.9)	46/242 (19.0)	23.5 (15.6 to 29.1)	0.45 (0.32 to 0.64)	<.001
Follow-up, 18 mo	83/253 (32.8)	49/243 (20.2)	11.6 (2.6 to 17.9)	0.65 (0.45 to 0.92)	.02
Follow-up, 24 mo	22/226 (9.7)	15/204 (7.4)	2.3 (–4.5 to 5.9)	0.76 (0.39 to 1.47)	.41
Zinc deficiency, zinc <64.7 μg/dL[c]					
Baseline, 6 mo	223/370 (60.3)	217/370 (58.7)			
End, 12 mo	237/383 (61.9)	186/342 (54.4)	5.2 (–7.6 to 15.7)	0.92 (0.75 to 1.12)	.40
Follow-up, 18 mo	212/359 (59.1)	187/337 (55.5)	4.0 (–8.9 to 14.5)	0.93 (0.75 to 1.15)	.51
Follow-up, 24 mo	179/313 (57.2)	167/290 (57.6)	1.6 (–13.1 to 13.2)	0.99 (0.79 to 1.25)	.94
Vitamin A deficiency, RBP <0.7 μmol/L[c]					
Baseline, 6 mo	8/384 (2.1)	4/380 (1.1)			
End, 12 mo	5/388 (1.3)	2/349 (0.6)	0.3 (–4.7 to 1.1)	0.77 (0.13 to 4.60)	.77
Follow-up, 18 mo	4/363 (1.1)	5/346 (1.4)	–0.4 (–5.7 to 0.8)	1.38 (0.31 to 6.16)	.62
Follow-up, 24 mo	9/314 (2.9)	10/291 (3.4)	–0.7 (–8.7 to 1.8)	1.25 (0.39 to 4.03)	.70

Abbreviations: RBP, retinol binding protein; RR, rate ratio.
SI conversion factors: To convert ferritin to picomoles per liter, multiply by 2.247; to convert zinc to micromoles per liter, multiply by 0.153.
[a] Mean difference between groups (with 95% CI), adjusted for values at baseline (age 6 months).
[b] *P* value for comparison between groups, adjusted for values at baseline (age 6 months) and adjusted for infection (excluding cases with α1-acid glycoprotein concentration >1 g/L or C-reactive protein concentration >5 mg/L [to convert to nanomoles per liter, multiply by 9.524]).
[c] Adjusted for infection (excluding cases with α1-acid glycoprotein concentration >1 g/L or C-reactive protein concentration >5 mg/L).

at 12 and 18 months but not at 24 months. The risk of moderate iron deficiency anemia, as measured by an Hb level lower than 10.0 g/dL and a ferritin concentration lower than 12 ng/mL in the absence of infection, was reduced in the intervention group at 12 months by 70% (RR = 0.30; 95% CI, 0.18-0.52; $P < .001$) and at 18 months by 55% (RR = 0.45; 95% CI, 0.02-0.23; $P = .02$) but not at 24 months (RR = 0.70; 95% CI, 0.29-1.72; $P = .44$). The risk of non–iron deficiency anemia was also reduced at 12 months by 50% (RR = 0.50; 95% CI, 0.33-0.76; $P = .001$) but not at 18 months (incidence RR = 1.11; 95% CI, 0.67-1.83; $P = .67$) or 24 months (incidence RR = 0.76; 95% CI, 0.43-1.34; $P = .34$).

ANTHROPOMETRY

There was no statistically significant difference between the intervention and control groups for any of the anthropometric variables at any time (**Table 6**). The prevalence of underweight and stunting increased steadily from ages 6 to 24 months.

COMMENT

We have shown that home fortification with micronutrient Sprinkles along with IYCF education in rural Cambodia reduces anemia and iron deficiency compared with IYCF education alone. We achieved high adherence rates, confirming the acceptability of micronutrient powders found in other studies,[9,19] and have shown that the program can be implemented through existing government health structures. Furthermore, our study strengthens the findings from the Cambodian foodlet trial[20] that children with a genetic Hb disorder can use iron supplementation and micronutrients effectively to reduce anemia.

As recently called for,[21,22] our study reported on the prevalence of deficiencies of iron, zinc, and vitamin A in the absence of infection, as well as anemia prevalence. We also followed up with the children for 12 months after intervention and show the effectiveness of Sprinkles on a population with genetic Hb disorders. We provided IYCF education to both arms, which promoted the consumption of animal-source foods and adequate quantity and frequency of complementary foods. This design may have resulted in a smaller observed difference between our groups for anemia and no statistically significant difference in growth.

Efficacy trials in Cambodia and elsewhere have shown that Sprinkles are as efficacious as iron drops in reducing anemia, with fewer adverse effects and better acceptance.[9] In a meta-analysis, anemia risk (Hb level <10.0 g/dL) was halved, although the effects on plasma zinc and vitamin A levels were mixed.[9] Our effectiveness study showed a 24% risk reduction in any anemia (Hb level <11.0 g/dL) and a 57% risk reduction in moderate anemia (Hb level <10.0 g/dL) at 12 months. The prevalence of anemia was significantly lower in both groups at ages 18 and 24 months, consistent with the age-related pattern seen in the Demographic and Health Surveys in Cambodia[2,3] and elsewhere.[23] Other follow-up studies have been restricted to those children whose anemia was successfully treated[24-26] or have ignored the characteristic age-related improvements in Hb level.

There was a low prevalence of iron deficiency at age 6 months (baseline, Table 5) based on low serum ferritin values in the absence of infection. The prevalence of iron deficiency in both groups had increased by age 12 months. This age-related increase has been reported in other Sprinkles studies, in Cambodia[8] and elsewhere,[27] and is attributed to increased iron requirements of the growing infant and insufficient available iron from non-

Table 6. Growth Impairments as Secondary Outcomes

Anthropometric Measure	No./Total No. (%)		Difference, % (95% CI)[a]	*P* Value[b]
	Control	Intervention		
Underweight[c]				
Baseline, 6 mo	78/529 (14.7)	66/528 (12.5)		
End, 12 mo	79/498 (15.9)	83/504 (16.5)	−2.4 (−6.1 to 1.2)	.19
Follow-up, 18 mo	100/492 (20.3)	107/481 (22.2)	−2.8 (−7.4 to 1.8)	.23
Follow-up, 24 mo	139/499 (27.9)	116/490 (23.7)	3.2 (−2.7 to 9.1)	.28
Stunting[d]				
Baseline, 6 mo	60/530 (11.3)	70/528 (13.3)		
End, 12 mo	95/498 (19.1)	92/504 (18.3)	3.5 (−0.7 to 7.8)	.10
Follow-up, 18 mo	146/492 (29.7)	164/481 (34.1)	−2.2 (−7.6 to 3.3)	.44
Follow-up, 24 mo	196/499 (39.3)	180/490 (36.7)	0.7 (−5.9 to 7.4)	.83
Wasting[e]				
Baseline, 6 mo	27/528 (5.1)	23/526 (4.4)		
End, 12 mo	38/498 (7.6)	36/504 (7.1)	−0.3 (−3.5 to 2.9)	.84
Follow-up, 18 mo	39/492 (7.9)	30/481 (6.2)	1.4 (−2.2 to 5.1)	.43
Follow-up, 24 mo	24/499 (4.8)	28/490 (5.7)	−0.2 (−3.2 to 2.6)	.85

[a] Mean difference between groups (with 95% CI) and adjusted for values at baseline (age 6 months).
[b] *P* value for comparison between groups, adjusted for values at baseline (age 6 months).
[c] Weight for age less than −2 *Z* score.
[d] Length for age less than −2 *Z* score.
[e] Weight for length less than −2 *Z* score.

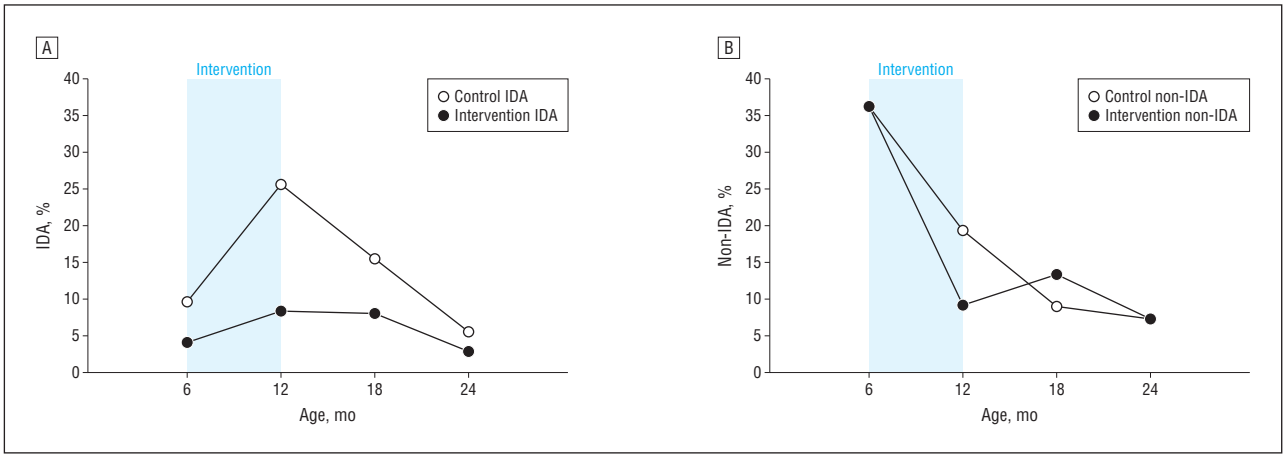

Figure 2. Prevalence of iron deficiency anemia (IDA) (hemoglobin level <10.0 g/dL [to convert to grams per liter, multiply by 10.0], ferritin concentration <12 ng/mL [to convert to picomoles per liter, multiply by 2.247] in the absence of infection) (A) and non-IDA (hemoglobin level <10.0 g/dL, ferritin concentration >12 ng/mL) (B) by study group. There were significant differences in the prevalence of IDA at 12 and 18 months ($P < .001$ and $P = .02$, respectively) (A) and in the prevalence of non-IDA at 12 months ($P = .001$).

milk foods.[28] Importantly, the increase was significantly less in the intervention group compared with the control group, confirming a treatment effect of Sprinkles. This treatment effect persisted at 18 months but not 24 months. In the Cambodia efficacy trial,[8] Sprinkles were given for 12 months from ages 6 to 17 months, with reported reductions in anemia (Hb level <11.0 g/dL) and moderate anemia (Hb level <10.0 g/dL) of 38.5% and 13.8%, respectively. The prevalence of iron deficiency at 18 months in this earlier study (albeit not adjusted for infection) was much lower compared with our findings (13.8% vs 21.7%, respectively), suggesting that perhaps giving Sprinkles for longer than 6 months is more effective in reducing anemia and iron deficiency.

While much of the impact on anemia at 12 months was through the relative reduction of iron deficiency, this is the first Sprinkles study to our knowledge to show an effect on non–iron deficiency anemia for moderate anemia (**Figure 2**). This positive effect on non–iron deficiency anemia was observed only at 12 months and did not persist at 18 or 24 months.

We used serum ferritin to detect iron deficiency in our population, as recommended by the World Health Organization.[29] The soluble transferrin receptor level, elevated in iron deficiency and unaffected by inflammation or infection,[30] was elevated in children with certain genetic Hb disorders, consistent with reports elsewhere.[31] Hence, the soluble transferrin receptor is of limited use as a biomarker of tissue iron levels in our population.[32]

We found a significant but small increase in serum zinc concentration due to Sprinkles at 12 months (Table 4). Moreover, although not statistically significant, there was an 8% reduced risk of zinc deficiency at 12 months. Notwithstanding the higher zinc content (as zinc glu-

conate) of our Sprinkles compared with that used earlier (10 vs 4.5 mg, respectively),[27] the prevalence of low serum zinc concentrations in the intervention group at 12 months was still above the level (>20%) indicative of population zinc deficiency.[11] Such a modest response in serum zinc concentration despite the high prevalence of zinc deficiency and stunting among the infants at baseline is disappointing (Table 5 and Table 6). However, our finding is consistent with earlier reports for zinc fortificants in cereal-based porridges compared with aqueous supplements.[33] Poor zinc absorption arising from either high-phytate cereal-based porridges fortified with zinc or interference by iron fortificants has been implicated,[11] although in our study the fortified complementary foods were rice based with low phytate content[34] and an iron to zinc ratio (1.25:1) proven to not adversely affect absorption of zinc fortificants.[35]

The overall prevalence of vitamin A deficiency was very low at any time (<3.5%), with our RBP levels corresponding to the serum retinol range known to be homeostatically controlled.[36] Therefore, our study did not show a significant reduction in vitamin A deficiency due to Sprinkles (Table 5). A national micronutrient survey in 2000 showed a 22% prevalence of vitamin A deficiency,[37] although recently the coverage of the national vitamin A supplementation program in Cambodia has improved, which may account for this discrepancy.[2,3,38]

The lack of positive growth response due to Sprinkles was disappointing but consistent with other micronutrient Sprinkles studies.[9,21]

This study provides clear evidence supporting the rollout of Sprinkles as a micronutrient intervention in Cambodia and similar settings. Because the observed reduction in anemia was not sustained beyond the intervention period, a critical question is the optimal duration of Sprinkles implementation. Research indicates that the first 2 years of life are the period of greatest vulnerability[39] and the most effective period for nutrition interventions. Our findings and the results of efficacy trials of Sprinkles given beyond infancy provide a compelling rationale to sustain Sprinkles at least until age 18 months and preferably until age 24 months to cover the period of greatest vulnerability, after which the prevalence of anemia and iron deficiency has been shown to decline. It seems reasonable that such a policy be adopted immediately and monitored to confirm ongoing benefits of Sprinkles in children.

Accepted for Publication: April 3, 2012.
Published Online: July 16, 2012. doi:10.1001/archpediatrics.2012.1003
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Financial Disclosure: None reported.
Funding/Support: This work was supported by the A2Z Micronutrient Project, Academy for Educational Development, US Agency for International Development, Cambodia Health Sector Support Projects I & II (World Bank, Department for International Development, Australian Agency for International Development, United Nations Children's Fund, United Nations Population Fund, and French Cooperation), World Health Organization Cambodia, and Global Alliance for Improved Nutrition. Dr Jack is a recipient of the Lady King Scholarship, University of Otago, Dunedin, New Zealand.
Role of the Sponsors: The A2Z Micronutrient Project advisors reviewed the study protocol. No sponsors had any role in the design or conduct of the study; collection, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.
Online-Only Material: This article is featured in the *Archives* Journal Club. Go to http://www.archpediatrics.com to download teaching PowerPoint slides.
Additional Contributions: We thank the children and their caregivers, families, and communities of Svay Rieng Operational Health District who participated enthusiastically in this study. We acknowledge the Provincial Health Department, Operational Health District and Health Centre staff, and especially the Village Health Support Groups for their active roles in implementing this study. We acknowledge the dedicated team of surveyors and phle-

botomists, the Svay Rieng Provincial Hospital, Mom Chandara, MSc, and the National Institute of Public Health Laboratory, National Paediatric Hospital Laboratory, and their staffs for the use of their facilities. The Good Food for Children Study Steering Committee, the National Nutrition Program, and Helen Keller International Cambodia staff played a crucial role in overseeing the implementation of this study, data collection, and data entry. We acknowledge the support of Robert Johnston, MPH, and Phil Harvey, PhD, for their work in the earlier stages of the study and Juergen Erhardt, PhD, and Karl Bailey, PhD, for their laboratory expertise. Finally, we acknowledge the National Institute of Public Health and National Maternal and Child Health Centre of the Ministry of Health Cambodia for their support of this study.

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